canusa

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United States
Spruce Budworms
Program

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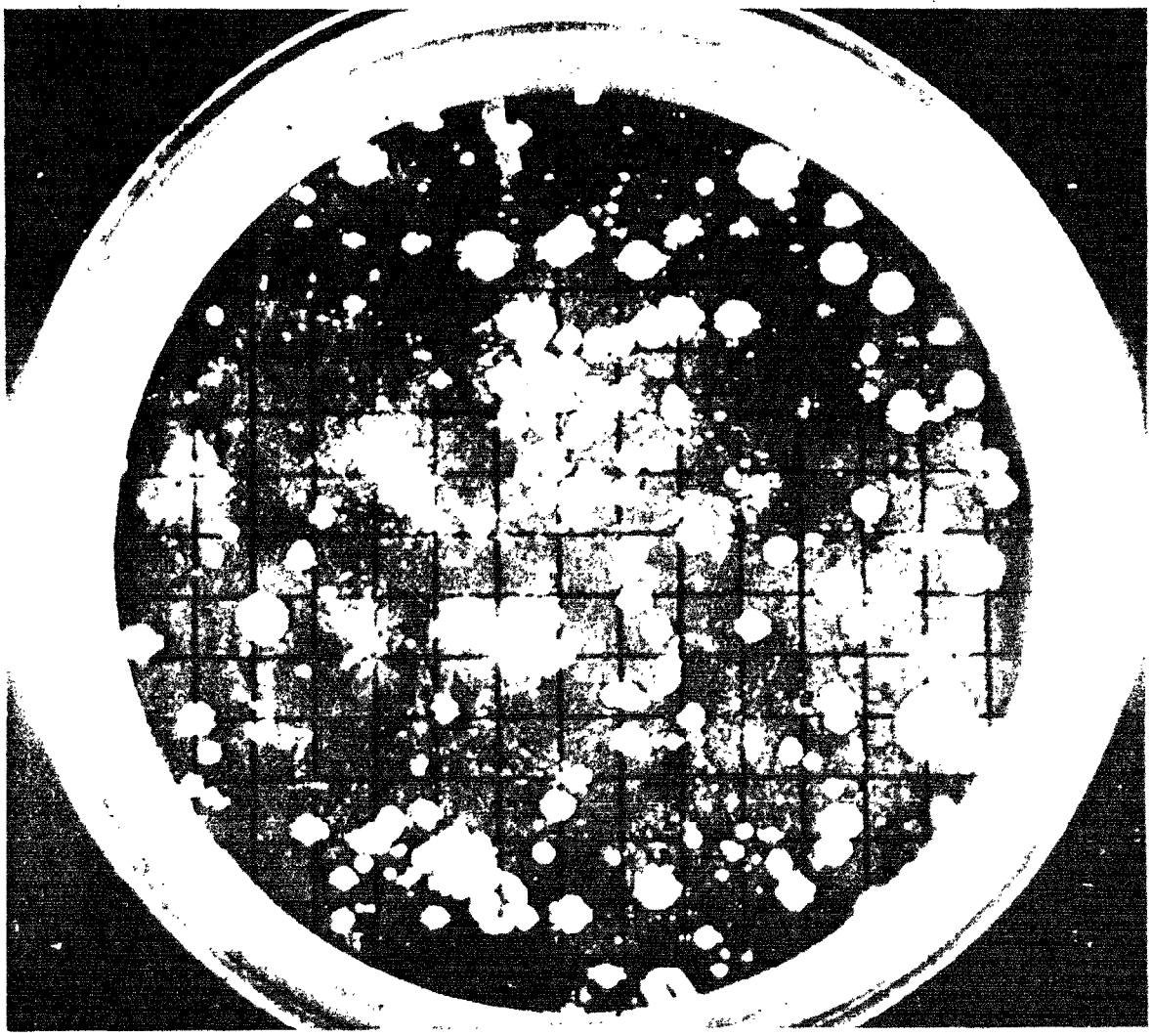
Proceedings

Symposium: Microbial control of of spruce budworms and gypsy moths



PREFACE

The Canada-United States Spruce Budworms R&D Program (CANUSA) was created in 1977, when the United States Department of Agriculture and the Canadian Department of Environment agreed to cooperate in an expanded and accelerated research and development effort. CANUSA was aimed specifically at the spruce budworm in the east and western spruce budworm in the west. The broad objective was to design and evaluate tactics and strategies for not only controlling budworms but also managing budworm infested forests, thus helping forest managers attain their objectives in an economically and environmentally acceptable manner.

The spruce budworm and the gypsy moth are the two most damaging insects to ever infest North American forests. Both are now, and have been for several years, in outbreak status, with no good hope for relief in sight. Each of them has been the target for millions of acres of aerial insecticide sprays and, since most of the insecticides used have been chemicals, there has been increasing opposition to the programs from groups concerned for the environment.

CANUSA and the Northeastern Forest Experiment Station have supported much research and development work on microbial sprays to suppress high populations of these two insects. State and Provincial land managers on both sides of the border are showing increased interest, and acceptance of microbial agents as a substitute for, or as a supplement to, chemical sprays. *Bacillus thuringiensis* (*B.t.*) and, to a lesser extent virus (for gypsy moth), are currently the only registered microbial insecticides available for use by the forest pest manager. Great improvements have been made in the technology for each of these two microbials in the past five years and further strides are expected in the near future. The use of microbial sprays in forest pest control will increase as opposition to chemicals becomes more intense and performance of the microbials further increases. Land owners realize that intelligent forest management must involve protection of their investment in the resource, which under insect outbreak conditions translates into direct control with aerial sprays.

The goal of the symposium was to present state-of-the art information on the development and use of microbials insecticides to pest control officials, researchers and university personnel. This was one of a series of workshops and symposia organized as part of the technology transfer effort of the CANUSA Program. We hope the information contained in the following papers will be of interest and use to forest protection people and forest managers everywhere.

DAVID G. GRIMBLE
FRANKLIN B. LEWIS

Co-Chairmen and Symposium Coordinators

PROCEEDINGS

SYMPOSIUM: MICROBIAL CONTROL OF SPRUCE BUDWORMS AND GYPSY MOTHS

Sponsored by:

CANADA-UNITED STATES SPRUCE BUDWORMS R&D PROGRAM

NORTHEASTERN FOREST EXPERIMENT STATION, USDA FOREST SERVICE

April 10 - 12, 1984
Windsor Locks, CT

Each contributor submitted camera copy and is responsible for the accuracy and style of his or her paper. The statements of contributors from outside the U.S. Department of Agriculture may not necessarily reflect the policy of the Department.

PROGRAM

Tuesday, April 10, 1984

OPENING Remarks: Denver P. Burns, Director, Northeastern Forest Experiment Station, USDA Forest Service, Broomall, PA

Purpose of Symposium: F. B. Lewis, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

USER'S EXPERIENCES - GOOD AND BAD

SPRUCE BUDWORMS

CANADIAN USER PERSPECTIVES ON Bt USE for PROTECTION AGAINST SPRUCE BUDWORMS.
H. J. Irving, Managing Director, Forest Protection Ltd., Fredericton, N.B.

OPERATIONAL EXPERIENCES with Bt in the Eastern United States.
Henry Trial, Jr., Director of Survey and Assessment, Old Town, ME

Four engine aircraft experience in the application of Bacillus thuringiensis against the spruce budworm in Quebec.
Louis Dorais, Quebec Spray Program Coordinator, Quebec

Review of foliage protection spray operations against the spruce budworm with Bacillus thuringiensis Kurstakii from 1980 to 1983 in Nova Scotia and New Brunswick, Canada.
E. G. Kettela, Forestry Officer, Maritimes Forest Research Centre, Fredericton, N.B.

Comparison of Bt formulations against the spruce budworm.
Lew McCreery, Imants Millers and Dennis Souto, Forest Pest Management, USDA Forest Service, Durham, NH; and Bruce Francis, Director of Forestry, Passamaquoddy Indian Forestry Department, Princeton, ME

GYPSY MOTHS

Pennsylvania's experiences with microbial control of the gypsy moth.
James O. Nichols, Chief, Division of Forest Pest Management, Pennsylvania Bureau of Forestry, Middletown, PA

Microbial control of the gypsy moth in recently infested states: Experiences and Expectations.
Timothy C. Tigner, Virginia Division of Forestry, Charlottesville, VA

Some negative aspects of using Bacillus thuringiensis Berliner in operational programs against the gypsy moth (Lepidoptera: Lymantriidae).
John D. Kegg, Chief, Bureau of Entomology, New Jersey Department of Agriculture, Trenton, N.J.

Overall aspects of B.t. in forest service cooperative gypsy moth suppression projects.
Noel F. Schneeberger, Forest Pest Management, USDA Forest Service, Morgantown, WV

RECENT FIELD RESEARCH EXPERIENCE

Spruce Budworms

Recent field experiences with Bacillus thuringiensis in Canada and research needs.
Oswald N. Morris, Agriculture Canada Research Station, Winnipeg, Manitoba

Recent field research experiences with Bt against spruce budworm in the eastern U.S.
John B. Dimond, Professor of Forest Entomology, University of Maine, Orono, ME

Field tests with a highly concentrated Bacillus thuringiensis formula against spruce budworm, Choristoneura fumiferana (Clem.) (Lepidoptera: Tortricidae).
W. A. Smirnoff, Laurentian Forest Research Centre, Sainte-Foy, Quebec

Status of viruses as biological agents for spruce budworms.
J. C. Cunningham, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ont.

Erynia radicans potential mycoinsecticide for spruce budworm control.
Richard S. Soper, ARS Incect Pathology Research Unit, USDA.

The effect of Erynia radicans on food consumption, utilization and fecundity by the spruce budworm, Choristoneura fumiferana.
Abdul K. A. Mohamed, Lucius Lewis and Denise Lewis, Biology Department, Jackson State University, Jackson, MS

GYPSY MOTHS

Recent field studies on the use of Bacillus thuringiensis to control the gypsy moth (Lymantria dispar L.).
Normand D. Dubois, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

Interactions between microbial agents and gypsy moth paratsites.
Ronald M. Weseloh, Connecticut Agricultural Experiment Station, New Haven, CT

Gypchek[r]: Past and future strategies for use.
J. D. Podgwaite, Northeastern Forest Experiment Station, USDA Forest Ervice, Hamden, CT

Recent field research using microbial insecticides against gypsy moth.
Lawrence P. Abrahamson and Donald A. Eggen, State University of New York, Syracuse, NY

Wednesday, April 11, 1984

New Research Developments (What more is needed)

Selection of new more potent strains of Bacillus thuringiensis for use against gypsy moth and spruce budworm .
Normand R. Dubois, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

Chitinase producing B.t. strains.
Haim B. Gunner, Matthew Zimet and Sarah Berger, Department of Environmental Sciences, University of Massachusetts, Amherst, MA

The biochemistry of the protein crystal toxin of Bacillus thuringiensis.
Paul G. Fast, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ont.

Selection of active strains of the gypsy moth nuclearpolyhedrosis virus.
M. Shapiro and E. Dougherty, Agricultural Research Service, Otis, MA

Pathways of nucleopolyhedrosis virus infection on the gypsy moth, Lymantria dispar.
K. S. Shields, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

Enzyme immunoassays for detection of gypsy moth nuclearpolyhedrosis virus.
Michael Ma, Department of Entomology, University of Maryland, College Park, MD

Genetic engineering of microbial pesticides.
Bruce C. Carlton, Professor of Molecular and Population Genetics, University of Georgia, Athens, GA

New Developments in Commercially Produced Microbials

Recent developments (1980-1983) in the application of Bacillus thuringiensis for forest insect control.
Robert J. Cibulsky, Abbott Laboratories[1]

Developments on commercially produced microbials at Biochem Products.
John Lublinkhof and Douglas H. Ross, Biochem Products, Montchanin, DE

Commercial production of microbials by Reuter Laboratories, Inc., for control of the gypsy moth and the spruce budworm.
F. D. Obenchain, Reuter Laboratories, Gainesville, VA

Recent developments in the Zoecon Corporation and the thuricide[r] forestry formulations.
William R. Beck, Zoecon Corporation, State College, PA

DISCUSSION PANEL - FORMULATION AND APPLICATION OF MICROBIALS FOR SBW AND GM CONTROL

Co-Chairmen: Jack A. Armstrong, Canadian Forestry Service, Ottawa, Ontario

William G. Yendol, Professor, Pesticide Research Laboratory, The Pennsylvania State University, University Park, PA

Panel Speakers: Bruce McGauley, Supervisor Pest Control Section, Ontario Ministry of Natural Resources, Maple, Ontario

Chester M. Himel, Professor, Entomology Department, University of Georgia, Athens, GA

David B. Smith, Professor, Agricultural and Biological Engineering Department, Mississippi State University, Mississippi State, MS

Michel Pelletier, f. eng., Quebec-Energy and Resources Department, Entomology and Pathology Service, Quebec, Quebec

Paul G. Fast, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario

John W. Barry, Program Manager of U.S.D.A. Forest Service, U.S. Army Cooperative R&D Program, Davis, CA

THURSDAY, APRIL 12, 1984

DISCUSSION PANEL - FIELD TEST DESIGN AND DATA ANALYSIS

Chairman: Daniel M. Schmitt, Program Manager, Canusa/US Spruce Budworms Program, USDA Forest Service, Broomall, PA

Panel Speakers: William E. Waters, Professor of Entomology and Forestry, University of California at Berkeley, Berkeley, CA

B. Leo Cadogan, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario

Gerald S. Walton, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

Coffee

DISCUSSION PANEL - STANDARDIZATION OF BIOASSAYS AND POTENCY DETERMINATIONS

Chairman: Howard T. Dulmage, Insect Pathology Research Unit, USDA, Brownsville, TX

Panel Speakers: Clayton C. Beegle, Insect Pathology Research Unit, USDA, Brownsville, TX

Norman R. Dubois, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

W. A. Smirnoff, Laurentian Forest Experiment Station,
St-Foy, Quebec

SYMPOSIUM WRAP-UP

Peter W. Orr, Staff Director, Forest Pest Management, USDA Forest Service, Broomall, PA

Frank B. Lewis, Northeastern Forest Experiment Station, USDA Forest Service, Hamden, CT

[1]No written paper submitted.

DENVER P. BURNS

Opening Remarks

Thank you, Dave (Grimble). Let me begin by saying that it is particularly fitting that this group meet at this point in time. First, it a fair guess that in the 1984 field season more forest acreage will be treated with microbial insecticides than in any previous year. Second, the research effort devoted to all phases of microbial insecticide formulation and application appear to have arrived at a unique stage when recent R&D is finding ready application -- I am thinking of B.t. -- current R&D, particularly on viruses, fungi, and parasites, is well along in development, and the possibility of genetically tailored biological insecticides is far more than a gleam in a microbiologist's eye. Momentum -- and that's what this group has -- incites achievement.

I also note with satisfaction that our private sector colleagues have a prominent place on the program. Their contributions and viewpoints, both formal and informal, in exchanges with other scientists provide a sense of "wholeness" to the meeting which reflects the informal network binding this group together.

In the same vein I also want to welcome our Canadian colleagues -- many of whom played key roles in the CANUSA program, and those scientists from distant states. Indeed, the geographic diversity of the group and its scientific composition -- engineers, physicists, chemists, and an array of biological expertise -- demonstrate how clearly effective interdisciplinary R&D can be when mutual respect and common goals guide the enterprise.

Another interesting feature of this Symposium is that it brings together experience and expertise on two major forest defoliators, budworm and gypsy moth. The Northeastern Station, while continuing its budworm effort after the CANUSA Program ends in the U. S., will be increasing its level of gypsy moth R&D. Consequently the Microbial Symposium will be of special interest to some of our scientists, and I am happy to say the Station will be publishing and distributing the Proceedings.

In brief I am looking forward to the deliberations of this meeting, so let's get on with it. Thank you.

SYMPOSIUM ON MICROBIAL CONTROL OF SPRUCE BUDWORM AND GYPSY MOTH

PURPOSE OF SYMPOSIUM

F. B. Lewis

Project Leader, Center for Biological Control of Forest Insects and Diseases, Northeastern Forest Experiment Station, Hamden, CT 06514.

The principal purpose of this Symposium is to take advantage of the CANUSA experience, which has engaged so many of us recently, to share and place in the context of our total experience, the current situation regarding microbial insecticides and their use. The exercise will be useful not only for information exchange -- the traditional purpose of symposia -- but also to sharpen and hone future research program planning for microbial insecticide research.

There are many similarities with the microbial component of the 1974-78 gypsy moth R. D. A. Program. Thus the combining of the two in this symposium, many here participated in that effort. Indeed in microbial R & D there are many common goals and problems, regardless of the target insect.

We hope the program - admittedly full packed - will stimulate discussions, friendly disagreements, and at the conclusion a clearer idea of where we stand, what has been done, what remains to be done, and the approaches to be taken. Hopefully a continuation of scientist to scientist interaction we have observed recently will be stimulated and the importance of the problems remaining will be underscored for the administrators present. We certainly hope the International cooperation fostered during the CANUSA program will serve as a model and that this conference will facilitate even more and better cooperation.

A section of this conference will present new research, where it is going, and how it should benefit microbial implementation against these two major insect pests. We will be hearing from the Industrial people and where they see their products and thrust are going. And most importantly we will be hearing from the users - those on the firing line - and how they assess microbials in meeting their problems.

Our workshop sessions will be slightly different than most and hopefully will capture all the various points of view. They are designed to stimulate open discussion. These workshops will be recorded "in toto" so that the Chairperson will have all statements, comments, opinions in hand to prepare a thorough exposition of the topic area and not miss the casual, but important comment from the floor.

We hope to have the Proceedings of this conference in your hands within a short time. This will depend upon the speakers and workshop chairpersons having their camera-ready copy in quickly if they are not already turned in.

Have a good meeting. We hope you find it enlightening, stimulating and valuable to your sphere of interest.

CANADIAN USER PERSPECTIVES ON BT USE FOR

PROTECTION AGAINST SPRUCE BUDWORM

H.J. Irving

Managing Director, Forest Protection Limited,
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Abstract

Reference is made to a recent inter-provincial review of the performance of present-day B.t. viz a viz conventional chemicals. The argument is presented that in the Canadian context its practical acceptability to resource managers remains highly jurisdictionally-specific for reasons over and above conventional technical assessments. The New Brunswick situation is reviewed as a case in point.

I want to say at the outset that the invitation to take part in this symposium was received with pleasure and surprise. Surprise because I believe this is the first or at best the second unsolicited opportunity ever extended to the New Brunswick B.t. user community to say something on its own behalf about the B.t. issue. The closest exception, in Boston a year ago, was also welcomed but was not the open forum that this one is.

I was pleased because it comes at a juncture in the evolution of attitudes and practice in budworm control when generalization about such questions as the viability of B.t. as an alternative to conventional insecticides is no longer enough. I confess to be a bit sensitive about this because in the past year or two it has been driven home to me very forcibly that New Brunswick's performance with B.t. and its alleged attitude toward it is not held in much esteem in some circles. I can agree that there is some justification for this by comparison with the record of our neighbors in Quebec, Maine and, indeed, Nova Scotia. But what I find particularly disturbing and what needs to be challenged is the accusation that this reflects an ignoble pro-chemical, anti-biological mindset that stubbornly ignores superior wisdom elsewhere. To believe that is to ignore a long record of at least decent, self-propelled attention to the question and to reveal a lack of appreciation of the most compelling reasons that ultimately come into play in the performance of individual jurisdictions.

What I was asked to address here, as the program states, are good and bad B.t. experiences in the eastern Canadian scene. My first intention was to approach this in a way that would underscore the point that judgments of good or bad, acceptability or unacceptability cannot be generalized in the Canadian context, or indeed across international boundaries. Outside experience can be an invaluable guide and technically-qualified advice absolutely imperative. But ultimate responsibility for choices of action or inaction as the case may be are the responsibility of the individual jurisdictions because it is there that accountability lies. Without pointing the finger more in one direction than the other Canadian forestry has historically experienced some difficulty with this distinction at both federal and provincial levels.

With these convictions in mind I had decided to attempt here a synopsis of the good and bad B.t. experiences in the four Canadian jurisdictions judged by the forest managers of those jurisdictions, but without editorial comment. As luck would have it I have been spared that job by the timely issuance of the proceedings of an invitational B.t. seminar organized by my New Brunswick colleague, Rod Carrow, last September. For those who have not received copies it contains assessments of B.t. user experience over the past three years in Ontario, Quebec, New Brunswick, Nova Scotia and Maine. The assessments are made on the basis of four criteria: efficacy, cost, operational peculiarities, and environmental status. This was the first such forest manager-oriented seminar to be held on B.t. in Canada and I particularly commend to you the summary provided by Dr. Carrow on pp.85-90. Capsulized, it says the following.

> (1) Cost remains the major obstacle to greater use.
>
> (2) There is promise of continuing decline in the cost of the product.
>
> (3) Application, and therefore over-all costs cannot be presented in a way that permits valid comparisons between jurisdictions. Relative cost of B.t. vs chemical use by jurisdiction provides a more meaningful statistic.
>
> (4) Recent product and application developments appear to be producing better and more consistent protection, particularly the use of higher BIU rates.
>
> (5) Certain inherent characteristics of B.t. ensure more difficult application problems.
>
> (6) The narrower timing window is a fact of life.
>
> (7) The faith of B.t. developers and users in its greater environmental safety is given insufficient and inconsistent recognition in regulatory circles.

From this applicator's position there is absolutely nothing to disagree with in this assessment. But where Dr. Carrow and I might part company would be in the validity of one of the stated objectives of his seminar, namely "to develop a consensus on the potential for expanded use of B.t. against spruce budworm". In my

pinion, as long as significant advantages to hemical usage remain, until B.t. usage becomes egislated, and while outbreaks continue to exist n their current scale, such consensus is an nrealistic expectation. What must be realized y the more technically-oriented community of .t. promoters is that the four areas of concern ddressed in Dr. Carrow's seminar, which are hose also conventionally addressed in eneralized reviews by the Canadian Forestry ervice and CANUSA do not constitute all of the ecessary ingredients of the decision making rocess in any jurisdiction. It is those extra, ighly jurisdictionally-specific factors that efy the development of the jurisdictionally-ransferable models of B.t. or chemical usage hat some managerially-detached opinion seems to avisage. Perhaps the following table will llustrate the point.

Perspective on Protection Need and Action
Eastern Canada 1983

	Ont.	Quebec	N.B.	N.S.
-S Infestations M/ha	9000	21000	5300	600
sessed need tensive otection	Minimal	High	Maximal	Mounting
rayed M/ha	3	1254	1495	21
Infestation Sprayed	0.04	6	28	3.5
t. Usage M/ha	3	46	10	21
B.t. Usage	87	4	0.7	100

It might also clarify the managerial rspective, and correct some misconceptions as ll, to examine the New Brunswick story as a se history.

When I took over the management of Forest otection Limited nine years ago I took over a raying program already designed and ready to . It was the largest, most complicated and st costly program attempted up to that time - 7 million hectares at a cost of 8.5 million llars. It had also, as I came to learn, been signed under the tightest budgetary constraints any previous program. One way of stretching e dollars was to resort to lower dosages. It s a gamble taken in the worst possible year. some of you will recall, and as the literature stifies, 1975 proved to be a year of such eptionally virulent budworm activity that eptionally poor spray results were experienced only throughout New Brunswick but in Quebec Maine as well. The results were reminiscent an earlier cost-cutting experiment in 1956; h case had to be followed by the largest grams ever undertaken up to that time, and in case of 1976 probably the largest program ever. At $15.9 million for 3.9 million hectares it was an experience that has left both government and industrial sponsors very distrustful of chancy and unproven tactics.

As it happens one of the five insecticides used in 1975 was B.t. at a single application of 8 BIUs on 1500 acres. It was an independent initiative of my predecessor who was eager to get B.t. experimentation underway in New Brunswick as well as Quebec. Forest Protection Limited had helped Dr. Smirnoff organize some of his first field trials three or four years previously and had volunteered to substitute B.t. on the watershed of the City of Saint John's water supply. It was turned down, incidentally, because of public apprehension about "germs".

As reported by Ed Kettela at the 1976 Abbott symposium the 1975 trial produced better results than a single chemical application and only slightly less protection than a double. Although I had had nothing to do with the venture I soon became aware that B.t. could be a very touchy subject. The Abbott proceedings also record the late Dr. J.J. Fettes' opinion that such ad hockery was scientific mockery and that such user initiative represented dissipation of effort. Perhaps that is why that result has yet to appear on any CFS score card of "positive" results.

But more important to the case history is that that was the one and only decision taken for or against operational B.t. use by the manager of Forest Protection Limited. To understand the implications of this it is necessary to realize that Forest Protection Limited is a government-industry consortium with the sole purpose of conducting spruce budworm spraying on behalf of its sponsors. Shares in the corporation are owned 90% by the province, 10% by industry. Representation on the Board of Directors to which I am responsible is preponderantly Department of Natural Resources. Until the Department of Natural Resources took over responsibility for biological surveys and assessments in 1983 company decisions relied upon estimates and advice provided by the Canadian Forestry Service through the Maritimes Forest Research Centre. Whatever opinion I, as Managing Director of Forest Protection Limited have had about the viability of B.t. usage in New Brunswick has closely reflected that advice. Under the new Crown Lands and Forests Act industry decides upon and pays for protection on its freehold land: the Department of Natural Resources is responsible for Crown lands and small private ownerships.

As I have said, the evident inadequacy of spraying in 1975 greatly alarmed its sponsors. One major industrial sponsor stated publicly that the formulations used in that year "were no more effective than dishwater". This, combined with the revelation of the Reye's Syndrome hypothesis pushed budworm management onto the floor of the provincial legislature with demands that the government ensure sufficient research and development to resolve the problem. It commissioned Dr. Gordon Baskerville to advise on budworm control alternatives and a substantial

research and development budget was erected from federal/provincial shared-cost funds. My Board of Directors authorized a Forest Protection Limited research and development budget of up to 3% of total spray budgets.

The way in which this funding came to be applied reflected in large part Dr. Baskerville's advice that the only foreseeable alternatives to conventional protection practice were B.t. and pheromones and that the best pay-back for immediate research and development investment would be in improved technology for conventional practice. Forest Protection Limited then pressed ahead with already-existing endeavors to develop a provincially-based interest and competence in spray delivery technology which has included B.t. usage. The Department of Natural Resources directed some funding to CFS research on budworm dispersal and modelling and deliberated how resources should be directed toward the B.t. and pheromone areas. The decision came down in favor of pheromones. Influenced somewhat by the puzzling antipathy toward user initiative in B.t. trials exposed at the Abbott symposium, and a great deal by the heroic B.t. investments being made by Quebec, it was reasoned that the wiser course would be to concentrate our province's support on pheromone R&D. That recommendation was accepted but in retrospect we realize that it was politically and otherwise not the best. It has been misinterpreted as a lack of faith in B.t. without earning much appreciation for some heavy investments in the pheromone alternative. On the B.t. scorecard, by all means score that episode as a New Brunswick mistake.

So much for that regret, how else does the New Brunswick B.t. record stack up? If numbers mean anything we have sprayed a few more hectares than Ontario, about one quarter as many as Nova Scotia and a pittance compared to Quebec. We have attempted to protect more private woodlots than the others and have severely tested B.t.'s suitability for very sick trees in the process. In more healthy and less difficult targetting situations I believe our record for success or failure by CANUSA standards is not that much different from equivalent experience elsewhere. The tentative indication at this point is that B.t. performed as well or better than fenitrothion for woodlot protection last year. This is encouraging in one way but cold comfort in another, because neither result was that good. As for attitude, this applicator for one has not doubted for some time that given a break with some highly unpredictable and uncontrollable variables, the right application technology and enough money, B.t. could give decent protection. That seemed evident enough in 1975 and from what could be observed elsewhere. We have not been entirely derelict in testing its usability for our needs, witness our efforts in 1979, 1980, 1982 and 1983. But we have been equivocal about spending money in the same old way again and again with no likelihood that we would be the wiser whatever the result. We have not been unwilling to pull our weight in R&D that we could believe in, witness our activity in delivery technology and in shared-cost fundamental research with CANUSA and the CFS.

So what's the problem? As I have already stated it comes back to some totally non-scientific facts of managerial life. It is much easier to accept a greater risk of failure, a higher per hectare cost, and be environmentally noble under an Ontario model than a New Brunswick one. There is no problem accepting limited forest protection with B.t. in Nova Scotia, when it is the only insecticide permitted. It must be a wonderful experience to our colleagues in Quebec to be told to use B.t. for 40% of their 1984 program and that the extra funding will be provided to maintain the same size program. Resource managers in New Brunswick phantasize about getting on the side of the angels too so it cannot be with much pleasure that our industrial managers have concluded that they can afford only chemicals on their private holdings in 1984 and that government managers once again have regretfully settled for less woodlot protection and B.t. usage than they had hoped for. It's a tough decision to opt for a hectare of B.t. spraying with the knowledge that it will deny protection to an extra hectare or two somewhere else.

Let me conclude with these thoughts. Surely much of the advocacy for B.t. in the past few years has been the kind of wheel spinning Jim Fettes warned against in summing up the Abbott symposium in 1976. Did not the CANUSA core test fit the description of the sort of scientific ad hockery he so disparaged? Some spray practitioners may have needed the evidence it provided to convince themselves that B.t. could be made to work - in New Brunswick it merely begged the real questions. Is it not regrettable that some salesmanship from scientific circles, of all places, should resort to inaccurate and unproved disparagement of chemicals while these remain so critically essential to the most hard-pressed users? Is it not still as evident as it was to Jim Fettes, that the kind of fundamental research that is needed to fully understand successes and failures has remained sadly neglected and that most of the advances made since have been principally by commercial development and empirical trial and error? So, though it may seem to be a strange reversal of roles, let a spray manager now take up the cudgel for more fundamental research as well as for better understanding of the other fellow's position.

Literature Cited

Carrow, J.R. (Ed.) B.t. and the spruce budworm. Proceedings. Seminar held in Fredericton, New Brunswick, Sept. 8, 1983. New Brunswick Dept. Natural Resources, Fredericton, N.B. 90 pp.

Anonymous. Bacillus thuringiensis in forest pest management. Proceedings of the North American Symposium on the Microbial Control of the Spruce Budworm. Jan. 19-22, 1976, Montreal, Quebec. Abbott Laboratories Ltd. Montreal P.Q. 148 pp.

OPERATIONAL EXPERIENCES WITH BT IN THE EASTERN UNITED STATES

Henry Trial, Jr.

Director of Survey & Assessment, State Department of Conservation, Box 415 Airport Road, Old Town, ME 04468

Bacillus thuringiensis (B. t.) has been used operationally in the eastern U. S. since 1978 with most applications occurring in Maine. Changes in B. t. dosage rates, volume, cost, spray aircraft, and use patterns will be discussed. Evaluation of B. t. dosage in the east has consisted of variable results with 8 B.I.U. treatments in 1978 through 1980, to consistently good results with 12 B.I.U.'s for 1981 to 1983. Application technology has changed from high volumes in 1979 to undiluted or neat applications in 1983. Spray delivery systems have changed from boom and nozzle equipped small helicopters to small fixed-wing aircraft equipped with rotary atomizers. Improved efficacy has significantly broadened the B. t. use pattern.

Bacillus thuringiensis (B. t.) has been used as part of operational spray projects in the eastern United States since 1978. Numerous formulations and spray regimes have been employed since 1978 resulting in a substantial evolution in B. t. use patterns and spray technology. Major factors in the operational evolution of B. t. have been insecticide, dosage, volume, aircraft, spray delivery systems, spray weather guidelines, use patterns, and costs. Highlights of the evolutions of these factors in the eastern United States will be discussed in this paper.

Most operational use of B. t. in the eastern U. S. has occurred in Maine as part of the State run program or as part of other smaller operations. Most of this paper will deal with these Maine experiences. In an attempt to avoid repetition, evolution in the operational use of B. t. will be presented year by year. Changes for each of the factors mentioned above will be described for the year under consideration.

The first large scale use of B. t. in an operational program in the eastern U. S. occurred in Maine in 1978. The operation was based at Codyville in eastern Maine and covered about 22,000 acres of forest. Spraying was done with four Bell 47 series helicopters equipped with conventional booms and 80-02 nozzles. Thuricide 16B was sprayed at 8 B.I.U.'s per acre with a finished spray volume of 80 fluid ounces per acre. The operation was timed to begin at the peak of the 3rd instar, however some blocks were not sprayed until the 5th instar. The predominate host tree in the spray area was balsam fir.

Cost of the 1978 application was difficult to assess, but was certainly in excess of $15.00 per acre; more than four times the cost of Sevin-4-Oil applied in 1978.

The treatment area near Codyville was chosen for several reasons that reflect the beginning of a B. t. use pattern. First, the infestation level, host condition and population level, was moderate. This type of area was preferred because B. t. efficacy was not expected to equal that of chemical insecticides. The Codyville blocks included many streams, ponds and marshes and relaxed buffer policies with B. t. increased forest coverage compared to a chemical operation. Small helicopters were chosen to remove B. t. from the main airport operation since operational problems were expected and because short ferry distance with helicopters was expected to lessen the impact of high spray volumes (80 ozs.) needed. Also, the low, slow helicopters were expected to provide better deposit with a highly volatile water based B. t. formulation.

Results in 1978 were acceptable foliage protection with population reduction far less (70 to 85%) than expected from chemicals (85 to 95%). Some 1978 blocks were considered to have

only fair foliage protection. The lower level of protection in these areas was thought to be the result of poor application and wet weather following spraying.

Results of the 1978 B. t. operation were good enough to promote the further use of B. t. in Maine in 1979. Operational spraying of B. t. in 1979 covered about 38,000 acres. Thuricide 16B was used at the same 8 B.I.U.'s and 80 fl. oz. volume as in 1978. Cost of the 1979 operation was very similar to the 1978 operation.

Infestation conditions in some 1979 blocks were significantly different from conditions in 1978. As in 1978, most 1979 blocks had moderate host conditions and population, however, some blocks treated in 1979 had extreme prespray host damage and high populations. These blocks, located near Old Town, were chosen to provide information about B. t. applied under more severe infestation conditions.

The 1979 program included the use of small, single engine fixed-wing aircraft to spray some B. t. blocks, although most of the B. t. operation employed Bell 47 helicopters. Use of the fixed wing aircraft and helicopters provided an opportunity to compare the two types of aircraft and, to use B. t. in a conventional airport setting rather than a remote heliport. Both helicopters and fixed-wing aircraft used in 1979 were equipped with conventional boom and nozzle spray systems.

Operational buffering standards were formalized for B. t. in 1979. Small fixed-wing aircraft and helicopters could spray B. t. to within 1/4 mile of a house whereas 1/2 mile buffers were required for the same aircraft spraying chemicals. B. t. could be sprayed to the edge of a stream or pond, but chemicals used in 1979 required a buffer of 150 to 500 feet around water. These formal criteria made B. t. use in some areas extremely advantagous and in some areas it was the only material that could provide adequate coverage.

Results of the 1979 B. t. project were extremely variable. Results on blocks in western Maine, treated with helicopters, ranged from poor to good. Poor results were blamed on rain after treatment and late application. Results with fixed-wing aircraft varied from poor to fair. All blocks with high populations and severe tree conditions had poor results. Wet weather was not the cause of failure in the Old Town areas. These failures were attributed to poor spray deposit from the fixed-wing aircraft and high budworm populations in the blocks.

Results of the 1979 project led to two restrictions for B. t. usage. First, rotor-wing aircraft were thought to provide better spray deposit and were the favored aircraft for future applications. Second, B. t. would not be recommended in areas of high budworm population or extreme tree condition.

Operational use of B. t. in 1980 covered a much larger area (200,000 acres) than 1978 or 1979. In 1980 B. t. was sprayed as a huge demonstration project sponsored jointly by the USFS and the Maine Forest Service. Technology used was basically the same as that employed in 1978 and 1979 except that the size of the project dictated the use of more productive aircraft and use of more than one insecticide.

Large jet helicopters (Bell 204, 205, and 212) were first used for B. t. in 1980. These aircraft were capable of carrying B. t. loads of 200 to 400 gallons compared to 55 gallons carried by Bell 47 aircraft. In addition to the larger capacity, these jet helicopters were faster and sprayed wider swaths. The jet helicopters were equipped with standard boom and nozzle spray systems.

Dipel 4L was used operationally for the first time in 1980. Dipel 4L was used at 8 B.I.U.'s in 80 ozs. of final volume, identical to past applications of Thuricide 16B. Thuricide was sprayed at 80 and 128 ozs. of final volume in 1980 to determine if the high volume (128 ozs.) would improve consistency of results.

Due largely to economics of scale and insecticide competition, B. t. costs decreased somewhat in 1980. Costs were $10.07 to $10.51 per acre for 80 oz. applications compared to a cost of more than $15.00 in 1978 and 1979.

Blocks chosen for B. t. in 1980 were selected on the basis of proximity to human habitation, open water in spray block, proximity to sensitive areas and moderate infestation conditions.

Results of the 1980 project were disappointing. Foliage protection and larval reductions were extremely inconsistent and not all block failures could be explained. An encouraging aspect was uniformally good deposit from large jet helicopters. Tests did show that Diepl 4L produced results equivalent to Thuricide 16B. The two spray volumes of Thuricide 16B used did not produce significantly different results.

Due largely to inconsistencies with 8 B.I.U.'s in 1978 to 1980, a number of dosage variations were tried operationally in 1981 (Table 1). Products used in 1981 were Thuricide 16B and 24B and Dipel 4L. Spray volume ranged from 80 to 120 ounces. The most important change in 1981 was the use of high dosages of B. t. as a single application of 12 B.I.U.'s and two applications of 8 B.I.U.'s. Dosage was increased to obtain more consistent results from a range of weather and infestation conditions.

Several other aspects of the B. t. program did not change in 1981. Large jet helicopters were used, and B. t. blocks were selected as before. Buffers and restrictions remained the same. The B. t. area remained relatively large,

covering 126,000 acres.

Table 1. Results of B. t. Treatment Variations By Area For the 1981 Maine Spruce Budworm Control Project

Treatment	Area	Host	% Unadj. Red.	% Def.	% Fol. Saved
Dipel					
8 B.I.U.'s					
80 oz.	18-1	F	95.5	87	13
		S	91.5	36	2
	13-8	F	88.6	61	32
	22-16	F	97.4	58	30
		S	93.3	13	25
	SW*	F	95.8	14	14
		S	94.1	12	10
Thuricide					
8 B.I.U.'s					
80 ozs.	18-2	F**	87.9	71	29
		S	88.8	18	20
Dipel					
12 B.I.U.'s					
120 ozs.	17-1	F	91.8	(24)34	31
		S	85.2	(18)24	14
	5 Pl.	F	97.4	31	61
	**	S	96.9	23	58
Thuricide 24B					
12 B.I.U.'s					
96 ozs.	7 Pl.	F	95.3	26	60
		S	95.2	22	43
Dipel					
16 B.I.U.'s					
8+8					
80 ozs.	5 Pl.	F	95.7	50	42
		S	96.5	17	28

* Pre counts less than 10
** Pre counts 25 or more
Number in () represents omitting of a sample line which received heavy rain shortly after spray.
All areas sprayed with Bell 204 and 212 Helicopters.

In 1981 cost of an 8 B.I.U. application decreased to a range of $7.37 to $8.75 from over $10.00 in 1980 due to stiff insecticide competition, more concentrated B. t. products, and lower application costs. The cost of a 12 B.I.U. treatment ranged from $11.73 to $12.89 depending on spray volume and insecticide used.

Results of the 1981 project were encouraging (Table 1). While results in 8 B.I.U. areas varied from poor to good, results with 12 B.I.U.'s and two applications of 8 B.I.U.'s were consistently good. Two areas treated with 12 B.I.U.'s had good results even though budworm populations in these areas were high. Population reduction and foliage protection in 12 B.I.U. areas were not significantly different from similar areas sprayed with chemical insecticides.

It was first noticed in 1981 that B. t. was producing good results on red spruce especially at the 12 B.I.U. rate. Red spruce protection was a growing concern and chemical treatment had been only marginally effective.

The 1982 B. t. program was generally planned to confirm 1981 results, to re-evaluate small fixed-wing aircraft, to operationally assess Thuricide 32LV and Bactospiene, and to treat operationally sensitive areas with an environmentally safe product. About 89,000 acres were treated with spray variations listed in Table 2.

Table 2. Results of B. t. Treatment Variations By Area for the 1982 Maine Spruce Budworm Control Project

Treatment	Area	Host	% Unadj. Red.	% Def.	% Fol. Saved
Dipel 4L					
12 B.I.U.'s					
120 ozs.	M72	F	95.3	36.0	44.1
Heli.		S	87.5	28.7	22.3
12 B.I.U.'s					
96 oz.					
Thrush					
w/Micro.	J5	F	98.0	10.7	25.1
		S	98.4	5.7	21.6
Thuricide 32LV					
12 B.I.U.'s					
96 ozs.					
Thrush					
w/Micro	J5	F	98.7	17.3	18.5
		S	98.9	9.7	17.6
*Bactospiene					
12 B.I.U.'s					
96 oz.					
Heli.	B13	F	91.3	12.0	22.8
		S	91.9	8.0	21.5

* This area received rain within 4 hours of application.

Most products were applied at 96 ozs. per acre, but some Dipel 4L was sprayed at 120 ozs. per acre. Cost of a 12 B.I.U. application at 96 ozs. per acre were the same as in 1981 for

Thuricide and about $2.00 more for Dipel 4L. Cost of applying a 12 B.I.U. treatment with a small fixed-wing aircraft rather than a jet helicopter was about $2.00 less per acre.

Fixed-wing aircraft used in 1982 were equipped with Micronair rotary atomizers rather than boom and nozzle systems. Previous tests had shown improved B. t. deposit from this type of equipment. Spray deposit results from the 1982 Maine operations showed that deposit from Micronair equipped Thrushes and jet helicopters were similar in terms of overall block coverage and droplets per unit area.

Efficacy results for 1982 confirmed 1981 results with 12 B.I.U. applications (Table 2). All 12 B.I.U. variations evaluated had good foliage protection and population reduction was similar to reductions with chemicals. Comparisons between Dipel 4L and 32LV showed no significant efficacy difference. Results with Bactospiene were inconclusive due to poor spray weather.

The effect of spray volume was compared in 1982 using Dipel 4L at 120 and 96 ozs. of final spray volume. No significant difference was found between the two volumes.

Protection of red spruce with B. t. was evaluated in 1982. Single 12 B.I.U. applications of Dipel 4L or Thuricide 32LV were found to be comparable to split applications of Sevin-4-Oil for protection of red spruce. B. t. applications were able to prevent about half the defoliation expected on the red spruce assessed.

Efficacy in 1982 on blocks treated with Micronair equipped small fixed-wing aircraft was equal to efficacy achieved with boom and nozzle equipped jet helicopters. Fixed-wing results in 1982 led to a change in the favored status held by helicopters. The decision was made to choose 1983 aircraft on the basis of price. Rotary atomizers would also be required.

A new material, volume changes, and additional evaluation of B. t. efficacy on spruce were considered during the 1983 Maine program. In addition to the 119,000 acre State run program, B. t. was applied on lands owned by the Penobscot and Passamaquoddy Indian Tribes and as a part of a large private project.

Maine added Dipel 6L to its list of operational materials (Table 3). Spray volume was reduced to 64 ounces or less for all insecticides used by the State except Thuricide 24B. Dipel 4L, Dipel 6L, and Thuricide 32LV were also applied undiluted at 48, 32, and 48 ozs. respectively. International Paper Company applied Dipel 6L and 8L undiluted in about 25% of their 1983 project. Baskahegan Company used Thuricide 32LV on their private project. The Penobscot and Passamaquoddy Indian Tribes used a variety of B. t. products on a 40,000 acre project. Finally, the State of Vermont conducted a 1,700 acre project using Thuricide 32LV, Bactospiene, and Dipel 6L. All 1983 applications in the eastern U. S. were at 12 B.I.U.'s. All applications in Maine were done with small fixed-wing aircraft at costs varying from $5.00 to $8.00 per acre. Cost reductions were greatest in small private projects primarily due to low project overhead. Insecticide costs were significantly lower in 1983 due to competition.

Table 3. Results of B. t. Insecticide Treatment Variations by Area For the 1983 Maine Spruce Budworm Control Project

Treatment	Area	Host	% Unadj. Red.	% Def.	% Fol. Saved
Dipel 6L					
12 B.I.U.'s					
64 ozs.	G46	F	73.0	47.1	25.7
		S	85.5	58.7	13.5
	M82	F	93.6	44.6	43.6
		S	97.4	26.0	45.3
		H	89.3	34.1	33.8
	M72	F	81.3	12.3	18.1
		S	90.4	13.8	16.6
	M121	F	78.7	26.1	33.4
		S	79.7	20.0	41.6
		H	97.8	6.3	26.4
Dipel 4L					
12 B.I.U.'s					
48 ozs.					
Undil.	M 5	F	94.5	15.1	36.1
		S	96.4	16.4	16.4
Dipel 6L					
12 B.I.U.'s					
32 ozs.					
Undil.	M10	F	87.5	28.4	22.8
		S	86.6	20.9	11.9
Thuricide 32LV					
12 B.I.U.'s					
64 ozs.	G26	F	91.3	52.2	20.6
		S	97.4	34.1	38.1
	B5	F	88.6	45.6	27.0
		S	93.7	59.1	13.1
Thuricide 32LV					
12 B.I.U.'s					
48 oz.					
Undil.	B13	F	85.4	56.9	34.3
		S	96.9	24.3	47.9
Thuricide 24B					
12 B.I.U.'s					
96 ozs.	J12	F	91.2	33.4	45.6
		S	91.8	13.1	38.0

Table 3 Continued.

Treatment	Area	Host	% Unadj. Red.	% Def.	% Fol. Saved
Bactospiene 12 B.I.U.'s 64 ozs.	G27	F	86.7	45.6	27.2
		S	81.9	42.8	29.4

All areas were treated with Thrushes or M18 aircraft with Micronairs

Efficacy of all 1983 budworm treatments was down because of poor spray weather and unusual larval/host synchrony. However, nearly all B. t. applications conducted in 1983 were successful. Results of the Maine B. t. treatments (Table 3) were again comparable to chemical treatments. Some blocks received only fair foliage protection, but these blocks were sprayed late when much defoliation had already occurred.

Undiluted applications of Dipel 4L and 6L and Thuricide 32LV were as effective as 64 oz. applications made under similar conditions. Spray deposit with undiluted materials was comparable to deposit with 64 oz. applications.

Protection of red spruce with B. t. was good for the third consecutive season. Defoliation on spruce was unusually severe in Maine in 1983, but B. t. applications were able to reduce defoliation to 50% of expected in most blocks.

To summarize, the six years of operational B. t. usage have led to substantial changes in B. t. usage patterns in Maine. It seems appropriate to end this paper with the current status of B. t. application in Maine and the eastern U. S..

Dosage - The 12 B.I.U. dosage is generally accepted in Maine and the eastern U. S.. This rate has a nearly perfect record of success over three years of use. A change from 12 B.I.U.'s to a lower rate would require significant evidence.

Material - Currently available materials favored for use in the eastern U. S. are Bactospiene, Dipel 4L, 6L, and 8L, and Thuricide 32LV and 48LV.

Volume - Low spray volume is generally accepted. Volumes of 64 ozs. have been proven as effective as high volumes used in the past. The Maine State program favors undiluted applications at 48, 32, or 24 ounces depending on which product is used.

Cost - B. t. costs have shown large reductions since 1978 due to competition, concentrated products, and lower emission rates which lower application costs. B. t. costs are now close to the cost of Sevin-4-Oil, but remain significantly higher than the cost of Matacil or Zectran.

Aircraft - Small fixed-wing aircraft are now preferred as a less expensive, but effective alternative to helicopters.

Spray Delivery System - Micronair atomizers are preferred to boom and nozzle systems.

Efficacy - Foliage protection and population reductions on spruce with 12 B.I.U.'s of B. t. have been comparable to chemical results. Results with B. t. on fir are not usually different from chemical results, but in an exceptional year, chemical results of fir can be significantly better.

Timing - B. t. has been effective from the mid 4th to early 6th instars. Early timing is restricted by need for bud expansion to provide a foliar target. Spray applications during late instars are restricted by the amount of prespray damage.

Operational Considerations - Undiluted B. t. materials do not present any unusual operational problems. However, bulk shipment would be an improvement.

Usage Patterns In Maine

1. Can be used on any level of budworm population
2. Often favored on areas with a high spruce content (70% spruce)
3. Not favored on fir in critical condition
4. Favored in wet areas where chemical buffers hinder spray coverage
5. Favored near environmental hazards
6. Favored near human habitation
7. The percentage of the Maine spray project treated with B. t. has shown a gradual increase since 1978.

Year	Approximate Acreage	Percentage of Operation
1978	20,000	1
1979	38,000	2
1980	200,000	15
1981	126,000	11
1982	89,000	10
1983	119,000	14
Planned 1984	250,000	30

Operational Weather Guidelines

1. Should not be sprayed when cool, wet weather is predicted shortly after spray

2. Best results are expected when warm, dry conditions are expected for a day or two after spray

3. Weather requirements are more restrictive than those for contact chemicals due to the quicker action of chemicals

FOUR ENGINE AIRCRAFT EXPERIENCE IN THE APPLICATION OF BACILLUS THURINGIENSIS AGAINST THE SPRUCE BUDWORM IN QUEBEC

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Introduction

I want, during this presentation, to give you a spray program coordinator point of view on Bt and try to explain why things are always different in Quebec. Not alays better but always different, even in the application of Bacillus thuringiensis where 4 engine aircrafts were used to control the spruce budworm, Choristoneura funiferana (Clem.), infestation.

First, a background of the situation is required: background of the Quebec forest and background of our perception of Bt. In order to progress in our approach to use Bt, some KEY FACTORS and GOALS have been established. This brought us into two major periods in the use of Bt: the 1971-1980 period where we learned to live with Bt and its constraints, and the 1981-1983 period where some of the constraints have been solved to a certain degree. Finally, the 1984 project is discussed.

Background

To fully understand the approach, it is important to know that 95% of the Quebec forests are crown lands which are leased to forest industries for wood supply. In order to keep the industrial activity going, one of the tasks of the Quebec Department of Energy and Resources is to organize protection programs against any forest enemies. In the perspective of spruce budworm infestation which attacked softwood species, the major component of the Quebec forest economy, this meant protecting a very large area during a short period of time, the period of the year where the insect was active (6 weeks), in parts of the province where roads and airstrips were scarce. This situation led us to organize spraying programs on large blocks of continuous forests on crown lands, while other approaches like salvation cutting were organized on more accessible but patchy private woodlots. In this strategy, the use of large aircrafts with large capacity payloads was the only alternative available to achieve adequate protection.

The major species attacked by the budworm in Quebec is balsam fir. White spruce and, to a minor degree, red and black spruces are also attacked but being not an important part of the softwood volume, they are not considered in our approach with respect to timing, efficacy and damage.

Programs were initially conducted in western Quebec where the infestation was first reported but by the mid seventies, as the infestation moved from west to east and covered the whole province, the treatments were concentrated in eastern Quebec, where the most important softwood forests were located.

The fact that Bt is a living organism had a major influence on our techniques used to evaluate spray deposit and on mixing procedures. Petri dishes with gelose were used on the ground to evaluate deposit in term of colonies/cm^2, without any reference to size of droplets because the addition of dye (needed for drop sizing) could affect the viability of the spores. Likewise, the addition of water for final formulation was done just prior to application, to avoid activation of the spores.

Key Factors and Goals

In order to evaluate progress achieved thru years with Bt, the treatments had first to be characterized, then some KEY FACTORS were chosen to evaluate efficacy, GOALS were set up as bench marks for what we considered adequate results.

The important points chosen for the characterization of treatments were:

- areas treated (hectares)
- type of Bacillus thuringiensis (oil and water basis)
- concentration of the initial formulation (BIU/litre)
- volume used (litre/ha
- dosage (BIU/ha)
- final formulation (%)
- type of aircraft used (altitude (meter)
  (speed (Km/s)
  (capacity (litre)
  (swath (meter)
  (pressure (litre/s)
- navigation system
- spraying system
- weather condition
- timing (Insect and shoot development)

The KEY FACTORS that were examined to evaluate the efficacy of the treatment were:

- Pre spray population (larvae/45cm branch)
- Residual population (pupae/45cm branch)
- Larval mortality (at peak pupae)
- Previous damage (years of severe defoliation)
- Annual defoliation
- Deposit (number of colonies/cm^2)
- Synchronization of insect and shoot development.

GOALS

The treatments were conducted to reduce stress on the trees and to keep the forest alive. Experience with chemical insecticides told us that, as long as the annual defoliation was kept below the 50% threshold, a treatment could be considered as adequate and successful.

THE 1971-1980 PERIOD: Learning to live with Bt and its constraints

When the spruce budworm infestation was first reported in Quebec during the late sixties, Bacillus thuringiensis (Bt) was the only biological alternative to chemical insecticides. Under the technical advice of the Laurentian Forest Research Center, the Quebec Department of Energy and Resources initiated in 1971 some experimental work with Bt.

On a trial and error basis, it soon appeared that the application of Bt was more sensitive than the application of chemical insecticides. Detailed treatments conducted from 1971 to 1980 have been described in detail in an earlier presentation (Dorais and al. 1980) and the major conclusions that could be drawn from that period were that:

- good results with Bt have always been associated with good deposit. A spray deposit of 20 colonies per cm^2 collected on petri dishes was the rule of thumb to consider a treatment to be effective;

- spray volume required to achieve such a deposit varied from 4.68 (for single engine) to 7.0 l/ha (for four engine aircraft). Under those circumstances, a dosage of 20 BIU/ha could adequately control population levels up to 25 larvae/45cm branch;

- timing also appeared to be very critical. As a stomach insecticide, Bt has to contaminate the annual foliage and has to be ingested in order to kill the larvae. No treatment could be done prior to bud flare and, on a block basis, a treatment conducted within 10 days after the bud flare could be considered as adequate. On a spray program basis, like in eastern Quebec, 20 days was the usual maximum period available per year to apply Bt for proper timing;

- products manufactured became more concentrated thru time (16 to 32 BIU/USG) to suit forestry needs: these more concentrated formulations increased the plane efficiency per year and the area treated could then be increased (Table 1;

- since spores are activated after 12 hours in water, the final formulation was prepared only when the block was ready and when we were absolutely sure that the product could be applied. 30 minutes of mixing time was then required prior to application. This procedure limited the use of Bt to one load per spray period;

- oil base formulations diluted in water required a higher volume to be sprayed than water base formulations, because the amount of water needed in the final formulation had to be at least equal to the amount of oil to avoid inverted emulsion;

- planes used thru the years were: Stearman, TBM Avenger, CL-215, Constellation L-749, DC-6B, and DC-4G. The larger planes came into use to improve spray load capacity.

The 3 major constraints that limited our confidence in Bt at the end of this period were:

- the poor efficacy against high populations (population > 25 larvae per 45cm branch)

- the poor plane productivity (efficiency) per year

- and the cost of the treatment; still 3 times more expensive than a double application of chemical insecticides

Resulting Spray Policy

Even as all efforts were oriented to increase the area treated annually, results obtained were highly variable and the use of Bt was limited to highly sensitive areas where chemical insecticide could not be applied.

Based on experience the norms followed to achieve good results with Bt were:

- population level > larvae/45cm branch tip
- dosage 20 BIU/ha
- volume 4,68 l/ha for single engine aircraft
  7,0 l/ha for four engine aircraft
- deposit 20 colonies/cm^2
- timing 0 to 10 days after bud flare
- cost of treatment (1981): $9.48 for chemical treatment and $31.43 for biological treatment.

Based on the payload of a DC-4G (20 000 lbs) at the rate of 7 l/ha, one plane could normally treat (over a 20 day period) 24 000 hectares per year compared to 125 000 hectares (x2) with chemical insecticide.

The 1981-1983 Period: Solving The Bt Constraints

During the period of 1981 to 1983 some new highly concentrated formulations have been developed and tested in the field. It was expected that the droplets produced, being more concentrated, could kill the insect faster than the former diluted formulations and could therefore reduce the damage caused. At the same time, a smaller amount of material being applied per hectare would increase the annual plane productivity.

Tests were conducted with one of these concentrated formulations (FUTURA 51 BIU/USg). Applied in a volume of 2.5 l/ha, this concentrated formulation for 2 years in a row gave very good larval mortality plus an overall striking degree of foliage protection, even against population levels as high as 35 larvae per 45cm branch tip. These observations changed completely the picture of our aerial spraying programs with Bt. The major changes were:

- limitations for the concentrated Bt, regarding population levels, seemed to be identical to chemical insecticide; concentrated Bt in fact performed as well as chemical insecticides against population levels as high as 35 larvae/45cm branch tip;

- dosage of 20 BIU/ha provided adequate results in tests conducted in Quebec with FUTURA while in other provinces or states a dosage of 30 BIU/ha seemed to give more consistent results; 20 BIU/ha was considered then as a marginal dosage that could be successful if other factors like timing, deposit, etc. were favorable. Volumes lower than 2.37 l/ha never gave adequate deposit with 4 engine aircraft, so dosage was chosen in order to respect the 2.37 l/ha volume limit;

- deposit of 10 to 15 colonies per cm^2 could be considered adequate; since droplets were more concentrated, a fewer number was required for good results, compared to diluted material. Meanwhile, in order to improve spray technology, an experiment was carried out to correlate deposit with foliage protection (without success!). This raised questions about the colonies per cm^2 in petri dishes as a valuable technique, and left the question about ideal size and concentration of droplets that should be generated (the smaller size droplets collected on foliage contained a lethal dosage) unresolved.

- oil-base concentrated formulations when applied undiluted (neat) have an advantage over water-base formulations, with respect to payload. Oil being lighter than water, a higher volume could be carried by the plane per load compared to water-base formulation. On the other hand, plugging problems have been encountered with oil-base material when water, created from condensation in the tank, produced an inverted emulsion with oil (Table 2);

- because concentrated formulations were applied undiluted, mixing procedures were simplified. The product was stable by itself and required only agitation to make an homogeneous suspension prior the application. Without elaborate mixing procedures just prior to application, two loads per spray period could be planned, weather permitting, the same as for chemical insecticide applications.

The 3 major constraints that were discussed from the previous period were reduced to a certain degree with the new concentrated formulation. That is:

- improved efficacy against high larval populations

- an increase in plane efficacy each year (by 2 to 3 times)

- the cost of treatment was still 2 times more expensive than the cost of a double application of chemical insecticide, but the cost of the B.t. product has been decreasing for 2 years in a row, as a result of larger scale economies based on more confidence in the reliability of B.t.

Planned 1984 Spraying Program

After 13 years of research and development to utilize Bt on large scale budworm spraying programs, the Quebec Department of Energy and Resources decided that the major improvements achieved in the three past years warranted giving Bt an honest try; thus, 300 000 of the 800 000 ha contemplated for 1984 will be treated with Bt (200 000 ha with undiluted material and 100 000 with diluted material).

The criteria now recognized to achieve good results with Bt are:

- population level: < 35 larvae/45 cm branch tip

- deposit: 10 to 15 colonies/cm^2

- volume: 2.37 l/ha (undiluted) to 4.68 l/ha (diluted)

- dosage: 20 to 30 BIU/ha

- timing: 0 to 10 days after bud flare

- cost (1983): $10.99 for chemical treatment
  $26.86 for biological treatment

So, in 1984, based on the payload of a DC-4G (20 000 lbs) at the rate of 2.5 l/ha, one plane could treat over a 20-day period a total of 65 000 to 83 000 hectares, compared to 125 000 hectares using chemical insecticides (Table 2).

Planned 1984 Experimental Program

In order to keep improving Bt control for spruce budworm, our research priorities for 1984 have been established as:

- to develop a reliable spray deposit technique which can be correlated with foliage protection;

- to investigate the ideal droplet size and concentration (i.e. the smallest droplet which contains a lethal dose);

- to test in the field some formulations of Bacillus thuringiensis Kurstaki (isolate HD-1) concentrated at 64 BIU/USg and a new isolate (NRD-12), potentially more toxic for the budworm than the former HD-1.

Conclusions

Technological development of new formulations which increased efficacy of Bt and plane efficiency, the growing pressure of the larval population that survives in the forest to cause increasing tree mortality, and the collective history created about chemicals by uniformed people, are the factors which have contributed to greater use of Bt. B.t. still remains the only operational biological alternative to chemicals for spruce budworm control.

The growing use of Bt generated an important diversified expertise. Research programs, like CANUSA, have helped by a joint effort to sort out the good and bad experiences, but the important thing is that a climate of confidence has been created towards B.t. by users and producers. With all this interest generated, major improvements are still to come in the near future: increased toxicity and effectiveness at a lower cost.

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Table 1.--Comparison of aerial applications of Bacillus thuringiensis cconducted in Quebec from from 1972 to 1980

Year	Area treated (ha)	Concentration (BIU/USg)	Volume (/ha)	Aircraft used
1971	80	16B	18,72	Stearman
1972	4000	16B	18,72	TBM Avanger
1973	120	26B	4,6	CL 215 TBM Avanger
1974	3250	26B	4,6	DC 6B CL 215
1975	91000	16-26-36	2,3 - 4,6	DC 6B
1976	120	32B	4,7	S-55T Helicopter
1977	NONE	---	---	---
1978	13300	32B	9,36 4,68	DC 6B Ag Cat B
1979	17030	32B	4,68 - 7,0	L-749
1980	22180	32B	7,0	L-749

Table 2.--Alternative treatments of Bacillus thuringiensis for 1984

Product	Concentration (BIU/USg)	Dosage (BIU/ha)	Volume (ℓ/ha)	Plane productivity (ha/year)
Thuricide 32LV and Novabac-3	32B	20	2.37 (neat)	70 040
		20	4.68 (diluted)	42 740
		30	3.55 (neat)	46 760
		30	7.01 (diluted)	28 580
Dipel 88	32B	20	2.37 (neat)	83 540
		20	5.85 (diluted)	33 000
		30	3.55 (neat)	55 780
		30	8.84 (diluted)	22 500
Futura TM	51B	20	2.5 (diluted)	65 600
		30	3.75 (diluted)	43 740
Thuricide 48LV	48B	20	1.58 (neat)	105 060
		30	2.37 (neat)	70 040
Dipel 132	48B	20	1.58 (neat)	125 320
		30	2.37 (neat)	83 540

REVIEW OF FOLIAGE PROTECTION SPRAY OPERATIONS AGAINST THE SPRUCE BUDWORM WITH BACILLUS THURINGIENSIS KURSTAKII FROM 1980 TO 1983 IN NOVA SCOTIA AND NEW BRUNSWICK, CANADA

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Spray operations against the spruce budworm have been conducted in New Brunswick in 1980, 1982 and 1983 and in Nova Scotia from 1980 to 1983. The results obtained in terms of foliage protection have been extremely variable. Attempts to pinpoint the causes for this variable result suggest that a number of factors are responsible. These are weather during and after spraying and sampling problems.

Introduction

The purpose of this paper is to review the usage of Bacillus thuringiensis kurstakii (B.t.k.) in foliage protection programs against the spruce budworm (Choristoneura fumiferana Clem.) in New Brunswick and Nova Scotia and attempt to pinpoint the resaons for the frequent failures in foliage protection. Operational foliage protection spray programs using commercial formulations of B.t.k. were conducted in Nova Scotia from 1980 to 1983, and in New Brunswick in 1980, 1982 and 1983. In Nova Scotia B.t.k. was the only material allowed for use against the spruce budworm on large tracts of forests while in New Brunswick both B.t.k. and chemical insecticides were used. In New Brunswick B.t.k. has been used only in the woodlot protection program (along with chemicals) in an attempt to provide protection to select select stands of fir and spruce.

There is a trend to increased use of B.t.k. throughout eastern Canada so it is important to try and understand why the results are so erratic. This type of performance plus the higher cost of spraying B.t.k.'s offset the environmental advantage of them, hence there is a reluctance to use more B.t.k.

Assessment Of Efficacy

In both Nova Scotia and New Brunswick efficacy in terms of foliage protection is estimated by comparing the level of defoliation in treated and unsprayed control plots with similar pre-spray spruce budworm population densities. Pre-treatment densities of larvae are determined for one 45cm. branch tip per sample tree. The branches are taken from the mid-crown area of the selected trees.

In Nova Scotia defoliation for each sample tree (fir or spruce) is assessed by classifying the level of defoliation on each of forty shoots per tree using the Fettes method(Dorais and Kettela, 1982). In New Brunswick defoliation on balsam fir (Abies balsamea Mill.) is estimated for the whole tree crown using methodology developed by Webb(1956). However for spruce (Picea sp.) defoliation is estimated as either nil, light, moderate or severe. In the case of the spruces it is the authors opinion that both methods used are inadequate in estimating defoliation on spruce, particularly the red/black hybrid complex.

Unsprayed control data are used to generate graphs showing the relationship between larval population density and resultant defoliation. These relationships need to be examained each year as the degree of defoliation caused by a given population of larvae varies from year to year on both species of trees (Fig. 1&2).

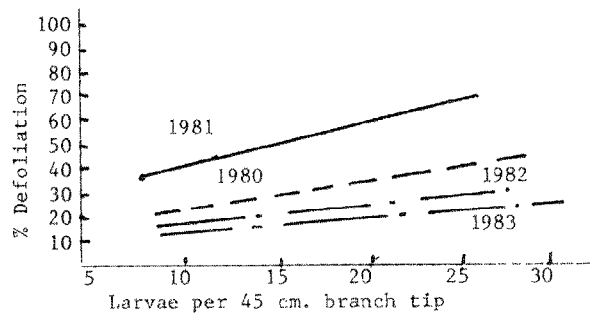

Figure 1. Defoliation as a function of early instar spruce budworm larvae per 45 cm. branch tip on red/black spruce in the Maritimes region of Canada.

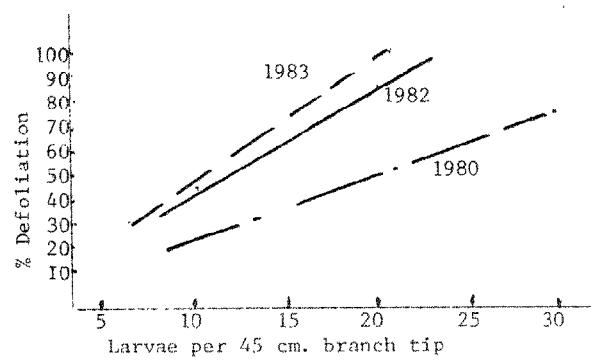

Figure 2. Defoliation as a function of early instar spruce budworm larvae per 45 cm branch tip on Balsam fir in the Maritimes region of Canada.

It is apparent from Figures 1. and 2. that fewer budworm larvae cause more defoliation on balsam fir than on spruce.

Foliage protection due to treatment can be estimated a number of ways; first as simply the difference between expected defoliation and observed defoliation, secondly by using Abbott's formula(Dorais and Kettela, 1982) which is

$$\frac{\text{expected defoliation} - \text{observed}}{\text{expected defoliation}} \times 100\%$$

and thirdly as the difference between observed defoliation and 100%. This last method estimates total foliage retention **which though** very important to the tree is a poor measure of efficacy. Essentially it is possible to generate three estimates of foliage protection from the same set of data. This is an important consideration when arbitrary values for good foliage protection are established such as those in New Brunswick and Nova Scotia.

Two important factors that influence efficacy are the stages of development of larvae and shoots at the time of spray application.At this point in time there is some disagreement as to the appropriate timing for good foliage protection on fir and spruce. It is generally accepted that the shoots on balsam fir should be starting to flare when B.t.k. is applied. This more or less coincides at the peak of fourth instar development. In Nova Scotia , where considerable areas of red/ black spruce are sprayed,spraying is commenced when the buds of spruce have started to swell and the bud caps have fallen off. This more or less coincides with development of larvae between fifth and sixth instar. Delays in spraying caused by weather when this type of late timing is used can result in poor foliage protection. Apparently adequate foliage protection of spruce has been obtained at a considerably earlier timing of B.t.k. sprays. How this works is not understood.

Results Of B.t.k. Applications In New Brunswick

1980

In 1980 10 521 ha were treated with either Thuricide 16B or Novabac-3 at a dosage rate of 20 B.i.u's per ha in 11.5 l per ha.Three spray systems were used ; fixed wing aircraft with boom and nozzle spray equipment, fixed wing aircraft with Micronaire rotary atomizers and a rotary wing aircraft equipped with boom and nozzle spray equipment.

Of the 251 woodlots sprayed 148 were sampled to determine efficacy of spraying. Results of this investigation are detailed by Kettela and Steel (1984). Essentially , the results in terms of foliage protection were extremely variable. From 40 to 60% of the woodlots had a detectable level of foliage protection, depending on the region in New Brunswick. Analysis of the data showed no clear relationship between efficacy and timing aircraft type, spray system used or forest composition and condition. Interestingly red/black was apparently better protected than balsam fir. In light of experiences in 1983 with red/black spruce sampling the result noted in 1980 may be due simply to inadequate sampling of spruce. Further, the mediocre result may have been due to the dilution rate of B.t.k.

1982

In 1982 4 000 ha of forest were treated with B.t.k., 3 200 ha operationally with Dipel 88 and 800 ha in a test program with Novabac-3 and Bactospeine.

With respect to the Dipel 88 treatment one half of the area was treated at 20 b.i.u. per ha and the other half at 30 b.i.u. per ha. Sampling for efficacy was concentrated in that area treated at 30 b.i.u. per ha. The results (Table I) showed that although there was excellent larval mortality foliage protection was very poor.

Table I. Results of spraying Dipel 88 undiluted at a rate of 30 B.I.U per ha in New Brunswick,1982.

	Budworms/ 45 cm branch		Development Index		Defoliation %	
	larvae	pupae	shoots	larvae	Observ.	Expt.
Balsam Fir						
Treated	19	0.4	5.0	4.5	70	84
Control	20	2.6				
Red Spruce						
Treated	18	0.3	1.5	3.4	50	50
Control	16	0.5				
White Spruce						
Treated	17	0.9	5.0	3.8	75	75
Control	25	1.5				

Easy explanations for this result are not forthcoming. By all measures conditions for spray application were excellent,weather conditions were good, before, during and for days after the spray, and shoot and larval development were optimum.

1983

In 1983 some 10 117 ha of irregular shaped woodlots of from 25 to 500 ha were treated with Novabac-3 at a rate of 30 b.i.u./ha in 4.7 l/ha. The bulk of the area treated was located in the Fredericton area(central N.B.), with small areas located in southern NB by the Fundy Coast, and in northern NB south of Campbellton. Spraying was done with small agricultural type fixed wing

aircraft equipped with Micronaire AU 3000 rotary atomizers.

Spray efficacy was estimated from pre-spray larval counts on both balsam fir and spruce (usually red/black spruce), and post spray estimates of defoliation. Three blocks in northern N.B. were sampled for just post spray defoliation. Development of shoots and larvae were estimated from the appropriate graphs. A summary of the results is shown in Table II. Red/black spruce data is omitted because of its inconclusive nature.

Table II. Summary of spray results with B.t.k. (Novabac-3) on woodlots in New Brunswick in 1983.

Shoot Development Index	Pre-spray Larvae per 45 cm branch	Defoliation % observed	Defoliation % expected
3.1	2.2	80	10
3.1	4.7	80	30
3.1	4.9	30	35
3.1	5.2	80	40
3.1	12.3	60	72
3.4	7.5	10	60
3.4	8.1	100	61
3.4	9.8	20	65
3.4	13.6	100	78
3.9	3.8	60	20
3.9	5.7	60	45
3.9	7.7	60	60
3.9	20.4	10	90
4.0	11.5	30	67
4.4	11.1	50	66
	spray blocks in northern NB		
4.5	high	20	70+
4.5	high	10	70+
4.5	high	30	70+

Of the eighteen blocks sampled only 10 had a detectable level of foliage protection. There appears to be a weak relationship of better foliage protection with increasing shoot development. Examination of weather records showed that all the blocks sprayed in the Fredericton area were treated during a cold wet spell where there was rain most every day and mean daily temperatures of 16°C. It is suspected that this weather inhibited larvae from feeding and retarded shoot growth. Further, the irregular shape of the blocks made it difficult to spray the areas accurately. Similarly poor results were obtained on woodlots in the Fredericton area treated with the chemical fenitrothion. However, the three spray blocks in northern N.B. seem to have been adequately protected. Post spray weather for these blocks was generally warm with no rain for at least two weeks.

Results of B.t.k. Spraying in Nova Scotia

An experimental spray program to provide foliage protection to balsam fir and spruce was carried out in 1979. This program was apparently very successful and set the stage for operational usage of B.t.k. against the spruce budworm in Nova Scotia. Initially spray operations were concentrated on Cape Breton Island but were expanded to include red spruce forests in Cumberland and Colchester County in western Nova Scotia. From 1981 to 1983 spray operations were concentrated there. Although considerable data exist on spray effects on balsam fir, it is the spruce data that is particularly interesting. Details on area sprayed, costs and related items are presented in a report edited by J.R. Carrow (1983).

The spray format followed for the past three years has not varied, although a variety of B.t.k. formulations were used. Spray applications were started when red spruce buds started to swell and lose their bud caps. Larval development on average was between the fifth and sixth instar. Ag-cat spray aircraft with standard boom and nozzles were used. Swath width was a nominal 30 meters per aircraft and they flew in formations of two or three planes. The dosage used was 20 b.i.u./ha in 4.7 l/ha.

Of the spray blocks examined here, four were sprayed for the past two years, two for the past two years, and two for the first time in 1983. This provides an opportunity to examine protection applied consistently over time (Table III). Blocks were selected for protection based on tree condition, stand type and egg-mass infestation. Timing and weather are not overriding factors for all blocks were sprayed within guidelines established for the program.

It is evident from Table III that there was a large difference between observed defoliation and expected defoliation in 1981, indicating that the B.t.k. application provided a measurable level of foliage protection. However in 1982 and 1983 the difference between observed and expected defoliation was considerably less. This does not mean that there was a decrease in the effectiveness of the B.t.k. applications in 1982 and 1983. In fact, in view of the guidelines set down for adequate foliage protection, this is the case. Figure 2 shows clearly that less defoliation was caused by similar larval densities in both 1982 and 1983 than in 1981. Observed defoliation was generally low in all blocks in all years. This problem with spruce is a recurring thing and greatly affects the way data on spray efficacy are analyzed. Similar results were obtained on spruce in New Brunswick in 1983. This spruce sampling-interpretation problem must be acknowledged particularly when analyzing data from experimental spray trials. It would be very easy to draw the wrong conclusion on efficacy.

In general the efficacy of B.t.k. against the spruce budworm has been mixed with good results in 1981 but apparently poor results in both 1982 and 1983.

Table III. Results of spraying B.t.k. at 20 b.i.u per ha on selected red spruce stands in Cumberland and Colchester Counties in Nova Scotia from 1981 to 1983.

Location and Year	Pre-spray Larvae per 45 cm branch	Defoliation	
		Observed	Expected
Advocate			
1981	18	18	49
1982	37	28	41
1983	26	27	29
Shulie			
1981	19	16	52
1982	38	29	42
1983	14	24	16
Moose River			
1981	17	15	47
1982	14	8	18
1983	4	9	4
Economy			
1981	9	8	36
1982	10	5	11
1983	9	7	10
Welton Lake			
1982	33	30	39
1983	21	21	23
Eatonville			
1982	31	30	39
1983	6	7	7
Chicnecto			
1983	20	21	22
Sand River			
1983	33	14	37

Conclusions

Looking for the clues as to why B.t.k. at times gives poor results , or apparently poor results is a frustrating task. The reasons for failure can range from poor timing,to bad weather following application, block size and configuration, and an inability to separate cause and effect because there is no apparent difference between treated and non-treated stands of trees.

Certainly, in the New Brunswick experience in 1983 cold wet weather seemed to be the cause for the poor result in those blocks sprayed in central N.B. Delaying spraying to take advantage of forecasted good weather seems to be at best a very hazardous option. The longer you wait to apply B.t.k. consigns at least some of the foliage you want to protect to the budworm. This is particularly so when budworm larval populations are very high.

Sampling and data interpretation problems appear to be particularly acute with red/black spruce. Sampling systems currently being used are apparently not sensitive enough to at times clearly show the differences between treated and non-treated areas, This is true for areas treated with chemicals as well.

Studies that are to be reviewed elsewhere in this symposium point to deposit as a key factor in the performance of B.t.k. as well as the concentration of B.t.k. in each drop.Surely development of more stable formulations with stickers and more potent Bt. strains will remove some of the chance out of protection operations with B.t.

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COMPARISON OF BT FORMULATIONS AGAINST THE SPRUCE BUDWORM

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SUMMARY

The Passamaquoddy Indian Forestry Department treated 40,300 acres in Maine in 1983 using Bt to protect red spruce and eastern hemlock from spruce budworm damage. The post treatment evaluation indicated that the protection objectives were achieved. In cooperation between the Passamaquoddy Indian Forestry Department and two commercial Bt suppliers, Abbott Laboratories and Zoecon Corporation, four commercial products were applied as eight field formulations. Where possible, these treatments were compared for effectiveness. The results show an average defoliation of 23 percent and population reduction from 864.5 budworms per 100 square feet of foliage to 62.5 budworms, or an average of 94 percent population reduction. The undiluted field formulations gave significantly ($P = 0.05$) less defoliation and greater population reduction than the diluted formulations. A method for spray deposit assessment using spruce foliage is presented.

INTRODUCTION

McCreery (1983) reported high spruce budworm populations and heavy past damage on about 40,000 acres of Passamaquoddy Tribal Trust lands. The Passamaquoddy Indian Forestry Department (PFD) requested and obtained spruce budworm suppression funds. USDA Forest Service, Durham Field Office staff provided technical assistance to the PFD in implementing and evaluating the suppression project. An earlier report summarized the suppression project accomplishments (McCreery and Francis 1983). In this report, various Bt field formulations used on the project are compared.

PROJECT AREA

The project was conducted in SBW-infested spruce and hemlock stands on Tribal Trust land in Townships T4ND BPP, T5ND BPP, T5R1 and Indian Township, Maine. These lands, most of which were only recently purchased by the Tribe, have been infested by SBW for many years. Only a small portion of the lands has been protected in the past.

The total area sprayed was about 40,300 acres. This area was in 26 blocks ranging from 500 to 4,000 acres. Blocks were delineated on the basis of biological need for treatment as well as topography and forest cover to aid in navigation.

METHODS

Insecticides

Four commercial preparations of Bt were used: Dipel 6L and 8L (Abbott Laboratories), and Thuricide 32LV and 48LV (Zoecon Corporation) (Table 1). All materials were applied in various field formulations from undiluted to 95 fl. oz. per acre to give 12 BIU's of active ingredient per acre. The only exception was Dipel 8L, which was applied undiluted at eight BIU's per acre (16 fl oz/acre). A sticker, RA-1990 (Rohm and Haas) was added at 2% rate of field formulation for all dilute applications.

Mixing and Loading

Mixing and loading services were provided by Chem-Pro of Oregon as subcontractors with Biegart Aviation.

Two semi-trailer tankers, each equipped with three compartments, were used to mix the insecticide. Dipel and Thuricide were mixed in separate tankers to avoid problems of incompatibility. Insecticide was unloaded from 55-gallon drums and stored in one of the compartments and water was stored in the other. The third compartment was used to mix the insecticide as needed. All liquids were metered as they were pumped to the compartments and to the aircraft. Insecticides were used within 24 hours after mixing. The sticker was added to the water before the addition of insecticide. No mixing problems occurred with the sticker.

Table 1.--Products used during the 1983 Passamaquoddy Suppression Project

Product	Rate (fl oz/acre)	Gallons Sprayed	Acres Sprayed
Dipel 6L	32	935	3740
	65[a]	5277	10344
	96[a]	3000	3960
	Subtotal	9212	18044
Dipel 8L[b]	16	110	900
Thuricide 32LV	48	741	1976
	64[a]	2295	4590
	96[a]	8764	11685
	Subtotal	11800	18251
Thuricide 48LV	32	782	3128
	Grand Total	21904	40323

[a] A sticker, RA-1990 (Rohm and Haas), was used with all dilute applications at a rate of two percent by volume.

[b] Dipel 8L was donated by Abbott Labs for use on this project.

Aerial Application

The aerial application was done by Precision Air of Foley, AL, as subcontractors to Biegart Aviation, Mesa, AZ. The aircraft were based at Princeton Airport, Princeton, ME. Spraying started on June 1, 1983 and was completed on June 9, 1983. The spray blocks were from 2 to 35 air miles from the airport.

Aircraft and Spray Equipment

Three aircraft each with a spray volume capacity of 300 gallons were used. One was a Thrush 600; the others were Snow Thrushes. All three were equipped with eight Mini-Micronair rotary atomizers (AU 5000). Atomizers were equally spaced along the boom, beginning 18 inches from the outboard end. Atomizer pitch was set at 45°.

Aircraft Calibration and Characterization

The aircraft were calibrated at Princeton Airport on the basis of data from spray trials in Foley, AL (O'Neal 1983). At the spray trials, Thrush 600 aircraft were flown at 110 mph, 50 feet above target at pump pressure of 40 psi. Twenty drops/cm^2 was the minimum standard for locating an effective swath at the Foley trial. Thuricide 48LV did not provide the 300 foot swath width at Foley, but later achieved that swath during tests at Princeton Airport. A swath width of 300 feet was used for all formulations.

Application

The three aircraft sprayed as a team. Team guidance was provided by a guide ship (Lincoln Air) equipped with a Loran C guidance system (Teledyne Corp.), which was used to locate team swaths. A monitor and spotter, both PFD employees, observed all applications from the monitor aircraft (Lincoln Air) 800 to 1000 feet above the terrain. The spotter called booms-on, booms-off over treatment areas. The monitor observed spray cloud behavior, team spacing, and weather conditions.

Meteorology

Weather conditions were monitored by ground personnel within treatment areas, the monitor aircraft, and through long range forecasts provided by the Maine Forest Service and Bangor International Airport Flight Service. The work plan specified these weather conditions as acceptable for Bt application:

a. Drip dry foliage within or in close proximity to spray blocks.

b. No rain forecast for four hours.

c. Windspeed >2 mph but <15 mph.

d. No inversion conditions present at or below 100 feet above canopy.

e. Air temperature forecast to be 55°F or higher within 36-48 hours.

f. Air temperature above 32°F at application time.

Larval and Bud Development

Larval and bud development were monitored at eight sites - five in the spray areas and three in the check areas. At each site, five codominant spruce were marked for development sampling. Each sample consisted of one 36-inch branch cut from the midcrown and brought to the field laboratory. Collections were made at three to five day intervals until spraying was completed. Laboratory examinations were completed on the same day as collections.

In the laboratory, all larvae (not more than 100) found on the branches were removed and their location recorded as:

1. Wandering on branches.

2. Mining in the needles.

3. Mining in the buds.

4. Other (loose in the bag, etc.).

Larval instars were determined from head capsule measurements and body coloration. Larval index was determined by the method described by Dorais and Kettela (1982). The results are summarized in Tables 2 and 3.

Bud development index is shown in Table 2. This system was developed by D. G. Mott, formerly of the PFD staff. The classes were defined as:

Class 1 - Tight buds, no green showing at the base of the bud.

Class 2 - Bud expanding, ≤2mm of green showing at the base of the bud.

Class 3 - Bud expanding, >2mm of green showing at the base of the bud.

Class 4 - Bud cap off.

Insecticide application was timed to start when most of the larvae had left needle mines (more than 50%).

Table 2.--Larval and bud index values for the Passamaquoddy Spray Project (first and last spray day), 1983

	June 1		June 9	
Location	Larval Index	Bud Index	Larval Index	Bud Index
T4ND 1	3.4	1.0	4.0	2.5
2	3.0	2.1	4.0	3.3
3	3.0	1.0	4.0	2.0
T5ND 1	3.0	1.0	3.8	1.2
2	3.5	1.5	4.6	2.5

Table 3.--Larval behavior during and after the Passamaqouddy Spray Project, 1983

	T4ND BPP			T5ND BPP		
Behavior	6/1	6/9	6/15	6/1	6/9	6/15
% Wandering	39	26	53	25	25	51
% In Needles	43	0	0	60	2	0
% In Buds	12	56	37	6	58	40
% Other[a]	6	18	10	9	15	9
	100	100	100	100	100	100

[a] Includes larvae webbed either on or near buds or found loose in the bags.

SPRAY EFFICACY EVALUATION

Samples and Sample Clusters

A total of 45 sample clusters were established in 10 treatment blocks. Each cluster was a line of 20 dominant/codominant spruce trees spaced about 50 feet apart, across the anticipated spray swaths.

Additionally, three check blocks were established in widely separated areas at least ½ mile outside the spray blocks. Each check block had four clusters established in a manner similar to the spray blocks. Despite these precautions, Bt deposits were found in two of the three check blocks.

Prespray Survey

Prespray samples consisted of one 18-inch-long branch taken from the midcrown of each sample tree. Collections were made two to three days before spraying. The branches were bagged, labeled and delivered to a laboratory for examination. The average number of larvae per 18-inch branch was determined in the laboratory.

Postspray Survey

Postspray samples included two branches, each 18 inches long, taken from the midcrown of the sample trees. The samples were collected about 40 days after spraying when larval feeding was completed. This coincided with 10 percent adult emergence. Again, branches from each tree were bagged, labeled and delivered to the laboratory. In the laboratory, the average number of budworm per branch and the degree of defoliation were determined from 25 shoots rated in 10-percent defoliation classes.

Spray Deposit Assessment

Spray coverage was evaluated at the sample clusters in nine of the ten treatment areas and in all the check areas. Foliage samples were collected from the midcrown and from the lower crown of each sample tree. In each treatment area, deposit assessment was made for five trees in each of the selected clusters. In addition, millipore filters were exposed on 3 foot stakes near sample trees. In each treatment area, five filters were set out in each cluster. The filters were set out 8 to 12 hours before spraying and picked up within six hours after spraying. Foliage samples were taken as millipore filters were collected.

About a month later, all deposit samples were placed on tripticase soy agar plates and the spray coverage determined. For foliage samples, 20 old needles from each branch were placed on agar. After incubation, the proportion of needles producing Bt colonies were determined. Sample colonies from each dish were examined under a microscope to confirm the presence of Bt crystals. The millipore filters were handled the same way, except that the number of colonies per square centimeter was determined.

Field Laboratory

Foliage examination was done in a laboratory operated by the PFD. Personnel included experienced workers. Branches were brought to the laboratory and kept refrigerated. They were examined within a week after collection.

RESULTS AND DISCUSSION

Efficacy

The average defoliation in treated areas was 23 percent. Spruce budworm populations declined in treated areas from 865 to 62.5 budworms per 100 square feet of foliage. Thus, the average spruce budworm mortality was 94 percent.

The results for each treatment are summarized in Table 4. The differences between treatment means were analyzed using Student's Newman Keuls test ($p = 0.05$). Spruce budworm mortality was significantly higher in undiluted Dipel 6L treated blocks than in undiluted Thuricide 48LV or diluted Thuricide 32LV treated blocks. Although the differences between diluted and undiluted Dipel 6L treated blocks were not significant with the Newman Keuls test, both budworm mortality and defoliation were significantly different based on a grouped t test.

No other treatment means were significantly different. The sampling method used 20 tree lines as clusters and did not give enough samples to detect mean differences of less than 10 percent with normal field variation.

A comparison was made between diluted and undiluted treatments of Dipel 6L and Thuricide 32LV combined. The undiluted treatments had significantly lower defoliation and higher budworm mortality than the diluted treatments (Student's t test, grouped data; $p = 0.05$).

Table 4.--Spruce budworm population changes and spruce defoliation in the Passamaquoddy Spray Project, 1983

Treatment	Rate (fl oz /acre)	Clusters	Deposit % Needles cov'd	Mean No Larvae/ 100 ft^2 pre ± se	post ± se	% Mortality ± se	% Defol ± se
Dipel 6L	32	5	32	664 ±81.7	12.4 ± 2.3	98 ± 0.5	20 ± 3.2
Dipel 6L	32	4	65	935 ± 116	5.6 ± 3.3	99 ± 0.3	13 ± 2.9
Dipel 6L	65	7	47	1090 ±73.3	14.7 ± 2.3	98 ± 0.4	28 ± 2.9
Dipel 6L	65	4	50	1412 ±25.5	82.6 ±16.4	93 ± 1.7	25 ± 2.9
Dipel 6L	65	4	--	918 ± 318	86 ± 7.0	86 ± 4.7	21 ± 2.7
Dipel 8L	16	3	53	821 ± 137	30 ±12.2	95 ± 2.7	18 ± 0.7
Thuricide 32LV	48	4	87	921 ±73.5	32.5 ± 7.2	96 ± 0.9	19 ± 4.4
Thuricide 32LV	64	4	97	972 ± 206	69.1 ±14.0	91 ± 3.5	16 ± 3.1
Thuricide 32LV	64	4	70	1742 ± 214	87 ± 6.6	95 ± 0.5	24 ± 2.8
Thuricide 48LV	32	6	43	304 ±50.7	23.5 ± 5.3	88 ± 5.8	17 ± 2.4
Check 1		4	5	969 ±22.4	115 ±20.2	81 ± 9.3	28 ± 2.9
Check 2		4	28	314 ± 119	142 ±31.4	57 ±10.3	32 ± 5.6
Check 3		4	0	471 ± 116	174 ±76.7	50 ±21.1	32 ± 2.9

Spray Coverage

Spray deposit in the treated areas indicated good coverage (Table 5). No significant differences in deposit were found between midcrown and lower crown needle samples (60.9 percent vs. 59.3 percent of needles with Bt). On millipore filters, the average count was 48.2 colonies per cm^2. However, one block (47) had an average of 171 colonies per cm^2, suggesting multiple coverage. Without that block, the average deposit was 32.9 colonies per cm^2.

Our work with aerial application of Bt over the past three years indicate that checks need to be sampled for Bt deposits. As in the previous years, the current year's checks received some

Bt deposits. Although, the amounts are considerably less than what is found in the sprayed areas, some budworm mortality from Bt is expected, and therefore, the checks do not represent truly untreated Bt areas.

Table 5.--Spray deposit assessment for the Passamaquoddy Spray Project, 1983

Treatment	Rate (fl oz/ac)	Block No	% Needles Producing Colonies: Mid-crown	% Needles Producing Colonies: Lower crown	Millipore Filters (Colonies/ cm^2)
Dipel 6L	32	72	36	28	24
		43	59	70	34
Dipel 6L +2% Sticker	65	45			
		48	52	48	19
		71	51	42	15
Dipel 8L	16	49	49	56	90
Thuricide 32LV	48	46	88	86	22
Thuricide 32LV + 2% Sticker	64	44	96	97	24
		47	73	66	171
Thuricide 48LV	32	73	44	41	45

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PENNSYLVANIA'S EXPERIENCES WITH MICROBIAL CONTROL OF THE GYPSY MOTH

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ABSTRACT

Pennsylvania's first experience with using Bt on insect control occurred in 1964. For the next 17 years, various projects were conducted, in cooperation with the USFS and industry, in an effort to secure operational status of Bt for gypsy moth suppression. This point was reached in 1982, and the Governor was convinced that the time was right to convert most gypsy moth suppression operations from chemical spray to Bt. In 1983, 326,000 acres of high-use areas were treated with Bt, making it the largest Bt operation on the gypsy moth undertaken to date. Suppression results were virtually on a par with formerly used chemicals, spraying objector problems dropped to near zero, and the per acre suppression cost was cheaper. Some problems with Bt still remain, and these are discussed.

My remarks in this presentation will be confined to Bt since we have no operational experience with other microbials in gypsy moth suppression. The gypsy moth virus material may at some future date prove to be operationally feasible, but it is my opinion that this is still a long way off. It doesn't match Bt in effectiveness, at least at this time.

Being an old timer with 27 years of responsibility for gypsy moth suppression in Pennsylvania, I cannot help but reflect for a minute on long-term trends. When I first came on board in 1957, DDT was under attack. The hue and cry was to switch to less offensive "soft" chemicals such as carbaryl or trichlorfon. This was finally done in the early 1960's, and for a period of time we had little or no problems with opposition to chemical spray programs. In the 1970's, however, these biodegradable chemicals came under attack. The insistence then was that we switch to Bt.

Two things should be obvious: 1) much energy has been expended uselessly in fighting the long-term and inevitable trend away from broad-spectrum chemicals. This energy should have been expended in accelerating the conversion to microbials, and 2) if you think back to your college days you might remember some professor saying that one of our ultimate goals in forest insect control must be the development and use of specific suppression materials that have little or no impact on other organisms, the environment, and on humans. We have entered this new era of microbial control on a large-scale within just the past two years. Work is now accelerating at a rapid rate, witness the number of new Bt formulations that become registered every year.

In Pennsylvania, I like to think that we have never forgotten our ultimate goals. My first contact with a microbial insecticide was back around 1960, when we made a concoction of virus to use on the European pine sawfly. It worked, but its use was soon halted by EPA.

Our work with Bt began in 1964, when we sprayed several hundred acres to test its control of the fall cankerworm. Results were variable to say the least. Over the next 17 years, our experimental work with Bt was primarily on the gypsy moth. Very few years went by without some Bt spraying, but results always left something to be desired. We could kill gypsy moths, but more than one of us suspected that a 1974 Bt spray operation on 35,000 acres did nothing more than prolong the outbreak, due to the number of healthy survivors. We also had the dubious honor in 1974 of having this large Bt operation temporarily stopped by EPA for possible violation of the terms of an experimental label. This necessitated a trip to EPA headquarters in Washington to straighten the matter out, and it made us wonder who our friends were.

At this point, I would like to pay tribute to a man who has devoted his entire career to the development of microbial insecticides. During these earlier years, he never gave up hope that someday Bt would become a viable replacement for chemical sprays. I'm sure he had many frustrating experiences, but his dedication to the task never left him. He is, of course, Dr. Frank Lewis. I don't know where we would be today with Bt if Dr. Lewis had not been on the job.

In 1981, some outstanding successes began with the newer Bt formulations. In 1982, we decided to conduct a large-scale project with Bt as part of our operational program. This was done on 32,000 acres of forested residential properties in southeastern Pennsylvania. Results were as good as with chemical spraying. Twelve B.I.U.'s were used in 128 ounces of solution per acre. A polyvinyl sticker was used over much of the area but was later abandoned due to its propensity to adhere semi-permanently to automobile finishes. We also augmented the Bt spray areas with *Apanteles* parasites with good results.

Following this successful operation with Bt, we felt the time was right to recommend a major changeover in our program from trichlorfon (Dylox) to Bt, at least in all forested residential areas. Trichlorfon was becoming too expensive, and chemical spray objectors were presenting us with severe operational problems. This recommendation was approved by the Governor who, in fact, directed us to make the changeover.

In 1983, we treated 326,000 acres with Bt, involving over 3,000 separate spray blocks of forested residential properties. Twelve B.I.U.'s

were used over most of the area, with gypsy moth densities of over 1,000 egg masses per acre in building populations receiving 16 B.I.U.'s and in some cases two treatments. The significant results of this project can be described as follows:

1. The spraying objector rate of 5-10% with previously used chemicals dropped to virtually zero with Bt. Environmentalists not only ceased their attacks but applauded the conversion. The mail that did come in was mostly from pest control officials and environmentalists in other states seeking Bt efficacy data and the reasons for our changeover. It was obvious that Pennsylvania's action influenced some other states to chart a similar course.

2. Overall costs were about $1.00 per acre lower than with previously used chemical sprays.

3. Control results with Bt were generally acceptable, with about 80% of the areas showing 70-93% larval mortality and defoliation below 30%. Overall egg mass reduction was not as good as with chemical sprays. Many areas with building populations still had unacceptable numbers of larvae, resulting in a continuing public nuisance problem. However, even in these latter cases, foliage protection was achieved.

4. In a large program, making two applications 7 to 10 days apart in critical areas presents excessive operational problems, which we must avoid if at all possible. In 1984, we will try 20 BIU's in these critical areas. An alternative for evaluation may be two applications of 12 BIU's on the same day. In general, our treatments with 16 BIU's were not significantly better than the 12 BIU treatments in the limited areas where they could be compared with equal populations. It should be noted that control problems also existed in many of these same critical areas with chemical sprays. Basically this occurs either because foliage is being consumed as fast as it is being produced, leaving little leaf surface to spray, or because later hatching larvae are being blown in a week or more after treatment.

5. Timing of Bt applications did not prove to be as critical as we had feared, but the spring of 1983 was unusual. We allowed ourselves two weeks to get the job done in each of three geographic regions of the state. Due to cool weather after general egg hatching and reduced feeding activity, we had at least three weeks for spraying in each area. In a few areas, poor control was traced to spraying too early. The ideal start time is when white oak foliage is one-third grown.

6. We attempted to evaluate spray stickers but could not come up with any concrete evidence that they improved control. In 1984, we will only use stickers if there is a threat of rain. Our plans also call for retreating an area if rain occurs within 4-6 hours of treatment.

7. Mixing Bt solutions proved to be a headache, primarily due to inadequate equipment by contractors and our need to monitor the mixing. This mixing process, on occasion, resulted in 18 hour work-days. We experienced no significant differences in the two Bt products used, Dipel 6L and Thuricide 32LV, in the matters of efficacy, cost, and ease of spraying.

8. Considerable work was done on solution rates. About three-quarters of the operational program was done with 128 ounces per acre, and the remainder with 96 ounces. No detectable differences in efficacy existed. Consequently, the 1984 program with 12 B.I.U.'s will be 96 ounces per acre. In 20 B.I.U. areas, we will use 128 ounces as insurance for good coverage. In 1983, we also conducted experiments on thirty-four 100 acre plots to test nine Bt formulations in reduced solutions and undiluted rates. Undiluted Bt, for the most part, gave unsatisfactory control, as did solutions under 96 ounces.

To reflect on where we are now with Bt in gypsy moth suppression, there is no doubt we have come a long way. However, we are not at a resting stage; perfection is the name of the game. The way to proceed should be fairly obvious. Ideally, a spray operation should utilize undiluted material in one application. I feel certain that this is within our reach, since there is no doubt that Bt will kill gypsy moths. Expedited research in spray technology must be implemented. For example, we are still using the same spray nozzles that were in use 30 years ago. Do we really know the best type of spray aircraft to use, its speed, its spraying altitude, its swath width, and atmospheric condition limits to achieve the best results? Do we know what kind of Bt coverage we're getting within tree canopies and how long it will be effective? These are the answers we now need.

I am convinced that Bt will be in our gypsy moth suppression operations for a long time. Outsiders may not appreciate the tremendous, time-consuming burden that has been lifted from our backs by not constantly being in battle with the anti-chemical spray groups and answering public concerns for safety of their health.

MICROBIAL CONTROL OF THE GYPSY MOTH IN RECENTLY INFESTED STATES: EXPERIENCES AND EXPECTATIONS

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Experiences and expectations concerning microbial control of the gypsy moth in recently infested states are summarized. Initial experience included mixed results, but expectiations remain optimistic. Public sentiment assures continued pressure for improvement in microbial control technology.

In states where the gypsy moth is a recent arrival, experience with microbial control is, of course, limited. But interest and expectations are high. Results of a survey in December, 1983, show that nine of sixteen recently infested states have already used microbials at least once, although only three have ever sustained heavy gypsy moth defoliation (Table 1).

TABLE I: Gypsy Moth Control In Recently Infested States[a/]

State	Has had Heavy Defoliation	Control Materials Applied			
		Broad Spectrum	Growth Regulator	Pheromone	Microbial
Arkansas	-	+	-	-	-
Delaware	+	-	+	-	+
Georgia	-	-	-	-	-
Illinois	-	+	-	+	+
Maryland	+	+	+	-	+
Michigan	+	+	+	+	+
Minnesota	-	+	-	-	-
Missouri	-	-	-	-	-
North Carolina	-	+	+	+	+
Ohio	-	+	-	-	-
South Carolina	-	+	-	+	-
Tennessee	-	-	-	-	-
Virginia	-	+	+	+	+
Washington	-	+	-	+	+
West Virginia	-	-	+	-	+
Wisconsin	-	+	-	+	+

[a/] Responses to a survey in December, 1983; + = yes, - = no.

Most conventional and experimental approaches to gypsy moth control have been used or tested in recently infested states. Microbials, especially Bacillus thuringiensis (Bt), are expected to constitute an important component of all future suppression programs. This apparent predilection for Bt reportedly stems more from reaction to public pressure than from absolute preference for its characteristics and performance. Among those who have used Bt where the gypsy moth is a relatively new problem, opinions of its efficacy and reliability range from approbation to deprecation. This divergence corresponds to the diversity of circumstances under which Bt has been applied--from attempts at eradication of incipient infestations to the suppression of outbreak populations.

Perceived environmental impact is a major consideration in gypsy moth suppression programs because of increasing public concern about the hazards of pesticide use. Public perceptions of the materials currently registered for gypsy moth control clearly place microbials in a preferential position, especially in comparison with broad spectrum insecticides. But efficacy, cost and consistency of performance are also important attributes, and the record for microbials in these areas has been erratic. Recent advances in the formulation of Bt, a concomitant reduction in application costs and apparent improvement in performance have maintained optimism about its usefulness; but reservations remain. Concern about efficacy and predictability of performance was mentioned repeatedly in survey responsen. Most recently infested states are still dealing with incipient populations, and the primary objective is gypsy moth mortality, rather than foliage protection or nuisance abatement.

Experience with gypsy moth nucleopolyhedrosis virus (NPV) was reported only from Michigan, Virginia, and Wisconsin. Even in these cases it was used on a very limited scale. In Virginia, a recent test of NPV applied to shade trees from the ground produced

encouraging results. Little consideration has been given to NPV by most states presumably because of its very restricted availability and uncertain performance record.

Microbial control is certain to remain an important issue as the gypsy moth extends its range. Expectations in recently infested states include increased use and improvement of microbial formulations. The prevailing attitude is generally one of optimism.

Some Negative Aspects of Using *Bacillus thuringiensis* Berliner In Operational Programs Against The Gypsy Moth (Lepidoptera:Lymantriidae)

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Operational programs to suppress gypsy moth populations in residential and recreational areas first began in New Jersey in May of 1980. *Bacillus thuringiensis* was used on approximately 17,000 acres applied at the dosage rate of 8 B.I.U.'s in one gallon of water per acre. Two treatments approximately one week apart were applied.

The insecticide *B.t.* was used previous to 1980 in pilot test programs, but because of the high cost of materials and erratic results the Department could not recommend it as the viable alternative to chemical pesticides. However, more recently with improved formulations and lower costs it has become more feasible to utilize *B.t.* in operational programs.

Despite *B.t.*'s increased efficacy over earlier formulations, there are still a number of negative aspects to consider when used in operational programs. One of the negative aspects of most concern is the narrow "spray window" available to properly control second and third instar larvae especially if there is a prolonged hatching period. However, even when it is applied when the larvae are the proper size, rainfall and/or prolonged cool weather can reduce its efficacy because of the material washing off foliage and the lack of feeding which occurs by the larvae during cool weather conditions.

Additionally, even though we have reduced the number of applications from two to one per spray season, it still is applied at the rate of approximately one gallon per acre which means more operational hours are necessary to complete the work than the low volume chemical insecticides.

Another negative aspect of major concern is the wide variability in efficacy depending on the health and vigor of the gypsy moth population. Building populations of the gypsy moth, where the egg masses are large and the numbers exceed 1,000 egg masses per acre, are much more difficult to control with *B.t.* than declining populations of the pest. Declining populations are found when the insect is under stress due to starvation and disease and egg masses are small. *Bacillus thuringiensis* tends to be more successful under these conditions. Because of this variability, the New Jersey Department of Agriculture occasionally has to re-spray areas containing high building populations because larval reductions were not sufficient to prevent reinfestation of these areas of the following season. This re-treatment sometimes amounts to nearly 50% of the spray block.

Although insecticide costs are greater for *B.t.* than for the chemical insecticides, the cost is not the major concern when towns make their selection. The effectiveness of the material seems to be the greater concern to town officials. The reason being is that there are certain notification and legal procedures which must precede the spray operation and if after all these requirements are met, the results are not good in terms of foliage protection and population control, the towns will sometimes switch to the more effective chemical insecticides the following season.

Another negative aspect of the *B.t.* spray is its slow knock-down ability. Residents in the sprayed area believed that once spraying is done, the larvae should rapidly disappear from the area and no further nuisance should exist. This rarely occurs when *B.t.* is used and some residents complain that the insecticide is not working.

One minor aspect of the insecticide *B.t.* that is considered negative is a fact that it is very difficult to see on the foliage. Therefore, if residents believe a skip has occurred, it is difficult to verify because the *B.t.* is virtually colorless on leaf surfaces.

Evaluating the efficacy of *B.t.* by the New Jersey Department of Agriculture has been done each season since its introduction in 1980 using tar paper bark flaps and comparing results of larval counts in treated and untreated areas at the same time. The best control of larvae during any one season occurred in 1982 when a 70% average larval reduction in all treated areas was accomplished. The lowest larvae reduction of caterpillars had occurred in 1983 when only 40% reduction in larval numbers were observed. Apparently, the woodlands where *B.t.* was used in 1983, contained extremely high and healthy populations of the gypsy moth and the results were not as good as in previous years.

Finally, the Department does recognize the fact that stickers can increase the residual life and effectiveness of the B.t. application. However, the stickers that work the best are also the most difficult to remove from car surfaces and does present a negative factor since most of the spraying is done in residential areas. For this reason, the Department has used stickers (Plyac or Nu-Film) which are able to be washed off car finishes more readily.

In conclusion, there are a number of negative aspects of using B.t. sprays in residential areas. However, in general, B.t. does not offer good foliage protection, and therefore, the objective of preventing tree loss in these areas is met. The New Jersey Department of Agriculture will continue to recommend and encourage the use of B.t. in spraying operations. However, we do feel towns should be given the opportunity to select the insecticide in a voluntary program, especially when all the aspects of B.t. are considered.

OVERALL ASPECTS OF *B. t.* IN FOREST SERVICE COOPERATIVE GYPSY MOTH SUPPRESSION PROJECTS

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Improvements in *B. t.* performance and cost, coupled with public concerns over human health risks have elevated *B. t.* to a viable alternative to chemical insecticides. Operational use of *B. t.* in recent years has demonstrated that while foliage protection can generally be achieved in most situations, gypsy moth population reduction cannot. Efforts are needed to improve *B. t.* performance in healthy, building gypsy moth populations.

Introduction

Bacillus thuringiensis (*B. t.*) has had a long and sometimes arduous journey in gaining acceptability and respectability in Forest Service cooperative gypsy moth suppression projects. The insecticide's slow acceptance stemmed not only from its high cost, special operational considerations, and erratic performance demonstrated in early field trials, but perhaps also from the misconception of *B. t.* as a replacement for chemical insecticides. Today, users realize that *B. t.* is not appropriate in all situations, and efforts are directed at finding the proper role for the insecticide.

B. t. Use in Cooperative Suppression Projects

The use rate of *B. t.* in cooperative gypsy moth suppression projects reflects an ebb and flow relationship, with increased or decreased use rates in 1 year directly related to the results of field tests or operational trials the previous year. Some of the earliest operational trials with *B. t.* in Forest Service cooperative gypsy moth suppression projects occurred in 1973-1975. The number of acres treated with the insecticide were small, and never exceeded 1.5 percent of the total project acreage (Table 1). Although foliage protection was generally acceptable in these trials, State cooperators expressed doubts about the prospect of achieving desired population reduction levels. Problem areas were identified in methods improvement, including application timing, dosage, and number of applications required (USDA 1974). During the next 4 years, 1976-1979, *B. t.* was not used operationally in cooperative gypsy moth suppression projects (Table 1), although field trials continued.

In 1980, *B. t.* operational use skyrocketed. The three States participating in cooperative suppression projects that year treated more than 37,000 acres with the insecticide or approximately 46 percent of the total project acreage (Table 1). Unfortunately, the results were dismal in most cases (USDA 1981). Operational use of *B. t.* dropped considerably in 1981 and 1982 (Table 1) as States directed resources to more economical and efficacious insecticides in wake of the increased gypsy moth activity throughout the Northeast.

Table 1.--*B. t.* use in Forest Service cooperative gypsy moth suppression projects 1973-1984.

Year	Total acres treated	Acres treated with *B. t.*	Percent treated with *B. t.*
1973	173,570	2,570	1.5
1974	252,844	2,305	0.9
1975	48,579	700	1.4
1976	42,270	0	0
1977	96,146	0	0
1978	169,880	0	0
1979	69,887	0	0
1980	81,370	37,663	46.0
1981	350,299	22,436	6.4
1982	726,757	66,470	9.1
1983	598,760	418,434	70.0
1984 a/	543,613	214,010	40.0

a/ Proposed.

A variety of factors returned *B. t.* a leading role in 1983 suppression projects with applications made to more than 400,000 acres or 70 percent of the total project acreage (Table 1). Current plans in 1984 call for *B. t.* application to 214,010 acres, representing 40 percent of the total acreage proposed for treatment (Table 1).

The acceptability and almost standard use of *B. t.* in cooperative gypsy moth suppression projects in recent years can be attributed to the following:

1. Methods improvement.
2. Cost reduction.
3. Public acceptance (and pressure).

Since 1980, rapid advances have been made in the methods improvement area. The introduction of new formulations of *B. t.* have eased mixing, loading, and application difficulties experienced in the past making *B. t.* more efficient to use operationally. More than any other improvement, the demonstration of acceptable performance with a single application at higher dose rates has elevated *B. t.* to a viable, cost effective alternative. Marketplace competition by *B. t.* manufacturers has also served to drive down material costs.

For example, material and application costs in 1983 cooperative suppression projects were as low as $5.50 per acre. Bids already received by the West Virginia Department of Agriculture (WVDA) for 1984 project work indicate that material and application costs could be near $6.00 per acre (personal communication, Alan R. Miller, WVDA). These costs are lower than all of the chemical insecticides except Dimilin.

Public concerns over human health risks have generated a tremendous appeal for the use of B. t. in place of the chemical insecticides. Pressure has been applied to public officials by local citizenry through State legislatures and the courts to ban or severely curtail the use of chemical insecticides. Comment letters received for and published in the current USDA Final Environmental Impact Statement for gypsy moth suppression and eradication projects (USDA 1984) are replete with questions on public health risks, particularly cancer and birth defects. Most of these letters support the use of B. t. instead of chemical insecticides. The recent increase in B. t. use, particularly in populated areas, can be largely attributed to public pressures, and States' responses to address these concerns.

B. t. and Project Objectives

Is B. t. adequately achieving the objectives of cooperative gypsy moth suppression projects? The track record of B. t. in the last several years is mixed. Comments from State cooperators and data in post-suppression evaluation reports indicate that except in extremely high gypsy moth populations, the objective of foliage protection can be achieved. Population reduction, however, is variable.

During the 1983 cooperative suppression projects, 418,434 acres comprising 70 percent of the total project acreage in 7 States were treated with B. t. These treatments were applied to a wide range of gypsy moth population levels (250 to more than several thousand egg masses per acre) in different phases of outbreak (building, static, declining) and provide a good evaluation of B. t. The results of 1983 suppression activities in Maryland, New Jersey, Pennsylvania and West Virginia demonstrate similar trends. Treatments in these four States accounted for 98 percent of all the B. t. used in cooperative suppression projects last year.

Maryland.--The Maryland Department of Agriculture (MDDA) treated 50,242 acres with B. t. in 1983. Foliage protection was achieved; however, approximately 42 percent of the treatment areas Statewide contain gypsy moth populations averaging more than 250 egg masses per acre and qualify for retreatment in 1984. Up to 90 percent of treatment areas which have never sustained defoliation, but contain building populations, qualify for retreatment in 1984 (personal communication, Robert Tichenor, MDDA).

New Jersey.--The New Jersey Department of Agriculture (NJDA) reported that 5,000 of 11,000 acres treated with B. t. last year need to be retreated in 1984. When B. t. is used in areas containing building gypsy moth populations in excess of 1,000 egg masses per acre, NJDA expects the need to retreat nearly 50 percent of the acreage the following year (personal communication, John Kegg, NJDA).

Pennsylvania.--The Pennsylvania Bureau of Forestry (PA BOF) treated more than 326,000 acres with B. t. in 1983, accounting for 88 percent of their total project acreage. Statewide, 25 percent of the treatment areas qualify for retreatment; however, this ranges from a low of 15 percent to a high of 84 percent. In areas where building gypsy moth populations were present, 44 percent of the treatment areas qualify for retreatment (personal communication, William Slippey, PA BOF).

West Virginia.--The West Virginia Department of Agriculture treated 16,735 acres with B. t. in 1983. These areas had never before sustained gypsy moth defoliation, but contained building gypsy moth populations averaging several hundred egg masses per acre. Foliage protection was achieved, but population reduction was variable, averaging 43 percent overall. In some areas, gypsy moth populations increased more than twice the pretreatment levels. A total of 9,775 acres of the area treated in 1983 (58 percent) may be retreated in 1984 (personal communication, Alan R. Miller, WVDA).

The overall experience with B. t. by State cooperators can be summed up by the conclusions drawn in Pennsylvania's 1983 post-suppression evaluation. In that report, it is stated that "treatments...in healthy-building populations gave inconsistent results. Overall, however, foliage protection was still achieved in the vast majority of areas." Furthermore, "Most treatment areas will not need respraying in 1984, the exceptions being most of those areas which exhibited healthy-building populations." (PA DER 1983).

Planned B. t. use in proposed 1984 cooperative suppression projects will drop to about 40 percent of total project acreage as State users reevaluate the most efficient role for the insecticide (USDA 1984). Pennsylvania, the largest user of B. t. in 1983, plans to treat only 49 percent of project acreage with the insecticide in 1984 while West Virginia's B. t. use will encompass only 16 percent of proposed treatment acreage. B. t. treatments in Maryland and New Jersey will be in between these figures.

It appears that 1984 will be a year of retrenchment as States evaluate new techniques with B. t. in building gypsy moth populations. Pennsylvania, for example, plans to pursue 20 BIU applications in healthy-building populations, and will also gather more operational data on reduced volume applications (96 ounces per acre) and various stickers. New Jersey plans to conduct similar activities. A concerted effort is necessary to continue methods improvement activities in order to improve B. t. performance in these building population situations.

For the foreseeable future, B. t. will continue to be the insecticide of choice in populated areas given the public mood concerning other treatment alternatives. State cooperators agree that single applications of 12 Billion international units (BIU) per acre in static or declining gypsy moth populations do an excellent job and can achieve both foliage protection and population reduction. Where gypsy moth populations are healthy and building, however, B. t. will lose favor unless performance improvements can be demonstrated.

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RECENT FIELD EXPERIENCES WITH *BACILLUS THURINGIENSIS* IN CANADA AND RESEARCH NEEDS

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The CANUSA working group on the use of B.t. against the spruce budworm has prepared a document entitled "Guidelines for the operational use of *Bacillus thuringiensis* (B.t.) against the spruce budworm" following six years of extensive cooperative field trials in Canada and the U.S.A. (Morris et al 1984). The document summarized below (Table 1) describes the current state of the art and is based on the concensus of a committee of scientists actively involved in the management of this insect pest in the two countries.

TABLE I. Technical guidelines for the operational use of *Bacillus thuringiensis* against the spruce budworm - summary

1. Potency: Be sure actual potency is as claimed on label.
2. Aircraft: Calibration essential. Use actual tank mix.
3. Mixing sequence: Water then sticker then B.t.
4. Sticker: As recommended by B.t. manufacturer.
5. Dye: None for operational use.
6. Dosage rate: 12 BIU/AC (30 BIU/ha)
7. Volume rate: .25 to 0.5 gpa (2.37 to 4.7 l/ha).
8. Application timing: When buds start to flare.
9. Ground droplet density: Aim for greater than 25 drops/cm^2.

There are several items in this summary and elsewhere which to my mind requires further study with a view to improving the effectiveness of B.t. I intend to discuss briefly these items, to present some recent field results, and to offer some recommendations for research.

But firstly an update on the operational use of B.t. against the budworm in Canada.

Status of Operation Trials

The summary presented in Table 2 shows quite clearly an increasing use trend between 1979 when the cooperative CANUSA program started and the proposed 1984 use. Between 1979 and 1980, the proportion of forests sprayed with B.t. was very low (1.5 to 2%). The proportion increased to 9% in 1983 and to 14.1% (proposed) in 1984. This represents a substantial increase of B.t. use between the beginning and the end of the co-operative program on the whole, a trend which I expect will continue.

Table 2. Approximate areas (ha) of forest operationally sprayed for spruce budworm control in Canada between 1979 and 1984 (proposed).

Year	Chemical Insecticides	B.t.	% by B.t.
1979 + 1980	4,024,227	83,773	2
1982	2,265,000	34,000	1.5
1983	86,650	7,600	9.0
1984 (Proposed)	2,094,900	343,000	14.1

A summary of the use trend by province (Table 3) shows that Ontario, Nova Scotia and Quebec were the largest users of B.t. between 1979 and 1984 (proposed) based on the percentage of their sprayed areas treated with B.t. New Brunswick also increased their B.t. treated areas but in terms of acreage actually treated with the microbial insecticide the increase was not large. Newfoundland dropped precipitously from 44% in 1979 + 1980 to 1% (proposed) in 1984.

Table 3. Areas (ha) operationally treated by chemical pesticides and B.t. against the spruce budworm in 1979 + 1980 and in 1984.

Province	1979 + 1980 Total	1979 + 1980 % Bt.	1984 (Proposed) Total	1984 (Proposed) % Bt.
ONT.	49,710	9.5	10,000	80.0
N.B.	3,252,360	0.01	1,930,000	2.1
NFLD.	35,619	43.9	71,300	1.1
N.S.	63,276	47.8	35,000	100
QUE.	791,377	4.2	815,800	32.6
TOTAL	4,191,773	2.0	2,862,100	12.2

Effect of B.t. on budworm biomass

It is generally acknowledged that the non-lethal effects of B.t. treatments are important additional criteria for judging the effectiveness of the microbial agent. This type of effect was recently demonstrated in the field against the budworm in balsam fir stands (Morris and Moore 1985). The data summarized below (Table 4) were gathered from experiments carried out at Riviere du Loup, Quebec in 1981. Note that larval biomass in B.t. treated populations were significantly lower than in the control and the Dipel-vehicle-alone treated populations. There was no difference between the check and the Dipel-vehicle plot. This plus the data on population reductions and defoliations demonstrate that the vehicle had no effect on the population as was suspected by one worker. We also found that the Dipel-vehicle had no demonstrable effect on the incidence of larval and pupal parasitism (Morris 1985).

Table 4. Effect of Thuricide 16B® and Dipel 8L® on spruce budworm larval biomass, larval population reduction and defoliation of balsam fir.

Treatments	Larval Biomass (Mg/100 shoots)	% Reduction	% Defol.
Thuricide 20 BIU in 9.4 L/ha	0.86	36a	20±11a
Dipel, 20 BIU in 9.4 L/ha	0.69	36a	21±16a
Dipel vehicle 25%, 9.4 L/ha	44.2	0b	66±16b
Untreated checks	34.2	0b	65±1ab

Potency

Several researchers and operators have received in the past from manufacturers B.t. products which were below labelled potency. Rarely does one have enough time between receipt of the product and the time to apply it to assess labelled potency. This problem, in addition to ensuring significant errors in experimental applications can create serious credibility gaps between the product user and the manufacturer. I believe some attempt has been made by the commercial companies to collaborate on the standarization of their product assays but as far as I know there is still no agreement among them. The problem occurred as recently as 1982 when product potencies as determined by suppliers were far in excess of actual potencies as determined by independent laboratories. I want to implore the producers to come to some general agreement among themselves on bioassay technique for monitoring their products. This technique should be one that is fairly easy to use by researchers, independent laboratories, and operators.

I believe that this bioassay problem is due not only to differences in basic technqiue but also to differences between test insects. Even "minor" genetic differences between and within insect populations may result in differences in their susceptibility to microbial agents. This admitedly makes a standard bioassay procedure rather difficult. One possible way of solving this dilemma is to complement insect bioassay with the enzyme-linked immuno absorbent assay (ELISA) for B.t. endotoxin. The development of this technique while not replacing insect bioassay entirely would be valuable not only for assaying the toxin content of commercial products but also for rapidly assaying tank mixes and field deposits over the short or long term at the ground or tree level. The method is rapid, specific, sensitive and reproducible and I believe, if used, could reduce the variability normally encountered in bioassays. I have no doubt that this has been considered by B.t. and virus producers, so I would be interested to know why it has not been developed to the stage required.

During the 1970's and 1980's, the commercial companies have made a valiant effort to manufacture more highly concentrated B.t. products and have been quite successful in doing so. Products of 48 BIU/US gal. have already been field tested and 64 BIU products are already on the market or will soon be. The obvious advantage to highly concentrated products relates to cost reduction. Much of the cost of earlier materials was due to bulk shipment and this could be reduced by shipping high concentrate products. The other advantage of such high concentrates is the fact that B.t. is most effective when the target trees are densely covered with droplets containing a high concentration of the toxicants.

Table 5 presents data (from Drs. Fast and Sundaram, FPMI) on the relationship between droplet density of B.t. on coniferous foliage and spruce budworm larval mortality. Note that larval mortality at the lowest dosage (8 BIU/U.S. gal) is highly variable regardless of

coverage. This supports my contention that 8 BIU /acre is only marginally effective for both spruce budworm and gypsy moth control.

However, just 3 drops/needle at 12 BIU/gal gave acceptable mortality. A 12 BUI/acre dosage rate is gaining general acceptance by operators. Thus if B.t. is commercially formulated in concentrated form so that the 12 BIU/acre will alow a high level of atomization (VMD circa 60 mm), low evaporation rate and dense coverage at the feeding site, then the cost-benefit relationship in forestry applications should be entirely acceptable.

Table 5. Relationship between droplet density on coniferous foliage and spruce budworm larval mortality following aerial application of Bacillus thuringiensis[a]

Concentration Sprayed	Drops/Needle	% Mortality (50 larvae)
2.1 BIU/l	1	20 (16-24)
(8 BIU/US gal)	3	38 (30-46)
	6	31 (8-70)
3.2 BIU/l	1	55
(12 BIU/US gal)	3	84
5.3 BIU/l	1	78 (70-88)
(20 BIU/US gal)	3	96 (92-100)
	6	100

[a]Courtesy of Drs. P. Fast and A. Sundaram (FPMI)

The question of the most appropriate droplet size for maximum effectiveness against the spruce budworm is also unanswered at this point. It would be useful to know what droplet size one could expect with a particular set of formulation, atomizer and weather conditions but we are still in a state of confusion on this score. To illustrate the problem, Table 6 gives the droplet analyses of undiluted Thuricide and Dipel of drops collected on Kromekote cards during aircraft calibration in 1982.

Table 6. Droplet analysis of spray deposits of undiluted Thuricide and Dipel collected on kromekote cards during aricraft calibration[a]

Criteria	Thuricide 48B	Dipel 48B	Thuricide 32B	Dipel 32B
Spread Factor Mean±SE	1.2±0.09	1.2±0.01	1.2±0.09	1.2±0.08
D_{min}(M)	<25	17	<25	17
D_{max}(M)	233	302	323	417
NMD (M)	50	41	155	163
VMD (M)	100	66	205	207

[a]Applied with AU3000 Micronairs

We see that the droplet maximum diameters (D_{max}) of undilated Dipel 48B and 32B were larger that those of undiluted thuricide 48B and 32B suggesting that the atomization of Thuricide was superior to that of Dipel. Note, however, that the differences between NMD and VMD (which reflect uniformity of the droplet spectrum) were identical for the two products (i.e. 50 um) indicating that the atomization of the sprays were in fact similar, in spite of Dmax differences. This unexpected similarity is a bit of a conundrum but may have something to do with the viscosities of the products. Viscosity will directly affect droplet size range produced during atomization and also affects indirectly droplet size at the impingement site because it affects the rate of evaporation during descent of the droplet. Dipel acts like a typical non-Newtonian fluid. The apparent viscosities of such fluids can be dramatically reduced depending on sheer rate. Research is needed with non-Newtonian products to determine the most suitable apparent viscosities for a variety of nozzle types and sheer rates. Some has already been done but to my knowledge has not been published. Such information would help to predict drop-size spectra for a variety of spray systems.

Lastly, when 32B and 48B preparations are compared, the difference between NMD and VMD for Dipel 48B is 25 but is 44 for Dipel 32B. The reason for the apparent difference in behavior between 32B and 48B products is not clear to me. Perhaps manufacturing representatives can explain this.

Stickers

In the CANUSA technical guidelines, we recommended that a sticker be used in all presently manufactured B.t. product tank mixes. We could not find enough well documented information on the effectiveness of various stickers for aerial application and on their compatibility with B.t. to make any general recommendation. We were also aware that different types of formulations may require different types of stickers. We therefore, suggested that operators should follow the recommendation of the product manufacturers.

Table 7. Some stickers used in the aerial applications of microbial agents over forests.

Product	Sticker	Conc./US gal.
Thuricide, Dipel	Chevron Dupont	2.365-3.785 Ml
	Spread sticker	2.42 ml
	Biofilm	4.73 ml
	Rhoplex B60A	75.7 ml
NPV	Biofilm	4.73 ml

Table 7 presents some stickers commonly used in aerial applications of microbial agents over forests. None of these have been fully accepted by operators or researchers in terms of their effectiveness. There is evidently an information gap here which B.t. producers in particular would be well advised to explore. Acrylic stickers (e.g. Rhoplex) are now entering the market and they do appear to be superior to older types of stickers in terms of effectiveness. Other acrylics like 5% Bond and 5% Acrylucoat are highly effective under laboratory conditions (Win McLane, Otis Air Base) when applied with B.t. or NPV against gypsy moth on red oak seedlings. There are, however, several criteria which should be assessed before a sticker is recommended:

1. It must be determined that the sticker does not delay or inhibit the availability of the microbial agent when it is ingested by the target pest. It should be easily degradable in various liquid environments simulating the insect gut contents of a variety of pest species.

2. It should be non-toxic for the microbial agent. Some microbial agents seem to be particularly sensitive to chemical agents. Microsporiadia in my experience is one such group and nematodes another.

3. Its use should not be limited to certain water types or qualities because the operator often has little choice in terms of the water he must use as diluent.

4. Its chemical nature should not be altered significantly under conditions of high shear produced by aircraft spray systems. In 1982, the use of Rhoplex in B.t. tank mixes in Canada put out of action our aircraft flow meter and gummed up the spinning disc cage of our Micronairs delaying our first-application by one day under good weather conditions. Since polymers tends to introduce thixutropic properties to a liquid formulation, some knowledge of their effect on viscosity-shear rate would be helpful for producing desired drop-size spectra during aerial application.

In summary, it seems to me essential that the physical, chemical, biological and toxicological properties of stickers should be tailor-made to achieve maximum spray efficiency with microbial insecticides.

Tracer dyes

The CANUSA guidelines recommended that no tracer dyes be used in operational applications, however, if a dye is needed the recommendation of the manufacturer should be followed. Dyes are sometimes called for in semi-operational and often in experimental trials. As far as I know, the commercial suppliers are not fully agreed on an acceptable tracer dye. A large number of dyes have been tested as B.t. tracer, but, as in the case of stickers, very little information exists on their compatibility with microbial agents, their chemical stability following release from the aircraft, the

Footnote:

NMD = the diameter that divides the number of droplets into two equal groups - 50% of the droplets have a diameter above the NMD and 50% below.
VMD = the droplet size diameter that divides the spray volume into equal parts - 50% of the volume is in droplets below the VMD and 50% above.

maintenance of their integrity on the surface of foliage and their environmental acceptability.

Table 8 gives a list of the most commonly used tracer dyes for aerial applications of microbials over forests.

Table 8. Some tracer dyes used in aerial applications of microbial agents over forests.

Product	Dye	Con./US gal
Thuricide,Dipel[a]	Rhodamine B	3.785 g
	Nigrosine	7.57 g
	Brilliant Sulphoflavine	3.785 g
	Erio Acid Red X B400	0.095 g
Tussock Moth-NPV	Rhodamine B	3.785 g
	BSF	3.785 g

[a]Water-based

Table 9 presents data on the compatibility of the dyes with B.t. under laboratory conditions (Morris 1980).

Of the 5 dyes shown, only Rhodamine B had any apparent deleterious effect on spore viability but the half-life was so long, that this effect is considered practically insignificant. Rhodamine B, however, has been reported to induce mutations in Salmonella and DNA damage in hamster ovarian cells (Nestmann and Kowbel 1979; Nestman et al 1979) making it suspect environmentally.

Table 9. Decay rates of viable spores of Thuricide 16B diluted 1:1 in water and exposed to 0.1% of various tracer dyes for 21 days.

Dye	Decay Constant (R)	1/2 life-days (95% CL)
Thuricide alone	0	-
Rhodamine B	0.036±0.015	19(10-150)
Brilliant Sulpho Flavine	0	-
Nigrosine	0	-
Erio Acid Red X B400[a]	0	-

[a]No significant decay for 69 days.

I have used successfully Erio Acid Red XB, a red fluorescent dye chemically related to Rhodamine B (4,5 dihydroxy-3-(P-nitro benzyl) azo 12,7 naphthalene disulphonic acid disodium salt. It is not mutagenic for bacteria. Toxicity data from Ciba Geigy indicate rat oral acute toxicity of 5000 mg/kg; 96 hr fish toxicity-LC_{50}, 1000 gm/l; bacterial activity - no inhibition at 300 mg/l.

Table 10 gives data on the toxicity of EAR for spruce budworm larvae using a foliage dipping technique. No significant toxicity to the larvae could be detected even at a concentration 1000x that used in field tank mixes (25 mg/l).

Table 10. Toxicity of Erio Acid Red XB 400 for L_4-L_5 larvae of the spruce budworm; 5 replicates.

Conc. of Dye (g/l)	Pooled No. of larvae	Adjusted % Mortality
0.025	109	0
0.25	106	3.6
2.5	115	0
25.0	103	0
Check	99	-

Table 11 gives data on the bioassay of Thuricide with and without the dye against L_5 larvae. The "needle inoculation" method was used in which known dosages of B.t. were fed to the larvae on balsam fir needles. The dye had no apparent effect on larval mortality. Because this dye is fluorescent, it has been used successfully in detecting very small droplets of B.t. on coniferous foliage under fluorescent optics. It should be tested with other microbial agents.

Sunlight screens

Several workers have demonstrated that B.t. and other entomopathogens are rapidly inactivated by sunlight and some have shown that the residual activity of some of these agents can be enhanced by the addition of

Table 11. Bioassay of Thuricide 16B with and without Erio Acid Red XB400 (0.25 g/l) against 5th instar spruce budworm[a].

Dosage/larva	No.	Adjusted % Mortality	Statistics
3.1 IU	49	49	
3.1 IU + EAR	49	49	x^2=2.026, IDF
31.0 IU	49	88	
31.0 IU + EAR	49	93	x^2=0.544, IDF
Check	49	-	

[a]Single needle inoculation method

sunlight-screens to microbial formulations. Nearly all the published reports so far relate to agricultural systems and only a few of them have done their tests under natural sunlight. Most recently Shapiro et al (1983) demonstrated that benzylidine sufonic acid enhanced the persistence of NPV 3-fold under sunlamps in the 320 to 400 nm range. We designed experiments to find protective materials which could be added to commercial products or to tank mixes intended for aerial application over forests.

Table 12. The effect of sunlight screens on the residual insecticidal activity of Dipel 36B applied to 2 replicate white spruce trees.

Formulation	Slopes[a]	R1/2 in Kcal/ cm^2[b]	Absorb Max. (nm)
Dipel alone	-0.202a	1.8	270
1% DS49+CYASORB + 0.5% CMC	-0.148ab	2.4	282,320
1%DS49 = 1% UVITEX + 0.5% CMC	-0.087ab	4.1	282,330
DS49 + 0.1% EAR			282,320
+ 6% MOLASSES	-0.069b	5.3	400,565
1% DS49 + 0.1% EAR + 1% PVP + 0.5% CMC	-0.129ab	2.8	282,328 565

[a]Estimates followed by the same letter are not significantly different at 5% level (SNK)
[b]R1/2 represents the amount of radiation required to reduce the potency by 50%.

The data in Table 12 summarizes the results of experiments designed to test the effect of sunlight screens on the residual activity of Dipel 36B applied to white spruce trees. Of the four formulations of sunscreens tested, DS49+ EAR+ molasses had the largest number of absorbance peaks within the solar wavelengths, the highest R/2 (the amount of solar radiation measured in $KCal/cm^2$ required to reduce the potency of B.t. by 50%) and a slope that was significantly different from that of Dipel without sunlight screens.

This sunscreen formulation was aerially tested against the spruce budworm in balsam fir stands. The results are summarized in Table 13.

Table 13. The effect of the addition of sunlight screens[a] to commercial Bacillus thuringiensis (Thuricide 32BX) on larval population reduction of and defoliation by the spruce budworm in balsam fir stands.

Dosage rate BIU/ha	% Larval Population Reduction at Days Post-application 14	21	Percent Defoliation[b]
10	0b	9b	69d
10+screen	67a	79a	39ab
20	63a	68a	45bc
20+screen	67a	88a	55cd
Check I	0b	0b	71d
Check II	0b	0b	89d

[a]1% Uvinul DS49 (Sodium 2,2'-dihydroxyl-4,4'-dimethoxy-5-sulfobenzophenone) +0.1 Erio Acid Red B 100 (sodium salt of formyl-m-benzenedisulphonic acid).
[b]Defoliation values followed by the same letter are not significantly different at 5% level.

In these tests molasses was omitted from the mixture because of the already high sugar content of the Thuricide used.

The results indicate that at the lower dosage rate of B.t. (10 BIU/ha), the effectiveness of Thuricide containing the sunlight screens was significantly greater than that of Thuricide without the screens. The screening effect was apparently masked by the higher level of active ingredient at the 20 BIU/ha application rate. Thus, it may be possible to reduce the dosage rate applied by extending the residual life of the B.t. on forest trees.

In summary, while the effectiveness of currently produced B.t. has improved greatly over that of the older products, greater effort on the part of manufacturers to formulate their products with sunlight screens to enhance residual activity seems fully justified to me.

Table 14. Effect of single vs. double application of the same dosage rate on population density reduction in and defoliation of balsam fir stands.

Treatment	Colonies per cm^2	% Population Reduction[b]	% Defoliation
Dipel 2x10	82	91ab	35a
Dipel 1x20	42	84ab	24a
Thuricide 2x20	71	81ab	35a
Thuricide 1x40	77	95ab	36a
CH-I	--	0c	71b
CH-II	--	0c	89d

[a]Dipel population density/100 shoots, 23 and 24; Thuricide, 23 and 27.
[b]Final post-spray corrected for pupal mortality. Values followed by the same letter are not significantly different at 5% level (ANOVA, Tukey's).

Single vs. double applications

Double or split applications of B.t. are generally accepted as necessary in mixed stands of white spruce, Picea glauca and balsam fir, Abies balsamea, but the strategy of applying split applications of B.t. in unmixed stands has been in question. Aerial applications of Dipel and Thuricide in spruce budworm infested balsam fir stands were designed to answer this question. The results summarized in Table 14 indicate that split applications are not significantly more effective than single ones of the same dosage as measured by defoliation and population reduction (Morris 1984).

Dosage and Volume

The question of the most effective and appropriate dosage and volume for aerial application against the spruce budworm was the subject of a cooperative field research project between Canada and the U.S.A. two years ago. In Canada, doses of 10, 20, 40 and 80 BIU in 9.4 l/ha and 30 BIU in 2.4, 4.7 and 9.4 l/ha were applied against budworm populations ranging in density between 10 and 36 fourth instar larvae per 45 cm branch in balsam fir stands. The results of the dosage test (Table 15) show that while larval population reduction and defoliation were statistically similar between 20 and 40 BIU/ha, considering the acceptable limits of 75% population reduction and 50% defoliation of current year's growth, the 20 BIU/ha treatment was only marginally acceptable. When the values were adjusted for differences in population densities by calculating the ratios of larval density to population reduction and % defoliation, it is apparent that the efficacy of the treatments increased with dosage applied. This supports the recommendation of 30 BIU/ha as being likely to give more consistently acceptable results than 20 BIU/ha.

Results of the tests on volumes (Table 16) indicate that the volumes applied between 2.4 and 9.4 l/ha had no significantly different effect on population reduction and defoliation. In fact only the treatment at 2.4 l/ha gave less than 50% defoliation. These data support the CANUSA recommendation of applying B.t. in volumes betweem 2.4 and 4.7l/ha.

Table 15. Population reduction and defoliation in balsam fir stands treated with various doses of Thuricide 32B in 9.4 l/ha.

Dosage BIU/ha	Larval density/45 cm branch	Population reduction (%) 21 days post-application	Defoliation (%)	Ratio: Larval density/pop. red.	Ratio: Larval density/% defol.
Check	14	1 d	80 b	14.0	0.18
10	16	9 cd	69 b	1.8	0.23
20	12	68 ab	45 a	1.8	0.27
40	16	95 a	36 a	1.7	0.44
80	36	95 a	61 b	0.4	0.59

Table 16. Population reduction and defoliation in balsam fir stands treated with various volumes of Thuricide 48B at 30 BIU/ha.

Volume/ha	Larval density/45 cm branch	% population reduction 21 days post-application	% Defoliation	Ratio: Larval density/pop. reduction	Ratio: Larval density/% defoliation
Check	18	75 a	86 a	0.24	0.21
2.4	10	68 a	45 b	0.15	0.22
4.7	17	72 a	59 ab	0.24	0.29
9.4	16	76 a	56 ab	0.21	0.29

[a]Three replicates

To summarize this discussion as a whole, I offer the following recommendations:

1. Every effort should be made by B.t. manufacturers to guarantee the potency of their product to the satisfaction of their clients.
2. Manufacturers of B.t. ought to continue their already valiant efforts to formulate their products in highly concentrated form in order to reduce application costs to users.
3. Stickers should be well researched for their effectiveness, bioavailability of microbial agents with which they are mixed and their compatibility with various formulations and spray equipment before they are marketed or recommended.
4. While tracer dyes for B.t. are not generally recommended for operational B.t. sprays, they are sometimes needed for semi-operational and experimental trials. Such dyes should be non-toxic to target insects, to the microbial agents with which they are mixed and should be environmentally acceptable. Erio Acid Red is suggested as an acceptable dye.
5. I recommend that a concerted research effort be put in place by B.t. manufacturers to find effective sunlight screens which can be added to their commercial formulations or to tank mixes.
6. Information on the most effective droplet sizes for spruce budworm control is sparce. More research is needed in this area.

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RECENT FIELD RESEARCH EXPERIENCE WITH B.T. AGAINST SPRUCE BUDWORM IN THE EASTERN U.S.

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Recent testing in the eastern U. S. has led quickly to the adoption of 12 BIU/acre as the best operational dosage rate, with operational spray emission rates reduced to a quart or less per acre. Some recent work suggests that older larval instars of the budworm are highly susceptible to B.t. sprays, and the effective "spray window" can be broadened when budworm populations are moderate. New studies on second-year benefits of B.t. treatments are producing negative results.

Introduction

Development of B.t. for spruce budworm control has occurred rapidly and dynamically. In only 4 or 5 years B.t. has changed from a little used, expensive material, of questionable reliability, to one which is now perceived to compete favorably with chemical insecticides in quantities used (in some jurisdictions at least), in cost, and in reliability. The improvements in technology, made by the manufacturers of B.t. and tested by cooperating research groups, have rapidly become operational. These operational changes will have been discussed by other speakers, and we need not dwell on them at length.

The principal tests involved:

1. Comparisons of the major, competing formulations of B.t. (Walton and Lewis 1982, Dimond et al. 1981, Reardon et al. 1982, Grimble and Morris 1983, Morris 1981). These found no significant differences in efficacy.

2. Comparisons of three dosage rates and two dilution rates of B.t. sprays (Lewis et al., in press), as well as results of operational testing (Morris 1982), suggested that 12 billion International Units (BIU) per acre of B.t. produced more reliable results than the prevailing 8 BIU dosage, and should become the standard operational dosage. It was also concluded that spray emission rates of one gallon/acre provided no advantage over 0.5 gallon/acre.

3. Tests of applications of undiluted or "neat" B.t. (Dimond 1982) with spray emission rates as low as 1 quart/acre, were highly successful. This was followed by operational usage, with success, in 1983. Undiluted, or similar low-volume applications, are scheduled for wide use in 1984.

4. A combination of laboratory and field tests (Fast and Dimond 1984) suggested that the later larval stages of the budworm, through early sixth instar, are readily killed with B.t. It is no longer necessary to believe that a narrower "spray window" must be used with B.t. than with chemicals. Later applications, aimed at fourth and fifth instars, are likely to provide best results when budworm populations numbers are not unusually high.

5. In cooperation with Normand Dubois of the USDA, Forest Service, we field-tested one of his new strains of B.t., found in the laboratory to be more efficacious against the spruce budworm. Dubois will present these data in his contribution to this Symposium.

Prolonged Benefits of B.T. Treatments

For the balance of this presentation, I will summarize some unpublished information dealing with the question of second-year benefits of B.t. applications, sometimes called "carry-over effects". The latter term is a poor choice since it implies a possible persistence of B.t. organisms in substantial amounts within the budworm population or on the host trees for a year or more.

While some spores apparently can carry over to a second year (Reardon and Haissig 1983, 1984) there is no evidence that these are in sufficient quantities to affect budworm populations. Second year benefits could be explained, however, by the frequent observation that survivors of B.t. treatments often produce undersized, and presumeably unhealthy, pupae and moths (Morris and Moore 1983, Smirnoff 1983, Dimond and Spies 1980). It has been suggested that the generation of insects descending from these stunted survivors may have a low survival potential (Morris and Moore 1983, Smirnoff et al. 1974).

Evidence supporting second year benefits of B.t. applications has been circumstantial until recently. Dimond and Spies (1981) noted significantly less defoliation in small, 100 acre plots treated the previous year, than occurred in nearby control plots. They speculated that population reductions due to B.t. treatments were unlikely to have persisted to a second year because of the small sizes of plots and the high dispersive tendencies of both larvae and moths of the budworm (Greenbank et al. 1980); but, they had no counts of budworm numbers in the plots. Morris (1977) noted that over 2 to 3 years after treatment, both defoliation by and oviposition rates of spruce budworm were lower in treated plots than in untreated plots. In neither of the two cases above was it possible to identify any specific cause for these observations.

In further studies in 1981 (Dimond et al., unpublished[1]), we sampled budworm populations and damage in operational spray blocks (3,000 - 15,000 acres) treated by the Maine Bureau of Forestry the preceeding year. Seven blocks were selected, which had received satisfactory protection from B.t. applications in 1980, (Millers 1980), along with seven, nearby untreated areas. Samples of early-instar larvae, late-instar larvae, early pupae, and late pupae (= early adult) were collected in all plots. Insects were counted and instars determined. The latter step was to compare developmental rates between treatments. Subsamples of 200 insects from each collection in each block were reared in the laboratory to measure survival rates. Insects dying were examined for presence of entomopathogens. Defoliation rates were also checked on all blocks, using the standard Fettes system (Sanders 1980).

The results of these observations were inconclusive. Sprayed blocks began the season with somewhat higher mean numbers of insects than control stands (Table 1). However, the control stands had slightly higher numbers in the late collections. This suggests lower survival in the treated blocks, however, the differences were small and not statistically significant.

Percent of insects that were dead in collections were always slightly higher in the four treated blocks in the Molunkus area than in controls (Table 2). But, this was not true in the Caribou area. None of the differences were significant.

Percent of insects that died during rearing, for reasons other than emergence of parasitoids (Table 2), showed no significant differences. Causes of death (Table 3) showed a preponderance of microsporidian infections and unidentified deaths. Bacterial, viral, and fungal infections were rare, and B.t. was not recovered. There were no differences between treatments.

Developmental indices[2] (Table 4) showed treated and untreated populations in the Molunkus area to be equally advanced in the first collection, but sprayed populations were significantly delayed in the three later collections. In the Caribou blocks, development was significantly advanced in the treated blocks in the first collection, but with no difference in subsequent collections. Both cases suggest a statistically significant retardation of development of the treated populations, however, the differences were very minor, and perhaps of questionable ecological impact.

Pupal weights (Table 5) were different between areas, probably due to different insect densities in the two areas. But, there were no differences between treatments.

Defoliation (Table 6) in 1981 was greater or equal to levels in 1980, and there were no differences between treatment.

These findings were perplexing. Differences between treated and untreated plots were few and very minor in magnitude. If the differences were real, they were too small to explain the differences in defoliation rates seen the year earlier (Dimond and Spies 1981). But, on the other hand, we did not detect differences in defoliation (Table 7) in this series of blocks. We had no data to explain the earlier observations because prolonged benefits of B.t. treatments were not evident in the plots studied.

To further compound perplexity, we had one additional set of data, also taken in 1981. Defoliation observations were made in a series of 100 acre plots, 25 treated and 5 untreated that were sprayed experimentally with B.t. in 1980 (Dimond et al. 1981). These showed the same results (Table 7) that we had seen the year before; protection from defoliation appeared to persist for a second year. It is unfortunate that we had no population data from these small plots. We had chosen to study the large State blocks, believing that events there would be less influenced by redistribution of sprayed and unsprayed populations after treatment.

Similar studies continued in 1981[3]. Overwintering larvae were collected in April, 1982 in four State operational blocks that had been treated in 1981, and in four nearby untreated sites of similar character. Collections were made by pruning 25 balsam branches, 1 per tree, in each plot and forcing the larvae from hibernation. Larvae were counted as they emerged, and these were reared through to the pupal stage on artificial diet (Grisdale 1970). Plots had been treated with B.t. at 12 BIU/acre, and this was the first time that this dosage had been used operationally in Maine. No further collections were made on these plots because all were retreated with B.t. in 1982. Observations on the reared insects included development rates, survival rates, and pupal weights. Significantly more insects emerged from the untreated branch collections than from the treated branches (Table 8). There were no significant differences in rates of development or survival or in pupal weights.

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[1] Dimond, J.B., D.G. Boucias, and C.J. Spies, 1981. Prolonged effects of Bacillus thuringiensis treatments on spruce budworm: field observations in Maine 1981. Office report. Entomology Department, University of Maine, Orono, 04469, 23 p. Report available from the author.

[2] A developmental index for a population containing 10% instar 2, 60% instar 3, and 30% instar 4 is calculated as (10 x 2) + (60 x 3) + 30 x 4)/ 100 = 3.2. A lower value of the d.i. indicates a younger age structure in the population.

[3] Dimond, J.B. 1983. Final report on a contract to continue studies of prolonged effects of B.t. applications on spruce budworm. Office report, Entomology Department, University of Maine, Orono, 04469, 12 p. Report available from author.

Table 1.--Numbers of spruce budworms collected in 1981 in operational spray blocks treated with B.t. the preceeding year and in unsprayed stands, mean and range (parentheses).

Block	Collection period - insects per 18 inch branch			
	Early larval	Late larval	Early pupal	Late pupal
Molunkus area sprayed	39(27-50)[a]	28(23-34)	15(11-18)	6(2-8)
Molunkus area unsprayed	30(24-37)[a]	30(25-40)	18(14-24)	6(4-10)
Caribou area sprayed	20(11-32)[b]	-- --	12 (9-15)	8(5-10)
Caribou area unsprayed	17(16-18)[b]	-- --	10 (9-11)	8(7-9)

[a]Mean and range of 4 replicates.
[b]Mean and range of 3 replicates.

Table 2.--Percentage of spruce budworms that were dead in collections or died during rearing (in parentheses).

Block	Collection period - % of insects			
	Early larval	Late larval	Early pupal	Late pupal
Molunkus area sprayed	10.0(16.8)	6.8(17.0)	3.2(11.8)	12.4(26.2)
Molunkus area unsprayed	9.9(15.1)	3.5(13.1)	3.1(11.3)	7.2(28.0)
Caribou area sprayed	18.3(15.7	-- --	8.2(22.1)	10.9(2.8)
Caribou area unsprayed	18.8(15.2)	-- --	10.6(22.5)	11.4(1.6)

Table 3.--Results of tissue analyses of dead larvae in collections and those dying during rearing.

Block	Numbers examined	Diagnosis of larvae, %				
		Bacteria	Virus	Microsporidia	Fungus	Unknown
Molunkus area sprayed	235	5	2	43	7	43
Molunkus area unsprayed	180	1	7	43	6	44
Caribou area sprayed	238	2	1	42	3	54
Caribou area unsprayed	168	3	1	49	8	39

Table 4.--Developmental indices of spruce budworm populations in blocks sprayed with B.t. the previous year and in unsprayed stands, mean and range (parentheses).

Block	Collection period			
	Early larval	Late larval	Early pupal	Late pupal
Molunkus area sprayed	3.4(2.9-3.9)	5.1(4.9-5.2)[a]	5.9(5.8-5.9)[b]	7.3(7.1-7.5)[b]
Molunkus area unsprayed	3.3(2.9-3.8)	5.3(5.1-5.4)	6.0(5.9-6.0)	7.6(7.5-7.7)
Caribou area sprayed	4.4(4.3-4.6)[b]	-- --	6.4(6.2-6.4)	7.0(6.9-7.0)
Caribou area unsprayed	3.9(3.8-3.9)	-- --	6.4(6.2-6.6)	7.0(6.9-7.0)

[a]Significant difference, $p < 0.10 > 0.05$
[b]Significant difference, $p < 0.05$

Table 5.--Weights of pupae collected in blocks sprayed the previous year with B.t. and in unsprayed stands, mean and range (parentheses).

Block	Females	Males
Molunkus area sprayed	28.1(27.0-30.3)[a]	14.2(13.8-14.7)
Molunkus area unsprayed	25.5(22.3-27.7)	14.4(13.9-14.8)
Caribou area sprayed	32.3(31.2-32.9)	16.4(16.1-16.8)
Caribou area unsprayed	31.5(30.1-33.6)	16.4(15.8-17.1)

[a]Each mean is derived from approximately 200 insects.

Table 6.--Percent defoliation of balsam fir on blocks treated in 1980 with B.t. and in unsprayed stands 1980 and 1981, mean and range (parentheses).

Block	1980	1981
Molunkus area sprayed	43(35-52)	69(55-81)
Molunkus area unsprayed	52(29-73)	71(63-81)
Caribou area sprayed	51(26-89)	79(62-89)
Caribou area unsprayed	86(79-94)	84(82-88)

Table 7.--Percent defoliation on small plots treated in 1980 with B.t. and in unsprayed plots, 1980 and 1981; means of five replicates.

Treatment	1980	1981
Dipel 1 gpa[d]	56 a[e]	27 a
Dipel 1/2 gpa	60 a	27 a
Thuricide 1/2 gpa	64 a, b	38 a, b
Dipel 1/4 gpa	65 a, b	25 a
Thuricide 1 gpa	77 b, c	46 b, c
Untreated	86 c	70 b, c

[d]All treatments at 8 BIU/acre but with different dilution rates.
[e]Means sharing the same letter are not significantly different, $p > 0.05$.

Table 8.--Observations made in 1982 in four operational spray blocks treated the previous year and in four comparable untreated stands, mean and range (parentheses).

Observation	Treated blocks	Untreated blocks
Numbers of larvae emerging from collected branches	175(95-282)[a]	378(274-494)
Days from emergence until pupation - males	21.3(16.8-23.0)	21.3(17.5-24.5)
- females	23.7(19.1-27.2)	23.9(20.6-26.3)
Percent survival of reared larvae	45 (28-56)	36 (31-41)
Weights of surviving pupae - males	42 (37-49)	44 (32-55)
- females	71 (55-87)	69 (49-93)

[a]Significant difference between treatments, $p > 0.05$.

Before drawing any conclusions from these results, one additional study should be reviewed. Reardon and Haissig (1983) studied 1-year post treatment densities of budworms and defoliation levels in a series of small plots in Wisconsin (Reardon et al. 1982). One year after treatment, populations and damage of the budworm were significantly lower in the treated plots than in controls. Populations declined on all plots in 1981, but the amount of decline was uniform among treatments. Development rates, as indicated by the distribution of larval instars in the collections, and pupal weights were not different. They attributed the reduced defoliation in 1981 as reflecting a persisting reduction in insect population, rather than any "carry over" influence of the B.t. treatments.

We have evidence in three cases (Morris 1977, Reardon and Haissig 1983, Table 8 of this report) that population reductions due to B.t. treatments persisted into a second year. This becomes the most credible explanation of reduced defoliation in B.t.-sprayed plots a year after treatment. We also have one case where population reductions did not persist into the next generation (Table 6). Data available on reduction in population survival of spruce budworms in the generation after treatment are largely negative (Tables 2-5, Table 8, Reardon and Haissig 1983) or show only very small differences. On the basis of results to date, there seems little evidence that the offspring of survivors of B.t. treatments are of less than normal vigor or have reduced potential for survival.

If the conclusions above are true, it follows that population reductions due to any agent, biological or chemical, may persist beyond the generation of treatment, and that prolonged benefits of B.t. treatments may not be a unique attribute of B.t. It may be significant that the three cases where population reductions persisted following B.t. treatments (Morris 1977, Reardon and Haissig 1983, Table 8), and two cases where only defoliation benefits have been reported (Dimond and Spies 1980, Table 7), have occurred in areas of declining population trend. Where there was no evidence of prolonged benefits of B.t. (Table 6), population were high or increasing (Table 1). There may be less redistribution of budworm populations, due to dispersal, and less re-invasion of sprayed plots, in declining populations.

There are delayed benefits of B.t. treatments in that survivors may be stunted, leading to lower survival of pupae and lower egg-production of moths. But, it now appears unlikely that any effect continues into the next generation.

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FIELD TESTS WITH A HIGHLY CONCENTRATED *BACILLUS THURINGIENSIS* FORMULA AGAINST SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

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Field tests were conducted in 1980, 1981, and 1982 with a new *Bacillus thuringiensis* formula called Futura. Tests were conducted with a Grumman AgCat aircraft on 40 ha forest blocks and with a DC-4G aircraft on larger forested areas. Futura which is able to disperse the required 20 x 10^9 I.U./ha for spruce budworm control in 2.5 L/ha proved to be physically and bacteriologically stable and easy to mix. Also it assured a deposit of up to 80% emitted volume, a larval mortality in the range of 70 to 90%, and a foliage protection of about 70%. Because of its low-volume dispersion property and other qualities it is a most valuable alternative to chemical insecticides for spruce budworm control.

Introduction

Investigations with *Bacillus thuringiensis* carried out during the past 10 years, have shown that this entomopathogen can be used effectively for the operational control of spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae) (Smirnoff 1978, Smirnoff 1979, Smirnoff 1980a). This work led to the development of a compact, efficient, and economical *B. thuringiensis* suspension which could be dispersed at the dosage of 20 x 10^9 I.U. in a final volume of 2.5 L/ha (Smirnoff 1981). This preparation, called Futura, was tested during calibration tests (Smirnoff 1980b, c) and field tested in 1980, 1981, 1982, and 1983 (Smirnoff *et al.* 1982, Smirnoff and Valéro 1983).

Materials and Methods

All stands were composed of 70% balsam fir, *Abies balsamea* (L.) Mill., with white spruce, *Picea glauca* (Moench) Voss, trembling aspen, *Populus tremuloides* Michx., and white birch, *Betula papyrifera* Marsh., as remaining species. These stands were 55 to 65 years old and considered adequate for the requirements of pulp and paper industries.

The tests were conducted in the Chicoutimi region of Quebec on 40 ha blocks of forest severly infested with spruce budworm. Three blocks (one each year) of 3 500 ha, were treated by the Quebec Department of Energy and Resources in the Rivière-du-Loup region of Quebec. Three sample plots, consisting of transects with 5 marked trees, one chain apart, were established in each 40 ha block and eight in a nearby untreated area. Nine sample plots were established in the 3 500 ha treated blocks and eight in a nearby untreated area.

Samples were taken from the upper part of the median section of trees. To assess larval mortality, two samples were made before, and four after treatment in each of the sample plots. Post-treatment samples were collected 10 days after spraying, at peak L_6 and again at 90% adult emergence. For determining defoliation, the methods of Fettes (1950) and Dorais and Hardy (1976) were used. Also, assessment of the current year growth mass was carried out. Tree mortality, efficiency of treatment sequences, loss of wood volume, and stand improvement were assessed in 0.04 ha sample plots established in each treated block and in the untreated area.

The 40 ha blocks were treated with a Grumman AgCat aircraft equipped with a boom and nozzle spray system composed of flat fan (T8004) nozzles. The 3 500 ha blocks were done either with a DC-4G or a Super Constellation L-749 aircraft equipped with a boom and nozzle spray system composed of 110 open type nozzles evenly distributed on each boom in 1981 and 1982, and close to fuselage in 1983 to improve deposit. The spray system on both aircrafts had been calibrated extensively to optimize deposit[1]. For the three years the dispersions were carried out from June 3 to 15, temperature varied between 1°C and 14°C, relative humidity between 60 and 100%, and winds were always below 10 km/h.

The method of Smirnoff (1980b, 1982) was used to assess deposit in number of viable spores per square centimetre. This implies distribution of containers with peptonized water at 30 m intervals along a transect perpendicular to flight lines.

Concurrently, biochemical analyses were conducted on residual pupae from blocks treated with *B. thuringiensis* and from the untreated area to determine any residual effect of *B. thuringiensis* on the spruce budworm populations (Smirnoff 1983). Measurements were also made on residual pupae from blocks treated with chemical insecticides to obtain comparative values.

Formulas used:
1.- Futura [2]: 15% *B. thuringiensis* primary powder (80 000 International Units/mg); 44.8% Sorbo (an aqueous 70% sorbitol solution); 33.7% water; 6.5% inert ingredients; and 1/1 600 Chevron sticker.

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[1]/Smirnoff and Valéro, submitted, Environment Canada, Canadian Forestry Service Res. Notes.
[2]/Biochem Products, Montchanin, Delaware, USA

Each hectare was sprayed with 1.5 L of the above preparation diluted with 1 L of non-chlorinated water and 1.56 mL of Chevron sticker.
2.- Thuricide 48B [3]: 2 parts Thuricide 48B; 1 part non-chlorinated water; and 1/1600 Chevron sticker.
3.- Thuricide 48LV [3]: 50 parts Thuricide 48LV; 20 parts Sorbo; 30 parts non-chlorinated water; and 1/1600 Chevron sticker.

Results

Field tests

1980. In 1980 spruce budworm populations in the region of the field station, Chicoutimi, Quebec, were rather low and tests aimed mainly at comparing Futura with the Thuricide + sorbitol formula used at 4.7 L/ha in large field tests. Results revealed that deposit with Futura was good with 348 756 viable spores per square centimetre over a theoretical emission of 445 000 spores per square centimetre meaning that 80% of emitted number of spores were deposited at ground level. Larval mortality was 72.2% and defoliation 7.45%. These figures compared advantageously with results given by the Thuricide + sorbitol formula and were of course much better than those obtained in untreated plots. Futura dispersed well, provoked a suitable larval mortality, and protected foliage sufficiently. The next step was to test it in severely infested stands supporting various degrees of defoliation.

1981. In 1981 three series of field tests were carried out. The first treated 7 x 40 ha plots with Futura at 2.5 L/ha (20 x 10^9 I.U./ha) using a Grumman AgCat aircraft. The second test treated 895 ha with Futura at 2.35 L/ha (18.8 x 10^9 I.U./ ha) by means of a Super Constellation L-749 aircraft. The third test, carried out simultaneously with the first test as a compagnion, involved treatments using Thuricide 32B/sorbo/water/chitinase/Chevron (20 x 10^9 I.U./ha in a final volume of 4.7 L/ha) by means of a Grumman AgCat aircraft. Results are summarized in Table 1. They show that Futura dispersed by the Grumman AgCat was able to yield a good larval mortality and foliage protection. Larval mortality was 72.0% 10 days after treatment dispersed at 2.5 L/ha compared to 70.3% with Thuricide + sorbitol, dispersed at 4.7 L/ha. Defoliation with Futura averaged 44.1%, but some of the blocks were in a desperate situation after several years of severe defoliation. Thuricide + sorbitol yielded a 67.0% defoliation for a stand in an acceptable condition (Table 1).

Dispersion of Futura by means of the Super Constellation aircraft at 2.5 L/ha yielded a 75.0 to 96.9% larval mortality for a pretreatment population averaging 27.5 larvae per 45 cm with a peak at 46.2 larvae per 45 cm (Table 1). Defoliation was 25 to 40% in plots with 10 to 20 larvae per 45 cm and 50 to 60% in plots with 25 to 35 larvae per 45 cm.

Finally, foliage potential (buds formed in the fall) increased in all treated plots and decreased in untreated plots (Table 1). At the end of the 1981 field season it was concluded that Futura was able to kill spruce budworm and protect foliage with half of the volume of the *B. thuringiensis* formulas used precedingly, 2.5 L/ha instead of 4.7 L/ha.

1982. Five 40 ha blocks were treated with Futura (20 x 10^9 I.U. in 2.5 L/ha); one 40 ha block with Thuricide 48B (20 x 10^9 I.U. in 2.5 L/ha); another 40 ha block with Futura (30 x 10^9 I.U. in 3.75 L/ha); and one more with Thuricide 48LV (30 x 10^9 I.U. in 4.7 L/ha). The aim was to determine the efficiency of Futura in different conditions of stand deterioration and population levels and compared it with a higher dosage of an other formula. Results are summarized in Table 2. Also, a 3 500 ha block was treated with Futura by means of a DC-4G aircraft.

The number of *B. thuringiensis* viable spores initially emitted was 445 000 per square centimetre for a dosage of 20 x 10^9 I.U./ha and 667 500 per square centimetre for a dosage of 30 x 10^9 I.U./ha. In blocks treated with Futura at 2.5 L/ha for a dosage of 20 x 10^9 I.U., the number of viable spores deposited varied from 269 830 to 406 821 spores per square centimetre and averaged 351 848 per square centimetre. Deposit was 268 862 spores per square centimetre with Thuricide 48B. In blocks treated with a dosage of 30 x 10^9 I.U./ha, deposit was 520 300 viable spores per square centimetre for Futura (3.75 L/ha) and for Thuricide 48LV (4.7 L/ha), 608 388 spores per square centimetre. Deposit with Futura dispersed with a DC-4G was 222 400 viable spores per square centimetre (Table 2). Deposit varied from 60 to 90% of the emitted number of spores during treatment with the Grumman AgCat aircraft.

Larval mortality established 10 days after treatment averaged 91.5% in blocks treated at 20 x 10^9 I.U./ha compared with 95.3% in blocks treated at 30 x 10^9 I.U./ha showing that higher dosage has little effect on spruce budworm mortality. Defoliation of current year shoots averaged 13.4% in blocks treated at 20 x 10^9 I.U./ha and was 24.7% in blocks treated at 30 x 10^9 I.U./ha. In untreated areas defoliation was 100% (Table 2).

The current year growth mass also indicates the valuable protection resulting from the use of Futura at 20 x 10^9 I.U./ha. For each 100 cm branch, 49.0 g of current year shoots were protected while only 1.2 g remained in untreated plots (Table 2). Results using the DC-4G were satisfactory (Table 2). Also, calibration tests conducted in the fall of 1982 with the DC-4G aircraft revealed that an increase of 50% in deposit of *B. thuringiensis* can be obtained by distributing the 110 nozzles on the DC-4G close to fuselage, 55 on each side.[4]

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[3] Zoecon Inc., a subsidiray of Sandoz Inc., Palo Alto, California, USA

[4] Smirnoff and Valéro, submitted Environment Canada, Canadian Forestry Service, Bi-Mon. Res. Notes.

Table 1. Results of field tests with *Bacillus thuringiensis* against the spruce budworm in 1981

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
				Grumman AgCat				
Futura	20.0	2.5	283 000	24.9	—	34.7	26.5	93.3
Futura	20.0	2.5	342 850	30.1	—	58.8	17.1	84.5
Futura	20.0	2.5	381 600	17.2	—	38.9	18.6	70.5
Average-Futura	20.0	2.5	335 816	24.0	—	44.1	20.7	82.8
Thuricide 32B/ Sorbo/Water	20.0	4.7	413 000	22.2	—	67.0	17.3	85.9
Untreated a/	—	—	—	25.2	—	96.4	4.8	39.4
				Super constellation L-749				
Futura b/	18.8	2.35	295 000	10-20	—	25-40	15.1	76.9
Untreated c/	—	—	—	29.5	—	98.7	0.7	6.6

a/ Average for 8 sample plots; b/ Average for 9 sample plots; c/ Average for 5 sample plots.

Table 2. Results of field tests with *Bacillus thuringiensis* against the spruce budworm in 1982

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
				Grumman AgCat				
Futura	20.0	2.5	336 380	28.0	0.14	21.9	33.4	77.8
Futura	20.0	2.5	384 780	17.3	0.13	12.5	46.6	77.9
Futura	20.0	2.5	269 830	20.2	0.19	19.6	42.9	77.8
Futura	20.0	2.5	406 821	24.8	0.37	12.9	67.4	81.1
Futura	20.0	2.5	361 427	12.2	0.13	10.3	54.7	85.3
Average-Futura	—	—	351 848	20.5	0.19	13.4	49.0	79.9
Thuricide 48B	20.0	2.5	268 862	11.8	0.09	8.8	67.4	68.4
Futura	30.0	3.75	520 300	22.7	0.26	22.7	41.2	91.1
Thuricide 48LV	30.0	4.7	608 388	36.8	0.24	26.7	48.3	84.1
Untreated a/	—	—	—	25.7	0.24	100.0	1.2	4.6
				TC-4C: Four engines				
Futura b/	20.0	2.55	222 400	31.8	0.19	24.9	43.7	80.1
Untreated c/	—	—	—	30.1	0.28	100.0	0.6	10.2

a/ Average for 8 sample plots; b/ Average for 9 sample plots; c/ Average for 5 sample plots.

1983. The field tests were aimed at determining the theoretical minimum dosage that can be used with Futura. Thus, Futura was dispersed at 2.50, 2.14, 1.43, and 0.71 L/ha (35.0, 30.0, 20.0, and 10.0 ounces/acre). Also, a new device called "pyramid" was developed to determine spreading of B. thuringiensis dispersion in a tree for each dosage tested and for each flight. Petri dishes with nutrient agar medium and containers with peptonized water were placed at different levels and depth in the tree crown and under it. Some 2 000 analyses were carried out and preliminary results reveal: there is no relation between the number of viable spores per square centimetre and the number of droplets per square centimetre; evaluation of the number of viable spores dispersed is the only valuable figure for deposit assessment; deposit at different levels in the tree varied with height of dispersion (flight altitude).

Results of field tests are just being analysed and some of them are reported in Table 3. It can only be said at this time that it seems possible to reduce the volume of Futura provided that purer B. thuringiensis concentrates be obtained, i.e. concentrates with less inert material. This would permit droplets with a larger number of spores, but still provide the 20 x 10^9 I.U./ha required for spruce budworm control.

Also, a 3 500 ha block was treated with Futura at 2.5 L/ha by means of a DC-4G aircraft. Although pretreatment population was high, 50.6 larvae per 45 cm or 0.31 larvae per bud, larval mortality was 94.1%, treatment efficiency (Abbott 1925) 78.9%, and defoliation between 40-50%.

Results of Long-Term Studies

Observations carried out during the last 5 years in blocks treated with different treatment sequences, in the B. thuringiensis treated blocks, and in the untreated area, revealed an average of 255 dead trees per hectare in treated blocks and 706 dead trees per hectare in the untreated area. Thus B. thuringiensis treatments saved 451 stems per hectare. Tree mortality in treated blocks was 12% and it was 40% in the untreated area. This represents the protection of some 29 metric tons of foliage per hectare (12.91 tons per acre) over a 5 year period. Studies continue to determine protection in terms of volume of wood per hectare.

Results of the biochemical analyses conducted during the past 8 years revealed that residual pupae of C. fumiferana surviving in B. thuringiensis treated areas have greatly reduce potential activity compared with that of pupae from untreated areas, as shown by their weight, calcium level, and total protein content as well as by their hydroxybutyrate dehydrogenase (αHBDH) and phosphatase activities. B. thuringiensis has a residual effect on the surviving C. fumiferana populations, involving a marked decline in the energy potential which is seen in practice as a reduction in the reproduction ability of the residual pest population (Smirnoff 1976, 1983). Conversely, the weights of calcium and total proteins of pupae from areas treated with Fenitrothion (organophosphate) are higher than in untreated pupae. Because αHBDH is of great importance during metamorphosis and adult development, this higher level is significant. It thus appears that the organophosphate treatments encourage the survival of a

Table 3. Results of field tests with Bacillus thuringiensis against the spruce budworm in 1983

Formulas	Dosages x 10^9 I.U./ha	Volume L/ha	Deposit viable spores/ cm^2	Pretreatment Populations		Current year growth defoliation %	Mass of current year growth/100 cm branch g	Buds formed (for the next year) %
				Larvae/ 45 cm branch tip	Larvae/ bud			
				Grumman AgCat				
Futura	20.0	2.50	349 841	12.3	0.08	32.8	36.0	84.2
Futura	17.0	2.14	142 000	21.1	0.24	55.9	18.6	78.5
Futura	16.3	1.43	143 456	19.9	0.19	54.8	12.4	73.4
Futura	8.0	0.71	135 475	18.8	0.19	49.8	22.1	79.7
Untreated a/	—	—	—	20.9	0.28	93.1	2.7	33.2

a/ Average for 8 sample plots.

vigorous C. fumiferana residual population with a high potential activity; in addition, such treatments ensures that more foliage is available for the survivors the following year. Moreover, the emergence of very vigorous populations of C. fumiferana could contribute to the formation of permanent spruce budworm epidemics in areas repeatedly treated with chemical insecticides, as suggested by the epidemiological studies conducted by Blais (1974).

Discussion and Conclusion

The results showed that the high concentrated and low volume B. thuringiensis formula which provides a dispersion of 20 x 10^9 I.U. in a final volume of 2.5 L/ha, assures good control of C. fumiferana populations and adequate foliage protection. The low-volume required, 2.5 L/ha, meets objectives imposed on cost of material and treatment, because this suspension can be successfully dispersed by means of large capacity aircrafts. Futura formula requires little mixing at the airport, is transported in a concentrated form, and dispersed in a final volume lower than that of the twice yearly dispersions of chemical insecticides presently used against spruce budworm. The conservative sorbitol allows storage of the ready to use Futura, including water, for several months on field sites. Sorbitol is a technical sugar and its osmotic pressure is higher than that of bacterial spores preventing fermentation and contamination. Under laboratory conditions, sorbitol keeps Futura's physical properties at a temperature of -22° C for several months. In 1983, Futura was dispersed in flight temperatures as low as -7° C and results showed that its physical properties were not affected under these field conditions. Also, B. thuringiensis should no longer be considered as a possible way of budworm control, but as an operational method with the same "ready to use" property as all other insecticides presently used against spruce budworm. An efficient control of C. fumiferana can be obtained with 20 x 10^9 I.U./ha. Therefore why use 30 x 10^9 I.U./ha (10 x 10^9 I.U. more) to obtain 10.4% less protection? Is it not a costly waste of material? This was confirmed by B. thuringiensis core tests conducted within CANUSA. These tests also showed that dispersion of B. thuringiensis at 30 x 10^9 I.U./ha is not more efficient than that of 20 x 10^9 I.U./ha (F.B. Lewis, personal communication). B. thuringiensis will be used by foresters for the operational control of C. fumiferana if its use remains operationally and economically valid and realistic. As with any method of control, the successful use of B. thuringiensis relies on the strict respect of technological recommendations (Smirnoff 1980d), which means use of the recommended formulas, dosages, final volume per hectare, and proper calibration of the spray system of the aircraft.

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STATUS OF VIRUSES AS BIOCONTROL AGENTS FOR SPRUCE BUDWORMS

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Abstract

Aerial spray trials with a variety of viruses have been conducted between 1971 and 1983 with 2 656 ha (65 plots) treated to control spruce budworm in Ontario and Quebec and 424 ha (6 plots) treated to control western spruce budworm in British Columbia. Generally, results have been inconsistent and less than satisfactory, but research continues in an effort to develop a viral insecticide for spruce budworm population regulation.

Introduction

Four different types of insect viruses have been isolated from *Choristoneura* spp.; nuclear polyhedrosis viruses (NPV), granulosis viruses (GV), cytoplasmic polyhedrosis viruses (CPV) and entomopoxviruses (EPV). NPVs and GVs are classified as Baculoviruses subgroup A and subgroup B, respectively. All these viruses share one common feature, the virus particles are occluded within proteinaceous bodies. When ingested by the insect host larva, the protein is solubilized in the alkaline gut juices, virus particles are released, penetrate the midgut cells and the infection process is initiated. Viruses isolated from various *Choristoneura* spp. are listed in Table 1. Generally, it has been found that they are cross-infectious within the genus, although all the permutations of cross-infectivity tests have not been conducted.

Some of these isolates may contain one and the same virus, but, to date, ones which have been analysed biochemically and compared are all distinct. Restriction endonuclease enzyme (REN) fragment patterns of *C. fumiferana* and *C. occidentalis* NPVs showed they were distinct, but closely related and *C. murinana* NPV was more distantly related (Rohrmann *et al.*, 1982). The inclusion body proteins (polyhedrins) of these three viruses and *C. diversana* NPV were indistinguishable when analysed by peptide mapping and polyacrylamide gel electrophoresis (PAGE), but, using the same techniques, the patterns of the virus particle proteins were all distinguishable (B.M. Arif, personal communication). *C. fumiferana* EPV and *C. conflictana* EPV were virtually indistinguishable when analysed by PAGE, but were closely related although distinct using REN. *C. fumiferana* and *C. biennis* EPVs were distinct when analysed by PAGE (B.M. Arif, personal communication).

Table 1: Viruses isolated from *Choristoneura* spp.

SPECIES	VIRUS			
	NPV	GV	CPV	EPV
C. biennis				X
C. conflictana				X
C. diversana	X			X
C. fumiferana	X	X	X	X
C. murinana	X	X		
C. occidentalis	X	X		
C. pinus pinus	X			
C. retiniana		X		
C. rosaceana	X			

The most intensively studied of these budworm viruses is *C. fumiferana* NPV and considerable progress has been made in constructing a physical map of the viral genome (Arif and Doerfler, 1983, 1984). This virus has also undergone extensive safety testing in mammals and birds and no ill effects were observed (Valli *et al.*, 1976). If *C. fumiferana* NPV is ever deemed to be an effective and economical alternative to chemical pesticides for spruce budworm population regulation, little additional research is required to prepare a petition for its registration in Canada.

Naturally occurring virus epizootics have never been observed to date in budworm populations although viruses are quite common at very low levels usually with less than 5% of the population infected (Cunningham, unpublished). Initiating and maintaining a virus epizootic in a budworm population is, therefore, a considerably greater challenge than doing so in populations of such insects as gypsy moth, *Lymantria dispar*, and Douglas-fir tussock moth, *Orgyia pseudotsugata*, which are often decimated by naturally occurring NPV epizootics. Spraying these species with NPV advances the time of a population collapse, thereby reducing defoliation and tree mortality.

Since 1971, NPV, GV, CPV and EPV have all been tested in aerial spray trials either alone or in various combinations. Combinations were not tested by choice, but because contamination with a second, or even another two viruses, occurred during virus production. With rigid quality control procedures, this problem has almost been eliminated, although contamination of our NPV product with CPV continues to be an intermittent occurrence.

Field trials have been conducted with viruses to regulate populations of both spruce budworm *C. fumiferana*, and western spruce budworm, *C. occidentalis*, and such parameters as dosage, timing of application, tank mix and adjuvants, spray equipment, droplet size, impact on different species of host trees and persistence of viruses from year to year have been studied. The life cycles and behavior of both species make them difficult candidates for regulation with viruses. Larvae are virtually

hidden until budflush, by which time they have reached the fourth instar or larger. Smaller instars are more susceptible to virus infection than larger ones; also viruses are relatively slow to kill the host and when larger instars are infected only those which ingest a lethal dose of the actual spray deposit will die. There is no time for secondary infection to occur before the onset of pupation. Secondary infection occurs when larvae die from viral infections, disintegrate and release more infectious inclusion bodies on to the foliage. Sprayed at budflush, viruses are really being used as slow stomach poisons, no epizootic occurs in the year of application and little or no foliage is saved.

It was hoped that, following an application at budflush, a sufficient number of dead larvae would remain overwinter on the foliage to serve as a source of inoculum and initiate a virus epizootic in the next generation. Virus does persist from one year to the next, but subsequent incidence of virus infection has never been spectacular and, at present, this cannot be considered a viable pest management strategy. Budworm larvae make little or no contact with each other unless populations are extremely dense. This situation is not conducive to the transmission of a pathogen.

Application of viruses on second instar larvae, as they emerge from hibernacula, is an attractive concept. These tiny larvae are highly susceptible to virus infection, there is ample time for secondary infection to occur and, if there is a hardwood overstory, leaves have not flushed at this time of year and a good spray deposit on conifers can be obtained. However, timing is critical and there are numerous logistical constraints such as freezing temperatures, snow in the forest and impassable, unpaved roads.

Virus Production

Viruses only grow in living cells, so it is necessary to propagate them either in susceptible insect larvae or susceptible cell cultures. *C. fumiferana* NPV can be grown in cell culture (Arif *et al.*, 1976), but, at present, it is not economically feasible to produce in cell cultures the quantities required for field trials. Hence, insect larvae are used.

C. fumiferana was originally selected as the host for production of EPV, NPV and GV, but in recent years *C. occidentalis* has been used as the host for production of NPV and GV; it is more susceptible to infection with both viruses than *C. fumiferana;* less inoculum is required, there is less loss to pupation and *C. occidentalis* larvae spin less silk webbing facilitating faster harvest of dead and heavily infected larvae.

Budworm larvae are small and not ideal hosts for virus producton. A mature, NPV-infected sixth instar larva yields 5×10^8 polyhedral inclusion bodies (PIB) and this yield drops on a production line basis. Virus production is labour intensive and, therefore, expensive. A search has been made for a larger host larva for production of budworm viruses. Intensive efforts to use saltmarsh caterpillar, *Estigmene acrea*, for this purpose were unsuccessful (Shapiro *et al.*, 1982) and although *C. fumiferana* NPV infects neonate cabbage looper, *Trichoplusia ni*, larvae and wax moth, *Galleria mellonella*, larvae, it does not infect large larvae required for virus production (Stairs *et al.*, 1981). The search for an alternative host continues.

Budworm viruses which are currently being produced in *C. occidentalis* larvae are *C. fumiferana* NPV and *C. occidentalis* GV. Large fifth and early sixth instar larvae are placed on artificial diet which has been sprayed on the surface with virus inoculum. When NPV is used, dead and heavily infected larvae are harvested in 10 days and, with GV, harvest time is 12 days at 22°C and 50% RH. Dead and diseased larvae are frozen, lyophilized and ground to a fine powder by mixing lyophilized material with an equal weight of dry ice and processing it in a Waring blender. Batches of material are tested to determine the number of inclusion bodies per gram. Our NPV product, which is currently being produced, contains 10^{10} PIB/g and our GV product 8×10^{12} capsules/g. A count is made of the total number of aerobic bacteria per gram and a check is made for the presence of mammalian pathogens. This is done by culturing in selective media and by intraperitoneal injection of samples into mice.

Spruce Budworm

The first field trials with spruce budworm viruses were conducted in Ontario in 1959 and 1960 when small trees were sprayed with NPV and GV (Stairs and Bird, 1962). In 1969, a range of dosages of NPV was applied on individual small trees at 2 to 3 day intervals throughout the larval period (Bird and McPhee, 1970). In 1970, an EPV isolated from 2-year cycle spruce budworm, *C. biennis* (Bird *et al.*, 1971), although slow acting, was found to give high levels of mortality in the laboratory at much lower dosages than viruses tested previously. It was thought that the EPV would prove to be a highly effective biocontrol agent and the first aerial spray trials were conducted with EPV in 1971 (Bird *et al.*, 1972). Since then aerial spray trials have been conducted almost every year and a total of 65 plots with a combined area of 2 656 ha have been treated with viruses or chemical insecticide/virus combinations in Ontario and Quebec between 1971 and 1983. A list of these treatments is given in Table 2 and a summary of treatments between 1971 and 1980 and their impacts are described by Cunningham and Howse (in press).

The following methods have been used to assess treatments; (a) deposit was monitored on Kromekote® cards, (b) prespray and pupal counts were made from samples from treated and check plots and population reduction due to treatment calculated using a modified Abbott's formula (Abbott, 1925),

(c) incidence of virus infection was determined either by microscopic examination of samples of larvae from the field or by rearing collections of larvae and examining those which died, (d) adult emergence on treated and check plots was compared and (e) defoliation estimates were made. Follow-up studies were conducted in most of the trials to determine the impact of the virus treatment in the year or years following the year of application.

Table 2: Spray trials with viruses on spruce budworm in Eastern Canada.

YEAR	TREATMENT	NO. OF PLOTS	TOTAL AREA (HA)
1971	EPV (+NPV+CPV)[a]	6	6.5
1971	NPV (+CPV)	2	4.6
1972	EPV	3	508
1972	NPV	3	268
1972	Fenitrothion/ EPV (+NPV)	1	16
1972	EPV (+NPV)	1	16
1972	Fenitrothion/ NPV	1	16
1973	NPV	9	295
1974	NPV	2	526
1975	NPV	5	318
1976	NPV	3	120
1977	NPV	5	108
1978	NPV	7	134
1979	NPV (+CPV)	6	74
1979	GV	1	6
1979	Orthene/NPV	1	40
1980	GV	1	10
1980	NPV (+CPV)	3	42
1981	NPV	3	108
1983	NPV	2	40
	Totals	65	2,656

[a]Viruses in brackets are contaminants in the material applied.

In 1971, the EPV which was applied was found to be contaminated with low levels of both NPV and CPV and the NPV applied was contaminated with CPV. In 1972, pure EPV was applied, but also in this year, it was found that carry-over of NPV from one year to the next was much better than with EPV, and further trials with EPV were suspended. From 1973 to the present, all efforts have concentrated on developing an effective strategy for the use of NPV and only two small plots have been sprayed with GV.

Dosages of NPV have ranged from 2.5×10^{10} PIB/ha to a double treatment with a first application of 3.4×10^{12} followed by a second application of 2.3×10^{12} PIB/ha. The most commonly used dosage has been 7.5×10^{11} which, on some occasions, has given acceptable levels of population reduction (Cunningham and Howse, in press). When populations are monitored on both white spruce and balsam fir hosts, generally better results are obtained on white spruce. A variety of tank mixes and stickers has been used. Until recently, most were aqueous and contained 25% v/v molasses, but in the last two years an emulsifiable oil (Dipel 88® blank carrier vehicle) has been used, with the virus applied in 25% oil and 75% water. Emitted volumes have ranged from 4.5 to 28.2 L/ha, but most applications were made at 9.4 L/ha. Both boom and nozzle and Micronair® rotary atomizers have been used. Generally, coarse droplets with a stain diameter of 200 to 400 um have given better results than droplets with a diameter less than 100 um. This is a very important point which should be clarified before further applications are contemplated.

Reference has already been made to timing of application and most sprays have been applied at budflush when larvae were in the fourth and fifth instars and sometimes even some sixth instar were present. Results from ground spray applications of heavy dosages of NPV on individual trees at, or prior to, second instar emergence have demonstrated that secondary infection can occur (Bird and McPhee, 1970; W.J. Kaupp, personal communication). Four attempts have been made to aerially spray second instar larvae as they emerge from hibernacula and only one was considered to be timed correctly. Our experience in Ontario, near Sault Ste. Marie, has been that weather generally stays warm in late April and that emergence is over a two to three day period; when the first larvae are observed, it is time to spray and the problem has been applying the spray too late, after larvae have mined into needles and buds. In 1983, in Gaspé, Quebec, weather was cool and larvae emerged slowly over a prolonged period. When larvae were observed on the foliage, NPV was applied, but it remained cool after the treatment and results were disappointing (Cadogan, Kaupp and Cunningham, unpublished).

Carry-over of virus from one year to the next has been monitored in most plots. Following application of NPV contaminated with CPV on two isolated stands of mature white spruce in 1971, levels of virus infection were monitored until 1977 (Cunningham *et al.*, 1975; Cunningham and Howse, unpublished). In the year of application, maximum levels of 21% NPV infection and 28% CPV infection were found in weekly samples of larvae from one plot and 46% NPV infection and 35% CPV infection from the second. Population reductions due to treatment were 69 and 80% respectively, but there was no detectable saving of foliage in either plot. In subsequent years, levels of CPV declined; it disappeared from one plot in 1973 and from the other in 1975. Microscopically detectable maximum levels of NPV in weekly samples of larvae from the first plot each season between 1972 and 1977 were 28, 17, 6, 21, 8 and 3% and from the second plot were 24, 14, 6, 9, 0 and 2%. By 1977, the spruce budworm population had declined to a very low level. Although not spectacular, the NPV had a considerable impact on the spruce budworm population over this period, reinvasion of these isolated stands did not occur and the virus probably exerted sufficient control of spruce budworm to reduce defoliation and thereby prevent tree mortality. Subsequent efforts to repeat this experiment and duplicate these results have proved unsuccessful to date.

The problem of CPV contamination of NPV stocks was solved between 1973 and 1978, but returned in 1979 and 1980. Several interesting observations were made on this contaminated material. Firstly, the levels of CPV contamination have been low and the ratio of NPV:CPV PIBs ranged from 178 to 400:1 (W.J. Kaupp, personal communication). Inspite of the low levels of CPV, the incidence of CPV was usually greater than NPV when freshly produced material was applied in the field. When lyophilized, CPV contaminated material is stored for more than one year, the CPV virtually loses all activity and is barely detectable after application of the NPV.

Two small trials were conducted with GV; in 1979, a 6 ha plot was sprayed with 2 x 10^{14} capsules/ha when larvae were in the fifth and sixth instars and, in 1980, a 10 ha plot was sprayed with 6.4 x 10^{13} capsules/ha when larvae were in the second instar. Results were inconsistent and there was no relationship between population reduction due to treatment and foliage protection (Cunningham and Howse, in press).

There has been considerable interest in using combinations of chemical insecticides and various pathogens as integrated pest management strategies and this topic has recently been reviewed by Jaques and Morris (1981). Two attempts have been made to use this strategy for spruce budworm control. In 1972, the following treatments were compared: a low dosage of fenitrothion (18g AI/ha) alone, NPV (7.2 x 10^{11} PIB/ha) alone, EPV contaminated with NPV (1.8 x 10^{11} inclusion bodies/ha) alone, fenitrothion followed by NPV, and fenitrothion followed by EPV (+NPV). Generally, better results were obtained with the chemical/virus combinations than either chemical or virus alone. There was better foliage protection in the year of application in the plots treated with virus alone or fenitrothion/virus combinations than with fenitrothion alone and this foliage protection was also recorded the following year (Morris *et al.*, 1972, 1974). In 1979, a 40 ha plantation containing both black and white spruce trees was treated with acephate (Orthene®) at 560g AI/ha when larvae on white spruce were in the fourth instar and with NPV at 7.5 x 10^{11} PIB/ha 8 days later when larvae were in the fifth and sixth instars. Significant foliage protection was obtained in the year of application and in the following year (Cunningham and Howse, in press).

Although these two trials showed promising results, this is a line of research which is not being actively pursued. In attempts to initiate and maintain a virus epizootic, the aim has been to kill as many larvae as possible with virus so as to introduce a large virus inoculum pool into the environment. When used in forestry, it is felt that the addition of even low dosages of chemical insecticides reduces this virus inoculum pool and detracts from the appeal of using an environmentally acceptable biocontrol agent.

Western Spruce Budworm

When the pathogenicity of NPV and GV were compared in spruce budworm and western spruce budworm, the western species was considerably more susceptible to both viruses (Cunningham *et al.*, 1983a) and it appeared that western spruce budworm is a better candidate for regulation with viruses than the eastern species. Aerial spray trials were conducted in 1976, 1978 and 1982; 6 plots have been treated with a combined area of 424 ha (Table 3).

Table 3: Spray trials with viruses on western spruce budworm in British Columbia.

YEAR	TREATMENT	NO. OF PLOTS	TOTAL AREA (HA)
1976	NPV	1	20
1978	NPV	3	60
1982	NPV	1	172
1982	GV	1	172

In 1976, an application of 2.5 x 10^{11} PIB/ha on Douglas-fir trees gave disappointing results (Shepherd and Cunningham, unpublished). In 1978, three plots containing Douglas-fir trees were treated with a dosage of 7.5 x 10^{11} PIB/ha. Fifteen days post-spray, levels of NPV infection, determined microscopically, were 55, 87 and 25% and population reductions due to treatment were 0, 26 and 48% respectively (Hodgkinson *et al.*, 1979). In 1979 and 1980, surveys were conducted on two of the plots, the third being abandoned because of a local population collapse. In 1979, NPV infection levels were recorded as 18 and 45% and in 1980 as 7 and 17%. In 1979, population declines, compared to 1978 levels, were recorded at 51 and 62% compared to increases of 46 and 201% in corresponding check plots. Unfortunately, this trend was reversed in 1980 with populations more than doubling in the treated plots compared to substantial declines in the check plots (Shepherd *et al.*, 1982). It was felt that small plots may be unsatisfactory when long-term studies are contemplated because immigration of moths may have a profound effect on population densities.

In 1981, small Douglas-fir trees were sprayed with 3 dosages (10 fold differences) each of NPV and GV when larvae were at the peak of the third instar. The lowest dosage of GV at 1.5 x 10^{12} capsules/ha had a marked impact, but the lowest dosage of NPV at 2.5 x 10^{9} PIB/ha had none, and it was considered that GV may be a better candidate as a biocontrol agent than NPV (Cunningham *et al.*, 1983b). A similar test was conducted in the US in 1981 when individual grand fir trees were treated with NPV and GV at 3 dosages (2 fold differences) at two application dates, when larvae were 50% second instar and 50% third and also when they were 10% second, 53% third and 37% fourth instar (M.J.

Stelzer and D.W. Scott, personal communication). They found no significant differences between the two viruses or the three dosages or any interaction between virus type and dosage. However, the GV provided better control when applied at the early timing compared to the late timing which probably reflects the longer incubation period of the GV compared to the NPV. They do not feel that either virus warrants further development since commercial preparations of *Bacillus thuringiensis (B.t.)* are available.

Following the results of the NPV aerial spray trials in 1978 and results of the ground spray applications in 1981 in B.C., it was decided to treat two large plots, one with NPV and one with GV to determine the long-term impact of these two viruses. This was accomplished in 1982 and two 172 ha plots containing Douglas-fir trees were sprayed at budflush when larvae were at the peak of the fourth instar. The two viruses were compared on the basis of weight of lyophilized material and 9 kg was applied to each plot in a volume of 9.4 L/ha using a tank mix containing 25% emulsifiable oil (Dipel 88® blank carrier vehicle) and 75% water. The actual dosage of NPV was 5.4×10^{11} PIB/ha and the dosage of GV was 1.7×10^{14} capsules/ha. A high level of control was not anticipated in the year of application, but it was hoped that dead larvae remaining overwinter on the foliage would release sufficient viable virus to initiate an epizootic in 1983.

Preliminary analysis of data shows that the NPV had a greater impact than the GV on western spruce budworm in 1982 with 52% population reduction due to treatment compared to 35%. In samples of larvae reared individually in the laboratory, 66 to 80% died from NPV and 59 to 62% from GV; successful adult emergence was 53% in the check plots, ranged from 6 to 13% in the NPV-treated plot and from 12 to 24% in the GV-treated plot. Both viruses had an impact in 1983, but it was less than in the year of application. Population reduction attributed to NPV carry-over was 35% and attributed to GV carry-over was 15%. Two samples from treated and check plots were reared until death or adult emergence. Successful adult emergence was 46 and 46% from the NPV-treated plot, 50 and 42% from the GV-treated plot and 61 and 73% from the check plots (Otvos, Cunningham and Kaupp, unpublished). These results certainly cannot be regarded as dramatic, and it appears that relying on virus carry-over from one year to the next to regulate the insect population is not a viable pest management strategy.

Conclusions

Presently, lack of commercial interest in producing viruses is a major stumbling block in their development. All virus production in North America is at government establishments and the one company, Sandoz Inc., which marketed a viral insecticide, has discontinued production. If production in cell cultures becomes a viable alternative to using insect larvae, several pharmaceutical companies, with expertise in production of viral vaccines, would probably be interested in this market. The cost of producing budworm viruses in insect larvae at the pilot plant level is extremely high. It is difficult to quote an accurate figure, but an NPV dosage of 7.5×10^{11} PIB/ha probably costs in excess of $300/ha. This could be justified economically if virus persisted sufficiently well in the environment so as to hold the population below an economic threshold for several years or if it spread from treated to untreated areas allowing zebra stripe applications (widely spaced swaths) to be made. NPV and GV have persisted from one year to the next following some, but not all, applications. However, the incidence of infection has not been, except in rare circumstances, sufficiently high to regulate the spruce budworm population. To date, applications of viruses at budflush have virtually been applications of a very slow acting, expensive, stomach poison which sometimes gives a high level of population reduction with little or no foliage protection.

If one wants to use a non-chemical agent for spruce budworm population regulation, an obvious choice is *B.t.* which is readily available commercially at a competitive price. Numerous trials and operational applications have shown *B.t.* to be effective against spruce budworm. Considerably fewer trials with *B.t.* have been conducted on western spruce budworm, but satisfactory results have also been obtained on that species (M.J. Stelzer and D.W. Scott, personal communication).

Viruses are extremely efficient pest management tools when they act in a truly biological manner, and are probably superior to applications of *B.t.* By truly biological, one means that the virus treatment initiates an epizootic, secondary infection occurs, virus is spread from treated to untreated areas by such agents as predaceous, parasitic and scavenging insects and also, perhaps, by birds and the virus may persist from one year to the next if the insect population is not completely controlled in the year of application. The most spectacular viruses studied to date are those infecting European spruce sawfly, *Gilpinia hercyniae*, European pine sawfly, *Neodiprion sertifer*, and redheaded pine sawfly, *N. lecontei* (Cunningham and Entwistle, 1981). *B.t.* is not effective against sawflies. Douglas-fir tussock moth NPV is another outstanding virus which initiates an epizootic in the year of application. Applied on first and second instar larvae, only 10 to 30% may become infected by ingesting the spray deposit, but secondary infection causes collapse of the population (Cunningham, unpublished). Unless an excellent deposit is obtained with *B.t.*, it is not as effective as NPV for regulation of Douglas-fir tussock moth.

However, it would be most unwise to discontinue research on alternative methods of spruce budworm control and rely entirely on *B.t.* Pest managers should have a variety of options from which to choose. Presently, there are three companies in North America producing *B.t.* and problems of availability seem unlikely. There is neither evidence

of insects becoming resistant to *B.t.* nor reports of any undesirable side-effects following the use of *B.t.* Currently, viruses cannot be recommended for spruce budworm population regulation and can only be regarded as being at the experimental stage of development.

Improved methods of controlling spruce budworm with viruses are constantly being considered, although the life-cycle and behavior of spruce budworms are major insoluble problems. A continuing search is being made for more virulent virus isolates. If viruses could be kept in an infectious state on the foliage for prolonged periods, their efficacy would be greatly enhanced. Also spraying before second instar larvae emerge from hibernacula may then become a feasible strategy. A search for such tank mix adjuvants as stickers and UV protectants is being made for other viruses and other microbial pathogens and a breakthrough in this field could revitalise research on budworm viruses currently on hand. The epizootiology of spruce budworm viruses is not well studied and a better understanding of the virus - insect host - forest environment could give the answer to why applications of viruses are less than satisfactory. Perhaps the answer is spruce budworm behavior, but it may be a factor which can be modified or manipulated with relative ease.

Lastly, by far the most exciting aspect of current spruce budworm virus researh is that of recombinant DNA technology. It is conceivable that the virulence, host range and resistance to UV light can be altered once the genes controlling the various viral functions are identified. Considerable progress has been made in constructing a physical map of the spruce budworm NPV genome. The next step is to produce a transcriptional map which ascribes a function to different regions of the DNA (B.M. Arif, personal communication). Genetic studies have revealed that alfalfa looper, *Autographa californica*, NPV is very closely related to spruce budworm NPV and that these two viruses probably evolved from a common ancestor. A recombinant of these viruses may yield a virus which can be propagated in cabbage looper larvae, a large, easily reared Noctuid, but which still retains its infectivity to spruce budworm (B.M. Arif, personal communication). Speculation on the potential use of genetically manipulated Baculoviruses is limitless, but it will probably be several years until such viruses reach the stage of field testing. Such hurdles as public opinion, safety testing protocols and regulatory agencies will have to be overcome.

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ERYNIA RADICANS AS A MYCOINSECTICIDE FOR SPRUCE BUDWORM CONTROL

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Abstract

The entomopahtogenic fungus Erynia radicans, has been under investigation for several years as a possible alternative to chemical control of the eastern spruce budworm. A commercial production method has been developed which allows the formulation of this pathogen as a mycoinsecticide. A standardized bioassay method was used to select strain RS141 as the most potent isolate. The SD_{50} was found to be 11 conidia/mm2 with a slope of 2.21 against 6th instar larvae and a ST_{50} at 20 C of 3.5 days. When the mycoinsecticide is applied in the field, conidia were produced for up to 5 days during wet periods. Temperature below 10 C seem to limit the fungus activity. It is recommended that the research continue with emphasis on epizootiological investigations and selection of low temperature strains.

Introduction

The entomopathogenic fungus Erynia radicans (Brefeld) Humber, Ben Ze'ev et Kenneth (syn. Entomophthora sphaerosperma Fres.) has been recognized as an important pathogen of the spruce budworm Choristoneura fumiferana Clemens, over much of its range (MacLeod 1956, 1963; Otvos et al. 1973; Vandenberg and Soper 1975, 1978).The prevelance of mycosis caused by E. radicans has been reportedby these authors as ranging from a low background infection of 1% to over 90%. In Newfoundland, E. radicans also attacks the hemlock looper, Lambdina fiscellaria (Guen.) causing extensive mortality. Phenological studies (Vandenberg and Soper 1978) showed that in Maine there were four species of entomopathogenic fungi which attacked the spruce budworm. These included an imperfect fungus Hirsutella gigantea Petch, and three entomophthoralians Conidiobolus sp., Entomophaga aulicae (Reichardt in Bail) Humber and E. radicans. Of these, E. radicans was the first to cause mortality each spring being most prevalent during the 5th larval stage. The other two Entomophthoralians did not generally occur until the 6th instar or pupae. H. gigantea was never abundant making it impossible to draw any conclusions concerning its phenology. However, spruce budworw larvae killed by H. gigantea from the previous season, were occassionally found in the spring on spruce and fir branches. When these specimens were placed in petri dishes at high RH, the fungus would sporulate. Since it would be important to initiate disease early in the spruce budworm population cycle to prevent defoliation, E. radicans was chosen for further study as a potential biological control agent.

The Pathogen

The spruce budworm pathogen, E. radicans, undergoes a typical entomophthoran life cycle. It survives during the winter in the resting spore state. These spores are multinucleate when newly formed. As they mature, the spore wall thickens and the number of nuclei are gradually reduced until the spore becomes bi-nucleate. At this stage the spore has entered dormancy and can not be readily germinated. McCabe et al. 1984 (in press) describe this process and give presumptive evidence that these nuclei fuse a few hours prior to the breakdown of the spore wall during the germination process. If this is in fact the fusion nucleus, then it represents the sexual stage of the fungus. Perry and Latge (1982) reported obtaining very high germination of Conidiobolus obscurus after storage at 4 C for several months. Perry et al. (1982) obtained similar results with E. radicans (reported as Zoophthora radicans) resting spores.

Under field conditions, the resting spores are likely found in the forest litter or on the tree bark. Evidence from other closely related fungi, Erynia canadensis (Macleod, Tyrrell et Soper) from the woolly pine needle aphid, Schizolachnus piniradiatae (Davidson), would indicate that the resting spores may not survive exposure to very low winter temperature (Wallace et al. 1976). On return to favorable conditions for germination, the resting spores produce numerous germ conidia which are actively discharged and become air borne. Infection takes place when these spores come into contact with a susceptible host. It has been demonstrated in the laboratory that the

fungus produces predominantly conidia when infecting 2nd, 3rd, and 4th instar spruce budworm larvae and resting spores from infected 5th, 6th instar larvae, pupae, and adults. The primary conidia are responsible for the current season transmission of the mycosis. These spores are also air borne and cause infection on contact. They have the ability to produce secondary spores of two types. If the spore fails to reach its host, it usually produces a capillospore which is almond shaped and forms at the end of a very thin tube. The capillospore is produced at right angle to the substrate on which the conidia has landed. This positions the secondary spore for better contact with any larva which might walk over the surface of the plant. The second spore type is morphologically similar to the primary conidia except it is smaller. It is also discharged and can become air borne. Conidial formation requires the fungus to either be in contact with free water or at 100% RH.

Development

The basic concept for use of E. radicans as a biological control agent for the spruce budworm was application as a mycoinsectcide. This requires the solution of several well defined critical problems. First it is necessary to select a strain of the fungus with high pathogenicity and virulence. Once an appropriate strain has been identified, the second step is to develop a commercially acceptable method of production. The least expensive production method for microorganisms is the use of deep tank fermentation technology. Fortunately E. radicans was known to be easily grown on liquid media. The final step in this process is the selection of an appropriate formulation.

Strain Selection: Central to the development of any microbial agent for insect control is the ability to determine pathogenicity and virulence. Initially it is necessary to choose a suitable strain for development and then monitor its quality during production. Traditionally, dose-mortality responses have been analyzed for SD_{50} and ST_{50} as a measure of strain effectiveness. Since "between-experiment" variability is generally high, a standard strain is selected to be included in all bioassays. The potency of the test material is then compared relative to the standard. In general, it is necessary to keep all sources of variation to a minimum so dose-mortality will reflect only pathogen response. The two major sources of variability are the host and the pathogen. It was determined in early experimentation that budworm larvae infected with chronic levels of microsporida would result in erratic E. radicans bioassays. When these larvae were stressed by infection from the fungus, they succumbed quickly. In most instances, diagnosis would reveal large numbers of protozoan spores and very few hyphal elements. A protozoan free colony was established and used to bioassay the fungal strains. The test insects were further standardized by using only 6th instar larvae which had moulted within the previous 24 hrs. Initial bioassays with E. radicans depended upon producing conidial showers from mycelial mats (Vandenberg and Soper, 1979). Although it was possible to obtain SD_{50} values, doses were highly unpredictable. It was found that spores were distributed unevenly over the infection court and the rate of sporulation could change rapidly over a brief interval (Table 1).

Table 1: Conidia of E. radicans Produced From Mycelia Mats at 15 min. Intervals

	Time			
Rep	15	30	45	60
1**	3(0.5)	4(0.7)	2(1.6)	2(0.9)
2*	10(2.3)	30(20.3)	37(25.1)	36(26.0)
3	13(10.1)	13(4.8)	18(9.5)	25(12.1)
4**	3(1.8)	6(4.0)	11(8.6)	28(16.1)

Mean with Standard Deviation
* Significant at 5%
** Significant at 1%

Clearly from these typical data, significant changes in sporulation rates can occur within a 15 minute exposure while the high standard deviations are indicative of an uneven distribution. To overcome this problem, experiments were conducted to develop techniques which would allow exposure of budworm to precise and controlled quantities of conidia. The first problem was to produce a stable, viable and uniform suspension of conidia. Ten wetting agents were screened for ability to wet the spores without effecting their ability to germinate and infect insects (Table 2).

Table 2: Effect of Wetting Agents on Conidia of E. radicans Conidia

Agent	Ability to Wet	Effect
Agrimul SS	Fair	Toxic
Agrimul 26	Poor	None
Atplus 401	Good	Toxic
GAFAC RE610	None	Toxic
Sellogen W	Poor	Toxic
Sponto 500T	Fair	Toxic
Triton X100	Excellent	Toxic
Tween 80	Fair	None
Tween 20	Fair	None

The best combination proved to be an aqueous solution containing a suspending agent, 0.1% Atmos 300, and 0.25% Tween 80. Storage tests showed high viability was retained for at least 48 hrs at either 4C or 20 C. Beyond that period, secondary germination within the suspension made determination of primary conidial survival difficult. In one test, however, it appeared that over 40% survived at 4 C for 9 days. For the purposes of the bioassay dose preparation, it was only necessary for the conidia to survive a few hours between collection and utilization. The standardized technique was as follows:

1. Mycelial mats formed as described above under production, are inverted over a petri dish (100 mm) containing approximately 5 ml of the Atmos 300 / Tween 80 solution. The conidia which are showered into the solution are collected every 2 hours by pipetting off the suspension and are then stored at 4° C to prevent germination.

2. The conidia are pooled from all dishes and centrifuged at 1000 rpm in a swinging bucket rotor for 10 minutes.

3. The spore pellet is resuspended in Atmos 300/Tween 80 to the desired volume using a vortex mixer.

4. The spore suspension is forced through a 200 mesh screen 10 times to reduce spore clumps.

5. Spore counts are made with a haemocytometer to determine final dilution. With current spray techniques, 0.5 ml of 1 x 10 to 6th conidia/ml yields a dose of approximately 100 spores/mm².

6. The spray apparatus consists of a modified JJ nozzel (Spray Systems Inc.) in which the normal spray input opening is plugged and replaced by a chromatography injection port. The top of the spray nozzel is drilled and tapped to accept the port. Air pressure is controlled, using a standard regulator and a flow valve, to 700 gm/cm².

7. A plexiglass box with a 100 mm diameter opening directly in the target area of the spray is placed at approximately 15 m from the spray nozzel. The opening is covered with a screen and a vacuum is placed on the box using an ordinary vacuum cleaner connected by means of a tube on the side. This serves to immoblize the test insects and results in a more uniform spray pattern.

8. Test insects consist of 40 6th instar budworm larvae per dose which are placed under the spray nozzel together with a cover slip.

9. Although approximate dosage is determined by varying the number of conidia in suspension, the actual dosage sprayed on the insects is determined by counting 10 random fields on the cover slip and expressed as spores/mm².

10. Five dosages are selected to bracket the SD_{50} and the experiment is repeated 4 times.

11. The insects are placed at high relative humidity without food for 24 hours following exposure to the conidia; at which time they are returned to normal rearing conditions. All larvae are reared individually at 20 C with a 16 hour photophase. They are checked daily for mortality and the dead insects are examined microscopically for disease. The assay is terminated in 6 days.

The standard E. radicans isolate is RS141. This strain has a common SD_{50} of 11.0 conidia/mm² and a slope of 2.21 against 6th instar spruce budworm larvae (Table 3). The ST^{50} for the standard is 3.5 days at 20° C with a range of 2 to 4 days. Comparison with RS491, for example, showed the standard to be 4.5 times more potent. In fact RS141 proved to be the most active strain tested and was thus selected for development as an mycoinsecticide.

Table 3: Response of Spruce Budworm Larvae (L_6) to Conidia of E. radicans

Assay	SD_{50}	95% C.I.	Stand. Error
1	9.3	5.2-15.1	0.45

2	10.2	6.0-16.4	0.59
3	11.1	6.5-18.0	0.54
4	13.4	6.2-21.1	0.52

Common SD_{50} = 11.0 S.E. = 1.76
Common Slope = 2.21 S.E. = 0.26
Heterogenity = 1.21 G(0.95) = 0.07

Production: Strains of E. radicans were kept in 8 % glycerol in ampules frozen under liquid nitrogen. This served as a source of material for production experiments. The ampules were removed from storage and the fungus rapidly thawed in 35 C water. The fungus mycelium was then transferred to mycological agar containing 1% yeast extract. When the fungus was actively growing, it was transferred to 500 ml shake flasks containing 75 ml of sterile liquid medium. Several liquid media are suitable but one which was frequently used was: corn syrup 40 ml; yeast extract 10 gm; peptone 10 gm; water 950 ml. The medium was adjusted to pH 6.8. The fungus was incubated at room temperature (25 C) on a rotary shaker at 300 rpm. When the fungus entered log growth phase, usually in 96 hrs, it was used to inoculate fresh media. A 10% inoculum level was used, viz. 7.5 ml of suspended mycelium was added to 75 ml of fresh medium. When the fungus reached log growth phase again, usually in 48 hrs, it was used to inoculate 25 l fermentation vessels. The inoculation rate was also 10%. The vessels were autoclavable plastic carboys which had been modified to permit compressed air to be bubbled through the medium from the bottom by means of a plastic tube. The fermentation process was allowed to progress to the end of log growth phase, usually 36 hrs, and then the mycelium was harvested. The harvesting process consisted of filtering the mycelium onto cheese cloth in a specially constructed box which allowed the liquid to be removed by vacuum. The fungus was washed several times with deionized water to remove residual media. The mats were then placed on wire racks and allowed to incubate for an additional 2-3 hours. At this stage the fungus was dried according to a process now being considered as a public patent (McCabe and Soper 1982 patent pending).

Growth Chamber Studies

Methods: Preliminary experiments with E. radicans against spruce budworm were conducted on potted balsam fir (55 cm) under controlled environmental condiditons. The trees were kept at 4 C until required for tests. Several weeks prior to the experiments, the trees were removed from cold storage and placed in the greenhouse to promote bud growth. Each test consisted of 50 4th or 5th instar laboratory reared larvae. These were placed on the potted trees and allowed to establish. The trees with the insects were placed in a spray chamber and treated with a standard dose of the mycoinsectide. The dosage was either 1 gm (10 to 7th conidia) or 10 gm (10 to 8th conidia). The fungus was applied with a 8002 T-jet fan spray nozzle placed approximately 40 cm from the top of the tree. The tree was rotated about 2.5 revolutions on a turntable during the spray period. A sample of the fungus was assayed for ability to produce conidia. Following treatment, the balsam fir trees were placed in environmental chambers set at various temperatures according to the experimental protocol. Relative humidity was that of the ambient conditions in the growth chambers or was raised to ca. 100% by placing plastic bags over the trees. Following 24 hrs of exposure to the test condiditons, the insects were removed from the trees and placed individually in cups on artificial diet. They were checked daily for disease. The experiments were terminated 5 days post application and all insects examined for the presence of infection.

Results: An experiment was conducted to determine how soon after spraying the insects became infected. In this experiment, 10 trees were sprayed with 1 gm of fungus powder in 50 ml of water. Following application, 50 4th or 5th instar larvae were placed on each tree. They were then placed at 100% RH at 20 C in a 16 hr photoperiod. The budworm were removed from each of 2 trees at 0, 5, 24, 30, and 48 hrs and reared individually. The infection observed at 0 and 5 hrs (2%-3%) probably came from spores which were carried into the rearing dishes on the exposed budworm. Most of the infection occurred between 5 and 24 hrs (28%) although an increase was observed at 48 hrs (41.5%) when the experiment was terminated.

Low overnight temperatures of 4 and 10 C for 8 hrs were also investigated for their influence on infection levels in the growth chambers. These were compared to a constant temperature of 16 C in a 16 hr photoperiod. In addition, the tests were designed to compare high (100%) and moderate (85%) RH during the day and 3rd instar vs 5th instar. The results show that moderate RH during the day did not influence the infection rate. However, 5th instar larvae were more vulnerable to

infection than 3rd instar larvae under these test conditions. Overnight temperatures between 10 and 16 C resulted in similar infection levels (36,0%--46.5%) while tests conducted at 4 C produced reduced infection rates (3/0% -16.5%). The general conclusions drawn from these and similar data were:

1. E. radicans is capable of causing significant spruce budworm mortality when applied as a mycoinsecticide.

2. Significant infection occurs within 24 hrs of application under conditions of high relative humidity.

3. Infection can take place under early spring conditions in Maine despite low night-time temperatures (4 C). However, infection rates are much higher at more elevated minimum daily temperatures (13 C).

Field Performance

Field Methods: Observations were made under field conditions to determine the influence of microclimatic conditions on the revival of the powdered mycelium of E. radicans and its ability to produce conidia. The study site was located in Township 34, Maine at the intersection of St. Regis stud mill road and logging road 30 00 00. The area consisted of an even age stand of balsam fir with trees about 3 to 6 meters high. The microclimate was monitored using data logging devices (Datapod, Omnidata, Logan, Utah) which automatically recorded information on EPROM computer chips. These were changed at weekly intervals and read directly into a Prime computer (USDA, ARS Beltsville, MD) by means of a computer terminal located at the field station. The information included leaf temperature, leaf wetness, rainfall, solar radiation, wind speed and wind direction. This data was summarized daily using SAS analysis procedures. A spore trap (Burkhard) which recorded the number of conidia collected per cubic meter of air per hour, was run continuously throughout the test period. The sore tapes were removed daily and the number of spores collected per hour were recorded by scanning under a compound microscope at 200x magnification.

The mycoinsecticide (100 gm) was suspended in one gallon of water with the aid of 8 g of a special adjuvant (Table 4).

Table 4: Adjuvant Developed for the E. radicans Mycoinsecticide

Ingredients	% by wt.	Supplier
Kelzan S	14.4	Kelco Co.
SGP 104	30.0	Henkel
PUP K-60	33.3	GAF Corp.
CMC	22.3	Hercules

The suspension was sprayed with a backpack mist blower (Solo Inc.). This was calculated to produce 10 to 9th conidia per tree. Application was made to each of 3 trees and replicated 4 times in a randomized block. Treatments were 1 = 4th instar, 2 = 5th instar, 3 = 6th instar and C = untreated check. Weekly samples of two 46 cm branches were collected from each sample tree. The budworm larvae were counted, removed and held for 6 days on artificial diet to check for infection. In addition, a sample of field collected budworm were exposed to conidia from the rehydrated fungus powder held at 100% RH for 24 hrs and then reared for 6 days to check the infectivity of the material.

Laboratory Methods: Powdered mycelia from the same production was used in both the field investigations and laboratory studies. The influence of temperature on revival of the mycelia and subsequent spore production was determined at 5, 10, 15 and 20 C. This was tested by placing 10 mg of powder on no. 3 Whatman filter paper in a 35 cm petri dish containing 2% water agar. The dish was inverted over an aqueous solution of 0.2% maleic acid plus 1.0% Triton-X 100 and sealed with parafilm. The water agar provided sufficient moisture to insure revival and sporulation, while the solution prevented germination. Spores were collected at 2 hr intervals, beginning at 4 hrs of incubation through 18 hrs, then continued at 26, 30 and 96 hrs. The spores were suspended in 10 ml of malic acid solution. A haemocytometer was used to count the number of spores in suspension. The treatment was replicated 4 times and the experiment was repeated 3 times. Mycelial mats were produced using the same method as in the patent process, but the fungus was allowed to sporulate rather than be immediately dried. These spores were used to test the influence of temperature on germination. Water agar (2%) in 35 cm petri dishes was exposed to the spore showers from the mats. The spores were covered with a glass coverslip to prevent resporulation, and then incubated at 5, 10,

15, and 20 C in both light and dark. Germination was determined under a dissecting microscope at 50x magnification by counting the number of germ tubes from a random sample of 100 spores. The treatments were replicated three times and the experiment repeated three times.

Results: The fungus was applied on June 3 (14.30 hrs), June 16 (12.00 hrs), and June 19 (18.00 hrs). There were intermittent rain showers during the first application. Nevertheless, the fungus powder did dry sufficiently to adhere to the balsam fir needles, but never dried to the point of hardness. The needles remained wet throughout the following 52 hours. Sporulation began at 23.5 hrs after application, with heavy conidial production between 23.00 hrs June 4 to 03.00 hrs on June 5. Sporulation stopped at 09.00 hrs and resumed again at 15.00 hrs, continuing uninterrupted until noon on June 6. Sporulation began again at 15.00 hrs and continued with varying degrees of intensity until the morning of June 9. The second application was during bright, sunny weather. The fungus particles dried rapidly to a very hard consistency. Sporulation did not occur until 20.00 hrs on June 18, 2 days following application. Samples of needles with fungal particles were removed from the sprayed trees and incubated at room temperature and 100% RH. All particles revived and produced spores. The delay in sporulation was caused by a thick coating formed by the protective adjuvant. It apparently took several days for the fungus to rehydrate and grow through the coating. Nevertheless, the fungus stayed alive during a very warm, dry period. The third and final application (June 19) was made during a warm evening with dew formation occurring within 2 hrs after spraying. Sporulation began very quickly and was most abundant from 01.00 to 06.00, stopping altogether at 09.00 hrs the next day. Sporulation was light the following evening in spite of the lack of leaf wetness. The experiment was terminated June 21.

The Spray applications were timed to correspond with peak 4th, 5th, and 6th instars. These targets were met, however, it is was also evident that the population in the test site was fairly low begining at 4.5 total larvae/46 cm branch and falling to about 1 at the end of the experiment. None of the budworm collected and reared showed any sign of infection. However, a significant percentage of the budworm exposed to conidia from the fungus powder in the field laboratory became infected. This indicated that either population density was too low, inoculum levels were too low, or some other environmental factor was limiting. Although all of these are possible, laboratory experiments indicated that low temperature may have been the problem. This is corroborated by the earlier growth chamber studies. It was evident that conidial production for rehydrated fungus powder was greatly influenced by temperature. Sporulation began in 4 hours at 20 C, 6 hrs at 15 C, 10 h rs at 10 C, and 16 hrs at 5 C, with total number of spores falling off drastically below 15 C. At 18 hrs for example, the previous 2 hrs had produced the following conidia (x 10 to 4th): 5 C = trace; 10 C = 3; 15 C = 12; and, 20 C = 21. Germination is also limited by temperature with the added complication that in the absence of light, spore production was further reduced.

Discussion

It is apparent that there is some factor in the environment which prevented E. radicans from infecting the budworm under field conditions. It was shown that the isolate selected for production (RS141) was capable of infecting 6th instar larvae at relatively low doses (11 spores/mm 2), and would kill them quickly (2-4 days) in the laboratory at 20 C. Further, the material formulated as a mycoinsecticide was shown to retain pathogenicity. There are several possible explanations. As discussed above, low temperature (10 C or below) increases the time required for the fungus to produce spores and then for the spores to germinate and cause infection. At 10 C the total time elapse would be about 24 hrs viz. 14 hrs for production plus 10 hrs for germination and penetration. This is unrealistic under field conditions. First of all, the fungus requires free water to rehydrate. This is usually present in the form of dew which forms overnight giving an activity period of 8 to 12 hrs although this time can be extended during rainy weather. Nevertheless, it is fairly common for the night temperature to fall below 10 C or at least be well below 20 C. Even though the field data showed sporulation occurred, there may not have been time enough for germination of the spores and penetration of the resulting infection tube before return to low RH. The second problem might have been the relatively low host population density, i.e. 2 to 4 larvae/46 cm branch. Finally, it was assumed that 10 to 9th

conidia per tree was a "high" dose. This may in fact have been relatively low, especially since the fungus particles were difficult to find and presumably lost 48 hrs after application.

Future Directions of Research

This pathogen, Erynia radicans, has been shown to cause extensive mortality in eastern spruce budworm populations without the intervention of man. The question is can this fungus be manipulated in some way to effect population control of the spruce budworm at other times. The answer is likely yes however inspite of the extensive research effort that has been conducted to date the research must be continued if we are to answer this question. The major problem of production has been solved and methods for bioassay developed. What remains now is to select an isolate that is suited to low temperature development and to conduct extensive epizootiological studies to determine the important factors which limits disease spread and intensity. There are researchable approaches to the problem of low temperature. Small quantities of the mycoinsecticide was produced from several E. radicans spruce budworm isolates chosen at random from the USDA, ARS culture collection. This material was incubated at 10 C as previously described and the number of spores produced determined at 6, 7, and 8 hrs (Table 6).

Table 6: Conidial Production from Various E. radicans Mycoinsecticides at 10 C

Strain	6	7	8
RS50	0	0	0
RS88	0	0	0
RS141	0	0	0
RS143	0.25	1.00	2.85
RS145	0	0	0
RS162	0	0	0.05
RS185	0.05	0.10	0.15

Conidia/100 mg x 10 to 4th

It is heartening to see that at least 2 isolates (RS143 and RS185) showed activity at 6 hrs, while RS141 used in field trials did not sporulate even at 8 hrs. Selection for low temperature activity can be conducted under laboratory conditions through a series of re-isolations and production experiments. Field investigations should continue with the objective of determining the "window of vulnerability" in the spruce budworm cycle to attack by E. radicans. Epizootiological studies should be initiated to develop a systems model for the Erynia radicans /spruce budworm interaction. This model could be used in simulation studies to develop an optimum strategy for using a mycoinsecticide against the spruce budworm.

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THE EFFECT OF ERYNIA RADICANS ON FOOD CONSUMPTION, UTILIZATION AND FECUNDITY BY THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA.

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Abstract

In food consumption and utilization studies, observations were confined to a period of 5 days after which mortality of Erynia radicans larvae began to occur. Mortality due to the fungus was 76 and 83% in male and female larvae, respectively. In control larvae the pupation rate was over 95%. Control larvae of both sexes consumed significantly ($P = 0.05$) more food than fungus treated larvae on day 4 and 5. Treated as well as control larvae showed a continuous gain in body weight up to day 3, following which treated larvae of both sexes gained significantly ($P = 0.05$) less weight than the control. Fungus infected larvae were also less efficient in converting ingested and digested food to body substances (ECI and ECD) on day 4 and 5 in females and on day 5 in males. Pupae of both sexes from treated larvae weighed less than the control. Fecundity of adults arising from fungus treated larvae was reduced by approximately 36.4% compared with the control.

Introduction

Erynia radicans (=Zoophthora radicans = Entomophthora sphaerosperma) is among the more promising of microbial agents for the control of the spruce Budworm, Choristoneura fumiferana and has a potential for commercial development as a microbial insecticide (Weatherston and Retnakaran 1975; Vandenberg and Soper 1979). However, an important drawback of mycopathogens in the control of insect pests is the time taken to kill the host. Generally, mortality of infected larvae occurs in 4-8 days after treatment (Mohamed et al. 1982) during which larvae continue to feed, presumably causing damage. Therefore, it would be benificial if it could be ascertained that fungal infection of the host can reduce food consumption. This would aid in assessing the suitability of the pathogen for use in insect pest management, particularly in a forest situation where the foliage can tolerate moderate feeding damage.

Most reported studies dealing with the control of insects with pathogens have dealt mainly with the direct effect on the host and have been evaluated in terms of percent mortality. Little attention has been focused toward the evaluation of the effect of infection on food consumption and the general reduction of the biological potency of the host (Jasic 1961). Ramakrishnan and Chaudhari (1974) studied the effect of a nuclear polyhedrosis virus (NPV) on the consumption and utilization of food by the Egyptian cotton leaf worm, Spodoptera litura. They found that food consumption and weight gain of virus infected larvae were significantly lower than the control. Drake and McEwen (1959) reported that the cabbage looper, Trichoplusia ni showed decreased food consumption following infection with NPV. Thomson (1958) showed that the microsporidian, Perezia fumiferanae retarded larval and pupal development, reduced fecundity and adult longevity of C. fumiferana. Larvae of Heliothis zea infected with Nomuraea rileyi showed significantly less food consumption and utilization than the control group (Mohamed et al. 1982).

The purpose of this study was to determine the effect of E. radicans on food consumption, utilization and fecundity of C. fumiferana.

Materials and Methods

Larvae and Fungal Cultures. - Overwintering second instar spruce budworm larvae were obtained from the Insect Pathology Institute, Saulte Ste. Marie, Ontario, Canada. Larvae were reared on Bio-Serv Spruce Budworm diet #9769, dispensed in about 15-20 ml aliquots into 30 ml plastic cups. Four to six larvae were placed in each cup and reared at 22.5°C with a photoperiod of 16 hr light and 8 hr darkness.

The culture of Erynia radicans was obtained from the laboratory of Dr. R. S. Soper, U.S.D.A. ARS, Insect Pathology Unit, Boyce Thompson Institute, Ithaca, N.Y. The initial sample of this fungus was isolated from a field collected spruce budworm larvae (Vandenberg and Soper 1979). It was grown on 70% modified egg-yolk medium (EY) in darkness at 25°C according to Soper et al. (1975.) Sporulation was induced according to the method of Vandenberg and Soper (1979). Several 250 ml flasks each containing 75 ml of CYP liquid medium (4% corn syrup, 1% yeast extract, 1% phytone peptone in distilled water) were inoculated with mycelia grown on EY, and continuously agitated in a controlled environmental shaker (New Brunswick Scientific Co. Inc.) at 25°C. When the culture reached the exponential growth phase (4-5 days), 5 ml of each culture was transferred to fresh CYP and allowed to grow for 2-3 more days. Then the medium was filtered and the remaining mycelia rinsed several times with sterile distilled water. After the excess moisture was drained off with paper filters, mycelia were placed in plastic Petri dishes (90 x 15 mm) and exposed to continuous fluorescent light for 24 hr at 20°C to stimulate sporulation.

Treatments

1. Food consumption and utilization. - Fifth instar larvae were used in all tests. The age of the larvae was determined according to the method of Retnakaran (1973). Larvae were

sexed as described by Koller and Leonard (1981). One hundred 5th instar larvae of each sex were placed in plastic cups individually and starved for 8 hr to empty their gut contents of residual food. Eighty larvae from each sex group comprised the untreated control group. A similar number of larvae were treated with E. radicans spores as described by Vandenberg and Soper (1979). The dose was estimated by exposing Petri dishes (100 x 15 mm) containing 2.5% sterile distilled water agar to sporulating cultures identical to those used in larval treatments. Spore concentration was estimated (spore/mm^2) by counting a random field in each of 10 areas in the dish (Vandenberg and Soper 1979). In preliminary tests, several groups of larvae were exposed to sporulating cultures at varying times (15 to 90 min.) An exposure time of 45 min. giving 39 ± 5.7 spores/mm^2 was the most appropriate in terms of mortality and survival of the larvae.

After treatment, larvae were held in individual Petri dishes (100 x 15 mm) lined with filter paper moistened daily with sterile distilled water. Each treatment was replicated 4 times with 10 larvae. Test larvae were held in an environmental growth chamber at 22.5°C with a photoperiod of 16 hr light and 8 hr darkness.

Larvae were individually provided with preweighed 5 mm diet plugs which were replaced daily. Food consumption and weight gain were determined by recording the initial weight of the larvae and diet, followed by recordings of the larval weight, feces and diet consumed every 24 hr. Data from these observations were used to determine food consumption and utilization. Diet consumed was corrected for moisture evaporation as described by Brewer and King (1979). The indices (Waldbauer 1968) to determine the efficiency of utilization were as follows:

a) Approximate digestibility which is the percentage of food ingested that is retained or utilized by the larvae:

$$(AD = \frac{\text{wt of food ingested} - \text{wt of feces}}{\text{wt of food ingested}} \times 100)$$

b) Efficiency of conversion of ingested food to body substances which is an overall ability of the insect to utilize ingested food for growth:

$$(ECI = \frac{\text{wt gain}}{\text{wt of food ingested}} \times 100)$$

c) Efficiency of converstion of digested food to body substances decreases as the digested food metabolized for energy increases:

$$(ECD = \frac{\text{wt gained}}{\text{wt of food ingested} - \text{wt of feces}} \times 100)$$

The data were analyzed by the analysis of variance and the Duncan's New Multiple Range Test (1955).

2. Fecundity. - One hundred 5th instar larvae of each sex were treated with E. radicans and placed in plastic cups containing diet. A similar number comprised the untreated control. Larvae were reared as described earlier and observed daily for mortality and pupation. Pupae were weighed within 24 hr of pupation and placed in the original cups. Adults used for oviposition were reared in individual containers (Stehr 1954). Branches of balsam fir, Abies balsamea[1] were used for oviposition. Each test was conducted over a period of 4-6 days, with daily removal and counting of egg masses. The number of eggs was determined by measuring the length and width of each egg mass as described by Bean (1961).

Results and Discussion

1) Food consumption and utilization. - Mortality of E. radicans treated larvae started on day 4 post treatment. Thus observations for food consumption and utilization were done for 5 days. On day 6 most of the treated larvae were dead. Mortality in fungus treated larvae was 76% in males and 83% in females. In the control 95% of males and 97.5% of the females pupated.

Table 1 shows the amount of food consumed by treated and control larvae. The amount of food consumed by treated larvae was significantly (P = 0.05) lower on day 4 and 5 post treatment for both sexes. Male and female control larvae showed a constant increase in food intake throughout the observation period. The average daily intake for the treated and control female larvae was 50.2 and 77.06 mg and for the males it was 60.4 and 75.9 mg respectively.

Table 1. - The effect of Erynia radicans on food consumption (mg) of Choristoneura fumiferana larvae.a/

DP[b]	Control	Fungus treated
Female		
1	67.3 ± 19.0a	59.4 ± 27.0a
2	66.2 ± 4.0a	60.9 ± 3.0a
3	60.7 ± 7.0a	51.5 ± 8.0a
4	94.2 ± 18.0a	40.2 ± 7.0b
5	96.9 ± 16.0a	39.0 ± 10.0b
Male		
1	56.3 ± 12.0a	61.8 ± 10.0a
2	71.4 ± 12.0a	78.4 ± 10.0a
3	67.4 ± 2.0a	57.3 ± 10.0a
4	90.6 ± 7.0a	54.9 ± 9.0b
5	93.9 ± 5.0a	49.3 ± 6.0b

a/ Means followed by a common letter horizontally do not differ significantly from each other (P = 0.05).

b/ Days post treatment.

The weight gain of treated and control larvae is presented in Table 2. Fungus treated larvae of both sexes showed significantly (P = 0.05) less weight gain on day 4 and 5. The average daily weight gain for the treated and control

1/ Specially ordered for us by a local garden and nursery center.

female larvae was 4.38 and 8.64 mg respectively. Similarly for the males the average weight-gain was 3.98 mg for the treated and 7.38 mg for the control group. The amount of feces produced in the treated larvae was significantly lower for both sexes on days 3, 4 and 5 (Table 3).

Table 2. - The effect of Erynia radicans on weight gain (mg) of Choristoneura fumiferana larvae.a/

DP[b]	Control	Fungus treated
Female		
1	5.8 ± 3.0a	4.9 ± 0.2a
2	6.4 ± 1.0a	7.5 ± 2.0a
3	6.3 ± 5.0a	4.8 ± 1.0a
4	10.9 ± 3.0a	2.3 ± 1.0b
5	13.8 ± 2.0a	2.4 ± 1.0b
Male		
1	3.7 ± 0.9a	3.0 ± 1.0a
2	7.4 ± 2.0a	6.1 ± 1.0a
3	8.5 ± 0.3a	5.2 ± 1.0a
4	7.6 ± 1.0a	2.9 ± 1.0b
5	9.7 ± 4.0a	2.7 ± 1.0b

a/ Means followed by a common letter horizontally do not differ significantly from each other (P = 0.05).
b/ Days post treatment.

Weight gain and fecal production are directly related to the amount of food consumed (Ramakrishnan and Chaudhari 1975). The decrease in the values of these parameters in treated

Table 3. - The effect of Erynia radicans on feces production (mg) by larvae of Choristoneura fumiferana.a/

DP[b]	Control	Fungus treated
Female		
1	4.1 ± 1.8a	4.7 ± 1.0a
2	7.3 ± 1.4a	6.9 ± 2.6a
3	12.0 ± 1.5a	4.6 ± 1.0b
4	15.0 ± 2.0a	2.7 ± 1.0b
5	17.6 ± 2.0a	2.2 ± 1.0b
Male		
1	4.5 ± 1.0a	4.0 ± 1.4a
2	4.3 ± 2.0a	4.4 ± 3.0a
3	7.5 ± 2.9a	4.3 ± 2.0b
4	13.2 ± 2.0a	3.6 ± 1.0b
5	20.0 ± 5.0a	3.7 ± 2.0b

a/ Means followed by a common letter horizontally do not differ significantly (P = 0.05).
b/ Days post treatment.

larvae can be attributed to reductions in food consumption of the larvae (Table 1). Reduction in food consumption, weight gain and fecal production in infected larvae have been previously reported by Mohamed et al. (1982) for H. zea infected with N. rileyi and by Ramakrishnan and Chaudhari (1975) in S. litura infected with NPV.

A more direct relationship between these parameters can be observed if we consider the total food consumption, total weight gain and total fecal production (Table 4). The total food consumption in male and female was significantly lower (P = 0.05) than that of the control. This

Table 4. - Total food consumption, weight gain and fecal production (mg) by larvae of Choristoneura fumiferana infected with Erynia radicans.a/

Parameter[b/]	Female		Male	
	Con	FT	Con	FT
FC	385.3a	251.0b	379.5a	302.0b
WG	43.2a	21.9b	36.9a	20.1b
FP	56.5a	21.1b	59.5a	20.0b

a/ Means followed by a common letter horizontally for male and female separately do not differ significantly (P = 0.05).
b/ Con = Control, FT = Fungus treated, FC = Food consumption, WG = Weight gain, FP = Feces production.

reduction in food consumption was approximately 20 and 35% for the treated male and female larvae respectively. Similarly, the total weight gain and fecal production were significantly lower (P = 0.05) in the treated larvae of both sexes indicating that these indices were directly related to the amount of food consumed.

2) Food utilization. - The approximate digestibility (AD) values are summarized in Table 5.

Table 5. - Approximate digestibility (AD) of the larvae of Choristoneura fumiferana infected with Erynia radicans.a/

DP[b/]	Control	Fungus treated
Female		
1	93.9 ± 11.4a	92.2 ± 7.6a
2	89.0 ± 10.8a	88.7 ± 12.7a
3	80.2 ± 11.8a	91.1 ± 16.7a
4	83.5 ± 8.3a	93.3 ± 11.2a
5	81.8 ± 12.8a	94.5 ± 13.6a
Male		
1	92.0 ± 8.4a	93.5 ± 11.2a
2	94.0 ± 8.7a	94.4 ± 14.9a
3	88.9 ± 11.1a	92.2 ± 13.7a
4	85.4 ± 12.6a	93.4 ± 9.8a
5	78.7 ± 6.9a	92.5 ± 6.2b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

No significant difference (P = 0.05) was observed between the control and the treated group in the female larvae. In males the AD for the control was significantly lower on day 5. The gross efficiency (ECI) and the net efficiency

(ECD) showed an identical trend (Table 6, 7). In the female treated larvae, both ECI and ECD values were significantly lower (P = 0.05) than the control group on day 4 and 5, while in the males the ECI and ECD of treated larvae were significantly lower on day 5 only.

Table 6. - Gross efficiency (ECI) of the larvae of Choristoneura fumiferana infected with Erynia radicans.[a]

DP[b]	Control	Fungus treated
Female		
1	8.6 ± 3.7a	8.2 ± 2.2a
2	9.7 ± 9.3a	12.3 ± 5.0a
3	10.4 ± 6.3a	9.3 ± 6.4a
4	11.6 ± 3.4a	5.7 ± 3.5b
5	14.2 ± 8.6a	6.0 ± 4.2b
Male		
1	6.6 ± 2.3a	6.3 ± 3.0a
2	10.4 ± 4.2a	7.8 ± 5.6a
3	12.6 ± 3.4a	9.0 ± 4.1a
4	8.4 ± 2.0a	5.3 ± 3.5a
5	10.3 ± 3.3a	5.5 ± 3.9b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

Table 7. - Net efficiency (ECD) of the larvae of Choristoneura fumiferana infected with Erynia radicans.[a]

DP[b]	Control	Fungus treated
Female		
1	9.2 ± 3.8a	8.6 ± 2.2a
2	10.9 ± 5.8a	13.9 ± 6.1a
3	10.7 ± 5.8a	10.2 ± 4.4a
4	13.9 ± 4.1a	6.2 ± 1.6b
5	17.4 ± 8.9a	6.4 ± 3.9b
Male		
1	7.1 ± 2.3a	6.8 ± 1.0a
2	11.0 ± 4.6a	8.2 ± 3.3a
3	14.2 ± 3.5a	9.8 ± 5.4a
4	9.8 ± 2.6a	5.7 ± 3.8a
5	13.1 ± 4.8a	5.9 ± 4.7b

a/ Means followed by a common letter horizontally do not differ (P = 0.05).
b/ Days post treatment.

3) Fecundity. - The purpose of this portion of this study was to determine a dosage that would produce a chronic and a sublethal effect that might result in the weakening of the host. Consequently, such an effect may cause a general disruption of the vital functions which could be manifested in reduced reproductive potential and shortened longevity (Gaugler and Brooks 1975). However, it was difficult to achieve such a sublethal dose. We tried various spore concentrations (6.3 to 38.1 spore/mm^2) as measured by the amount of time larvae were exposed (Vandenberg and Soper 1979) but we were unable to obtain sufficient number of adults for fecundity study due to low rate of emergence even though in some of the treatments (lower dosages) there was a high percent of pupation. Table 8 summarizes the results from one of the successful tests. In the fungus treated group the mortality was 55.6 and 63.2% in male and female respectively. Weight of control pupae was 75.2 ± 1.6 mg in males and 111.4 ± 2.6 mg in females as compared to 59.7 ± 2.0 mg for males and 97.7 ± 2.0 mg for females in the treated group. Fecundity expressed as mean number of egg/female was 92.8 in the control (9 pairs) and 59.0 in the treated group (2 pairs only).

Vandenberg and Soper (1979) showed that mortality of larvae of the spruce budworm treated with Erynia radicans occured in 3 to 6 days. Similarly, in our study larval death occurred in 4-6 days. During the infection stage, in this study, the food comsumption and utilization were significantly lowered in the fungus treated larvae as compared with the control on day 4 and 5. Also, pupae from infected larvae weighed less than untreated ones. Such effects are probably a result of depleted nutritional reserves and a reduced ability to assimilate food efficiently (Thomson 1958). Consequently, larvae will be less efficient at forming nutrient reserves for the pupae and adult stages.

In insect species such as the spruce budworm, where the adults do not consume food, nutritional reserves are dependent on the amount of nutrients accumilated by the larvae for the entire metabolism of the individual (Jasic 1961). Thus, if the reduction in food consumption and utilization in infected larvae occurs under natural conditions, adult females arising from diseased larvae will have even smaller reserves, since the individual must use a portion of the fat stored for general activity, thereby diverting a large portion of this reserve from egg production. This is important when considering the utilization of E. radicans in the management of an insect pest like the spruce budworm in a forest situation where the foliage can tolerate some feeding without serious economic damage.

Table 8. - The effect of *Eyrinia radicans* on the spruce budworm larval mortality, pupation, emergance and fecundity.[a]

Treatments	% Larval Mortality		% Pupation		Pupal wt (mg)		% Emergance		Eggs/per Female[b]
	M	F	M	F	M	F	M	F	
Fungus treated	55.6	63.2	44.4	36.8	59.7 ± 2.0	87.7 ± 2.0	9.4	8.0	59.0
Control	0.0	0.0	100.0	100.0	75.2 ± 1.6	111.4 ± 2.6	98.0	100.0	92.8

a/ Rate of larval spore exposure was estimated at 8.4 spore/mm^2.
b/ Based on the mean of only 2 pairs in the fungus treated group and 9 pairs in the control.

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RECENT FIELD STUDIES ON THE USE OF *BACILLUS THURINGIENSIS* TO CONTROL THE GYPSY MOTH (*LYMANTRIA DISPAR* L.)

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Efforts to improve the effective use of *B. thuringiensis* and reduce its application costs has stimulated the development of higher potency formulation. Recent field studies have shown that a single application at 30 BIU/ha (12 BIU/acre) is as effective as the split double application of 20 BIU/ha (8 BIU/acre) formerly recommended. Also results of using volumetric application rates of less than 9.6 L/ha (gallon/acre) are discussed.

Results of early studies on the field use of *Bacillus thuringiensis* (*Bt*) against the gypsy moth were encouraging but inconsistent. Aerial applications were often aggravated by incompatibility of *Bt* with its suspending medium (fuel oil) which often resulted in clogged nozzles and uneven spray coverage (Lewis and Connola, 1966). Development of standardization procedures based on insecticidal activity (Burges et al. 1967, Dulmage et al. 1971), formulation improvements, and the isolation and development of better and more potent *Bt* strains such as the HD-1 strain (Dulmage, 1970) all contributed to the resolution of difficulties associated with the *Bt* product and its application. Field studies that were done with these newer formulations (Thuricide 16B, Dipel WP, Dipel 4L) prepared with the HD-1 strain showed that *Bt* could be applied aerially to protect the foliage and reduce the density of the pest populations (Dubois and Lewis, 1981).

Until recently the recommended procedure for aerial use of *Bt* to control the gypsy moth was two applications, each at 20 BIU/hectar (8 BIU/acre) sprayed at cre/ a volumetric rate of 9.5 to 19 liters per hectare (1 to 2 gallons per acre). The first application was made when oak foliage expansion was about 50% and the majority of the larvae were still in the first instar. This treatment was repeated 7-10 days later when most of the larvae matured to second and third instar (Yendol et al. 1973, Lewis et al. 1974). Unfortunately the high cost of both the double application and the product itself discouraged general and large-scale use of *Bt* even in environmentally sensitive areas.

Two improvements of *Bt* products are contributing to the renewed interest for general and large-scale use of *Bt*; one, newer formulations are several times more potent and have longer residual activity than previously available; two, new strains of *Bt* that are significantly more potent that the HD-1 strain are continually being uncovered and evaluated for commercial development. It is becoming increasingly clear that as more and more strains of *Bt* are isolated, some are found to be more potent than the HD-1 strain against specific insect pests including forest and ornamental tree pests, agricultural pests, as well as disease vectors (Dulmage, et al., 1981).

Several studies were reported on the successful use of a single application of *Bt* compared to two applications previously recommended for use against the gypsy moth and spruce budworm (Morris et al., 1982, Morris, 1983, Reardon et al., 1982). Against the gypsy moth in particular, studies by different investigators (Table 1) have confirmed that one application at 30 BIU/hectare (12 BIU/acre) compared favorably to two applications at 20 BIU/hectare (8 BIU/acre). Regardless of the manufacturer or the formulation used (water or oil based), better foliage protection was achieved when *Bt* was applied once at the 30 BIU/hectare rate than when applied twice at/or lower than 20 BIU/hectare.

Table 1. Summary of *Bacillus thuringiensis* aerial spray tests conducted in Pennsylvania, Massachusetts and Connecticut comparing the effectiveness of single and double applications against instar II gypsy moth.

Applic. Rate	No. of Applic.	Popul. Trend	% Final Defol.	Ref.
10[c/]	1	1.55 R	25	McLane and Finney (1982)
10	2	1.10 R	41	
20	1	1.30 R	42	
20	2	1.38 R	38	
30	1	1.12 D	8	
30	2	1.60 D	5	
Control	-	1.75 R	70	
20[d/]	1	13.30 D	37	Andreadis *et al.* (1982)
20	2	15.00 D	15	
Control	-	6.50 D	61	
20[a/]	2	975.30 D[f/]	3.7	Andreadis *et al.* (1983)
30	1	21.30 D	5.6	
40	1	13.10 D	7.6	
Control	-	23.70 D	72.3	

[a/] rate of BIU applied at 9.5 liters per hectare

[b/] ratio of egg mass change: $\frac{\text{Post spray}}{\text{Prespray}}$ R = Rising population; $\frac{\text{Prespray}}{\text{Postspray}}$ D = declining population

[c/] Thuricide 16B (Zoecon, Inc.)

[d,e/] Dipel 4L (Abbot Laboratories)

[f/] Because of extreme variability, ratio is not significantly different from others.

In areas where the populations were on the decline, the trend was somewhat accelerated. Noteworthy is that in areas where populations were rising use of the single 30 BIU/hectare application rate reversed that trend and actually showed a decline in the egg mass change. Generally in these studies significant differences in relative larval densities were observed between treated and untreated blocks but differences between treatments were not significant. Furthermore, McLane and Finney

(1981) also showed that stickers effectively improved the residual activity of the Bt formulations. Eight days after application as much as 80% of the original insecticidal activity was retained on the sprayed foliage when acrylamide type stickers were incorporated into either oil or water based formulations. Without stickers insecticidal activity was reduced to 10 to 25% of its original activity. Undoubdedly the extended activity and improved physical formulation characteristics of these newer formulations contributed to the success of reducing application costs by eliminating the need for a second application. Slippey (1983) also reported that in declining populations, 30 BIU/hectare applications was as effective as higher dose rates (40 BIU/hectare) but when used against rising populations, results at 30 BIU/hectare were inconsistent.

Newer formulations of Bt designed for low volume application rates are from two to four times more concentrated than previously available. Such concentrated formulations contain from 12.7 to 16.7 BIU/liter (48 to 64 BIU/gallon) and permit the application of the 30 BIU/hectare rate at reduced volumetric rates. Against the spruce budworm, Dimond (1982) demonstrated that at 30 BIU/hectare, Bt could be used successfully at volumetric rates as low as 2.9 liters hectare (32 oz/acre). McLane and Finney (1982) reported that the same 30 BIU/hectare dose could be applied at a volumetric rate of 4.75 liters per hectare (64 oz/acre) against the gypsy moth and still provide excellent foliage protection and population reduction. Balaam (1983) also reported similar success of reduced volumetric rates at the same dose rate against gypsy moth infestations in New Jersey. The successful use of a single application of 30 BIU/hectare to control the gypsy moth must be applied when larvae are still in the second instar stage. Applications that are delayed until larvae are fourth instar will not be effective at 30 BIU/hectare. Higher dose rates of 40 to 50 BIU/hectare (16 to 20 BIU/acre) will only have a limited effect on the pest population (McLane et al., 1983).

Efforts to improve Bt formulations also include the development and evaluation of new strains. Andreadis and others (Andreadis et al., 1982) reported on the field evaluation of two experimental strains against a gypsy moth infestation. The strains, HD-243 and HD-263, were as efficacious as the commercially developed HD-1 as measured by relative larval densities and foliage protection. Noteworthy is that HD-243 was actually applied at half the dose rate of HD-1. Laboratory studies (Dubois unpublished data) had shown that HD-243 was about twice as potent as HD-1.

Recently a new strain of Bt labelled NRD-12 was isolated, which shows considerable promise for operation use (Dubois unpublished data). In a recent field study against a spruce budworm infestation it was significantly more effective than Thuricide® 32 LV. Both final defoliation estimates (12%) and the residual larval population (3.2 larvae per 45 cm branch tip) present in the block treated with SAN 415[1] were significantly lower than in the Thuricide® 32 LV treated block (25% and 7 larvae per branch tip) when both formulations were applied at 30 BIU per branch hectare. Laboratory studies of the SAN 415 formulation also show that NRD-12 may be more effective than the HD-1 strain against the gypsy moth. Results of parallel bioassays (Table 2) show the SAN 415 32 LV is 3.9 times more potent than Thuricide® 32 LV. Both strains were formulated at the same potency of 8.5 BIU per liter (Thuricide® 32 LV BIU per gallon). Whether this increased effectiveness will also be apparent under field operational conditions remains to be confirmed.

Table 2. Laboratory bioassays[a/] comparing HD-1 (Thuricide® 32 LV) and NRD-12 (SAN 415 32 LV) formulations against instar II gypsy moth.

	SAN 415 32 LV	Thuricide® 32 LV
Bt strain	NRD-12	HD-1
Serovar	H_{3ab}	H_{3ab}
Slope	5.37	3.73
S.E.[b/]	0.60	0.15
C.V.[c/]	0.19	0.07
LC_{50} (IU/ml)	45.20	173.7
S.E.	3.82	10.97
C.V.	0.15	0.11
Ratio[d/]	3.86	--

a/ average of 3 bioassays

b/ standard error

c/ C.V. coefficient of variation

d/ ratio of LC_{50} of Thuricide® 32 LV to SAN 415 32 LV

[1]Commercial formulation based on the NRD-12 strain. Mention of a commercial product does not constitute an endorsement by the USDA-FS.

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INTERACTIONS BETWEEN MICROBIAL AGENTS AND GYPSY MOTH PARASITES

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The parasite Cotesia melanoscelus attacks small gypsy moth larvae more successfully than large ones, and Bacillis thuringiensis retards the growth of caterpillars it does not kill. Together, both factors lead to higher parasitism by C. melanoscelus in areas sprayed with B. thuringiensis than elsewhere.

One of the major advantages of microbial agents over chemical insecticides is their specificity. They are usually benign to non-target organisms such as parasites and predators, and indeed many gypsy moth natural enemies are not harmed by treatments with microbial sprays. For instance, Dunbar et al. (1973) found that percent parasitism by a number of gypsy moth parasites was similar in check and Bacillus thuringiensis spray plots, and in another study the number of adult parasites caught in malaise traps during the year of treatment was usually the same in all plots (Reardon et al. 1979). However, in some cases parasite numbers were decreased by sprays. Reardon et al. (1979) and Andreadis et al. (1983) found that the pupal parasite, Brachymeria intermedia (Hymenoptera: Chalcididae), was not numerous in B. thuringiensis-treated areas. Ticehurst et al. (1982) showed that the tachinid parasites Compsilura concinnata and Blepharipa pratensis parasitized a lower percentage of gypsy moths in B. thuringiensis spray plots than in non-spray plots, and Kaya et al. (1974) noted that larvae of the predaceous beetle, Calosoma sycophanta, were less abundant in B. thuringiensis-treated as compared to untreated areas.

A decrease in parasite or predator populations in areas treated with a microbe does not necessarily mean that the agent has a direct effect on the natural enemy. Parasites and predators respond to host populations, which are generally different in treated and untreated plots. B. intermedia, for instance, seeks out high light intensities and so is usually most abundant in defoliated areas (Weseloh 1976b). Thus its numbers are often higher in check plots where most defoliation occurs. In field situations it is often not possible to tell whether the depressing effect of the agent is direct or indirect. Also, with one exception (to be detailed later), no laboratory studies have described the specific interactions between the most widely used microbial agent, Bacillus thuringiensis, and gypsy moth natural enemies.

A few workers have studied interactions between natural enemies and the gyspy moth nuclear polyhedrosis virus. Capinera and Barbosa (1975) showed that adults of the predaceous carabid beetle, Calosoma sycophanta, could pass viable virus polyhedral inclusion bodies through their gut without evident harm, and so may be a useful vector of this pathogen. Similarly, Raimo et al. (1977) demonstrated that the braconid, Cotesia (Apanteles) melanoscelus, could transmit virus from caterpillar to caterpillar when its ovipositor became contaminated, either artificially or from attacking diseased hosts. However, C. melanoscelus tends to avoid ovipositing in moribund hosts (Versoi and Yendol 1982), possibly restricting its usefulness as a vector.

Because such studies have not been extensive it is difficult to generalize about the impact microbial agents have on gypsy moth natural enemies. However, there is one interaction involving B. thuringiensis and the parasite, C. melanoscelus, that has received a substantial research investment.

In the early 1970's a number of field tests demonstrated that C. melanoscelus impact was enhanced in areas sprayed with B. thuringiensis. For example, in a 1972 spray test in Connecticut percent parasitism of field-collected caterpillars by C. melanoscelus averaged 1.0% in check plots and over 12 times higher (13.3%) in plots sprayed with Thuricide HPC or the experimental preparation IMC 90012 (Dunbar et al. 1973). The following year percent parasitism was 1.3, 7.0, and 20.6% respectively in check, Thuricide-16B, and Dipel WP plots. Also, an average of only 0.8 C. melanoscelus overwintering cocoons was located under each burlap band in check plots as compared to 4.7 in Thuricide and 8.1 in Dipel plots (Kaya, et al. 1974). Thus, the absolute number of parasite progeny as well as the proportion of hosts attacked increased in test plots. This is important because percent parasitism can be expected to increase in treated areas (assuming the disease has no direct effect on the parasite) even if parasites are not enhanced because low host numbers resulting from disease-induced mortality may result in a more favorable parasite:host ratio. Wollam and Yendol (1976) showed that this enhanced effect also occurred when 1000 mated female C. melanoscelus were released into plots which were also treated with Thuricide-16B. Counts of gyspy moth larvae under burlap bands as well as defoliation estimates were lower in plots receiving microbe and parasite than in plots receiving B. thuringiensis alone, C. melanoscelus alone or no treatments. This is the only study I know of where parasite releases

have been shown to actually reduce gypsy moth populations. It may be important that the effect occurred only when parasites were released in combination with B. thuringiensis.

The above reports show the potential which B. thuringiensis has for improving the effectiveness of C. melanoscelus. Because the combined effect was greater than the summed effect when either agent was present alone the phenomenon was a case of true synergism. Initially the causal mechanism was unknown. In early tests molasses was often used as an adjuvant in spray mixtures and may have attracted C. melanoscelus into the area. However, other parasites which should have responded to the molasses did not. Furthermore, I could not increase parasite activity by spraying from the ground with molasses. A clue to the mechanism involved occurred in the mid 1970's when I related C. melanoscleus parasitism rates to gypsy moth larval development (Weseloh 1976a). Percent parasitism by C. melanoscelus reached a peak when most hosts were in the second instar and declined thereafter, despite the fact that the parasite has two generations a year. First generation parasites emerge from overwintering cocoons when gypsy moth eggs are hatching and attack first instars. Their progeny develop without diapause and attack third and fourth instars. Clearly the first generation parasitized the most hosts in spite of the relatively large numbers of second generation adult parasites (as measured by sticky-panel catches) present when gypsy moths were fourth instars and larger. Results suggested that parasites did not successfully attack large caterpillars, and this was confirmed in laboratory tests. Because of the vigorous defensive movements and long hairs of older hosts, third and fourth instars were less frequently parasitized than were younger caterpillars (Weseloh 1976a).

I did not relate these results to the parasite-B. thuringiensis interaction until Ticehurst et al. (1982) found that, in addition to the increased parasitism by C. melanoscelus which occurred in Dipel-treated plots (32.3%, as opposed to 4.4% in the check plots), Dipel caused a one-week lag in development of surviving gypsy moths as determined by the appearence of pupae and adult moths. A similar lag was documented by Andreadis et al. (1983). This lag was caused by the gut-paralyzing effect of the delta-endotoxin of B. thuringiensis, which causes caterpillars to stop feeding at least temporarily (Heimpel and Angus 1959). Ticehurst, et al. (1982) suggested that this retarded development might enable C. melanoscelus to attack the small hosts it can successfully attack for a longer time, thus improving its effectiveness.

Laboratory work was done to investigate this. Ahmad et al. (1978) showed that B. thuringiensis-related mortality of larvae parasitized with C. melanoscelus was similar to that of non-parasitized gypsy moths, and they also concluded that there was no evidence for a direct, toxic effect of B. thuringiensis on the parasite. Weseloh and Andreadis (1982) confirmed these results and also showed that surviving, B. thuringiensis-treated second instars were retarded in development about two days as compared to controls. When presented with untreated larvae of the same chronological age as larvae exposed to B. thuringiensis three days before, female parasites attacked both hosts equally. However, if B. thuringiensis exposure had occurred ten days previously, untreated hosts were attacked substantially less frequently than treated ones. Ten days after treatment 97% of the treated larvae were still third instars while only 60% of the healthy ones were, so the former were noticably smaller than the latter. More importantly, a significantly greater number of parasites were reared from treated larvae than from untreated ones. Thus retarded development caused by B. thuringiensis is a plausable mechanism for enhancing the effectiveness of C. melanoscelus.

To test this hypothesis, C. melanoscelus and gypsy moth larvae were sampled in a 1981 aerial spray test using Dipel 4-L (Weseloh et al. 1983). As in the other tests mentioned, C. melanoscelus parasitism was only 1.2% in check plots and 6 to 12 times higher (6.9-14.8%) in treatment plots. Also, numbers of parasite cocoons under burlap flaps averaged only 4.0 per flap in control areas and from 13.2-20.3 per flap in treated plots. Caterpillar numbers were greatest and caterpillars were largest in the check plots. On June 11, only 5.2% of larvae were still third instars in the check areas, whereas 22% to 49% were third instars or smaller in treatment plots. Simple and multiple linear regression analysis clearly showed that percent parasitism was more closely related to the average caterpillar size per plot than to caterpillar numbers. This last point is important because, together with the larger numbers of parasite cocoons in spray plots, it shows that the increased parasitization was not due soley to changes in parasite:host ratios. Along with laboratory experiments, the test proved that the rate of gypsy moth development is critical to the field effectiveness of C. melanoscelus.

The importance of retarded host growth in improving the efficiency of C. melanoscelus led me to explore other circumstances where caterpillar development could be influenced. For instance, it is conceivable that low B. thuringiensis doses, lower even than those which cause mortality, might still retard caterpillar development. Also, feeding deterrents, by temporarily stopping feeding, might also be effective. In their field test Ticehurst et al. (1982) used Dipel 4-L at one-half the usual rate, but this still caused substantial mortality and no lower concentrations have to my knowledge been tested. Accordingly, in 1981 and

1983 I sprayed individual apple trees with B. thuringiensis concentrations ranging from the usual dose of 0.84 BIU to 0.035 BIU per tree (Weseloh, manuscript submitted). Other trees were sprayed with the mitacide Plictran (tricyclohexylhydroxystannane), an antifeedant for gypsy moths (Meisner and Skatulla 1975). Plictran is registered for use on apples, and was applied at the recommended rate of 0.22 g/l active ingredient.

Larval numbers were reduced below controls for all B. thuringiensis rates of 0.21 BIU per tree or more, but only for the highest dose was defoliation reduced. Plictran did not decrease larval numbers, but it and all the B. thuringiensis concentrations significantly retarded caterpillar development. Attempts to monitor parasitism in these areas were not successful in 1981 because of extensive inter-tree movement of caterpillars and in 1983 because parasitism levels, in general, were very low. But the link between developmental rates of gypsy moths in the field and parasitism by C. melanoscelus has already been proved, so it is likely that if parasite activity could have been monitored it would have been increased by the treatments.

Interactions between B. thuringiensis and parasites is not restricted to C. melanoscelus. Wallner et al. (1983) found in a laboratory study that retarded development of gypsy moths caused by B. thuringiensis enhanced parasitism by an exotic braconid, Rogas lymantriae, although a higher proportion of males emerged from infected larvae than from healthy ones. Also, Hamel (1977) showed that percent parasitism by two parasites of the western spruce budworm, Choristoneura occidentalis, increased in areas sprayed with B. thuringiensis. The two parasites, Apanteles fumiferanae and Glypta fumiferanae, attacked pre-diapausing budworms before sprays were applied. Parasitized larvae did not eat as much as healthy ones, and so probably avoided ingesting lethal amounts of B. thuringiensis. This is a different mechanism than operates with the gypsy moth parasites, where it is parasitism occurring after spray application that is enhanced.

Natural enemies may frequently be enhanced by applications of microbial agents, although few studies have been reported. Clearly, this is an area of research that needs more emphasis. Chemical pesticides might have similar effects, but chemical pesticides usually lack the specificity of microbial agents and kill natural enemies along with the target pest. Looking for side effects of pathogens on hosts could be important because such side effects can profoundly influence the effectiveness of natural enemies.

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GYPCHEK®: PAST AND FUTURE STRATEGIES FOR USE

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The development of the gypsy moth nucleopolyhedrosis virus product Gypchek, and strategies for its use, have been largely patterned after conventional pesticide technology. Prior to Gypchek registration with the Environmental Protection Agency in 1978, several field tests involving variations in virus product, application hardware, dose rates, timing and formulation were conducted. Further tests since 1978 have been promising, but nonconclusive, and 6 years after registration, Gypchek is not in commerical production. It is clear that if this product is to be seriously considered as either an alternative or adjunct to other control tactics, it must either be formulated in such a manner as to extend or amplify its activity on foliage, or it must be genetically manipulated to enhance its virulence.

As early as 1911, Reiff speculated that gypsy moth nucleopolyhedrosis virus (NPV) could be used to control the pest and some early field studies by Glaser and Chapman (1913) demonstrated its potential. In 1978 Reiff's prophecy was partially fulfilled with the EPA santioned registration of a gypsy moth NPV product Gypchek. This product was the fruit of several years of intense interdisciplinary research that ranged from understanding viral biochemistry and mode of action to testing and modifying viral delivery systems. The registration process has been bittersweet. A product that has great potential in gypsy moth management has been brought forward, yet in 1984, six years after registration, is not in commercial production. Why? The reasons will emerge as a brief chronology of Gypchek development and use are presented here.

Forest Service research on gypsy moth NPV began at the Northeastern Forest Experiment Station's Laboratory in New Haven, Connecticut in the early 1950's. A few, very limited field experiments[1] were conducted on individual gypsy moth infested trees treated with aqueous suspensions of macerated, virus-killed gypsy moth larvae. Results of these early studies were encouraging enough to test the virus in combination with Bacillus thuringiensis (B.t.) in a series of field trials in New York State, 1961-1963 (Lewis and Connola 1966). Virus used in these tests was from field collected (Connecticut) cadavers processed according to Rollinson and Lewis (1962). Since NPV was not used alone, these tests were ostensibly undertaken more for the hope of augmenting B.t. mortality than for assessing the efficacy of NPV. Results of these field tests, though showing some NPV effectiveness, were compromised by B.t. formulation and application problems, which made the evaluation of NPV effects even more difficult. What became apparent after 3 years of this type of testing was that B.t., in the same tank mix and sprayed at the same time as NPV, was antagonistic to the NPV infection process through its own mode of action, i.e., feeding inhibition through gut paralysis. Since NPV must be ingested to cause mortality, any feeding inhibitors in its formulation clearly reduce its effectiveness.

In 1963, NPV was tested alone on a 1-acre (0.4 ha) plot of mixed oak in the White Memorial Forest, Litchfield, Connecticut (Rollinson et al. 1965). Virus used was again from Connecticut field-collected cadavers (Rollinson and Lewis 1962) and was applied by truck-mounted mist blower at a rate of 4×10^{12} polyhedral inclusion bodies (PIB) in 4 gallons (15 l) of water-sticker tank mix per acre. Although data from this test is sketchy, it is apparent that the NPV was effective in reducing gypsy moth populations in the treatment plot; 96 percent egg mass (EM) reduction. No pretreatment EM counts were given for control plots whose post-treatment EM densities were 5 fold higher than those in the treatment plot. Results of this test were instrumental in securing a Forest Service research committment toward developing gypsy moth NPV as a microbial insecticide.

From these early field tests and from a variety of laboratory studies conducted on gypsy moth NPV and other insect viruses that followed, it became clear that first, gypsy moth NPV was not one of the more virulent insect viruses and second, that it remained active for only a few days following foliar application (Yendol and Hamlen 1973, Lewis and Yendol 1981). The implications of these findings relative to the development of Gypchek have been discussed in detail elsewhere (Lewis, 1981, Podgwaite 1984) but briefly, research focused on finding the most virulent gypsy moth NPV (the Connecticut strain, to date), developing a cost effective NPV production system (Shapiro et al. 1981), and finally developing a tank mix with sunlight protective properties.

By 1972 research had progressed to the point where further field testing was appropriate. A series of aerial tests were conducted in Pennsylvania between 1973 and 1978, the eventual year of Gypchek registration (Yendol et al. 1977, Wollam et al. 1978, Lewis et al. 1979). These field experiments evaluated various combinations of (1) a variety of NPV formulations (2) high versus low dose rates (3) one versus two applications (4) a variety of stickers, sunlight protectants and feeding stimulants (5) flatfan versus motorized nozzling systems and (6) morning versus evening application; all against a range of moderate to dense gypsy moth populations.

From an evaluation of all these tests emerged the current direct suppression tactic with Gypchek, i.e., two aerial applications, 7-10

[1] F. B. Lewis, Personal Communication

days apart, each at a prescribed rate between 1.0 and 5.0 x 10^{11} PIB per acre, against second stage gypsy moth larvae within moderately dense gypsy moth populations. The tank mix should include an appropriate sunlight-screen and sticker. Under optimal conditions, expected results using this tactic are (1) 50-80 percent EM reduction (2) < 55 percent defoliation and (3) the prevention of refoliation.

Results of further field tests[2] in Connecticut in 1981, using Gypchek in 4L® as well as in Protec®, although promising, were inconclusive. However, an aerial field test in Canada in 1982, using virtually the same Gypchek-4L formulation used in Connecticut in 1981, provided 95 and 90 percent EM reduction in treated plots compared to a 55 percent reduction and a 324 percent increase in EM in control plots. Treated plots averaged 20 percent defoliation compared to 45 percent for controls (Meating et al. 1983).

It is clear that there are some problems with Gypchek itself, as well as how it has been perceived, that have retarded its commercialization and its widespread acceptance as a microbial insecticide. The first is virulence. As mentioned earlier, gypsy moth NPV is of relatively low virulence when compared to several other insect viruses, e.g., the sawfly NPVs, many of which are 100 times as virulent against their respective hosts. Secondly, again reiterating, gypsy moth NPV is rapidly inactivated on foliage, losing most of its pesticidal activity within 2-3 days after treatment. Thirdly, erratic results from year to year, often due to the factors cited above, have done little to convince the user of its efficacy. Further, though the product is pesticidal only for the gypsy moth, and ultimately environmentally desireable, commercial producers are interested in developing products that will satisfy a broad market and to date have been hesitant to commit substantive resources toward developing products that will be used against only one insect. Finally, the users generally equate the performance of Gypchek with that of chemicals -- they expect equivalent results. Of course this is rarely acheived, and should not be expected. The product is essentially alive, requires 10-14 days to kill and has a narrow target window. It is unrealistic to equate its performance with a contact insecticide! The future of this product will hinge on its promotion as an adjunct to, rather than a substitute for, other pesticidal agents.

Toward this end some probing studies on alternate uses of this virus have been conducted. Parasite-NPV combinations have shown some promise. Raimo and Reardon (1981) found that the release of NPV-contaminated Apanteles melanocelus females resulted in almost double the incidence of NPV larval mortality in treated blocks compared to controls, while percentage parasitism was virtually the same in both. Podgwaite et al. (1981) introduced gypsy moth NPV into sparse gypsy moth populations by treating egg masses. In addition to an estimated 85-90 percent NPV mortality in larva hatching from EM so-treated, there was a 20 percent incidence of polyhedrosis in 4th-6th stage larvae in the year of treatment.

There are other researchable control tactics with Gypchek. These include (1) its use in sequence with other control techniques, e.g., with B.t., parasites, pheromones, sterile male moth release, and chemicals (2) the release of NPV-infected larvae into populations free of the disease (3) the release of NPV-contaminated predacious insects, mammals and birds (4) the use of attractants to lure larvae to contaminated baits and (5) the spot inoculation of this virus early in the developmental cycle of the gypsy moth or in its preceding generation.

Gypchek is at a crossroad in its development as a microbial insecticide. It is clear that if this product is to be seriously considered as an alternative to chemicals in any control strategy that involves broadcast application, then either it must be formulated in a manner that significantly extends its activity on foliage, or the virus must be manipulated genetically to enhance its virulence or perhaps increase its host range to make it more attractive for commercial development. This can be realized through a systematic research effort in the area of formulation and application, while integrating state of the art biotechnology into fundamental research on gypsy moth NPV.

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RECENT FIELD RESEARCH USING MICROBIAL INSECTICIDES AGAINST GYPSY MOTH

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Field research since 1978 using different formulations, dosages, and spray volumes of Bacillus thuringiensis Berliner (Bt) against the gypsy moth are reviewed. Problems associated with inconsistent results are discussed, with an emphasis on timing of application. Recommendations for proper use of Bt are presented along with suggestions for further studies on the use of Gypchek and development of better spray technology for both Bt and Gypchek.

Introduction

Two microorganisms are frequently used against gypsy moth, Lymantria dispar L; Bacillus thuringiensis Berliner (Bt) and a nucleopolyhedrosis virus (NPV). Field trials and research experiences with Bt in the 1960's and 1970's were summarized and reviewed by Harper (1974), Dubois (1981) and Cibulsky (1982) and will not be covered in this paper. NPV research is still centered on formulation and increased potency. Although NPV (GYPCHEK®) became commercially available in 1983 through Reuter Laboratories, Inc., it has not been used in the field except in research or plot studies. Lewis et al. (1979) and Lewis (1981) reviewed research on NPV and more recent summaries can be found in the 1981 and 1982 National Gypsy Moth Review Proceedings and in Laboratory Reports from the USDA-APHIS Otis Methods Development Center. This paper will concentrate on the recent studies and field experiences with Bt used against the gypsy moth.

Review of Field Trials 1978-1983

1978 and 1979

In 1978 and 1979 Bt was used operationally in suppression programs in only a few northeastern states. The two major Bt formulations applied aerially against the gypsy moth were Thuricide 16B® and Dipel-WP®. Most states relied on chemical insecticides, such as SEVIN® and Dylox®, to combat the gypsy moth because Bt was still trying to overcome its reputation as an environmentally desirable insecticide that produced inconsistent results. Field studies were conducted in New Jersey (McLane and Finney 1979) and New York (Abrahamson et al. 1981) with Thuricide 16B or 24B and results compared favorably with chemical insecticides in some cases and poorly in other cases. In New York State, where Bt was used operationally in several counties, it was applied at 8 BIU's/acre in a spray volume of 128 oz. Half of the treatment blocks were treated with a second application. Control was marginal when compared to results using chemical insecticides. Improper timing of application, poor weather conditions, lack of knowledge by spray applicators in the use of biological insecticides, inadequate mixing equipment and inconsistent spray deposition and droplet size were all factors that contributed to Bt's poor performance (Abrahamson et al. 1981).

1980

The gypsy moth defoliated over 5.1 million acres in the northeast during 1980. Spray acreage increased dramatically in all affected states, and the need for environmentally safe and effective insecticides intensified. Improved formulations of Bt were developed and tested. Wettable powders were no longer desirable and formulations of Dipel 4L, Thuricide 32B and/or Thuricide 16B were field tested in Massachusetts and Rhode Island (McLane and Finney 1980), New York (Abrahamson et al. 1981) and Pennsylvania (Fusco 1982a). Rates were still 8 or 8 + 8 BIU's/acre in a spray volume of 128 oz except for one test at 32 oz/acre. Foliage protection and reduction in larval and egg mass densities were satisfactory in many cases, however results were not always consistent and in some cases exceptionally poor. Again, the reasons for poor results could be attributed to a number of factors, the most common being improper timing of the application. Once the gypsy moth larval population reached late 3rd and early 4th instars the effectiveness of Bt diminished dramatically. It also became more apparent in 1980 that spray equipment, such as aircraft/nozzle configurations and mixing apparatus, and weather conditions played an important role in spray coverage and program results. Uniform spray deposition (20-25 drops/cm^2) and droplet sizes in the 100-200 micron range are necessary in order for Bt to control gypsy moth populations and/or protect foliage.

1981

Gypsy moth defoliation exceeded 13 million acres in 1981 and chemical insecticides were heavily used throughout the northeast. However, Thuricide 16B or Dipel 4L was used operationally in New Jersey, New York, Pennsylvania, Massachusetts and Maine. Bt use increased, but again results against dense populations were inconsistent when compared to chemical insecticides. Research on population reduction and foliage protection were conducted in Massachusetts with Thuricide 16B and 32BX (Burnham 1981 and McLane and Finney 1981) and in Connecticut with Dipel 4L (Andreadis et al. 1982). In Pennsylvania a Bt-parasite interaction study with Dipel 4L was also conducted (Fusco 1982b and 1982c). The above studies used the 8 or 8 + 8 BIU/acre rate in 128 oz spray volume applied aerially except

in Massachusetts where one and two applications of 4 and 12 BIU's/acre were also evaluated. One ground application study also occurred in Connecticut with Dipel 4L at the 8 BIU rate in a spray volume of 100 gallons/acre (Moore and Anderson 1982).

1982

Based on the results from 1980 and 1981, new concentrated formulations of Bt were developed for field testing in 1982 using higher BIU rates (12 and 16 BIU's/acre), and lower spray volumes (48 and 64 oz instead of 128 oz/acre). Tests were conducted in Connecticut with Dipel 4L at 8 + 8, 12 and 16 BIU's/acre in a spray volume of 128 oz (Andreadis et al. 1983); in Pennsylvania with Dipel 4L, Dipel 6L, Thuricide 32LV and Bactospeine at rates of 12, 12 + 12 or 16 BIU's/acre in spray volumes of 128 oz (64 oz for each of the 12 + 12 BIU/acre applications) (Fusco 1983a); in Pennsylvania by the USDA-APHIS with Dipel 4L, Dipel 6L, Thuricide 32LV and Thuricide 48B at rates of 8, 12 or 16 BIU's/acre in spray volumes of 48, 64 or 128 oz (McLane and Finney 1982); in New Jersey with Dipel 4L and Thuricide 32LV at the 12 BIU's/acre in a spray volume of 128 oz (Metterhouse and Balaam 1983a); in New York with Dipel 4L at 8 + 8 and 16 BIU's/acre in spray volumes of 128 oz (Glenister 1982) and a ground application study with Thuricide 32LV at the 12 BIU/acre rate in a spray volume of 100 gallons/acre (Eggen and Abrahamson 1983).

Overall results indicated that one application of these Bt formulations at 12 BIU's/acre in 128 oz spray volume provided foliage protection and reduced egg mass density in the spray blocks. The results from these studies set the stage in 1983 for a large scale suppression program in Pennsylvania with Bt.

1983

During 1983 a number of field studies were conducted throughout the northeast with different formulations of Bt at the 12, 16 or 20 BIU's/acre rate and a number of different spray volumes (24, 32, 48, 64 or 128 oz). These studies were conducted in New Jersey (Metterhouse and Balaam 1983b), Pennsylvania (McLane 1983, Fusco 1983b), and New York (Glenister 1983). Three Dipel formulations (4L, 6L and 8L), four Thuricide formulations (32LV, 48LV, 64LV and 64BX), Bactospeine and Futura were all field tested.

Operational programs using Bt in 1983 were conducted in Pennsylvania, New Jersey, Maryland and West Virginia. Thuricide 32LV, Dipel 4L or Dipel 6L at the 12 or 16 BIU's/acre rate in spray volumes of 64, 96 or 128 oz were evaluated.

The results from the field trials and operational programs indicated that when properly applied, 12 BIU/acre (or higher) in 96 or 128 oz spray volume gave results that were comparable to those obtained with chemical insecticides. Rates lower than the 12 BIU rate or 96 oz spray volume/acre gave variable degrees of foliage protection and egg mass reduction depending on local gypsy moth conditions and other operational factors.

The overriding factor that affected results in these field trials and operational programs was timing of the application so that it coincided with the 2nd and 3rd instars of the gypsy moth. As soon as a majority of the larval population reached the late 3rd and the early 4th instars efficacy dropped dramatically. Application rates of 16 or 20 BIU's/acre provided somewhat better results against 4th instars, but against 2nd and 3rd instars the results were not different than those observed with the 12 BIU's/acre rate.

Conclusions and Recommendations

1. The number one factor to be considered and closely monitored in any spray program using Bt is the developmental stage of the gypsy moth. Bt must be applied after egg mass hatch is completed and larvae are in the 1st-3rd instars. Leaf expansion should be at a minimum of 20 percent. As soon as the gypsy moth reaches the late 3rd to early 4th instar, higher rates of Bt and/or a second application should be considered. If not, application of a chemical insecticide may be necessary.

2. A single application of Bt at 12 BIU's/acre is recommended for operational use in areas with a declining population, or in areas that have building populations with less than 1000 large egg masses per acre. Multiple applications of 12-20 BIU's/acre or switching to a chemical insecticide should be considered in areas with high building populations (> 1000 large egg masses per acre).

3. More studies should be done to evaluate Bt on a range of gypsy moth population densities using low spray volumes with improved aircraft/nozzle spray application systems, such as Micronaire rotary atomizers, which give consistently uniform spray droplet sizes.

4. The newer Bt formulations are definitely better than the older formulations used in the 1960's and 1970's, and with improved application technology (stickers, nozzle types, spray equipment, etc.), results should be more consistent.

5. Research is continuing on newer and more potent Bt strains. The HD-1 strain now currently being used could be replaced by a more potent strain in the future.

6. NPV must be field tested more extensively in various formulations to determine its effectiveness against different population densities and to understand how this microbial can

best be incorporated into state IPM programs. All areas of spray technology must be studied with regard to NPV before it can be used on an operational level.

7. Additional research should be conducted on Bt-parasite interactions.

8. In large scale suppression programs, the effectiveness of a microbial insecticide depends on the amount of time, planning, manpower and monitoring that goes into the effort. The use of biological insecticides on an operational level requires proper application methods, otherwise the program is doomed to failure.

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SELECTION OF NEW MORE POTENT STRAINS OF BACILLUS THURINGIENSIS FOR USE AGAINST GYPSY MOTH AND SPRUCE BUDWORM

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The increased use of B. thuringiensis as an alternative to chemical insecticides has stimulated the search for more potent strains. Presently over 1100 strains have been isolated from worlwide sources. Some of these appear to be more effective than the commercially developed HD-1 strain against both the gypsy moth and spruce budworm. One strain in particular, when prepared as a standardized formulation was more effective than HD-1 against the eastern spruce budworm when compared under laboratory and field operational conditions. Laboratory bioassay data also indicates that against gypsy moth it may be approximately four times more potent than HD-1.

The selection of new B. thuringiensis (Bt) strains for development and use against specific insect pests can be a difficult and confusing task. The potency and toxin production of a given strain can be directly affected by the fermentation conditions used; not one single fermentation condition will be ideal for all isolates. Delta-endotoxins produced by different strains can differ quantitatively and qualitatively from each other and can have different activity spectra against a particular test insect. The spectrum of activity of a particular strain against different insect species, will vary such that a strain particularly potent against one insect species is not necessarily equally potent against another. Each strain has to be evaluated against each insect species.

Initial Studies: An understanding of the activity spectrum of Bt requires the evaluation of many strains against many different insect species. Because no single laboratory has the resources to conduct such an evaluation, several scientists from different laboratoris were organized into the International Cooperative Program on the Spectrum of Activity of Bacillus thuringiensis (Dulmage 1979). It was through the cooperation of this international program that about 300 uniformly prepared strains of Bt were recently evaluated against both the spruce budworm and gypsy moth. The results of this study will be reported here.

However, prior to this recent evaluation of Bt strains against the gypsy moth and spruce budworm the combined results of the first phase of this international research study, which included the screening of 350 strain preparations of Bt against 23 insect species was published in 1981 (Dulmage et al. 1981). The general conclusions were: (1) there is an interrelationship between insecticidal activity spectrum and strain serovar; (2) the same delta-endotoxin (crystal) can be produced by strains from more than one serovar; (3) clearly serologically different delta-endotoxins (crystals) i.e., K-1 and K-73, can be produced by strains of the same serovar; (4) against specific insect species some strains were more potent than the commercially developed HD-1 strain.

Specifically, against the gypsy moth (Dubois 1984a), there is a closer relationship between the crystal serotype and relative potency than between serovar and relative potency. Approximately 5% of the 350 strain preparations that were screened were considered very potent, and 16% were moderately potent. Of the 19 strains considered very potent 17 had a K-1 or K-1+ mixed crystal serotype and one each had a gal and aiz crystal serotype (from 64 and 35 representative strains respectively). Thirty three of the 57 strains that were considered moderately potent had a K-1 or K-1 + mixed crystal type. The influence of the type of crystal on potency can be particularly appreciated when we consider that most of the strains with a K-1 crystal type were of serovar $H_{3a\ 3b}$ var. kurstaki (49 strains), strains of the same serovar that produced a K-73 type of crystal (23 strains) were nonpotent or weakly potent. Forty-five strains of serovar H_1 variety thuringiensis that had a thu crystal type were also screened; only 9 of these strains were moderately potent and none were very potent. Numerous strains representing other serovars were also screened and most were weakly potent or nonpotent.

Relative to Trichoplusia ni and Heliothis virescens, strains that were very potent against the gypsy moth had a Tn/Hv ration of 1.91 which is a good indication that potency is associated with the K-1 type of crystal (Dulmage et al. 1981). Also strains that were very potent against gypsy moth were also very potent against T. ni but the reverse was not necessarily true.

The initial screening program identified some Bt strains that were as potent or more potent than HD-1 against specific insects. Two of these strains were field tested against a gypsy moth infestation and the results were encouraging (Andreadis et al. 1982). Morris and Moore (1983) evaluated 50 strains against spruce budworm larvae. Their selection for evaluation was based on the spectrum of activity of the selected strains against the Douglas fir Tussock Moth; none were more potent than the presently commercially used HD-1 strain.

Current Studies: For the present study, the LC_{50} (in µg/ml of diet) of 298 uniformly prepared strains was determined against both the gypsy moth and spruce budworm. Additionally the LT_{50} (at specific doses) of the experimental strains against the spruce budworm was also determined. The LC_{50} (and LT_{50}) of the HD-1 International Standard, HD-1S-1980, was determined against the same batches of larvae and used to compare the relative potency of the experimental strains.

The response of gypsy moth larvae to a specific preparation of Bt is generally consistent. Results of laboratory bioassays on

numerous batches of three generations of laboratory reared gypsy moth and spruce budworm larvae with the HD-1 standard, HD-1S-1980, are summarized in Table 1. The average LC_{50} is reasonably consistent between the generations (F_{23} to F_{25}) of gypsy moth that were used. Between batch and within generations variability (as measured by the C.V.) was higher than desirable but nonetheless acceptable. Slopes of the regression usually range between 4-5 (P = 0.05). Using the defined potency of 16,000 International Units of potency (IU)/milligram (mg) for the HD-1S-1980 standard, the overall LC_{50} for the three generations ranged from a low of 75 to a high of 105 IU/milliliter (ml) of diet. This is in good agreement with an earlier estimate of 96.2 ± 5.4 IU/ml (Yendol et al. 1973).

Table 1. Averages of the 1981, 1982, and 1983 bioassays with the standard HD-1S-1980 against laboratory stocks of instar II, Lymantria dispar L. and instar IV, Choristoneura fumiferana (Clem.)

Insect	YEAR		
L. dispar	1981	1982	1983
No. of bioassays	28	30	33
LC_{50} (µg/ml of diet)	5.25	4.95	5.99
± 95% C.L.[a]	0.72	0.47	0.55
S.E.[b]	0.35	0.23	0.27
C.V.	0.36	0.25	0.26
C. fumiferana			
No. of bioassays	6	18	22
LC_{50} (µg/ml) of diet)	4.84	4.75	6.88
± 95% C.L.	2.52	1.35	1.25
S.E.	0.98	0.64	0.60
C.V.	0.49	0.57	0.41

[a] C.L. - confidence limits

[b] S.E. - standard error

[c] C.V. - coefficeient of variation

Mortality data obtained from spruce budworm bioassays is often heterogeneous. Frequently, variation about the LC_{50} results in wide fiducial intervals which can actually be understated and will limit meaningful interpretation of the potency of a particular test strain relative to the standard. As such, estimates of the LC_{50} of a particular preparation are usually discarded when the calculated "g" value exceeds 0.2 (Finney 1964). The mean LC_{50} over three generations of spruce budowrm with HD-1S-1980 are also summarized in Table 1. Between generations, the LC_{50} is fairly consistent however between batch (within generation) variability can be large (note the high C.V.) such that, with all generations considered, the LC_{50} could actually range (P = 0.05) from 37 to 137 IU/ml of diet. Slopes of the regression usually vary between 2 and 3. Variability within and between bioassays with Bt against the spruce budworm has been a continuing problem for this and other investigators. Fast and Dimond (1984) and Fast and Régnière (1984) have reported higher LC_{50} and lower slope values in their bioassays with the HD-1 standard, HD-1S-1971 (precursor to HD-1S-1980) than reported here with HD-1S-1980. Some of these discrepancies could be accounted for by differences in diet and larval rearing conditions and the bioassay procedure used. The observation that larvae recover after exposure to Bt and the possibility of cycling of feeding inhibition and recovery could also contribute to the variability observed in bioassays with this insect. These investigators also reported that there was no significant difference in susceptibility between instar IV to VI. On the other hand Dubois and Squires (1970) had reported that in gypsy moth the LC_{50} of a Bt preparation could vary with larval weight and age.

The LT_{50} of spruce budworm with HD-1S-1971 as reported by Fast and Régnière (1984) was also higher than reported here (Table 2). Their estimate did not vary with larval age but did vary with Bt dose and length of larval exposure on the test diet. Present estimates of the LT_{50} with HD-1S-1980 over a two-fold dose range against instar IV larvae is remarkably consistent and within dose variability is very low.

Table 2. LT_{50} of different doses of the HD-1 standard, HD-1S-1980, against instar IV Choristoneura fumiferana (Clem.)[a]

	Dose in IU (µg)/ml of diet		
	200(12.50)	150(9.38)	100(6.25)
LT_{50} (days	4.9	4.6	4.1
95% range	4.3-5.6	2.9-6.7	3.6-4.7
C.V.	.19	.30	.14

[a] Larvae in constant contact for 8 days; 16:8 L:D photo period, 21°C. Average of 8 bioassays/

Most of the 298 strains evaluated in the present study were weakly potent or had no effect on the hosts. None of the 18 strains of serovar H_{14} var. isrealensis or the 14 strains of serovar H_{10} var. darmstadiensis had any effect on these two insect species. Numerous other strains had a limited effect on larval growth and effected very low mortality even at doses as high as 100 µg/ml of diet. Based on the response of these insects to the standard (Table 1) only those experimental strains with an LC_{50} of ≤ 1.99 ug (32 IU)/ml and those with an LC_{50} between 2.00 and 10.00 µg (32 and 160 IU)/ml of diet are summarized in Table 3; these LC_{50} values would indicate strains that are more potent than or equally potent as the HD-1S-1980 standard. The majority of the strains that are more potent than HD-1S-1980 against both insect species are primarily of serovar H_{3a3b} var. kurstaki and have a K-1 type crystal. A few strains of serovar $H_{4a\ 4c}$ var. kenyae and serovar H_7 var. aizawae are also very potent against both insect species. Of all the strains that are considered more potent than the standard against each insect species (16 + 10, LC_{50} ≤ 1.99 µg/ml) only four are equally more potent against both insect species. These are HD-262 (H_{3a-3b}-K-1), HD-545 (H_{3a3b}-K-1), HD-551 (H_{4a4c}-ken) and HD-562 (H_{3a3b} - ?). Against gypsy moth very

potent strains of other serovars worthy of mention are HD-287 ($H_{5a\ 5b}$ - K-1) and HD-854 (H_7 - ?) whereas against the spruce budworm HD-150 ($H_{5a\ 5b}$ - gal) and HD-120 (H_1 - K-1) are also worthy of mention as being specifically very potent. The observation that only four strains were equally very potent against both insect species and that there were a few strains uniquely potent against each insect further supports the observation made in the initial study that for each insect species there are some strains that are uniquely potent (Dulmage et al. 1981).

Table 3. Summary of the bioassay of HD strains considered more potent ($LC_{50} \leq 1.99$ µg/ml) or as potent ($LC_{50} \geq 2.00 \leq 10$ µg/ml) as the HD-1s-1980 standard against gypsy moth and spruce budworm.

		Gypsy moth LC_{50} - (µg/ml)		Spruce Budworm LC_{50} - (µg/ml)	
Serovar	crystal serovar	≤ 1.99	$\geq 2.00 \leq 10.00$	≤ 1.99	$\geq 2.00 \leq 10.00$
1	thu	0	5	0	1
	K-1 + thu	1	9	0	2
	K-1	1	2	1	0
	NI[a]	0	0	0	1
3a	ale	0	0	0	1
3a3b	K-1	5	19	5	6
	K-73	0	1	0	0
	NI	1	6	2	0
4a4c	ken	2	1	1	3
	NI	1	1	0	1
5a5b	K-1	1	0	0	0
	gal	0	2	1	0
6 sub	sub	0	0	0	1
6 ent	ent	0	1	0	0
7	aiz	1	6	0	5
	NI	1	2	0	2
8a8b	K-1	0	1	0	0
	NI	1	0	0	0
9	tol	1	3	0	1
	NI	0	2	0	0
12	NI	0	1	0	0
Unk[b]	thu + K-1	0	1	0	0
	aiz	0	1	0	0
	NI	0	4	0	1
Totals		16	68	10	25
Other Totals[c]		214		263	

[a] not identified

[b] serovar not determined

[c] strains non/or weakly potent

In addition to the 298 strains of Bt reported above, twenty other strains of Bt were also evaluated. These strains were isolated (Dubois unpublished data) from diseased spruce budworm larvae received as hibernaculum from FPMI at Sault Ste. Marie, Ontario. No other shipment of larvae received from FPMI either prior to or since that particular shipment exhibited such a high natural incidence of Bt infection. All the strains that were isolated except for one, were identified as serovar $H_{3a\ 3b}$ var. kurstaki; one strain was identifies as serovar $H_{5a\ 5b}$ var. galleriae (G. Donaldson USDA-ARS, Brownsville, TX 1983, personal communication). The serotype of the crystal of these new strain has not been determined. Three of these isolates labelled as NRD-8, NRD-10, and NRD-12, are 2 and 3.5 times more potent than a similarly prepared HD-1 (concentrate or formulated) against the spruce budworm and gypsy moth respectively. Also, unique with these three strains was their rate of kill against the spruce budworm. Regardless of the preparation, spore-crystal concentrate or standardized formulation, the LT_{50} of the three strains was almost half that of a similarly prepared HD-1 or of the international standard, HD-1S-1980 when compared at similar doses of µg/ml of diet. One of these strains, NRD-12, was commercially formulated and labelled as SAN 415 32LV. This formulation, standardized at 32 BIU/gallon, was then compared to the registered HD-1 formulation, Thuricide 32 LV®, against a natural spruce budworm infestation. The favorable results were summarized in a preceding presentation (Dubois 1984b). The microbial and pathogenic characteristerics of this strain are summarized in Table 4. The higher potency of NRD-12 compared to HD-1 is also apparent from bioassay with H. virescens but not with T. ni.

Table 4. Summary of the microbial and pathogenic characteristics of the NRD-12 Bacillus thuringiensis strain.

Source: Isolated by N.R. Dubois from diseased Choristoneura fumiferana (Clem.) larva at the NEFES-USFS laboratory at Hamden, CT, January 1981. Larva had been received from FPMI Sault. Ste. Marie, Ontario in December 1980

USDA Bacillus thuringiensis Culture Collection No. for NRD-12 is HD-945.
Serovar[a]: H_{3a3b} variety kurstaki, crystal antigen unkown.

Bioassay Characteristics:

	Comparative Potency (IU/mg) Trichoplusia ni	Heliothis virescens
HD-1 R641A:	39,800	15,400
NRD-12 (HD-945):	30,300±8980	32,700±7280

Lymantria dispar	NRD-12 (conc.)	HD-1S-1980	Ratio
Slope:	5.19	3.75	
LC_{50} (µg/ml)	1.75	6.14	3.51
LC_{50} (IU/ml)	28.00	98.2	
Choristoneura fumiferana			
Slope:	2.85	3.50	
LC_{50} (µg/ml)	1.75	3.60	2.06
LT_{50} (days) (3.0 µg/ml)	4.6	8.3	1.80
(6.6 µg/ml)	2.7	5.1[b]	1.91

[a] Serovar, data for T. ni and H. virescens provided by G. Donaldson and H. Dulmage, USDA-ARS, Brownsville, TX.

[b] LT_{50} based on a spore-crystal concentrate of HD-1: for LT_{50} of HD-1S-1980 at comparable dose refer to Table 2.

This similarity to HD-1 relative to its serovar and its potency against T. ni was also independently confirmed by Hostetter (D. Hostetter, USDA-ARS Columbia, MO, personal communication Dec. 1983). However, its spectrum of activity (and presumably that of NRD-8 and NRD-10) relative to gypsy moth, spruce budworm and H. virescens certainly indicates some toxin composition different from HD-1. Furthermore, its rate of kill (LT_{50}) against the spruce budworm is significantly faster than with any preparation of HD-1(at comparable doses) reported by others (Fast and Régnière 1984, Fast and Dimond 1984), or reported in this study. This insecticidal characteristic may be particularly advantageous in operational use of Bt (Fast and Dimond 1984, Morris, 1983).

Evaluation of Bt strains against insect pests is a continuing effort to search for new more effective strains. Presently, approximately 1,100 strains have been isolated from worldwide sources, and as with the NRD-series more are continually being isolated. This influx of new strains will undoubtedly increase as the potency of variants generated from genetic engineering efforts need to be evaluated.

Aside from the large grouping of the specific activity of serovar H_{14} against mosquitos and black flies, differences in activity among lepidopteran pests are more subtle. These differences such as the LT_{50} differences between NRD-12 and HD-1 relative to spruce budworm may have a greater impact on field effectiveness than the two-fold difference in LD_{50}. Whereas the almost four-fold difference in LD_{50} of these two strains relative to gypsy moth may be the insecticidal characteristic of greater significance. These observations along with the results of this survey, that only four strains were equally very potent against both insects and several strains were uniquely very potent against each insect species certainly suggest that serious efforts to improve the use of Bt by strain selection will require the evaluation of each strain against each insect pest.

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CHITINASE PRODUCING BT STRAINS

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Screening of 402 strains of more than 18 varieties of *Bacillus thuringiensis* showed chitinase to be inducible in virtually every serovar tested. Though the chitinase titre varied among strains, there was a strong correlation between enhanced lethality to spruce budworm, *Choristoneura fumiferana* (Clemens), and an increase in chitinase titre within the serovar. Strains grown in chitinase inducing medium gave a more rapid and higher kill than those grown in sporulation medium, and a faster kill than the currently used commercial strain.

Introduction

The sustained and growing assault on vast areas of spruce, balsam and Douglas fir forests by *Choristoneura fumiferana* (Clemens), the Spruce Budworm (SBW), has occasioned a variety of treatment regimens. Among these, and still representing an area of controversy, is the application of chitinase as an adjuvant in *Bacillus thuringiensis (B.t.)* treatments (Smirnoff 1974, 1977; Morris 1976). Indeed, little clear-cut evidence is available, at this time, on which to base a definitive opinion. Work in our laboratory (Dubois 1977; Daoust 1978) has demonstrated that the presence of microorganisms originally isolated from their insect host, induced in growth medium for chitinase production, and subsequently applied to leaf tissue, is associated with lesions produced in the peritrophic membrane of larvae which have ingested these chitinase active microorganisms. Ultimately, death occurs as a result of the generalized septicemia which follows spore and cell invasion through these perforations. Accordingly, it was decided to search among the various strains of *B.t.* for chitinase active cultivars. The underlying hypothesis was that such chitinase active organisms could, in hydrolyzing gut membranes, mediate spore entry and activity and, augmented by the activity of the crystal toxin on the epithelial cells of the gut, prove more lethal than non-chitinase active strains.

In the report which follows, we survey the incidence of chitinase activity in a wide range of *B.t.* crystal and serotypes, and indicate the relationship between chitinase activity, crystal type and lethal effectiveness to SBW.

Methods and Materials

Bacillus thuringiensis

Cultures of *Bacillus thuringiensis*, used in this study, were obtained from Dr. N. R. Dubois of the Center for Biological Control of the Northeastern Forest Experiment Station, U.S. Forest Service, Hamden, CT. These were maintained on nutrient agar slants and stored at 4^0C until needed. The various *B.t.* cultures were tested for chitinolytic activity by streaking on petri plates containing a mineral salts medium as described by Dubois (1977) in which 0.1% purified chitin served as the sole source of carbon. Replicate plates containing 0.2% casamino acids as well as chitin, were streaked with *B.t.* to assess the effects of this enrichment on the growth of the organism. Chitinolytic activity was estimated from the degree of clearing around each colony at 4, 6 and 12 days after inoculation.

Strains of *B.t.* were tested quantitatively for chitinase production colorimetrically. Chitinase titers were measured by the addition of 0.5 ml of 0.1M citrate buffer (pH 5.0) and 0.5 ml purified colloidal chitin (25 mg/ml) to 0.5 ml of supernatant from each test culture. The supernatant was obtained by centrifuging the whole culture for 15 min at 10,000 rpm in an RC-2 Sorvall refrigerated centrifuge. Chitin, one batch used throughout, was purified as described by Daoust (1978); broth culture techniques are described below.

The assay mixtures were incubated for one hour at 50^0C in a water bath. This temperature favors the action of the enzyme complex which attacks the chitin polymer, as opposed to those chitinase components which hydrolyze oligomer or dimer units (Weir 1978). Controls contained 0.5 ml distilled water instead of culture supernatant. Colorimetric determination of N-Acetyl D-glucosamine (NAG) levels, as a measure of chitinase titers, were made using a B & L Spectronic 21 colorimeter at 585 nm according to the method of Reissig *et al* (1955).

Cultures showing strong chitinolytic activity were selected for further testing in bioassays of SBW mortality. They were cultured in quantity using two media, one medium designed to amplify production of spores and crystals, and the other selected to enhance chitinase production while still permitting sporulation and crystal formation.

When maximal spore and crystal production was desired, flasks were prepared containing 100 ml of *B.t.* sporulation medium as described by Dubois (1968). Inocula were obtained from 24 hour cultures grown in trypticase soy broth (TSB), transferred again to TSB for 4-6 hours to obtain log phase growth and added as 1% of the volume of the medium in the sporulation growth flasks. These flasks were incubated at 27^0C for 3 to 5 days on a shaker table. Cultures were then examined microscopically for the presence of spores and crystals and only cultures which contained both were used in bioassays. Acceptable levels were considered to be 90% spores and crystals as compared to vegetative cells, and a 1:1 ratio of spores to crystals was virtually always found.

B.t. cultures were also grown in a chitin based liquid medium designed to increase the chitinolytic activity of the bacteria. It contained the mineral salts trace elements present in the solid medium used in the agar plates, and 3% crude chitin as the sole source of carbon.

The chitin was obtained from Pfaltz and Bauer, Stamford, CT. A single batch of crude chitin from this source sufficed for all experiments described in this report. The crude chitin was boiled 10 min in distilled water, followed by 5-6 distilled water rinses. After each rinse, the chitin was allowed to settle and the water was decanted by suction. The cleaned chitin was dried in an oven at 30^{0}C, and further ground for approximately 20 minutes (2-3 min pulses) in a blender.

TSB tubes (5 ml) were inoculated from slant cultures and incubated 20-24 hours at 25^{0}C, after which 1 ml from the tubes was inoculated into 99 ml of mineral salts, 3.0% chitin broth in 250 ml flasks. The flasks were incubated at 27^{0}C in the dark on a rotary shaker (115 oscillation/min).

Spruce Budworm

Cultures of *B.t.* were subsequently tested for virulence on SBW larvae which were reared as follows: hibernacula of SBW were obtained from the USDA Forest Service in Hamden, CT. and kept refrigerated. As needed, squares (approx. 1 cm^2) of cheesecloth containing the hibernacula were cut and placed on the underside of the lids of 1 oz plastic cups. The cups were filled approximately 1 cm high with "Bio-Serv" SBW diet medium containing aureomycin (Bio-Serv, Inc., Frenchtown, N. J.). The medium, its water and agar components sterilized to reduce contamination, was poured at about 120^{0}F, and allowed to solidify within the cups.

Cups were incubated at 25^{0}C (approx. 70% relative humidity) in 24 hours of light a day for approximately 3 weeks, or until larvae had attained fourth instar. Cups were incubated in an inverted position to take advantage of the larvae's photopositive behavior and to reduce mold infection in the cups. Larvae were transferred to new cups if they became overcrowded, if the diet medium was depleted or dried out, or if mold began to grow on the medium. Larvae were discarded if they showed any signs of infection.

The eclosure of SBW larvae is not synchronous, and in fact may span a week. Consequently, the growth of the larger larvae in a group occasionally was slowed down by sequestering them in a 12^{0}C incubator, permitting smaller larvae to catch up in size. Larvae in the three penultimate instars were used in bioassays. These larger larvae were separated into empty cups and starved for 24 hours at 12^{0}C prior to each bioassay. No more than 5 larvae were placed in a cup to reduce cannibalism.

Larvae were then sorted for size and instar, and transferred to replicate groups of 7 comparable individuals into prepared bioassay dishes. The bioassay dish consisted of an 8.5 cm plastic petri dish, floored with a piece of filter paper.

A small sprig of balsam fir was placed in each dish. Each sprig contained about 30 needles, and had a branch stem approximately 4 cm in length. In most experiments, the treatment to which the SBW larvae were subjected was applied on the surface of this balsam sprig.

For each treatment, balsam fir sprigs were washed in tap water, then dried at room temperature. Sprigs were next dipped into an aliquot of whole culture in beakers (usually 6 ml in a 25-50 ml beaker) followed by air drying for approximately one half hour. Triton X-100, a surfactant, was added at 0.1% to all treatments to enhance leaf wetting and adhesion by active agents.

Seven SBW larvae were added to each bioassay dish after a suitably prepared fir sprig was in place. They were allowed to feed (or not) on the sprig for 48 hours after which an untreated sprig replaced the treated one as a food source. Consumed sprigs were replaced as needed. Five to ten replicates of each dish were used, and controls dipped in sporulation medium or chitin broth were maintained. For most experiments, the number of viable bacteria (cells and spores), in the cultures employed was determined by the "drop plate technique" of Reed and Reed (1948). Five replicates of 0.01 ml from dilutions of cultures (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}) were delivered with a 0.1 ml Mohr pipet onto the surface of TSA plates. Plates were allowed to dry and were incubated in an inverted position at 27^{0}C for 20-24 hours. Bacterial counts were made using a Bausch and Lomb dissection microscope, and replicates were averaged.

For each experiment, specific details of numbers, replicates and variations in technique are presented in the results together with the data deriving from that experiment.

Results

As will be noted from Table 1, chitinase production is a characteristic widely distributed among the varieties of *B. thuringiensis* tested. Indeed few varieties were without a chitinolytic strain, and their absence in varieties of *B.t. sotto* and *subtoxicus* may simply reflect the minimal number of strains available for testing. Although the chitinolytic propensity seemed to cluster within specific crystal or serovar types (e.g. *kurstaki, entomocidus* or *darmstadiensis*) it was not by itself a predicator of lethal potential.

On the other hand, there was a clear correlation between chitinase titre and lethality (Figs. 1,2). In Fig. 1 are shown results obtained when 8 cultivars of the *kurstaki* variety (H3a3b) were tested for effectiveness against spruce budworm. Mortality corresponded very clearly with concentration of chitinase generated. In Fig. 2 are shown results achieved with repeated trials of the cultivar HD-110 (*B.t.* var. *entomocidus*) harvested at different periods under the same conditions and yielding different amounts of chitinase. In this case too, there is an unequivocal relationship between the amount of chitinase present and mortality.

Table 1. The production of chitinase by selected taxonomic serovars of *Bacillus thuringiensis*[1]

Varietal epithet	Serovar	Degree of Plate Clearing ++	+	+/-	0	Totals
kurstaki	H3a,3b	5	44	8	14	71
thuringiensis	H1	3	25	4	39	71
finitimus	H2	4	0	1	3	8
alesti	H3a	1	16	3	4	24
kenyae	H4a,4c	0	5	4	8	17
sotto	H4a,4b	0	0	0	1	1
dendrolimus	H4a,4b	6	1	2	1	10
galleriae	H5a,5b	1	43	12	19	65
entomocidus	H6	2	0	0	3	5
subtoxicus	H6	0	0	0	2	2
aizawai	H7	1	37	6	2	47
morrisoni	H8a,8b	0	5	3	6	14
tolworthi	H9	1	4	0	1	6
canadensis	H5a,5c	1	2	0	1	4
darmstadiensis	H10	6	4	0	3	13
toumanoffi	H11a,11b	0	1	1	0	2
israeliensis	H14	1	5	0	3	9
other	-	2	2	1	2	7
unidentified	-	3	8	0	6	17

[1] The degree of chitin-agar plate clearing was defined as follows: 0, no clear-zone visible in the agar surrounding colonies; +/-, a thin cleared ring around colonies just visible to the naked eye; +, a cleared ring around colonies about 1 mm wide; and ++, a cleared ring of 2 mm or more visible around the colonies on plate.

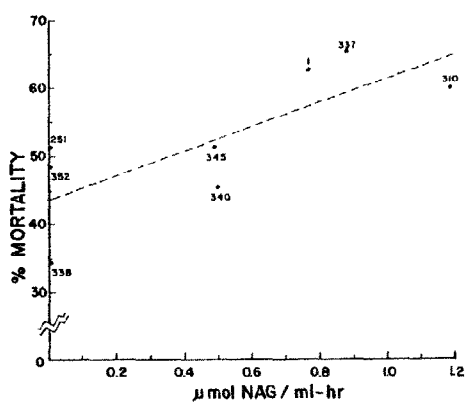

Fig. 1. Spruce Budworm mortality produced by 8 strains of *B.t.* var. *kurstaki* of varying chitinase titre. The strains were selected from one variety (serovar and crystal types, H3a,b and k-1, respectively) to minimize the effects of variables other than chitinase titre. All cultures were grown for 16 days in chitin broth medium. The initial number of larvae tested for each group was 35. Control mortality (not shown) was 5.7%. The correlation coefficient obtained for these points was r = 0.75; with 6 df, this gave 95% confidence limits by t-test. The number by each point is its HD number: HD-1 is the standard commercial strain.

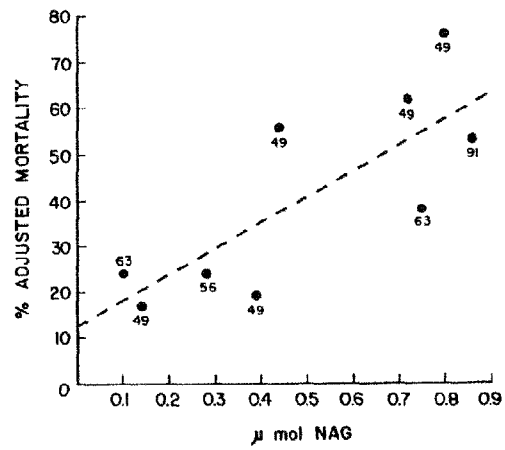

Fig. 2. The relationship of chitinase production in cultures of *B.t.* HD-110 (var. *entomocidus*) to mortality in spruce budworm.

The data points were calculated by means of Abbott's formula (1925) on mortality figures in each experiment to yield the "adjusted mortality" shown. The number by each data point gives the n for each experiment, the initial number of larvae in each treatment (and control) group. For these data, r = 0.785, significant at the 97% level of confidence by t-test with df = 7.

If one compares the overall efficacy of chitinase producing cultivars to that of the HD-1 strain currently in commercial use, the significant trend which emerges is the greater immediacy of kill achieved even though after 5 days the ultimate kill may be equivalent. It is clear (Fig. 3) that the presence of chitinase accelerates the general lethal action of *B.t.*

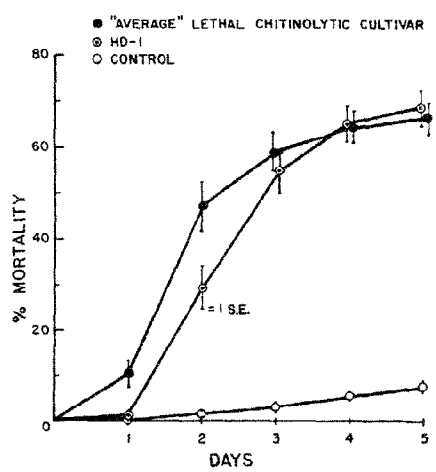

Fig. 3. Rate of kill of chitin grown *B.t.* strains compared with that of the commercial strain HD-1.

A composite of 9 separate experiments. The chitinolytic cultivars tested were HD's 7, 37, 48, 124a, 234, 287, 319, 340 and 539. Initial numbers per treatment group in each experiment ranged from 49 to 119, but each experiment was weighted equally to produce the average points

shown in the graph. The points for HD-1 and the controls are obtained from the same 9 experiments, and had the same initial n per group as their respective chitinolytic treatment groups.

The "chitinolytic cultivars" group results tested against that of HD-1 by ANOVA proved significantly different at the 0.05 level ($F = 4.92$, $df_1/df_2 = 1/80$). As the HD-1 actually produced higher ultimate mortality on day 5, this difference is plainly due to the distinctly higher mortality which the chitinolytic cultivars produced at the onset of the treatment exposure period.

That these effects are conditioned by the induction of chitinase is confirmed by the data in Table 2 and Fig. 4. The mortality achieved by cultivars grown in sporulation medium, i.e. in the absence of chitinase, is consistently and significantly lower than that achieved by the same cultures grown in a chitin medium for the induction of chitinase. As might be anticipated this, of course, did not hold true where the culture lacked the potential for inducible chitinases in significant amounts, the threshold level of available chitinase appearing at or above 0.6 µmol of NAG per ml-hour (see Table 2).

Table 2. A comparison of *B.t.* the lethality to spruce budworm of *B.t.* cultures grown in sporulation and chitin broth media, respectively.

Varietal	% mortality on 5th day: Sporulation medium	% mortality on 5th day: Chitin medium	Chitinase titre
HD-1	80.0	43.0	0.53[1]
HD-37	28.5	51.8	0.93
HD-48	44.8	44.8	2.04[2]
HD-110	32.6	36.7	1.15[2]
HD-124a	20.6	23.8	1.5
HD-198	63.2	36.7	0.33[2]
HD-198	45.6	47.3	0.4[3]
HD-224	21.4	42.8	0.68
HD-226	37.4	58.7	0.96[3]
HD-232	40.2	42.8	5.5[4]
HD-234	46.4	66.0	1.27
HD-287	41.0	71.4	1.4
HD-323	58.4	61.4	0.75[3]
HD-498	44.1	62.7	1.62[3]
HD-500	22.0	19.0	0.1
HD-539	51.7	46.4	0.6[5]

[1] = µMol NAG/hr/ml. Usually HD-1 grown in chitin medium killed over 60% by day 5 (see Fig 1).

[2] = in this experiment, control mortality = 22.8% on day 5 (over 2x normal).

[3] = sporulation culture tested alone. Chitin results are mean data from other experiments.

[4] = reading on chitinase probably aberrant.

[5] = in 4 other experiments, chitin cultures of HD-539 averaged 71.9% mortality and averaged over 1 in chitinase titre.

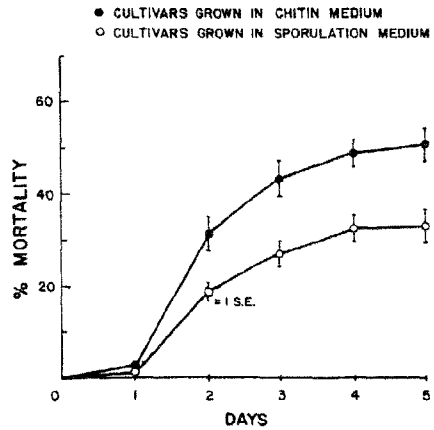

Fig. 4. Differential mortality to SBW achieved by cultures grown in chitin medium and sporulation medium.

This graph is a composite drawn from the data in Table 2. Cultivars HD-1, 198 and 500 have been omitted because of their low chitinase production, below 0.6 µmol NAG. The differences seen in HD-1 on Table 2 are extreme: see figs 1 and 3 for more usual values.

A further confirmation of the potential of chitinase active strains is shown in Fig. 5. In this instance, *B.t.* var. *galleriae* HD-287 (crystal type k-1) is consistently more lethal when induced for chitinase than commercial strain HD-1 or when grown in conventional sporulation medium. Again, as shown in Fig. 4, the significance of these results lies not only in the ultimate percentage kill achieved, but in the more rapid inception of kill and the minimizing of foliar damage this implies.

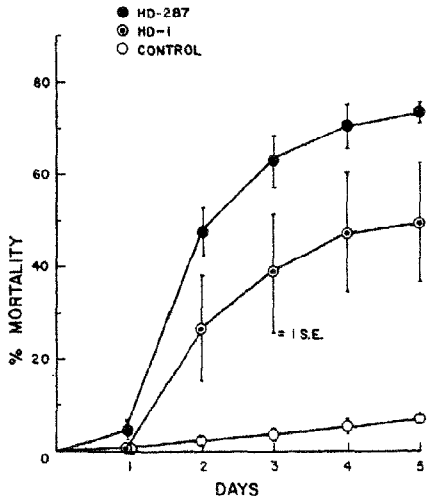

Fig. 5. Mortality to spruce budworm induced by *B.t.* HD-287, var. *galleriae* (crystal type k-1), and strain HD-1.

Composite data for 9 experiments. Although n, the initial number of larvae in each experiment

treatment group, varied from experiment to experiment (range = 42 to 70) data from separate experiments were averaged equally; it was felt that variation in mortality was due to larval batch tested and not random independent factors.

Data for HD-1 was drawn from only 4 experiments. In other cases, final mortality fell below 20% and was discarded as unrepresentative (see Fig. 3 for other HD-1 data).

This extreme variability in HD-1 lethality, as reflected in the S.E. shown here, is a common problem with this strain (N. Dubois, pers. comm.).

For 3 experiments (run as part of those above) *B.t.* HD-287 was tested as grown in sporulation medium. On day 5, mortality was $\bar{x}$ = 50.6% ± 6.84 (= S.E.).

Discussion

In contrast to the findings of Smirnoff and Valero (1977), the capacity for chitinase production would appear to be widespread and not confined to a varietal type. Within each varietal type there is, to be sure, a wide range of chitinolytic activity. For the *kurstaki* variety on which we report, the degree of enhanced lethality appears directly related to the titre of active chitinase. Certainly these results invite investigation of the relationship between chitinase and lethality within other varietal groups.

Although the phenomenon of chitinase enhanced lethality by *B.t.* to SBW seems unequivocal from the foregoing, further work is clearly required to elucidate the precise, operative mechanism. On the basis of the work reported by Dubois (1977), one may infer that there is an erosion of the peritrophic membrane. This facilitates entrance of spores as well as adventitious microflora into the larval haemocoel resulting in a broader and more diffuse disruption of various physiological and metabolic processes. Death occurs more rapidly in a population rendered more susceptible by these plural effects. A number of issues, however, remain speculative at this time. There is a discomfiting variability in the chitinase yield generated from the same culture grown under presumably identical conditions. This might suggest the presence of a catabolite repressor analagous to the cellulase system and a feedback mechanism that may inhibit the sustained production of chitinase. Additionally, it should be mentioned that the production of chitinase and the dissolution of the parasporal crystal and subsequent release of the toxic fraction represent antithetic pH demands: the pH optimum for chitinase lies at 5.5 to 5.8; for crystal dissolution above about 8. It is therefore remarkable that sites are present in the insect gut where both these agents may express themselves. However, there does appear to be a marked potential for chitinase activity at the peritrophic membrane of the Douglas fir tussock moth, *Orgyia pseudotsugata*. Studies by Brandt *et al* (1978) report that both chitinase and protease degraded the peritrophic membrane, releasing products of hydrolysis and effecting structural changes in the membrane. Although work in our laboratory showed that many strains of *B.t.* such as HD-287 do produce proteases, no clear-cut relationship between protease titres and mortality could be demonstrated.

A final question around which there has been much speculation has been our sustained inability to achieve any consistent enhancement of *B.t.* virulence with commercial chitinase. It might have been presumed that the lytic effect generated by this enzyme could have provided an additive measure of lethality in the same way provided by the chitinase induced in the chitinase active *B.t.* strains. Two explanations offer themselves for this failure: 1. the chitinase must be of a specific character capable of catalyzing the depolymerization of the chitin in the SBW gut; or 2. that the enzyme is operative only when membrane bound and responds to the presence of an additional factor provided by this linkage. The role of vegetative cells, always present in small numbers in treatment aliquots is also unclear. Though the event of increased lethality as a consequence of the presence of microbial chitinase seems certain enough, the precise details of its nature await further clarification.

Acknowledgment

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THE BIOCHEMISTRY OF THE PROTEIN CRYSTAL TOXIN OF *BACILLUS THURINGIENSIS*

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The crystal consists of dimeric protein subunits. The monomer peptide chains are held together in the subunit and the subunit in the crystal by disulfide and non-covalent bonds. The monomer peptide has a molecular weight of about 130 kdaltons which, in the presence of proteases, is hydrolyzed to a protease-resistant-protein of 65 kda that is toxic both to larvae by injection and to tissue culture cells and thus is the active toxin. The gene for this protein is on a plasmid which greatly simplifies the work of the genetic engineers. The gene has been partially sequenced and the secondary structure of this part has been predicted but not demonstrated.

Bacillus thuringiensis (Bt) is divisible into 20 varieties or serovars based on serological and biochemical tests on the vegetative cell. The protein that is responsible for most of the toxicity, and especially for the insecticidal activity of commercial preparations, is produced as a bipyramidal crystal during sporulation of the Bacillus (Fig. 1). The surface pattern observed is due to the regular positioning of rod-shaped molecules with dimensions of about 9 X 13 nm and a calculated molecular weight of 230,000 daltons (1 dalton is the mass of a hydrogen atom) (Holmes and Monroe, 1965). The shapes of the crystals vary from almost cuboidal to slender needle shapes depending on the serovar. Isolates can often be recognized by crystal shape alone.

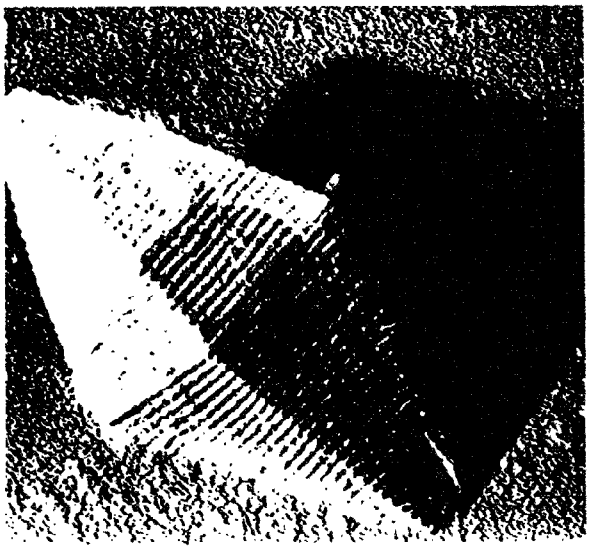

Figure 1. Electron micrograph of a parasporal crystal of *Bacillus thuringiensis* (Courtesy of C. Hannay)

Serology of the crystal broadly reflects the groupings referred to above but with considerable overlap (Krywienczyk et al., 1978, 1981). In this review I shall refer to serovars by their full names but crystal types by their first 3 letters, or in the case of *kurstaki* crystal types to K-1 and K-73, as suggested by Krywienczyk et al. (1978).

Many of the problems of dissolving and characterizing the crystal that have historically plagued the study of Bt crystal toxin have been overcome in the past 5-8 years. Crystals are now readily separated from spores (Milne et al., 1977, Sharpe et al., 1975) and can be freed of the proteases that are adsorbed to the crystal which have, in the past, caused confusion about the molecular weight of the constituent peptide chains (Chestukhina et al., 1978; Nickerson and Swanson, 1981).

The techniques for dissolution of the crystal are also now reasonably defined. The crystal is held together principally by intermolecular disulfide bonds of which there are as many per peptide chain as in wool keratin (Huber, 1982) and by noncovalent interactions such as salt bridges and hydrogen bonds. Highly alkaline conditions (pH 11-13) dissolve the crystal by alkaline hydrolysis of disulfide bonds but a considerable loss of toxicity proportional to the length of time in alkali occurs (Trumpi, 1976). Under less alkaline conditions (pH 9.5-11) dissolution will occur in the presence of disulfide reducing agents such as dithiothreitol (Dtt) or mercaptoethanol and toxicity is preserved. Under near neutral pH conditions denaturants such as ionic detergents, guanidine hydrochloride, urea, or chaotropic salts are required along with disulfide reducing agents and in most cases toxicity is totally destroyed. Huber (1982), however, describes conditions for the dissolution of crystals in guanidine hydrochloride in which toxicity is retained. The toxicity is also destroyed by heat as well as by denaturants, leading to the conclusion that secondary structure (helical or beta organization) or tertiary structure (packing of the helical or beta structural elements) of the crystal protein is required.

A protein with a molecular weight of about 230 kilodaltons (Kda) is obtained when the crystal is dissolved under carefully controlled conditions without reducing agents (Huber 1982). This is in good agreement with the molecular

weight calculated from the x-ray data (see above). In the presence of reducing agents a single molecule with a molecular weight of 130 kda is observed indicating that the unreduced molecule is probably a dimer. Although this has generally been the accepted interpretation, Huber (1982) was the first to present data clearly supporting this interpretation. The discrepancy in the molecular weights observed in the monomer and the dimer remains unresolved where molecular weights are determined by electrophoresis in SDS. Ultracentrifugation (Huber, 1982; Glatron et al., 1972) gave lower values for the monomer but the dimer molecular weight is exactly 2X that of the monomer. For the sake of clarity let us accept the 130 kda molecular weight in the balance of this discussion.

The amino acid compositions vary from serovar to serovar (Table I). The greatest variability is seen in the charged amino acids and glycine. The dicarboxylic amino acids aspartate and glutamate are present in unusually high amounts in all serovars, about half as their amides glutamine and asparagine (Glatron et al., 1972; Fast, 1981).

Table I. Amino acid compositions and minimal molecular weights

AA/type	*thu* (8)[a]	*fin* (2)[b]	*sot* (3)[c]	*ale* (3)[d]	*tol* (2)[e]	*gal* (2)[f]	*ken* (2)[g]	*and* (1)[h]	*kur-1* (1)[i]
Asp	14	14	13	14	15	16	15	12	14
Thr	8	8	7	8	9	9	8	7	7
Ser	8	9	8	9	12	11	9	8	9
Glu	13	12	15	14	12	13	14	15	13
Pro	5	6	8	6	6	7	5	7	4
Gly	10	15	9	12	16	11	9	10	8
Ala	8	13	7	8	11	10	8	8	6
Val	8	9	6	9	8	8	8	7	8
Cys	1	2	1	2	1	2	2	2	2
Met	1	1	1	1	1	1	1	1	1
Ile	7	8	8	8	8	8	8	7	6
Leu	11	11	13	11	10	13	13	11	9
Tyr	5	4	5	5	3	5	5	4	5
Phe	5	4	6	5	4	5	6	5	4
Lys	4	6	4	4	3	4	5	4	3
His	2	3	2	2	2	3	2	3	2
Arg	6	3	8	7	5	7	7	6	9
Try	1	1	1	1	ND	1	ND	1	1
min. mol. wt.	12820	13231	13231	11895	12796	14291	13042	13282	12400

Considerable controversy exists as to whether carbohydrate is bound to the crystal protein and is essential to toxicity. Several workers have reported concentrations of carbohydrate in the crystal ranging from 0.5% to 12% in crystals (Holmes and Monroe, 1965; Bateson and Stainsby, 1970; Bulla et al., 1977). Other investigators working with related isolates failed to detect carbohydrate in rigorously purified crystals (Lecadet, 1965; Huber et al., 1981). Treatment with carbohydrases to remove sugars or with immobilized concanavalin A to remove glycoproteins containing glucose and mannose (the sugars reported to be present) from solution failed to materially reduce toxicity (Fast, 1981). Amino sugars or sialic acids, often associated with glycoproteins, were not detected in the crystal (Fast, 1981; Bulla et al., 1977; Bateson and Stainsby, 1970). Whether the carbohydrates are covalently bound to the peptide chain or only adsorbed to it, they do not appear to participate in toxicity to insects. There is no lipid or nucleic acid associated with the crystal.

The monomeric 130 kda peptide chain is a protoxin that must be activated by proteolytic hydrolysis (Lecadet and Martouret, 1965; and many others). The activated product is generally a protease resistant protein (PRP) with a molecular weight in the range of 50-70 kda although smaller toxic fragments have frequently been reported but generally not well documented (Fast and Angus, 1970; Pendleton, 1973; Faust et al., 1974). Lecadet and Dedonder, (1967) isolated and partially characterized 2 small toxic peptides from 350mg of starting proteolytic digest. Work on this scale has not been repeated.

The amino acid composition of PRP is significantly different from that of the crystal. Amino acid compositions of PRP from 3 serovars are shown in Table II (Chestukhina et al., 1982). A major reduction in the amount of lysine and an increase in hydrophobicity in PRP over that of the protoxin is observed. In the light of Beegle's finding (Abstract, Ithaca meeting of the SIP, 1983) that the toxicity spectra of activated PRPs is the same as the spectra of the crystal, the differences in amino acid composition seen in the table are probably significant.

There are significant sequence differences in PRP of these serovars as well. SDS gels of cyanogen bromide cleavage products of PRP from 3 different serovars have been published (Chestukhina et al., 1982) (Fig.2). Cyanogen bromide cleaves peptides only at methionine. Because the products of cleavage have different molecular weights in the three serovars shown, sequence differences must exist and again are likely to affect toxicity spectra.

Table II. Comparison of the amino acid composition of the PRP of 3 serovars of *Bacillus thuringiensis* (from Chestukhina 1982).

Amino Acid	*galleriae* 65 kda (mol%)	*tolworthi* 65 kda (mol%)	*alesti* 70 kda (mol%)
1/2 cys	1.0	1.0	1.0
asp	11.7	11.7	11.1
thr	6.6	8.0	6.3
ser	11.8	9.8	9.4
glu	11.2	10.5	11.0
pro	5.1	4.8	5.7
gly	7.6	7.5	8.0
ala	6.2	5.7	5.7
val	4.9	4.4	7.3
ile	5.5	6.8	6.3
leu	8.5	9.3	9.8
tyr	3.6	3.9	3.1
phe	4.2	5.5	6.0
his	1.7	1.4	2.1
lys	1.3	1.0	1.0
arg	6.2	6.9	5.0
met	0.7	1.0	0.7
trp	0.7	0.6	0.6

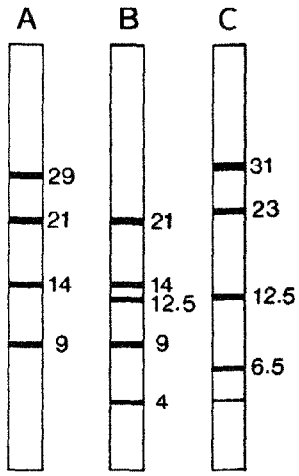

Figure 2. SDS-Page patterns of PRP of serovars (A) *tolworthi* (B) *alesti* and (C) *galleriae* after treatment with cyanogen bromide. Numbers are molecular weight in kda.

Recently the gene for the 130 kdalton peptide has been cloned and expressed in both *E. coli* and *Bacillus subtilus* (Schnepf and Whiteley, 1981; Klier et al., 1982). The expressed protein in both these hosts has been shown to be toxic to lepidoptera. The sequence of the first 333 residues of this protein from HD-1 Dipel have been inferred from the nucleotide sequence of the gene (Wong et al., 1983). In fig. 3 I have reproduced that sequence and have marked the hydrophobic and charged amino acids.

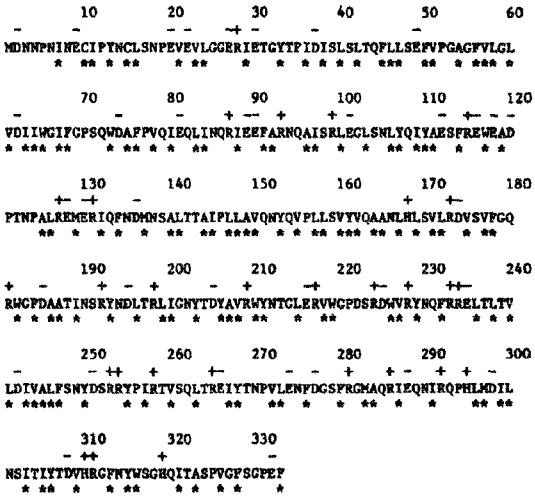

Figure 3. Amino acid sequence of the N-terminal 333 amino acids of the HD-1 Dipel crystal protein. Amino acids are designated by the single letter code. * designates hydrophobic amino acids. Charged amino acids are designated by + or -.

Techniques are available for predicting the secondary structure of a protein from sequence information (Chou and Fasman, 1978). I have applied these techniques to the crystal protein sequence and show the probable position of turns and of alpha helical and beta structures (Fig.4).

Because many of the turns are so tight, only 4-8 residues, it is likely that at least some of the beta structure is antiparallel. While such predictions of structure are of limited accuracy there are good indications that turns and secondary structure are highly conserved, even more highly than sequence, in homologous proteins. The availability of additional sequences, which are presently being derived, should greatly assist in identifying the structure(s) responsible for toxicity.

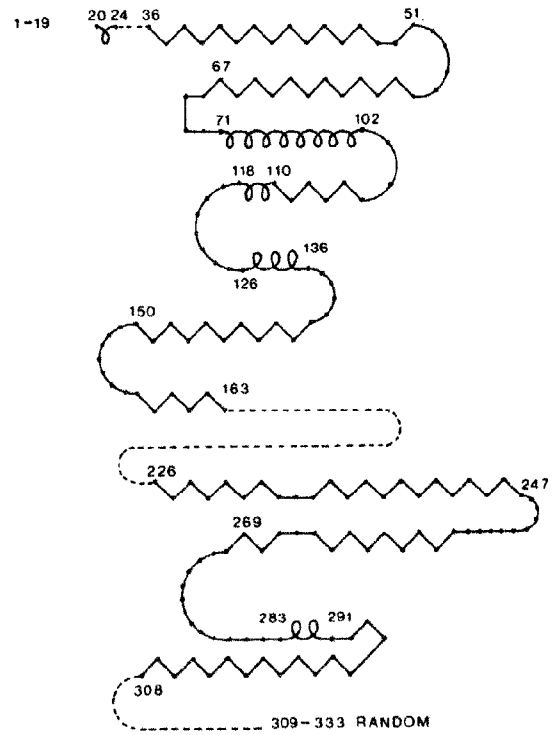

Figure 4. Secondary structure of the N-terminal 333 residues of HD-1 dipel crystal protein as predicted by the method of Chou and Fasman (1978). Zigzag indicates beta structure, coils indicate helices, and dashed lines indicate random structures. Chain reversal indicates predicted turns.

Utilizing a program developed to detect repetition of sequences, I have found no repeating sequences in these first 333 residues. This and the observation that the gene product has a molecular weight of 130 kda indicates that the subunit cannot be composed of aggregates of small peptides as previously suggested (Fast and Martin, 1980).

So what? The subtitle of this session is "Where do we go from here?" What I have reviewed in this paper is the barest of beginnings, but it is a beginning. We do not know what the structure of PRP is, nor even where it comes from in the sequence of the monomer. We do not know what the active structures are in PRP. We do not know how different isolates within a serotype with different toxicity spectra differ from each other, much less how toxins from different serotypes differ. We do not know why the toxicity of a single isolate varies so much between target insects; it may be because of differences in their proteases or their toxin receptors if there are such (I think there are!). In some cases we have hints, but nothing solid. When we know the answers to these questions then I predict that molecular biologists will be able to tailormake a crystal protein for each pest or crop, and Bt or related products could dominate the insecticide market for defoliating insects. The potential is enormous.

If Bt is to reach this potential it will be necessary to recruit and support much more fundamental work on crystal toxin. Because of its economic interest, its potential for much more benign pest control, and its potential for genetic manipulation interest is growing but support is meager. We need to convince our colleagues doing fundamental work on protein structure and molecular genetics that Bt is a good model for study. More particularly we need to convince the funding agencies of the value of such work so that it is supported. Significant growth in support of such fundamental studies is required to realize the potential of this organism for environmentally benign insect control.

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SELECTION OF ACTIVE STRAINS OF THE GYPSY MOTH NUCLEARPOLYHEDROSIS VIRUS

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The gypsy moth Lymantria dispar (linnaeus) has grown in economic importance as an insect pest over the past 75 years. From a localized infestation of a small geographical area of New England, the gypsy moth has spread to such an extent that is now found over much of the United States. Control measures are varied, but effective biological control is needed to control pest populations, especially in such sensitive areas as parks, residential areas, and municipal watersheds.

A nuclear polyhedrosis virus (NPV) of the gypsy moth has been registered for control purposes; however, efficacy is variable and borderline in control situations. Natural epizootics of the virus are extremely effective in destroying natural populations. The disparity in efficacy between natural virus outbreaks and artificial application of laboratory produced gypsy moth virus presents an obvious research problem for the selection of a more 'potent' strain of the virus. The approaches that we have taken involve: (1) in vitro strain selection; (2) evaluation of naturally occurring geographical isolates; (3) in vivo passage through L. dispar; and (4) in vitro and in vivo mutagenesis.

In vitro strain selection

Cell lines of ovarian origin were obtained by Dr. R. H. Goodwin of the Insect Pathology Laboratory (BARC-USDA). Using the IPLB-652Y cell line, a plaque assay was developed (Fig. 1), which not only allows for quantification of non-occuled virus but also allows for studying the replication and genetics of LdMNPV. Using the plaque assay, 13 plaque isolates were made and their virulence compared to the LDP-67 strain (U.S. Forest Service, Hamden, CT) (Table 1). No one isolate was more virulent than the standard (LDP-67); however, a few isolates (5-7D,14-4C) were close in activity. There was a wide range in activity with three samples giving no activity greater than 4250 times an LD_{50} for the LDP-67 isolate.

Isolation of DNA from the plaque isolates listed in Table 1 and digestion with restriction endonucleases (REN) showed that the twelve isolates could be separated into a least five groups based upon the profile of REN digests. Differences in the digestion profiles can be observed in Figure 2, which shows an EcoRI digest of the plaque isolates. Although many bands are common to the plaque isolates, obvious differences do exist. A summation of differences found among the twelve isolates using the restriction enzymes SAL I, BGL II, BAM HI, HInD III, and EcoRI are presented in Table 2. Utilization of the five enzymes tests produced five different groupings of isolates with common REN patterns. Use of additional restriction enzymes could potentially show further dissimilarities.

IPLB-652 cells Initial Density 10^5/cells/cm^2
↓
2 hr attachment remove medium
↓
Virus Dilutions made in KC52B medium
0.8 ml/25cm^2 flask
↓
1 hr incubation - gentle rocking
↓
Remove virus innoculum
↓
Add 3.0 ml KC52B medium containing 0.5% agarose
↓
Incubate 7 days @ 28°C
↓
Stain cell sheet with 0.01% neutral red
↓
Count plaques under an indirect light

Figure 1.--A plaque assay for Lymantria dispar MNPV using the IPLB-652 cell line.

Thus, comparison of the five groupings of plaque isolates having common patterns (Table 2) and virulence (Table 1) show that genotypic variants of L. dispar NPV do display differential virulence. These studies have shown that there is a diversity of genomic material within isolate LDP-67.

Naturally-occurring geographical NPV isolates

Differences in biological activity can be detected among geographical isolates of the same NPV species (Ossowski 1960, Smirnoff 1961, Chauthani et al. 1968, Shapiro and Ignoffo 1970), including the gypsy moth NPV (Magnoler 1970, Rollinson and Lewis 1973, Vasiljevic and Injac 1973). Our objective was to identify the most active isolates by comparing NPV isolates from North America, Europe, and Asia (Shapiro et al. 1984). Eighteen isolates of L. dispar NPV were harvested from both laboratory and field-collected larvae, and were compared to a Connecticut standard (LDP-67) is standardized bio-assays (Shapiro and Bell 1981). LD_{50}s varied from 1.7×10^3 polyhedral inclusion bodies (PIB)ml (=0.34×10^0 PIB/mm^2 of diet surface) to 5000×10^3 PIB/ml (=1×10^3 PIB/mm^2), a difference of about 2940-fold (Table 3). The most active NPV isolates generally originated from North America, the least active isolate originated from Asia (Japan). In the highest activity group, one

Table I. Bioassay of *L. dispar* Multiple Embedded Nuclear Polyhedrosis Virus Plaque Isolates

Plaque Isolate	LD_{50} (PIB/ml X 10^4)	Relative Efficiency (Percent)	Plaque Isolate	LD_{50} (PIB/ml X 10^4)	Relative Efficiency (Percent)
LDP-67	1.77	100.0			
5-7D	5.83	30.3	9-12	66.00	2.6
14-4c	6.57	26.9	7-109	125.35	1.4
12-4b	7.97	22.2	6-9a	7500	.02
11-13c	8.60	20.5	14-10b	7500	.02
13-7a	9.00	19.7	15-10c	7500	.02
10-13a	10.47	16.9			
16-13a	14.33	12.3			
8-12	15.00	11.8			

Figure 2

ECO RI DIGEST OF PLAQUE PURIFIED ISOLATES
OF THE LDP-67 STRAIN OF MLd NPV

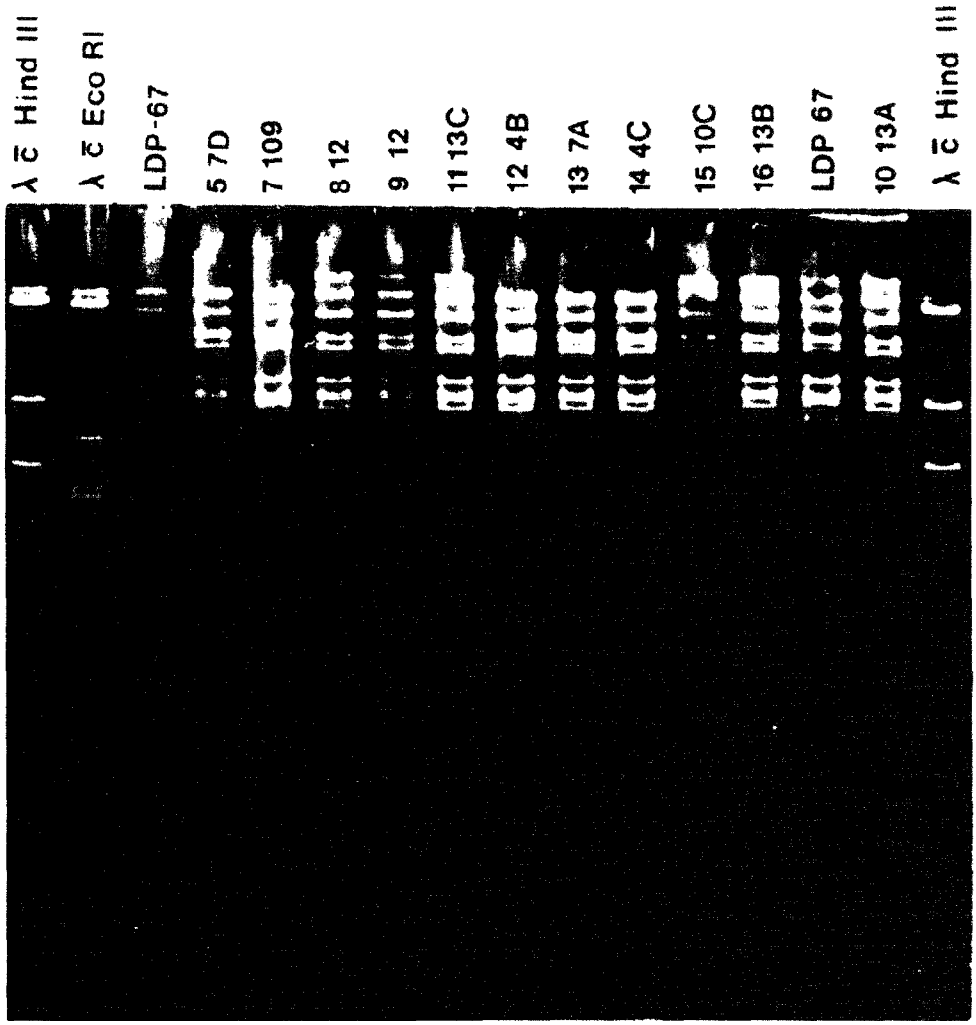


Table 2. LdMNPV Plaque Isolates: Summary of Grouping of Isolates According to REN Profiles

		Sal I	Bgl II	Bam HI	Hind III	Eco RI
LDP-67	(WT)	X	Y	Z	A	A
1D	5-7d	X	Y	Z	B	A
1F	7-109	X	Y	Z	A	A
1g	8-12	X	Y	Z	A	B
1h	9-12	X	Y	Z	-	B
1j	11-13c	X	Y	Z	A	B
1k	12-4b	X	Y	Z	B	A
1l	13-7a	X	Y	Z	B	A
1N	14-4c	X	Y	Z	B	A
1o	15-10c	X	Y	Z	-	B
1p	16-13b	X	Y	Z	A	B
1q	6-9a	X	Y	Z	A	C
1i	10-13a	X	Y	Z	C	B

Plaque Isolates with Common REN Patterns

aa	ab	ba	ac	cb
LDP-67	1g	1d	1q	2i
1f	1j	1k		
	1p	1l		
		1n		

Table 3.--Comparatige biological activities of L. dispar NPV isolates

NPV ISOLATE TESTED	LC_{50} (95% CL) PIB/ml x 10^3
QUEBEC 1	1.7(1.1-2.6)
ABINGTON, MASS.	1.8(1.0-3.3)
QUEBEC 2	2.4(0.8-6.4)
DIGHTON, MASS.	3.0(2.4-5.8)
BALD EAGLE, PA.	3.7(2.9-4.7)
LDP-67(CT STD)	4.8(4.0-5.8)
HOUGHTON POND, MASS.	5.0(3.3-7.6)
NEW HAMPSHIRE	6.8(5.3-8.8)
MIDDLEPORT, PA.	8.0(6.3-10.6)
ROMANIA	13.1(10.6-16.2)
SANDWICH, MASS.	21.0(14.9-29.6)
BRIDGEWATER, MASS.	21.4(13.4-33.7)
ORANGE, MASS.	27.3(17.3-43.5)
SERBIA-YUGOSLAVIA	28.8(23.3-35.6)
USSR	31.3(26.6-36.9)
SLOVANIA-YUGOSLAVIA	57.1(22.3-133.8)
DALMATIA-YUGOSLAVIA	59.3(48.7-72.2)
SPAIN	78.8
JAPAN	Greater than 5000

(From Shapiro et al. 1984).

isolate came from the United States (Abington, MA), and two came from Canada (Quebec 1 and 2). In the next activity level, one isolate came from Dighton, MA and one came from Bald Eagle, PA. Since these five isolates were significantly more active than the CT standard, they might be potential candidates for use as microbial control agents. The European isolates were in the sixth to eleventh activity levels. At a concentration of 5000x10^3 PIB/ml, the Japanese NPV caused only 16% larval mortality, whereas lower concentrations were not lethal (Shapiro et al. 1984).

Virus was purified from pooled cadavers from these experiments and the respectove viral genomes were purified and digested with a variety of restriction enzymes. As in the previous instance, there is a variation in bands of restriction fragments among the various geographical isolates, showing a great heterogeneity both intra-strain and inter-strain among L. dispar NPVs. Using a nick translated probe of the genome of LDP-67 to hybridize against a Southern blot of Figure 3, it was found that the greatest homology between LDP-67 and foreign isolates occurred among North American isolates, intermediate homology occurred with European isolates, and no homology occurred with the Asian isolate. Thus, reports stating the gypsy moth is an evolving species whose North American members now have different physiology, behavior, etc. could also pertain to the gypsy moth NPVs.

In vivo passage through Lymantria dispar

Eight virus isolates (USA-LDP-67; ROMANIA, SPAIN, USSR, YUGOSLAVIA-DALMATIA, SERBIA, SLOVENIA) were selected serial passage through second-stage L. dispar larvae. While the activity of the American isolate did not change during the four passages, activities of all other isolates increased significantly during the second passage (Table 4). Moreover, the activity of the Japanese isolate increased ca. 1000-fold during the passages. In general, activities became stabilized from the second to the fourth passage and were quite similar to that of LDP-67. The experiment was terminated after the fourth passage, since it was assumed, based upon the behavior of LDP-67, that further increase in activity would be minimal. While serial passage resulted in greater activity (see also Veber 1972, Shapiro and Ignoffo 1970), these isolates were still significantly less ($P<.05$) than the most active North American isolates (Quebec 1, Quebec 2, and Abington, MA) (Shapiro et al. 1984).

Since the NPV isolate from Abington, MA had greater biological activity ($P<.05$) than the LDP-67 standard, a concentrated effort was made to characterize this isolate, in terms of activity. The Abington isolate was serially passed, along with the LDP-67 isolate, at LD_{50} values. As was seen previously, serial passage of LDP-67 did not result in any change in activity, which indicated the stability of the isolate. Surprisingly, the activity of AB decreased during early passage through second-stage L. dispa larvae, but the NPV was still more active than the LDP-67 standard (Fig. 4). During subsequent passage of AB, however, activity increased to the original value, and remained stable during the last three passages. A sample of AB was then forwarded to the U.S. Forest Service Laboratory, Hamden, CT for testing. The high activity of this isolate was confirmed (W. Rollinson, personal communication) and large amounts of this isolate will be produced for subsequent field-testing.

Mutagenesis

In addition to identifying and monitoring natural genetic variation, artificially induced genetic variation is also being investigated. Over ten years ago, Reichelderfer and Benton (1973) reported that four treatments of an NPV (Spodoptera frugiperda) with 3-methylcholanthrene resulted in a nine-fold increase in virulence. A significant decrease in the Lt_{50} was observed during bioassays comparing treated and untreated virus. Moreover, similar results were observed with 1,2,3,4-dibenzanthracene, 5-bromodeoxyuridine,

Figure 3

GEOGRAPHICAL ISOLATES OF
LYMANTRIA DISPAR NUCLEAR POLYHEDROSIS VIRUS
DIGESTED WITH BAM HI

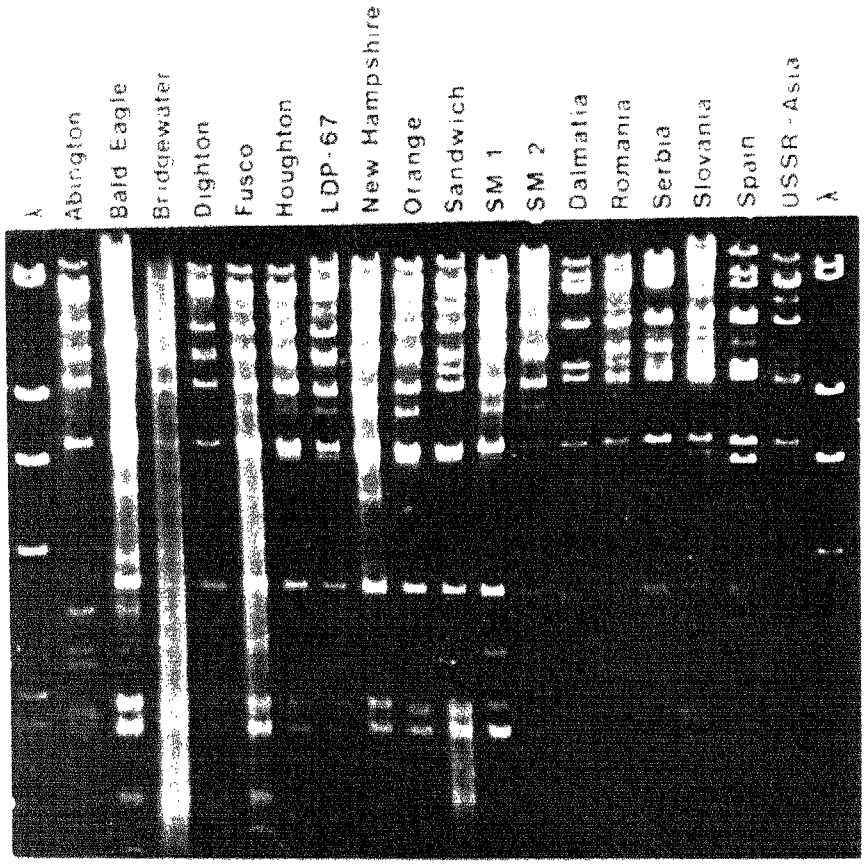
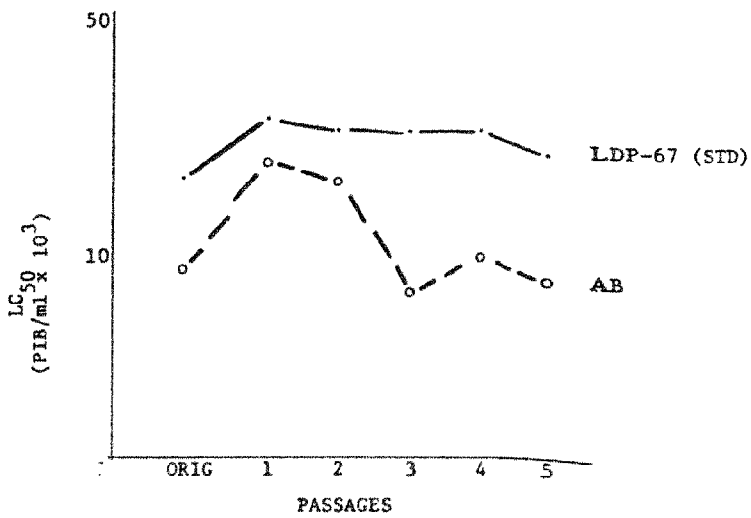


Table 4.- BIOLOGICAL ACTIVITIES OF NPV ISOLATES SERIALLY PASSED THROUGH *L. dispar* LARVAE

NPV ISOLATES TESTED	LC_{50} (95%CL) (PIB/ml x 10^3) AT PASSAGE 1	4
LDP-67 (STD)	6.26(3.98-9.58)	6.06(4.61-7/97)
SPAIN	94.6(41.2-200)	5.60(3.36-9.16)
ROMANIA	14.1(9.05-21.7)	5.82(2.42-13.2)
DALMATIA-YUGO	59.6(29.6-117.2)	6.10(4.30-8.60)
SLOVANIA-YUGO	57.6(25.9-120)	8.00(6.09-10.5)
SERBIA-YUGO	33.6(17.3-63.2)	9.95(6.35-15.4)
USSR	29.8(18.8-44.8)	5.87(4.47-7.67)
JAPAN	5000+	9.53(5.98-15.4)

(From Shapiro et al. 1984).

Fig. 4 SERIAL PASSAGE OF ABINGTON, MA NPV ISOLATE



N.B. ORIGINAL AB NPV ISOLATE WAS ALSO BIOASSAYED AT EACH PASSAGE, AND THE LC_{50} VALUES WERE 8.0, 8.3, 7.2, 9.6, AND 9.1 (MEAN VALUE = 8.44 x 10^2 PIB/ML).

and 5-iododeoxyuridine. In the case of the alfalfa looper (Autographa californica) NPV, however, no significant effects were noted with 5-bromodeoxyuridine or n-methyl-n'-nitro-n-nitrosoguanidine (McClintock 1980) in the cabbage looper, bollworm, tobacco budworm, or fall armyworm. A mutant of the alfalfa looper NPV (=HOB) was obtained in vitro after treatment with 2-aminopurine, which possessed increased virulence (Wood et al. 1981). In our investigations, in vitro experiments are now in progress uisng the mutagen 2-aminopurine. These experiments, along with co-infection studies with homologous and heterologous virions and genomes (co-transfection) to generate non-genetically engineered recombinant viruses should produce genomes having artificially or non-naturally occurring genetic variation. In addition, in vivo studies are now in progress using such mutagens as bromodeoxyuridine, bromodeoxycytidine, 2-sminopurine, ethyl methyl sulfonate, hydroxylamine, acriflavin, proflavin, and hydrogen peroxide, among others. More virulent strains are hoped for from these studies.

The empirical search for an array of genes which will produce a more virulent strain of LdMNPV has been or is now undergoing all classical and standard methods used to produce virulent strains of virus both in animal virology and in insect virology. Failure to produce such a strain does not imply that these methods be abandoned; however, the use of new strategies developed from the Biology revolution of the last decade should be considered. If lessons are to be learned from other viruses which have been manipulated by these methods, then a large amount of fundamental work such as described is necessary to intelligently modify current Lymantria dispa nuclear polyhedrosis viruses.

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PATHWAYS OF NUCLEOPOLYHEDROSIS VIRUS INFECTION IN THE GYPSY MOTH, LYMANTRIA DISPAR[1]

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Gypsy moth nucleopolyhedrosis virus polyhedral inclusion bodies dissolve slowly in host digestive fluids, in vitro. Infectious viral material is in the hemocoel two hours after ingestion of inclusion bodies. Hemocytes produce and release nucleocapsids throughout the course of infection, but in the fat body, nearly all nucleocapsids are enveloped and occluded.

Although the virus disease of the gypsy moth was reported as early as 1911 (Reiff 1911), very little attention has been given to the mode of action of the causative agent. This report deals with some of the steps in the pathway of nucleopolyhedrosis virus (NPV) invasion and infection in the gypsy moth.

First of all, we know that gypsy moth larvae can become virus-infected by ingesting polyhedral inclusion bodies (PIBs), but we don't know a great deal about what happens to PIBs once they've been ingested. A recent study of PIB dissolution in gypsy moth digestive fluid indicates that the dissolution process may be rather slow; significant dissolution occurred only after two hours incubation (Shields 1984a). We know that factors such as pH, ionic strength and enzymatic activity can be important in PIB degradation (Nordin and Maddox 1971, Paschke and Summers 1975, Wood 1980), and we know that some conditions, such as starvation, can influence these factors (Kawarabata et al. 1980, Pritchett et al. 1982). But we need to know more about the environment of the gut lumen; about how that environment fluctuates; and especially about those conditions that affect the ability of gypsy moth digestive fluids to dissolve PIBs.

With the dissolution of the PIB matrix protein, enveloped nucleocapsids are released in the gut lumen. Many of the nucleocapsids are carried with the food through the alimentary system and are defecated. But some nucleocapsids apparently find their way into the hemocoel. Exactly how nucleocapsids penetrate the peritrophic membrane and pass through the gut epithelium into the hemocoel has not been determined. However, hemolymph bioassays indicate that infectious viral material is in the hemocoel as early as two hours after ingestion of PIBs (Shields 1984a). Since virus is in the hemocoel so quickly, direct passage of inoculum nucleocapsids from the gut lumen to the hemocoel seems likely. This phenomenon has been reported for Autographa californica NPV in Trichoplusia ni (Granados and Lawler 1981).

For a systemic infection to proceed, once in the hemocoel, nucleocapsids must escape host defense mechanisms, enter susceptible cells, replicate, and progeny nucleocapsids must be released. Of the five types of hemocytes in gypsy moth larvae, three are susceptible to NPV infection - prohemocytes, plasmatocytes and especially coagulocytes. Coagulocytes are the first hemocytes to replicate virus and eventually are selectively depleted from the circulating hemocyte population. Granulocytes and spherulocytes do not seem to be susceptible to NPV.

Nucleocapsids appear to enter gypsy moth hemocytes by two routes. One is by fusion of the viral envelope with the hemocyte plasma membrane, releasing the nucleocapsid into the cytoplasm. Enveloped nucleocapsids also enter hemocytes by viropexis, an engulfment process. The fate of enveloped nucleocapsids that are engulfed is not certain. The vesicles containing the nucleocapsids may fuse with lysosomes and the nucleocapsids may be destroyed (Adams et al. 1977). However, it is possible that the virus might utilize this process for entry and subsequent release into the cytoplasm.

Nonenveloped nucleocapsids in the cytoplasm frequently are in close alignment with microtubules. This association has been observed in other NPV-host systems (Granados 1978, Granados and Lawler 1981) and Granados (1978) has suggested that microtubules might be involved in the movement of nucleocapsids from the cell surface to the nucleus. In some gypsy moth hemocytes, arrays of nucleocapsids and microtubules appear to extend from the cytoplasm into the nucleus (Shields 1984a, 1984b).

Virus morphogenesis in gypsy moth hemocytes is similar to that reported for NPVs in other species (Paschke and Summers 1975, Granados 1980). Nucleocapsids that acquire envelopes within the nucleus are occluded by polymerizing protein, forming PIBs. Increasing numbers of PIBs are seen in hemocytes during the course of the disease, but hemocytes do not produce as many PIBs as do the cells of the fat body (Shields 1984a).

Infection is seen in the fat body much later than in hemocytes, but eventually all of the cells of the fat body seem to be virus-infected. Nearly all of the nucleocapsids produced in fat body cells are enveloped in the nucleus and occluded. Very large numbers of PIBs are produced and few, if any, nucleocapsids are released from fat body cells.

In contrast, hemocytes replicate and release nucleocapsids into the hemocoel throughout the course of the disease. Nonenveloped nucleocapsids bud from hemocyte nuclei in long tubules of nuclear envelope. They may be released into the hemocoel by fusion of the

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[1]This is part of a dissertation prepared as part of the requirements for the Ph.D. degree, presented by the University of Connecticut, Storrs.

plasma membrane and outer lamella of the tubules, as was suggested by Nappi and Hammill (1975). Or, they might first be released into the cell cytoplasm through breaks in the tubule lamellae. In any case, many nonenveloped nucleocapsids are in the cytoplasm of infected hemocytes, and many of these nucleocapsids bud through the plasma membrane into the hemocoel. In so doing, they acquire a peplomer-modified envelope derived from the hemocyte plasma membrane. Adams et al. (1977) suggested that enveloped nucleocapsids bearing peplomers are responsible for secondary infection. If so, hemocytes play an important role in intercellular transmission of virus.

Little is known about the effectiveness of gypsy moth defense mechanisms against NPV, or about how changes in host metabolism might affect susceptibility to infection. A recent study shows that gypsy moth larvae, parasitized five days previously by Blepharipa pratensis, are resistant to NPV.[2] Perhaps B. pratensis myiasis activates anti-viral substances in the hemolymph of the host.

More research is needed on all of the factors that might be involved in determining whether NPV will invade amd kill the gypsy moth. Such information is necessary for prediction of viral epizootics and also could be useful in the development of improved viral pesticides.

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[2]Unpublished data, on file, Center for Biological Control of Northeastern Forest Insects & Diseases, Hamden, Connecticut.

Enzyme Immunoassays for Detection of Gypsy Moth Nuclear Polyhedrosis Virus

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Abstract

Enzyme-linked immunosorbent assays (ELISA) were developed for detecting gypsy moth (*Lymantria dispar* L.) nuclear polyhedrosis virus (NPV). They were used to detect the presence of NPV in hemoplymph samples collected from infected larvae. The incorporation of hybridoma antibodies with these procedures would make them even more specific for gypsy moth NPV detection. A pilot study of evaluating NPV spray with ELISA is also presented here. Potentially, these immunochemical methods have important applications in an IPM approach toward the control of gypsy moth.

Introduction

Nuclear polyhedrosis virus of the gypsy moth has received considerable attention as a biological control agent because it is (1) selective for lymantriids, (2) environmentally safe, (3) endemic among natural populations, and (4) persistent in the field after its introduction into a population (Doane 1970, Podgewaite and Campbell 1971, Lewis and Etter 1978, and Lewis 1981). With the recent registration of the European sawfly virus (Neochek) by the Environmental Protection Agency, there are four insect viruses (gypsy moth, Douglas-fir tussock moth and *Heliothis* NPVs) that need to be examined carefully as how they could fit into integrated pest management schemes of these important pests.

Detection of the gypsy moth nuclear polyhedrosis virus

In order to evaluate effectively the potential of these NPVs for controlling insects, reliable bioassays and analytical methods are needed for both laboratory and field studies. Numerous bioassay procedures have been developed for assaying the presence and the virulence of gypsy moth NPV. One of the disadvantage of bioassay is the long incubation time, usually 10-15 days, required to show any disease symptoms. During this time, polyhedral inclusion bodies (PIBs) can be seen as refractile particles, 1-10 microns in diameter, in the larval hemolymph. Microscopic identification of PIBs could shorten the diagnostic time. However, this method is only reliable in cases of acute infection. When microscopic diagnosis is performed in early stages (viruses present mostly in the non-occluded form) and low levels of larval infection, there is the possible difficulty of distinguishing a few PIBs from other particulate materials in the hemolymph.

Dubois (1981) reported that refractometer measurements of the hemolymph could be used to detect the presence of virus. Quantitative prediction of virus mortality could be made with accuracy in third instar larvae. However, this method is rather stadium-specific, since accuracy cannot be assured with fourth and fifth instars. Larvae just prior and after ecdysis and sample size of the first two instars also present problems for analysis.

Enzyme-linked immunosorbent assay (ELISA), since its development by Engvall and Perlmann (1971, 1972), has received wide application in the biological sciences. This method involves the detection of an antigen with enzyme labelled antibodies. The antigen-antibody complex is then detected by the enzyme label which changes the color of added substrate. The results are presented in the form of a quantitative optical density measurement. The advantages of ELISA over other immunochemical methods include: (1) fast test results (3-4 hrs), (2) simple colorimetric end point, (3) specificity for a given antigen, and (4) extreme sensitivity and reliability. ELISA methods have already received wide applications in modern agricultural research, particularly in diagnostics and epidemiological studies of plant and animal diseases. In recent years, different ELISA methods have been employed for the detection of insect baculoviruses (Kelly et al. 1978, Crook and Payne 1980, Longworth and Carey 1980, Langridge et al. 1981, Evans et al. 1981, Volkman and Falcon 1982). ELISA will undoubtedly be used more extensively in insect control programs involving microbials in the future.

Development of ELISA for gypsy moth NPV detection

Preparation of Specific Antibodies

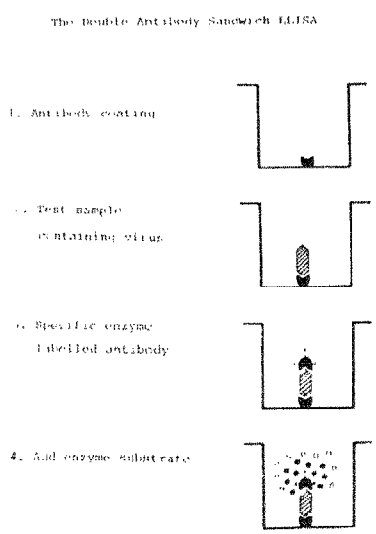
The most important requirement for an ELISA method is the development of a high titer antiserum against a purified preparation of antigen. Gypsy moth NPV PIBs were purified from moribund larvae following the procedure of Tompkins et al. (1981). Whole PIBs were dissociated in 0.1M Na_2CO_3 at pH 11 for 30 minutes, followed by pH adjustment to 7.3 with 2N HCl. New Zealand white rabbits and guinea pigs were immunized for antisera production. Antisera were only accepted if they detect 1 ug/ml of NPV at $1:10^5$

dilution. Immunoglobulin (IgGs) was purified by Protein A chromatography (Goding 1978) and the IgG concentration estimated spectrophotometrically with an extinction coefficient of 1.8 at 280 nm (McGuigan and Eisen 1968). The purification procedure is important for the production of antibody-enzyme conjugates with high specific activity and precison in the development of ELISA methods.

The Double Antibody Sandwich Method

Purified antibodies specific to the virus are coated or adsorbed onto a solid surface such as the well of a polystyrene or polyvinyl 96-well microplate (Fig. 1). A test sample, suspected to contain the viral antigen, is incubated with the antibody sensitized wells. All the hemolymph samples collected are first treated with pH 11 buffer followed by neutralization with acid. The viral antigens, polyhedrin and virions, are bound to antibodies developed with dissolved polyhedra. Enzyme-labelled specific antibodies are added after the test sample is removed. The antigen is sandwiched between two antibodies and the whole complex is bound to the surface of the well non-covalently. After a period of incubation, the enzyme conjugate is removed and is followed by the addition of the substrate. The color intensity is directly proportional to the amount of enzyme present in the sandwich complex, which in turn is proportional to the amount of virus present.

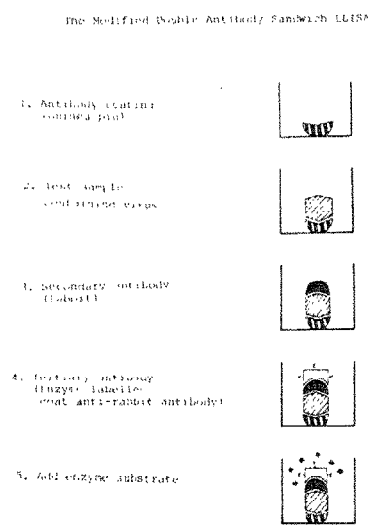
FIGURE 1



The Modified Double Antibody Sandwich Method

This method involves the development of antisera from 2 different animals (Fig. 2). The antibody used to sensitize the well is referred to as the primary antibody. After the addition of the test sample, antisera developed against the virus in a different animal is introduced. This is referred to as a secondary antibody. The virus is sandwiched between antibodies from two different animals. In the current studies, quinea pigs and rabbits were used. At this stage, either antiserum could be used as the primary antibody so long as the enzyme-antibody conjugate is directed at the secondary antibody. The antibody conjugated to the enzyme is referred to as the tertiary antibody which can be either goat anti-guinea pig or anti-rabbit antibody. These tertiary antibodies can be conveniently obtained from many commercial sources. When the sensitivity of the two ELISA methods are compared, the modified double antibody sandwich method is shown to be more sensitive. Detection limit of 50 ng/ml can be obtained consistently with the modified double antibody sandwich method. Since a secondary antibody is used, more than one enzyme-labelled tertiary antibody would link to the sandwich complex. With the presence of more enzymes present in the complex, the detection sensitivity is increased as a result.

FIGURE 2

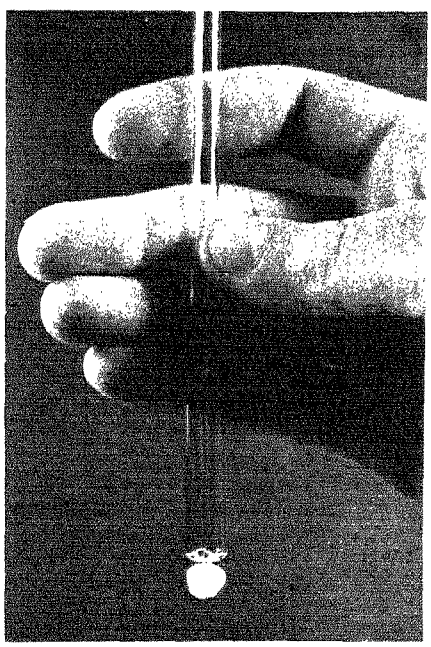


Plastic Bead ELISA

Despite the advantages of ELISA, it has not received wide acceptance in virus epizootiological studies because most field researchers usually lack the equipment or expertise to perform microplate ELISA

procedures. Hendry and Herrman (1980) have employed plastic beads as adsorptive solid phases for protein antigens. In the laboratory for this study (Ma et al. 1984), the relative merits of polystyrene and nylon beads were compared. The polystyrene bead was used in place of a microplate for ELISA detection of gypsy moth NPV (Fig. 3). The protocols developed for double antibody sandwich and modified double antibody sandwich ELISA can be easily adapted to the plastic bead procedure. There are several advantages in using plastic beads: (1) simple protocol which does not involve specialized equipment, (2) plastic beads are easily washed and ready for reuse. Cost is reduced considerably compared to that of disposable microplates. However, this method is not able to handle a large number of test samples which might be a disadvantage in certain situations.

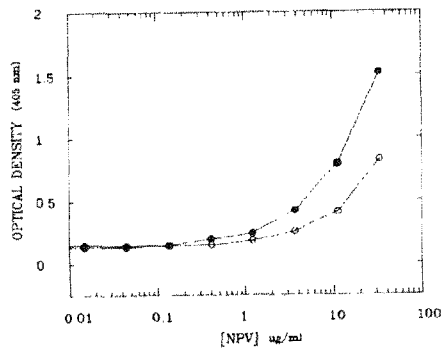
FIGURE 3



Detection of NPV in Infected Gypsy Moth Larvae

When we compared the sensitivity of the double antibody sandwich ELISA between the dissociated virus and whole polyhedra as antigenic preparations, the results showed that better sensivity can be achieved when the virus test sample is subjected to alkaline dissociation followed by pH neutralization (Fig. 4). There are two possible resons for this result. (1) When the antigen is in the dissociated

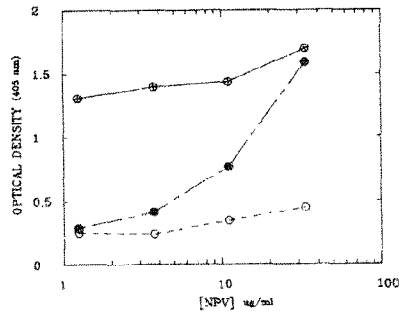
FIGURE 4



form, the antibodies recognize the epitopes of both the polyhedral and viral proteins. However, only those antibodies that recognize the surface epitopes of the polyhedra will react with the whole inclusions. (2) The affinity of proteins to the microplates is mainly a hydrophobic-hydrophilic type interaction. Since there are repeated washings in an ELISA protocol, some of the denser polyhedra may be washed off. A dissociated virus preparation consists of smaller protein fragments which would result in stronger attachments.

In order to look at the feasibility of ELISA for the detection of NPV in insects, the following experiment was performed. Fourth instar gypsy moth averaging 100 mg were used as experimenal animals. Lewis et al (1981) reported that the LC_{50} for early 4th instar larvae was found to be approximately 10^5 PIBs for the Pennsylvannia strain larvae. Ten 4th instar larvae were fed with a lethal dose of 10^6 PIBs/larva and all the hemolymph

FIGURE 5



samples were collected and checked for NPV with the double antibody sandwich method after one week. A negative control was provided by taking hemolymph from non-infected larvae at about the same age and assaying them for virus. The colorimetric reading of infected hemolymph, at 1:3 dilution, was found to be approximately one optical unit higher than the non-infected samples (Fig. 5). When the larvae were monitored at different times after ingesting a lethal dose of PIBs, the double antibody sandwich method can detect NPV in the hemolymph as early as three days post infection.

Case Study: Evaluation of Gypsy Moth NPV Field Application with ELISA

Dr. Tim Tignor (Department of Conservation and Economic Development, Division of Forestry, Virginia) conducted a pilot study on the evaluation of NPV spray applied to shade trees in 1983. Twenty white oaks (*Quercus alba* L.) on a small portion of the Conway-Robinson State Forest in Prince Williams County, Virginia were chosen for the evaluation of gypsy moth NPV spray. Trees ranged in size from 3.1 to 10.8 inches DBH and about 25 to 45 feet in height. Gypsy moth larvae were extremely difficult to find. Test trees were essentially uninfested. Larvae for spray treatment evaluation were reared from egg masses collected near the nothern Virginia/West Virginia border along U.S. Route 9 as the eggs were hatching on April 19. Larvae were held in the laboratory and fed with fresh oak leaves until most were second instars. On the afternoon of May 9, 200 larvae were placed about 20 feet above ground on each of the 20 test trees. Another 100 larvae per tree were added on the morning of May 12. Larvae were distributed among several branches.

Gypsy moth NPV was applied with a hydraulic sprayer to ten test trees on May 12th. Spray formulation consisted of 5.95 lbs of NPV powder blended well with a small portion of water and added to a larger volume of water containing 2 gallons of Rhoplex B60 A sticker, followed by 20 gallons of feed grade molasses and enough water to make 100 gallons. The virus concentration calculated to be approximately 5 X 10^7 PIBs/ml. Separation of treated and untreated trees ranged from about 50 to 500 feet.

TABLE 1. Numbers of larvae and pupae under burlap bands on NPV sprayed and unsprayed trees.

Date	5/18	5/23	5/26	6/3	6/9	6/16	6/23	6/30	7/7	7/15	7/21
Sprayed	23	32	28	0	0	0*	0*	1	0	0	2
Unsprayed	91	176	355	139	105	50	30	18	3	0	1

*Larvae on 6 of 10 trees; total number estimated.

Counts of larvae and pupae found under the burlap bands are shown in Table 1. Differences in larval numbers between sprayed and unsprayed trees were readily apparent from the first count on May 18. Larvae were noted on most sprayed trees throughout June, but none was found on sprayed trees during this period. After May 26, mean larval numbers declined steadily, and pupae were found on only a few trees. On May 26, all larvae under burlap bands were collected and frozen for later analysis by modified double antibody sandwich procedure described previously. One hundred larvae reared to the third instar were also tested for virus. Table 2 summarized the results. Larvae held in the laboratory yielded negative results. Only 18% of the larvae from untreated trees were infected compared to 64% of larvae from treated trees. When arbitrary categories were chosen to rank levels of infection, the difference between treatment and control groups was still greater. The heavily infected larvae have up to 50 ug/ml of virus. It is important to note that the survivors from the sprayed trees exhibit a higher level of infection. Most of these larvae would probably be dead and become a source of virus dissemination. The source of virus detected in insects from the unsprayed trees may be due to NPV drift from sprayed trees or possibly the breaking of "virus latency" under environmental stress.

TABLE 2 Virus infection levels of larvae from NPV sprayed and unsprayed trees as determined by the modified ELISA technique.

Infection Level 1/	Percent of Larvae 2/ Sprayed	Unsprayed	Laboratory
None	34	82	100
Light	14	9	0
Moderate	11	2	0
Heavy	0	1	0
Very Heavy	39	6	0

1/ Positive infection levels arbitrarily assigned to consecutively increasing optical density ranges of .05 units each.

2/ Numbers of larvae sampled. sprayed = 28, unsprayed = 292, laboratory = 100.

Development of Hybridoma Antibodies for the Gypsy Moth NPV

Limitions of Polyclonal Antibodies

ELISA may even be used as a quantitative tool in cases where the virus preparation is positively identified as gypsy moth NPV. Caution is advised because baculoviruses are shown to share similar antigenic characteristics using different serological methods such as radioimmunoassay, complement fixation and immunoprecipitin tests (Bilimoria et al 1974, Elliot et al 1977, and Carey et al. 1978). The presence of heterologous NPV virus in the test sample may be interpreted

as a small amount of gypsy moth NPV. However, there are examples of successes in using ELISA for diagnosis of viruses. Crook and Payne (1980) showed that ELISA can discriminate granulosis virus from Pieris brassicae , Agrotis segetum , and Cydis pomonella . ELISA is also capapble of distingusihing plant viruses which have been shown to have serological relationships by Ouchterlony double diffusion (Lister and Rochow, 1979, and Koenig 1978).

Since gypsy moth NPV is known to be specific for lymantriids only, it is therefore safe to assume that the hemolymph samples collected from infected larvae will yield positive readings only if the gypsy moth NPV is present. If we need to perform experiments such as the environmental persistence of NPV, an antibody preparation that is specific only to the gypsy moth is vital.

Rationale of Developing Hybridoma Antibodies to Gypsy Moth NPV

Conventional serological methods have been shown to be unreliable for the identification of Lepidoteran NPV (Hohmann and Faulkner 1983). The polyhedrin protein of these viruses share a lot of similar antigenic determinants. The antisera used in these studies involved the use of polyclonal antibodies which is a mixture of immunoglobulins to all the epitopes of an antigen. If two different antigens have a portion of the epitopes that are similar, crossreactivity would result.

Kohler and Milstein (1975) developed a method of making monoclonal antibodies that would only bind to one epitope. It is possible to develop specific biological probes for epitopes that are unique for that antigen. These antibodies are generated by somatic cell hybridization of mouse splenocytes and myeloma cells with polyethylene glycol which would result in the establishment of specific antibody producing cell lines. Each hybrid cell line synthesizes a homogeneous antibody that is highly specific for a single antigenic determinant or epitope (Yelton and Scharff 1981). This technology has the potential of locating antigenic determinants or epitopes that are unique for a particular antigen. In this case, monoclonal antibodies developed against these sites would be valuable for diagnostic work and the systomatics of NPVs.

In our laboratory, 35 different hybridoma cell lines producing monoclonal antobodies have been developed to the purified polyhedra of gypsy moth. We are in the process of characterizing these hybriodoma antibodies as to their immunoglobulin subclasses and affinity to the antigen. To pick out the monoclonal antibodies specific to the gypsy moth, at least two crossreactivities tests against other lepidopteran NPVs found in the forest ecosystem will be performed.

The first test involves the use of indirect ELISA to screen all the gypsy moth NPV monoclonal antibodies against purified preparations of European sawefly, tussock moth, spruce budworm and other NPVs found in forest environment. The second method, referred to as Western blotting, involves the separation of the purified polyhedra proteins of other NPVs using gel electrophoresis, followed by horizontal transfer of proteins from gel to a nitrocellulose membrane. The membrane would then be incubated with the monclonal antibodies developed for the gypsy moth NPV followed by histochemical staining to visualize the actual binding sites. If a clone of gypsy moth monoclonal antibodies binds only to the gypsy moth viral protein, these tests would confirm that a specific biological probe for a type specific antigenic determinant is located for the gypsy moth NPV.

Prospectus

The study of the gypsy moth nuclear polyhedrosis is important because of its effect on population dynamics and the decision to use Gypcheck as a possible component in an integrated management program. ELISA offers immediate help in studies involving the surveying of virus in gypsy moth populations, quality control of virus formulation, and the evaluation of NPV sprays.

There are additional important questions that need to be answered such as the elucidation of epizootiology, the environmental persistence and dissemination pattern of this NPV. Intelligent decision involving virus applications would be difficult if these basic knowledge are lacking. The development of a reliable protocol for environmental sampling of soil, host plant and non-target hosts would be an important step towards answering these questions. Solid phase radioimmunoassay will have to be developed because it offers the sensitivity of detection down to a few polyhedral inclusion bodies. Hybridoma antibodies will be incorporated in both radioimmunoassay and ELISA procedures to ensure specific detection of gypsy moth NPV and not other NPVs that might be present in the forest ecosystem. I am a strong believer that a lot of the procedure developed in this gypsy moth program will become standard protocols in many insect control programs involving microbials in the future.

Acknowledgement

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even initiated until the late 1970's, and are just now beginning to reveal where the toxin genes are localized and how they are expressed. For other biologicals the level of our basic understanding of their inheritance and mode of action is considerably less. Without detailed knowledge of their mechanisms, it is not surprising that efforts to develop these agents as alternatives to more conventional pesticides have been sporadic at best.

Despite the limitations and disadvantages of these biorational products, there is currently a great deal of interest, both from the basic science viewpoint as well as from the commercial side, in agents such as BT. A number of laboratories in the U.S. and abroad are currently focusing their attention on the genetics and molecular biology of BT and other biological pathogens, including other bacteria, viruses, and fungi. In addition, a number of new start-up biotechnology companies have announced plans to direct significant fractions of their efforts to development of new and improved biological pesticides. One such company is Ecogen, Inc., founded just a few months ago in Princeton, N.J., and with which I am especially familiar since I have recently agreed to become their full-time director of research and development beginning on June 1 of this year. The major thrust of Ecogen, Inc. will be to apply a multidisciplinary, integrated approach to the development of novel biopesticides, using modern technologies of genetics, protein chemistry, immunology, and cell biology.

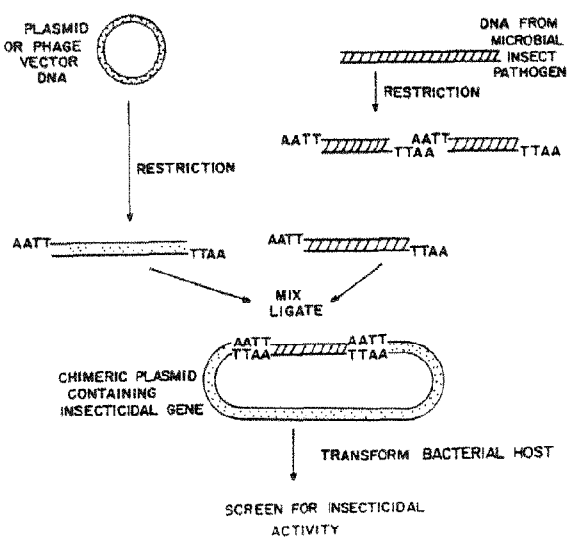

Fig. 1 Gene-splicing technology for molecular cloning of insecticidal genes.

What factors have led to the resurgence in interest in biological pesticides? Other than economic and environmental considerations, probably the major technological advance that has contributed to interest in biorationals is that of recombinant DNA-gene splicing methodology (Fig. 1). There are two factors of importance in this methodology. First is the ability to isolate a gene (or cluster of genes) controlling a given activity (e.g. pesticide synthesis) from the vast background of other genes and activities in any organism, regardless of its genetic complexity. Thus, in principle, a gene from a fungal cell can potentially be isolated (cloned) and its structure and regulation studied as easily as that of a bacterial gene. Second, once a gene has been isolated by molecular cloning, its structure can be readily altered (mutated) by a variety of molecular tricks that would be impossible by conventional mutagenesis procedures using intact organisms. For example, digestion of cloned genes with a combination of restriction endonucleases (enzymes which recognize and cleave specific 4- or 6-base sequences in DNA) identifies sites which can be specifically mutated by one of several methodologies recently developed for site-specific mutagenesis (Figs. 2 and 3).

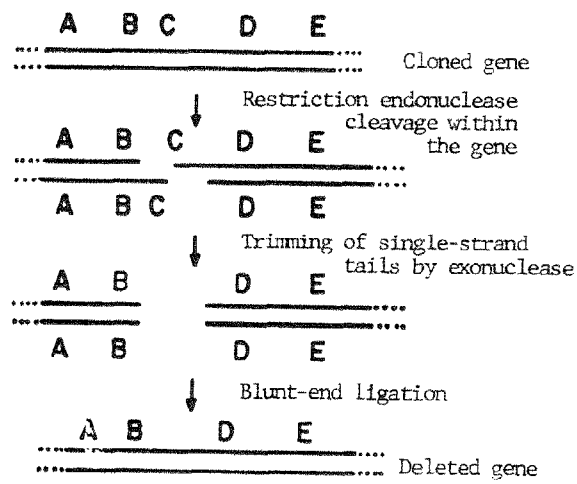

Fig. 2 Generation of deletions in an insecticidal gene.

These treatments can be carried out on a cloned gene *in vitro* (i.e., in a test tube) with much greater efficiencies than could be accomplished *in vivo* (in the intact organism). By these techniques one can thus change single bases in a DNA gene, delete blocks of bases, or add segments of DNA at will and observe the effects on expression of the gene. While these changes might also occur spontaneously *in vivo* for a non-cloned gene, their frequencies would be orders of magnitude less and their detection would be exceedingly difficult. Thus, the techniques of

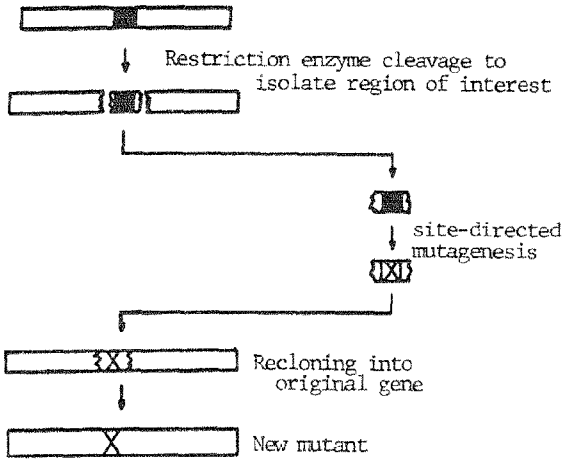

Fig. 3 Site-directed *in vitro* mutagenesis.

modern molecular cloning serve to greatly enhance the genetic sensitivity of mutational approaches. The isolation of individual genes by molecular cloning also makes possible the enhanced production of their products (usually enzymes or other proteins) by genetically fusing them to highly-efficient regulatory sequences (promoters). In certain instances, this manipulation can elevate a gene's expression several hundred-fold above its normal level of activity. Further, a gene which normally produces its protein product inside the cell, often a self-limiting process, can be fused to a small signal peptide coding sequence which allows the protein to be transported outside the cell. This construction can both increase the level of production of a particular gene product as well as facilitate its recovery because it is transported to the external medium.

How can the types of genetic manipulation described above be applied to the improvement and commercialization of biological pesticides? The possibilities are numerous. For example, molecular cloning of a gene which specifies an insecticidal toxin protein makes it amenable to yield enhancement by attaching the gene to a high-effiency promoter sequence, thereby markedly increasing the yield. The gene can also be altered by site-directed mutagenesis procedures to either broaden the spectrum of targets (e.g., different insects it will attack) or to increase its activity on a specific target. Cloning of two or more genes for distinct pesticide activities into the same host cell could generate a multi-purpose pesticide, having activities against two or more very different targets (e.g., two different insects, an insect and a weed, an insect and fungal disease,etc.). The relative ease with which such different gene combinations can be potentially constructed by recombinant DNA technology makes the possibilities virtually unlimited. The limitations fall to the imagination of the researcher and to the sensitivity of detection methods for identifying new gene combinations of interest amongst a background of hundreds, or even thousands, or new variants. In *Bacillus thuringiensis*, the discovery in our laboratories (Gonzalez *et al* 1982) of a natural, highly-efficient plasmid exchange system opens the way for constructing strains having novel combinations of insecticidal genes from different strains of BT, or other related strains. This plasmid transfer system has already been used to construct strains of BT having two toxin-coding plasmids from different parental origins, as well as to convert strains of normally non-toxic *B. cereus* into BT-like variants. These and other methodologies offer the possibility for producing new pesticides that can be rapidly tailored to individual locales and situations. For example, predictions of an outbreak of a particular plant disease and concomitant infestation of a particular insect on some important crop could dictate the construction of a bacterial, viral or fungal strain having the capabilities to control both pests. For some other crop or locale, a different combination might be called for. This type of rapid response to changing pest problems would be a significant departure from current planning in the pesticide field, which must be done years in advance due to the time required to develop new products.

Another future innovation in biological pesticides, as previously mentioned, is the possibility for creating pathogens having two different activities in the same strain. Smith, *et al* (1983) have recently shown that the genome of AcNPV, a baculovirus with insecticidal activity, can be used as a cloning vector into which foreign genes can be inserted and subsequently propagated and expressed in cultured insect cells. In the first experiments the gene they cloned was human β interferon, an anti-viral protein which is being clinically tested for activity on a variety of disease-causing viruses as well as for possible anti-cancer activity. Remarkably, the amount of interferon produced by the insect cell clones was much higher than that produced by *E. coli* bacterial cells, the most common host for propagation of cloned genes. This virus may be potentially useful in the future for cloning genes for BT toxins, generating a bifunctional pathogen having two distinctly different pathogenic activities directed at the same target insects.

Perhaps the ultimate accomplishment in developing new biological pesticides would involve incorporating their genes directly into the genome of a susceptible organism, thereby conferring the ability to synthesize its own pesticide and totally eliminating the usual delivery system. For example, it may be possible to transfer genes for bacterial insecticide toxins directly into the genetic backgrounds of various plants, enabling them to synthesize their own insecticidal material and bypassing the costly need for repeated sprayings. Genetic manipulations of this type may already be technically possible.

The soil bacterium *Agrobacterium tumefaciens* harbors Ti plasmids with potential as natural

vectors for the transfer of foreign DNA into plant cells (Chilton, 1983). Upon infection of a susceptible plant the bacterium induces formation of a crown gall tumor. Crown gall tissues synthesize a group of novel metabolites called opines, which are specified by the high molecular weight Ti plasmids in the Agrobacterium strain. Using modified Ti plasmids deleted in many or all of their tumorigenic functions it is now possible to molecularly clone a variety of homologous and heterologous gene functions and to express them in plant cells (Zambryski et al, 1983). Advances in the regeneration of whole plants from callus tissue derived from transformed plant cells promise to greatly expand the spectrum of plant and foreign gene combinations which can be constructed. The introduction of toxin-specifying genes from Bacillus thuringiensis and other microbial pathogens directly into plants may thus be possible in the very near future. Both plant viruses and bacterial vectors are already known which show promise for transporting foreign genes into plant cells. While many technical difficulties still remain to be overcome in accomplishing many of the suggested goals, it seems clear that the pesticide industry is poised on the threshold of a new era in its history.

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DEVELOPMENTS IN COMMERCIALLY PRODUCED MICROBIALS AT BIOCHEM PRODUCTS

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Biochem Products is part of a large industrial and scientific family - the Solvay Group. Solvay, headquartered in Brussels, Belgium is a multinational company with 46,000 employees worldwide. In the U.S., our working partners include a large polymer manufacturer, a peroxygen producer and a leading poultry and animal health products company. Biochem Products is a division of the latter - Salsbury Laboratories, Inc. Biochem Products in the U.S. was started in 1979. It is committed to research, development and marketing of microbial products for insect pest management. Our first product was Bactospeine® Wettable Powder. Then we obtained a registration for Bactimos™ Wettable Powder (our B.t.i. product). Since then, we have concentrated our efforts at developing and marketing various formulations of each.

With regards to forestry, we initially acquired Novabac-3 from another company and developed the Canadian registration of this product for use against the spruce budworm in forests and woodlands. This product contains 32 BIU per U.S. gallon. The registration was obtained in 1981. At the same time, we were developing a modified liquid formulation of B.t. in the U.S. known as Bactospeine Flowable Concentrate. Bactospeine FC contains 35 BIU per U.S. gallon. This product has a registration (registered in the U.S. in 1981) for use against approximately 40 different pests in forestry and agriculture including the spruce budworm and gypsy moth. In Canada, registration was received in early 1984 for Bactospeine-F (forestry) and Bactospeine-A (agriculture). In the past two years, our interests have been in reducing the total volume of spray mix required per acre and still achieve effective control. This objective has been achieved.

While the above were being developed, a cooperative research effort between Biochem Products and Environment Canada's Laurentian Forest Research Center led to the development of Futura® Flowable Concentrate. Futura contains 54.5 BIU per U.S. gallon. Futura is designed for effective low volume application. Highly successful and consistent results have been obtained with a 1.5 Liter per Hectare dosage rate in a total volume of 2.5 Liters per Hectare. Futura was registered in the U.S. in 1983 and in Canada in early 1984.

Future developments will be in undiluted applications of these products with the objective of providing economical and effective control.

COMMERCIAL PRODUCTION OF MICROBIALS BY REUTER LABORATORIES, INC., FOR CONTROL OF THE GYPSY MOTH AND THE SPRUCE BUDWORM.

F. D. OBENCHAIN

DIRECTOR OF RESEARCH AND DEVELOPMENT, REUTER LABORATORIES, INC., 14540 JOHN MARSHALL HIGHWAY, GAINESVILLE, VA 22065

REUTER LABORATORIES ANNOUNCES ADDITIONS TO ITS LINE OF MICROBIAL INSECTICIDES WITH THE 1984-85 INTRODUCTION OF A BACILLUS THURINGIENSIS, BERLINER, VARIETY KURSTAKI (HD-1, H-3A3B) WETTABLE POWDER FORMULATION. GYPSY MOTH NUCLEOPOLYHEDROSIS VIRUS, IN EXPERIMENTAL PRODUCTION SINCE 1982, IS SCHEDULED FOR COMMERCIAL INTRODUCTION AS A WETTABLE POWDER IN THE 1985-86 SEASON.

COMPANY HISTORY

REUTER LABORATORIES, INC., A VIRGINIA CORPORATION, HAS PRODUCED MICROBIAL INSECTICIDES AND OTHER BIORATIONAL PRODUCTS SINCE ITS ORGANIZATION IN 1974. ITS FIRST AND LARGEST SELLING PRODUCT IS MILKY SPORE POWDER, COMPOSED OF THE BACTERIAL SPECIES BACILLUS POPILLIAE DUTKY AND B. LENTIMORBUS DUTKY. IN THE ABSENCE OF IN VITRO FERMENTATION METHODOLOGIES, THESE BACTERIA MUST BE PRODUCED BY THE COLLECTION, INNOCULATION AND SUBSEQUENT INCUBATION OF THE WHITE GRUBS OF THE JAPANESE BEETLE, POPILLIA JAPONICA NEWMAN. THE ANNUAL PROCESSING OF THOUSANDS OF JAPANESE BEETLE GRUBS REPRESENTS A CONSIDERABLE INVESTMENT IN BOTH CAPITAL AND PROFESSIONAL EXPERIENCE AND IS THE BASIS FOR THE EXPANSION OF REUTER LABORATORIES INTO OTHER MARKET AREAS. THE INTRODUCTION OF THE JAPANESE BEETLE SUPER TRAP, COMPLETE WITH FLORAL AND SEX PHEROMONE ATTRACTANT BAITS, ALLOWS HOME OWNERS, ON AN INDIVIDUAL OR COMMUNITY BASIS, TO CONTROL JAPANESE BEETLE POPULATIONS WITHOUT THE USE OF CHEMICAL PESTICIDES.

IN 1980, REUTER LABORATORIES INTRODUCED A PRODUCT CONTAINING NOSEMA LOCUSTAE CANNING SPORES, A MICROSPORIDIAN DISEASE ORGANISM FOR THE CONTROL OF CERTAIN GRASSHOPPERS AND CRICKETS, TO THE HOME USE MARKET. SINCE 1981, THIS MICROBIAL INSECTICIDE HAS BEEN FORMULATED BY REUTER IN THE FREEZE-DRIED STATE, CRYOPRESERVED IN SERUM VIALS UNDER A NITROGEN ATMOSPHERE. THE NOSEMA SPORES ARE REHYDRATED AT THE POINT OF USE AND APPLIED TO A BRAN BAIT FOR THE CONTROL OF GRASSHOPPER OR CRICKET POPULATIONS ON SMALL PLOTS, GARDENS OR AGRICULTURAL AND RANGELAND AREAS.

IN ITS FIRST VENTURE INTO FERMENTATION MICROBIALS, BMC-BIOLOGICAL MOSQUITO CONTROL, A WETTABLE POWDER FORMULATION OF BACILLUS THURINGIENSIS BERLINER, VARIETY ISRAELENSIS (H-14), WITH 1750 INTERNATIONAL UNITS OF ACTIVITY PER MILLIGRAM OF FINISHED PRODUCT WAS INTRODUCED IN 1983 AND IS CURRENTLY MARKETED TO THE HOME CONSUMER IN A 4 OUNCE PACKAGE.

IN OCTOBER, 1983, CONTROLLING INTEREST IN REUTER LABORATORIES, INC., WAS ACQUIRED BY OTF EQUITIES, INC., A PUBLICLY HELD COMPANY WHOSE STOCK IS TRADED IN THE OVER-THE-COUNTER MARKET IN THE DETROIT, MICHIGAN, AREA. UNDER NEW MANAGEMENT, REUTER LABORATORIES HAS COMPLETED A PROGRAM OF PRODUCT AND MARKET ANALYSIS AND HAS BEGUN THE CONSTRUCTION OF EXPANDED OFFICE, INSECTARY, FERMENTATION AND PRODUCTION FACILITIES. WITH THE APPLICATION OF YEARS OF ACQUIRED EXPERIENCE AND EMPLOYMENT OF ADDITIONAL PROFESSIONAL STAFF REUTER ANTICIPATES THE INTRODUCTION OF AN EXPANDED LINE OF BIORATIONAL PEST CONTROL PRODUCTS.

BACILLUS THURINGIENSIS KURSTAKI WETTABLE POWDER

IN LATE 1984 REUTER LABORATORIES WILL RELEASE A WETTABLE POWDER FORMULATION OF B. THURINGIENSIS, BERLINER, VARIETY KURSTAKI (HD-1, H-3A3B) WITH 3200 INTERNATIONAL UNITS OF ACTIVITY PER MILLIGRAM OF FINISHED PRODUCT. LABELS SUBMITTED TO EPA FOR APPROVAL ARE IN FOUR COMMODITY AREAS:

1. BKT-VEGETABLE WORM AND CATEPILLAR CONTROL FOR USE ON GREENHOUSE AND GARDEN VEGETABLES AND FIELD CROPS,
2. BKT-LAWN MOTH CONTROL FOR USE AGAINST SOD WEBWORM AND RANGELAND CATEPILLARS,
3. BKT-FRUIT AND NUT TREE CATEPILLAR CONTROL, AND
4. BKT-SHADE TREE, ORNAMENTAL AND FLOWER CATEPILLAR CONTROL.

THE LAST TWO LABELS WILL SPECIFICALLY TARGET GYPSY MOTH LARVAE, AMONG OTHER APPROPRIATE LARVAL LEPIDOPTERAN SPECIES, WITH INITIAL TREATMENT RECOMMENDED WHEN LARVAE ARE YOUNG AND LEAF EXPANSION IS AT THE 40% STAGE. RETREATMENT WILL BE RECOMMENDED 7 TO 10 DAYS LATER. UNDER APPROPRIATE CONDITIONS OF POPULATION DENSITY AND AGE, THE SPRUCE BUDWORM WILL ALSO BE TARGETED FOR CONTROL ON THE FOURTH LABEL.

GYPSY MOTH NUCLEOPOLYHEDROSIS VIRUS WETTABLE POWDER

INITIAL PRODUCTION OF A FREEZE DRIED GYPSY MOTH NPV PRIMARY PRODUCT AT REUTER LABORATORIES OCCURRED IN 1982. SUBSEQUANT RESEARCH EFFORTS HAVE BEEN DIRECTED TOWARD PROPRIATORY IMPROVEMENTS IN VARIOUS AREAS OF PRODUCTION AND FORMULATION. VIRUS ACTIVITY IS EXPECTED TO BE MAXIMIZED BY CRYOGENIC MILLING AND PRODUCT EFFECTIVENESS EXTENDED BY THE USE OF NEW STICKING AGENTS AND UV PROTECTANTS. FIELD TESTS OF THE REUTER GYPSY MOTH NPV PRODUCT WERE SCHEDULED IN VIRGINIA FOR 1984 AS PART OF A COOPERATIVE PROGRAM. THAT STUDY IS NOW RESCHEDULED FOR 1985 DUE TO A SHORTAGE OF STATE FUNDS. PENDING THE OUTCOME OF THESE FIELD TRIALS AND OTHER ECONOMIC FACTORS, INTRODUCTION OF THE REUTER GYPSY MOTH VIRAL INSECTICIDE IS ANTICIPATED FOR THE 1986 SEASON.

ECONOMIC CONSIDERATIONS FOR COMMERCIALIZATION

VIRAL INSECTICIDES DO NOT HAVE AN ENCOURAGING REPUTAION WITH RESPECT TO COMMERCIALIZATION. IN THIS RESPECT THE ORIENTATION OF THE REUTER LABORATORIES MARKETING PROGRAM MAY MEDIATE IN FAVOR OF THEIR DEVELOPMENT. DUE TO THE CYCLICAL NATURE OF GYPSY MOTH POPULATION EXPANSIONS AND CONTRACTIONS, INSECTARY AND PRODUCTION FACILITIES FULLY UTILIZED FOR GYPSY MOTH NPV MANUFACTURE DURING YEARS OF PEAK DEMAND WOULD BE UNDERUTILIZED IN THOSE YEARS WHEN THE POPULATIONS WERE LOW. IF VIRAL INSECTICIDES WITH SUBSTANTIAL GROWER AND HOME OWNER APPEAL, AS FOR EXAMPLE THE GRANULOSIS VIRUS OF THE CODLING MOTH, COULD BE DEVELOPED AND SUCCESSFULLY MARKETED TO BOTH OF THESE CONSUMER GROUPS, THEN THEY COULD BE MANUFACTURED DURING PERIODS OF MINIMAL DEMAND FOR THE GYPSY MOTH PRODUCT. THIS ALTERNATIVE USE OF PRODUCTION FACILITIES APPEARS TO BE AN ESSENTIAL FACTOR WHICH MAY ENSURE THE ECONOMIC VIABILITY OF GYPSY MOTH NPV PRODUCTION.

RECENT DEVELOPMENTS IN THE ZOECON CORPORATION AND THE THURICIDE[R] FORESTRY FORMULATIONS

William R. Beck

Product Development Representative
Zoecon Corporation, A Sandoz Company
Crop Protection Division
323 East Park Avenue, State College, PA 16803

Sandoz is pleased to announce the acquisition of Zoecon Incorporated of the United States and Canada. Effective November 15, 1983, all Zoecon and Sandoz Crop Protection Division personnel, facilities and products will be combined under the Zoecon, Inc. name. Zoecon will be a wholly owned subsidiary of Sandoz Ltd. with major offices in Palo Alto, Calif. and Dallas, Texas.

Thuricide[R] forestry formulations will continue to be important products in the Zoecon product line. A. Temple Bowen will continue to handle government sales as the Thuricide Forestry Sales Manager for the United States and Lon Seymour will remain as product manager. Thuricide will be manufactured at our plant in Wasco, Calif. under the supervision of Tom Freaney.

William R. Beck, Product Development Representative, will continue to handle the forestry research and development in the northeast United States and eastern Canada.

In the past Sandoz has demonstrated the ability to combine its commercial expertise with government and university research programs in the development of innovative, environmentally safe products. This can best be illustrated by the diversity of the biological control agents that have been developed by Sandoz in recent years:

1. Elcar[R] Microbial Insecticide is a formulation of the Heliothis nuclear-polyhedrosis virus (NPV), registered for suppression and control of the tobacco budworm (Heliothis viriscens) and cotton bollworm (Heliothis zea). Elcar has no effect on beneficial arthropods found in the field.

2. Certan[TM] Bacterial Insecticide is a water dispersable concentrate of Bacillus thuringiensis Serotype 7 strain for the control of the Greater Wax Moth, Galleria mellonella. Certan[TM] has no detrimental effects on honey bees or honey production and the product may be applied directly to the honeycomb.

3. Teknar[TM] Biological Insecticide is a preparation of Bacillus thuringiensis Berliner, var. israelensis, Serotype H-14. It is selectively toxic to most species of mosquito larvae and blackfly larvae (Diptera: Culicidae and Simuliidae). Teknar is exempted from residue tolerance requirements and provides a unique opportunity for microbial control of mosquito and blackfly larvae without harmful effects on humans, domestic animals, wildlife, beneficial insects, fish or other aquatic life.

4. Thuricide[R] Microbial Insecticides are aqueous spray formulations based on the bacterium, Bacillus thuringiensis Berliner var. Kurstaki (b.t.) which is active against lepidopterous larvae, many of which are pests of shade trees and forests. Sandoz was the first company to successfully produce an aqueous formulation of b.t. capable of being applied by aerial application to the forest environment. Over the years Sandoz continued to improve the Thuricide formulation, progressing from the original Thuricide 16B to the present standard Thuricide 32LV and Thuricide 48LV formulations. This progression has resulted in formations that can be pumped through aircraft systems undiluted and with sufficient atomization to provide excellent droplet spectrums and deposit on the target foliage. Thuricide formulations may also be diluted with any amount of water desired enabling the applicator to adjust the actual spray volume to meet his needs.

Zoecon will continue this tradition of innovative research and development of biological control agents for agriculture and forestry. This is evidenced by the recent development of the NRD-12 strain of Bacillus thuringiensis. Zoecon was the first company to successfully formulate and field test this strain as SAN415 against the spruce budworm in Maine in 1983. The success of this program has triggered an expansive testing program in forestry and agriculture in 1984.

In addition to the search for new more effective strains of b.t., Zoecon is continuing to develop new formulations of Thuricide. The success of the Thuricide 32LV and 48LV aqueous formulations has encouraged Zoecon to continue to develop more concentrated formulations. Research plans for 1984 include the testing of a 64 BIU per gallon aqueous formulation of b.t. These new more concentrated formulations have demonstrated the ability to be applied undiluted at low volumes providing improved efficacy while reducing the cost of application.

While the Zoecon corporation has placed its emphasis on biological control agents that are compatible with their fermentation technology, the company will continue to be receptive to other microbial agents that have the potential for commerical development.

As of November 15, Sandoz, Inc. Crop Protection in San Diego has moved to the Zoecon location in Palo Alto, Calif.

The new address and phone number for Thuricide are:

Zoecon Corporation
Crop Protection Division
425 Sherman Avenue
P. O. Box 10975
Palo Alto, CA 94303
(415) 857-1130

For more information regarding these products, please contact one of the Zoecon sales representatives listed below:

Lon Seymour
480 Camino Del Rio South
San Diego, CA 92108

Temple Bowen
23 Sherbrook St.
Augusta, Maine 04330

Dick Schneider
8048 Lincoln Way West
P. O. Box 243
St. Thomas, PA 17252

Paul Bystrak
R. R. #2
Box 137
Berrien Springs, MI 49103

We are looking forward to providing you with the quality product and service that has long been a tradition with Sandoz Ltd. and will now continue under the Zoecon banner in the United States and Canada.

SYMPOSIUM ON
FORMULATION AND APPLICATION OF MICROBIALS FOR SPRUCE BUDWORM AND GYPSY MOTH CONTROL

J. A. Armstrong[1] and W. G. Yendol[2]

Introduction

This panel of experts from Canada and the United States has been brought together to discuss control techniques and strategies employed against these important defoliators - the spruce budworm and the gypsy moth. In selecting the panel we have chosen people with experience ranging from research to control. Our panel speakers are:

Bruce McGauley. - Supervisor, Pest Control Section, Ontario Ministry of Natural Resources. He will discuss the spruce budworm spray program in Ontario and the policies that government follows in organizing the spray program.

Michel Pelletier. - Quebec Ministry of Energy and Resources. He will speak about the spruce budworm program in Quebec, and the use of the large 4-engined spray aircraft.

Jack Barry. - USDA Forest Service. He has worked for many years on research programs aimed at furthering our knowledge of spray cloud movement and deposition.

David Smith. - Agricultural and Biological Engineering Department, Mississippi State. His specialty is in the area of droplet deposition on leaf surfaces and development of formulations and application technique to improve deposition.

Paul Fast. - Forest Pest Management Institute Canadian Forest Service. His research interests have been aimed at furthering our understanding of the mode of action of Bacillus thuringienses (Bt) and developing strategies and techniques to improve its efficacy.

Chester Himel. - University of Georgia, Athens. He has been involved in spray physics for a number of years, and today will discuss his research with Bacillus thuringiensis.

William Yendol. - Pennsylvania State University. He will bring to us his current experience in the field of aerial spray applications and will highlight some of the problems and some difficulties with aerial spray applications.

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[1] J. A. Armstrong, A/Program Manager, Protection Canadian Forestry Service, Environment Canada.
[2] W. G. Yendol, Professor of Entomology, Pesticide Research Laboratory, Entomology Dept., Pennsylvania State University.

I would like to take the opportunity to welcome our speakers and the audience to this discussion session. There will be opportunities for questions from the floor and we hope that with your active participation we will have an interesting and beneficial discussion. Additionally, it is desired that these discussions on formulation and spray technology will create further research interaction and more clearly focus on those mutually important problems.

SPRUCE BUDWORM SPRAY PROGRAM IN ONTARIO

B. H. McGauley, Pest Control Section, Ontario Ministry of Natural Resources, Ontario, Canada.

Today I would like to explain why Ontario has a smaller budworm program than the provinces and states to the east.

To explain Ontario's approach, I will outline the current spruce budworm policy. For the benefit of the American audience, I would point out that the Canadian provinces have the responsibility of managing the natural resources while the federal government's mandate is to undertake forestry research. Therefore, the policy that I am about to outline has been established at the provincial level and applies specifically to Ontario. The policy allows for three control objectives: epicenter suppression, to eliminate small defined insect epidemics; outbreak control, to contain larger infestations; and protection spraying, to protect the current year's growth.

In 1983, the spruce budworm infestation in Ontario spread across some 9 million hectares and the gypsy moth covered some 40,000 hectares. Therefore, the objectives of epicenter suppression and outbreak control were judged impractical and all spraying planned for 1984 is for protection. There are two categories of protection spraying: high value forests including provincial parks, plantations, seed orchards, seed production areas, wildlife habitat; commercially operable forests, i.e., those scheduled for liquidation within a five year period. The high value forests are usually identified by the provincial forest managers while the commercially operable forests are identified by provincial forest managers in consultation with industry foresters.

There are several constraints imposed on commercial forest protection spraying. The company must commit to harvest the forest within a five-year period, to initiate accessing the area within the year that spraying begins and complete road construction within three years. Protection spraying will by undertaken only three times in the five-year period. There are

other constraints, but those which I have identified here are the most important. These constraints help to ensure that short term protection spraying is combined with targeted and accelerated harvesting. The goal is to prevent the establishment of large areas of balsam fir, but at the same time ensure that the forest users have sufficient wood to maintain a viable industry.

SPRUCE BUDWORM SPRAY PROGRAM IN QUEBEC

Michel Pelletier, Quebec Ministry of Energy and Resources, 175 St. Jean, Quebec, Canada.

For this presentation I will give a general picture of our experiences with Bt in Quebec since 1974. We started in 1974, applying Bt using DC-6B and CL215 as spray aircraft. The tank mix was Thuricide 16B® plus sorbitol in a 50:50 preparation. There was no calibration of the aircraft as we would now use the word, rather we just checked the aircraft for flow rate and sent it off on its spray mission. Although the Bt sorbitol mix was a good non-volatile preparation, after pumping a few gallons the meter jammed. We had no idea exactly how much was added since we had to estimate visually. The spray nozzles were the standard spraying systems nozzle with the tip removed; this gave an open nozzle with a 3/8" orifice. Even this large orifice became plugged with the spray mix. In spite of these problems, we had sufficient success to encourage us to plan a large program for 1975.

Prior to the 1975 spray season, spray calibration trials were carried out at Barstow, California, with the assistance of A. P. Randall and staff of the Forest Pest Management Institute. The formulation finally selected was Bt:Sorbitol:water in the ratio 50:20:30. During the spray season we did more experiments; in fact 11 combinations to assess differing mixes, swath intervals, etc. We had no success at all. We realize now that we didn't have a consistant droplet spectrum to rely on or even knowledge of the required drop size to give good results. This experience so discouraged us that for 2 years no trials with Bt were carried out. In 1976 and 1977, Dr. Smirnoff of the Laurential Forest Research Centre was encouraged to participate and, although we don't know why, we had good results. Further tests were carried out in 1978. We realize that the major problem was aircraft calibration. At that time we really didn't know the best droplet spectrum, the desired deposit in terms of drops per unit area (degree of coverage), the volume per hectar required and the concentration of Bt required. We quickly determined that at an emission rate of 10 l/ha A treatment of 40 BIU/ha resulted in success. The mean deposit of Bt (measured in petri dishes) was 77 colonies/cm^2 (ranging from 31 to 122); budworm mortality was 97.6% compared to 63% in the untreated areas, and defoliation was only 30% compared to 73% in untreated check blocks.

This success encouraged us to continue, and in 1979 11,000 ha were treated using Dipel 88® (Dipel 4L®), Novabac® and Thuricide 32B®. The emission rate was reduced to 7.5 l/ha, but with the same dilution and the treatment was 30 BIU/ha. Dipel 88 was mixed with water to give a 40:60 mix ratio. When the open nozzles were used with 154 Tyre nozzles, a swath width of 150 m was obtained. Droplets were collected on Kromekote® cards, petri-dishes and Millipore filters. With Dipel 88-97% of the droplets collected on Kromekote paper were 0-75 microns and 5% over 150 microns. These assessments indicate that the sprays can be classified as being made up of small droplets. This nozzle arrangement has been maintained except with FUTURA®, where only 110 nozzles were used. With improvements we now have a 300 m swath. Calibrations were also done in 1983, and in 1984 we will be using from 1.6 to 2.5 l/ha. Thus, since our start in 1974 when we used 10 l/ha, we are now down to 2.5 l/ha. We still have the basic question - are we in the right range of emission? We don't know if the droplets we are producing are the correct size, if we are losing a lot of small droplets, or if some are too large. To be able to do a proper aircraft calibration, we must know the optimum drop size to kill a budworm and the optimum size of deposition. Without this knowledge our tests to compare different products are meaningless; we will not know why one product is doing a better job than another. As I see it, the most important thing we must do is to determine the best droplet size to kill the budworm and the best to ensure good foliar deposit.

AERIAL APPLICATION OF BACILLUS THURINGIENSIS IN THE WESTERN U.S.

John W. Barry, Forest Insect and Disease Management, Forest Service, USDA, 2810 Chiles Rd., Davis, California.

The following summarizes my remarks relating to aerial application of Bacillus thuringiensis (Bt).

Quality of Application. No pesticide has ever been better than its application. Quality of application is dependent upon a correct prescription for treatment and proper execution of the prescription. Once the entomologist specifies what is needed on the target foliage for the biological effect, the spray strategist can write a prescription for delivering the proper number and size of drops to the target. But I

emphasize, that the spray strategist must be told what is needed at the target. For the application to be successful, however, the applicator must be capable and willing to execute the prescription details, otherwise Bt, in all likelihood, will not be effective. The majority of aerial applicators are professional. Unfortuately, they may not be aware of new application technology, nor how to use it effectively.

Properly calibrated and clean application equipment are other important considerations. Calibration becomes more critical as the application rates decrease below one gallon per acre. Calibration includes checking to insure that the spray system has the correct number and size of nozzles for the desired flow rate, and that the system is clean. Calibration is basic and we shouldn't have to worry about these matters -- that's why we hire a professional. But we do worry and for good reason. We can do all the proper things and then the link breaks down with the applicator. I estimate that 75% of the pilots and even company operators don't know how to calibrate properly. Does this come as a surprise to you? It did to me. Fortunately, the epidemic of poor application is subsiding.

Technical Representative. Because technical representatives have a vested interest in their product, no one is more interested than the technical representative in seeing the product meet its expectations and the control project meet it goals. The technical representative has a responsibility of being well informed on product mixing, tank mix, stability, atomization, degradation, adjuvants, application, etc. The Project Officer should utilize this valuable source of information.

Tank Mixes. Tank mixes have been changing rapidly in the formulation, amounts applied per acre, and degree of dilution. We are now applying less than one gallon per acre and we are beginning to apply the formulation undiluted. Paul Fast, FPMI, has calculated the amount of toxin required for an LD_{50} for spruce budworm. Bt drops diluted more than 50% simply do not have enough potency to be effective. This insight has been a major breakthrough in understanding how to use Bt effectively. He further calculated that approximately two drops would be required per Douglas-fir needle. After aerial treatments of Bt tank mixes, applied at 96 to 128 oz/A, we observe approximately two Bt drops per spruce and fir needle. These two drops must contain sufficient toxin to provide a lethal dose to feeding budworm, and the lethal dose must be consumed before the effectiveness of the drops is lost through normal degradation.

The role and need for adjuvants in Bt tank mixes is not understood. We should be cautious in using an adjuvant until we understand its role regarding rainfastness, UV protection and impingement -- and until we know how to handle the adjuvant in the field.

Determining the Success of Bt Treatments. In the past Bt has been used and its effectiveness evaluated as if it were a chemical insecticide. Chemicals usually show results within a few days. This is not the case with Bt. Entomologists in the West and East have observed dramatic results with Bt in reducing egg masses, larval population, and defoliation the year following treatment. Unfortunately we seldom look at second year results. I submit that frequently those supposedly poor Bt treatments were indeed successful; we simply did not know how to measure our successes. There are other problems -- land managers do not know how to plan the use of an insecticide that gives benefits the year following treatment, and land managers in the West have little confidence in Bt.

Meteorology. Results of studies by Canadian and U.S. scientists in recent years have uncovered the mysteries of how atmospheric conditions influence spray deposit, spray drift, and drop impaction on target foliage. Spray meteorology is, however, a complicated process and cannot be treated adequately in this discussion. Prescribing the meteorlogical conditions for a particular treatment must consider the tank mix, application rate, spray release height, topography, forest characteristics (species, density, canopy and height), and drift. Reducing drift often is the overwhelming consideration, sometimes to the detriment of a successful project. As an example, if drift is acceptable we can use small drops to provide good coverage and wind energy to impact the drops on foliage. Results of field studies, wind tunnel tests, and modeling have provided information and an understanding of how meteorology relates to effective treatments. Significant advancements made in this area over the past 5 years allow us to prescribe meteorological conditions leading to successful applications.

Modeling Spray Behavior. The USDA Forest Service has two models that predict spray behavior. These models can be used effectively as a tool in planning spray operations. They can be used to select type and position of nozzle on spray booms, decide on release height, select meteorological conditions, and select the approximate tank mix and atomization. The models predict deposit on the canopy, on the forest floor, and off target. We need to adapt these models to predict the probability of achieving deposition under various atmospheric conditions and forest types. We need the capability to predict, for the land manager, the probability of project success so he can evaluate cost/benefits. The land manager makes the decision, we provide the information for the decision.

Spray Deposition. Studies have demonstrated that conifers are excellent scavengers of spray drops and that the majority of drops are collected by the upper crown. The smaller the drop the greater the chance that the drop will penetrate the canopy and deposit on the ground. The smaller particles (<20 μm) may enter, but eventually be ventilated out the canopy. Studies of spray deposition on the open ground vs on the forest floor show a higher percentage of large drops in the open (there was no vegetation for interception) and a higher percentage of small drops in the forest. On the other hand the majority of drops observed on foliage are less than 40 μm in diameter.

Examining foliage is an excellent way to evaluate the adequacy of spray coverage. Spray deposit papers should be used as a qualitative sampler to monitor quality of application and drift. Deposit papers are inexpensive and simple to handle. When a better method is developed (easier and less costly), this information will be made available.

Wind Tunnel Tests. Prior to the availability of lasers, wind tunnels were limited as a method to obtain information on nozzle atomization. Wind tunnels with particle measuring lasers can be highly effective in providing quantitative data on drop size. The wind tunnel provides a means of identifying the effects of nozzle type and orientation, boom pressure, airspeed, and tank mix on atomization. Information from wind tunnel tests is used for input to models that predict spray behavior and for developing spray prescriptions.

SOME IMPORTANT FACTORS TO BE CONSIDERED IN SPRAY TECHNOLOGY ASSESSMENT

David Smith, Agriculture and Biological Engineering Department, Mississippi State University, Mississippi State, MS.

Dosage mortality curves can be used in the field. By plotting the amount of mortality over deposit, an LD value can be obtained. If the degradation rate of the microbial product is also obtained, the established LD value can then be adjusted upward to compensate for this factor and, of course, this dosage level would be closer to the manufacturer's recommended dose.

There are dosage requirement differences between crop species. For example, to exemplify this, if we look at an adjuvant (this is a gustatory or feeding adjuvant on a soybean crop with Bacillus thuringiensis and Trichoplusia ni), we obtain about 78% mortality; on cotton 12.5% mortality; and cabbage 20.4% mortality. These relative mortality numbers change as you select different microbial-insect combinations on these same crops. For example, good dose-mortality response has been obtained with a cottonseed meal bait suspension applied on soybeans. On the other hand, PVA-carbon gave poor results. The point to be made is when the LD values are adjusted for degradation, formulation, crop, etc., we always fall short of the manufacturer's recommended dose. Thus, there appears to be a research gap that needs to be filled and this would be either in the application-formulation arena or the biological response of the pests.

There is need for some type of standard in many of our application trials. In the literature, one researcher will use one type of test at this location, while others will do different types of tests with entirely different equipment, different materials, etc. Thus, a poor basis of comparison results. Usually even the evaluation methodologies are different.

In the applications area, a number of different types of application studies have been conducted throughout the country. In considering the over-the-top type application, drift spraying has been done using droplets in the 30-50 micron range; with and without electrostatic charges; with oil, water, and also with oil/water mixtures. We have also tried to inject the material into the side of the soybean canopies because these materials are highly solar-sensitive.

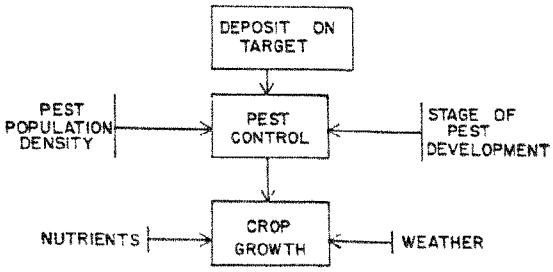
Vertical wedging in soybeans has also been tried. This technique forces leaves to the side so as to spray behind the wedge. We have also tried forcing the leaves vertically so as to spray in between the leaves as they are separating, again trying to penetrate the inner canopy.

The most promising technique is to simply bend the outer part of the canopy over and spray these areas as they are flipping back to an upright position. This increases canopy deposition about 20%. Probably the conventional over-the-top application for ground application is good as anything we have now.

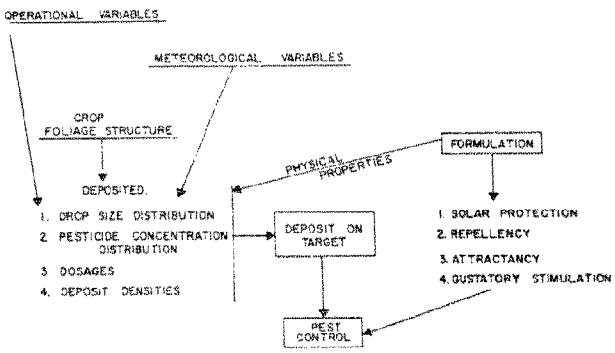
Deposit Variation. The literature indicates that both across-the-swath variations and down-the-row variations, with most of your ground applications, will have a coefficient of variation (CV) ranging from 20-25%, specifically with broadcast applications, on smooth surfaces, and not plant surfaces. When using the crop, the across-the-swath variations with ground applications results in CV's up to the 35% range. In aerial applications with forest canopies, many of these produce 35-50% CV values.

A coefficient of variation of 40% translates into a maximum deposit which is about 3.5 times greater than the minimum deposit within the same swath. There are also problems from an application perspective in getting the material into the inner canopy parts.

With reference to the following table:



We are primarily interested in crop growth with the inputs weather and nutrients being very important. Of course we are also interested in pest control and there are many variables which affect it. Let's first discuss the area of deposit on the target and, specifically, formulation.



First, consider formulation in terms of physical quantities; viscosity, density and surface tension. These properties can affect the droplet size distribution. Physical properties of the formulation can also affect its evaporation rate, and thus the amount of material deposited. Such properties as solar protection, repellency, attractancy, and gustatory stimulation may not affect the deposit on the target, but can affect the level of pest control.

The operational variables (such as pressure, nozzle size, nozzle type, flight height, flight speed, nozzle orientation) can affect the amount of on-target deposit. Meteorological variables (wind direction, wind speed, rainfall, temperature, humidity) also certainly have an effect. For a given crop, pesticide, pest and tank-mix formulation, there are four variables which can affect pest control (i.e., on-target droplet sizes, technical deposit, deposit density [no. drops/area] and tank-mix concentration).

Now let's consider aerial forest spraying at release heights of 50-200 feet above the crop. Drop size distribution, formulation and tank-mix concentration are generally reasonable well known.

Now, what do we know about these descending droplets that are volatile and evaporating, when they reach the canopy?

LOCATION	VARIABLES	KNOWLEDGE ABOUT VARIABLES
AT RELEASE HEIGHT.	1. DROP SIZE DISTRIBUTION	GOOD
	2. TANK MIX CONCENTRATION	VERY GOOD
	3. DOSE	VERY GOOD
AT TOP OF CROP:	1. DROP SIZE DISTRIBUTION	?
	2. PESTICIDE CONCENTRATION DISTRIBUTION	?
	3. POTENTIAL DOSAGE	?
	4. POTENTIAL DEPOSIT DENSITY	?
DEPOSIT ON CROP.	1. DROP SIZE DISTRIBUTION	REASONABLE
	2. PESTICIDE CONCENTRATION DISTRIBUTION	?
	3. ACTUAL DOSAGES	LIMITED
	4. ACTUAL DEPOSIT DENSITIES	REASONABLE
	PEST CONTROL	VARIABLE

Drop size distribution, very little; pesticide concentration inside the droplets, basically nothing (they have been evaporating) and we don't know the concentration or the potential dosage. For example, if we take an imaginary cube right above a tree, we do not know how to estimate the potential dosage. Potential deposit density (how many drops are in a cube above this tree) is also unknown. We know what left the aircraft, but the amount reaching a plane just above the target site is unknown.

There is some information available regarding deposited droplet size distributions and deposit densities. However, information about deposited pesticide concentrations and dosages is either severely limited or non-existent. Our research on row crops indicates that all 4, on-target variables are related to pest control. So when we have worked our way through these application problems, we eventually end up at our primary objective and that being pest control.

MEASUREMENT OF FOLIAR DEPOSITS OF BT AND THEIR RELATION TO EFFICACY

P.G. Fast, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario

E.G. Kettela, Maritimes Forest Research Centre, Canadian Forestry Service, Fredericton, New Brunswick

C. Wiesner, Research and Productivity Council, Fredericton, New Brunswick

Introduction

Interest in and discussion of the relationship between droplet spectrum emitted and droplet spectrum deposited, spray cloud behaviour, the relationship between droplets deposited and efficacy, and optimum droplet size, has increased in recent years and has resulted in a number of collaborative studies addressing aspects of these questions. The questions are particularly pertinent to operational use of Bacillus thuringiensis (Bt) preparations because Bt must be ingested, probably repeatedly, (Fast and Reginiere, Can. Ent. 116: 123-130; 1984) before it can act. Therefore, numbers of droplets deposited, their distribution on foliage, the probability of a larva encountering them, and the dose in a given droplet are important to efficacy. These considerations are as equally important to optimization of applications of contact insecticides, but they do not seem to have been adequately explored. The studies of spray cloud behaviour and deposition by the New Brunswick Spray Efficacy Research Group have established the optimum droplet size for deposition on coniferous foliage, as well as many of the parameters governing spray deposition. Fast and A. Sundaram, have begun the exploration of the effects of drop size, drop density (drops/needle or /cm. sq.) and Bt concentration on the efficacy of BT in laboratory bioassays on foliage. Here we present a narrative summary of the results of a field trial designed to measure the relationship of the number and spectrum of droplets deposited on balsam and spruce foliage in field applications to the emitted droplet spectrum and to the efficacy of BT. Space limits this discussion to a broad brush description of the experiment and the conclusions reached. A detailed description is in preparation. The experiment was jointly funded by Canadian Forestry Service, Forest Protection Limited, and USDA-Forest Service (CANUSA).

Formulation and Application

The Bt formulation chosen was Novabac 3 (32 BIU/gal) diluted to 6.3 BIU/l. and applied at the rate of 30 BIU/ha. Tracer dye Erio Acid Red XB 400 was added at 0.2% w/w, and a sticker, Rhoplex AC33NP (Rohm and Haas) was added at the rate of 2.0%. Studies by W. MacLean, USDA - Aphis, have shown this sticker to be much superior to Chevron sticker.

Laboratory bioassays established that neither the dye nor the sticker affected the toxicity of the Novobac 3.

Evidence from studies carried out in New Brunswick, Cranfield, and in the United States, show overwhelmingly that efficient impaction of drops and coverage of conifer foliage with ultra low volume applications can be achieved only with droplet sizes well below one hundred microns. Steps were taken to ensure maximum delivery in the range 30-50 u after evaporation. Wind tunnel tests performed by the International Centre for the Application of Pesticides (ICAP) in Cranfield, Great Britain, established optimum settings for the AU 3000 Micronair atomizers to maximize the number of evaporated droplets in the < 85μ range. A test spray run prior to spraying the blocks confirmed that 71% of all droplets found on needles were in the 25-75 μ range. Application was made by Cessna 188 flying swaths of 110 feet.

Two plots were sprayed. The first on 27 May, was treated when the balsam shoot development index was 3.8 and the larval index was 3.9 (indices as described by Dorais and Kettela 1982). Rain began within 4 hours of spraying and continued for the next 4 days, so no useful efficacy data was obtained but deposit measurement and foliar bioassays were performed. The second spray was applied 8 June when the balsam shoot index was 5.0 (open shoots) and the larval index was 4.8. Weather following this spray was sunny and warm. While spray weather was grossly the same in both sprays smoke from smoke bombs operated at canopy level showed the air to be much more turbulent during the second spray.

Atomization

Comparison of the volume fractions deposited on needles with the evaporated or target volume fractions reported in the ICAP study, shows that significantly more large drops were emitted than were found on foliage. Since the VMD was 85 μ, the 4% of droplets that were larger than 85 μ contained 50% of the Bt emitted. As drop size increases, this ratio becomes even more disadvantageous.

The effectiveness of Bt deposits on foliage is also directly related to the probability of an individual larva encountering and consuming a droplet, so deposit frequency is obviously a determining factor in efficacy. In the present case, the probability of encounter is approximately ten-fold higher for 30-85 μ drops than for drops over 85 μ in diameter.

Deposit

More droplets were deposited on fir needles than on spruce needles in both sprays, which may

reflect the inherently higher unit foliation of the spruce twigs (i.e. more and smaller needles). On the same species mean deposits were similar for the two sprays, 0.88 and 0.79 drops/needle on fir, 0.54 and 0.37 drops/needle on spruce. The median needle deposits were much lower, about 0.45 drops/needle.

Deposits on balsam fir buds differed significantly between the first and second sprays (.55 drops/bud and 2.8 drops/bud respectively), but on the basis of drops/cm^2 of bud, they were not significantly different, 6.0 and 6.9 drops/cm^2 of bud respectively.

Deposits on Kromekote cards did not correlate with or reflect foliar deposits, averaging 21.1/cm^2 in Plot 1 and 6.2/cm^2 in Plot 2.

Spectrum

Droplet spectra on needles of both balsam and spruce in both sprays were very similar with an average of 75% of all deposits falling in the 25-75 u range. The buds, which were expected to be somewhat inferior collectors of small droplets, in fact collected more in the 0-25 u range (20%) than the needles which averaged 6% in this range. At the present time deposit levels in the 0-25 u range are probably not important due to the low amount of bt carried in these drops; however, future highly concentrated formulations might change the toxicological significance of these size droplets.

Spectra deposited on Kromekote cards were significantly different from foliar spectra with greater numbers in the high diameter range reflecting the now well established differences in impaction efficiency between conifer foliage and large flat surfaces.

The Rhoplex AC33NP sticker appeared to give excellent adhesion in both dry and wet conditions. While no quantitative data on sticker performance were obtained, it was found that individual droplets on foliage were stable to manual handling and remained apparently intact even after prolonged exposure to rain. The dye was seen to wash out of the deposits which, however, remained intact. One question which remains is whether the spores and crystals were contained within the visible deposits or whether they tended to wash out like the dye.

Foliar bioassays

Foliage was collected from nine sites, and five terminal shoots from each site were bioassayed with 10 lab-reared newly-moulted vth instar budworm to give 50 larvae/site. The bioassay was identifical with that used for lab-sprayed foliage.

In the first spray, when the buds were still closed, there was no correlation between deposit and mortality. It was observed that larvae fed mainly inside the bud. This coupled with the low deposit (0.55 drops/bud) resulted in the lack of correlation. In the second spray a good correlation between mortality and deposit was observed. When the data were transformed and plotted as probit mortality and $\log_{10}$ drops/needle, a good regression was obtained with an R^2 of 81.5%. The 20% unexplained variation is probably due to within branch variation in deposit and the difference in deposition on old foliage, where the deposit was determined, and the new foliage where the larvae fed.

The LD_{50} (determined by bioassay) of 6.3 BIU/L Novobac applied at 4.7 L/ha having a droplet distribution with an NMD of 23 u then is just over 1 drop/needle. The median deposit experienced at the nine sites in this part of the study was 0.4 drops/needle. These bioassays clearly demonstrate the dependance of mortality in field applications on the foliar deposit, in terms of both drop size and drop density at a given concentration.

Field Evaluation of Efficacy

Observed survival and defoliation were corrected for expected effects based on prespray larval densities. Survival and defoliation in controls were separately plotted against prespray larval densities on the same sample trees, regressions derived, and the regression used to correct observed defoliation and survival on a sample by sample basis in the sprayed blocks. The corrected effects were then plotted against drops/needle. A clear relationship between drops/needle and both survival and defoliation was observed. Survival was noticeably decreased at 0.5 drops/needle and at the 6.3 BIU/L concentration the field LD_{50} was about 1.0 drop/needle which is in good agreement with the foliar assay and predictions from laboratory studies. Foliage protection (expected minus observed defoliation) also showed a relationship to drops/needle although, because of the low deposits observed (56% of samples had deposits of 0.5 drops/needle), the relationship was less clear than for survival.

No correlation was observed between deposit and total biomass/45 cm branch, biomass of buds/branch, or average pupal weight, because of extreme variation.

Several blocks in the same areas were sprayed under similar conditions with Bt at 12.8 and 14.0 BIU/L (Dipel 176 and Biochem Futura) again applied at the rate of 30 BIU/ha, but depsot was not monitored. These blocks gave significantly better control than the 6.3 BIU/L application used in the main experiment. Because the likelihood that budworm will encounter a droplet is low at the deposits measured on most branches, a higher concentration of Bt in those droplets will be expected to give more efficacious results. This hypothesis is to be tested in 1984 by relating deposit to field survival and defoliation at 32 BIU/gal, 48 BIU/gal and 64 BIU/gal concentrations.

No efficacy/deposit relationships were obtained on spruce in large part because the expected defoliation based on prespray larval counts was not observed.

STABLIZATION AND APPLICATION OF BT

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Introduction

This research report is concerned with: 1). A field application program using encapsulated Bt. The objectives of that program involved spray physics and the delineation of the critical parameters inherent in the delivery of Bt sprays to the microhabitat of the spruce budworm. The field application work was carried out jointly with Dr. Alam Sundaram and the Forest Pest Management Institute (Canada). Most of the data from the field studies will be published in a separate paper with Dr. Sundaram. 2). A laboratory program concerned with stabilization studies on pure Bt and BtI crystals. The laboratory studies on pure Bt and Bti crystals were carried out at the University of Georgia with the objective of providing a baseline system by which environmental parameters adverse to Bt (BtI) could be determined, quantified and controlled.

The application of pesticide sprays in forestry and agriculture, appears to be a simple procedure. It is widely assumed that each of the spray droplets in a spray cloud falls by gravity, over a time period determined by its mass (size), and that the downwind travel of droplets is directly related to that size (i.e., drift is directly related to droplet size). The final assumption of such simplistic concepts of pesticide sprays is that droplets can (fall) into the foliage system and kill target pests in their microhabitats. None of the above assumptions are correct, nor have any experimental proof. They are widely accepted. That widespread acceptance has had a devastating effect on pest management and ecosystem accountability. It is the single most significant cause of the present problems that beset the pest management industry. It provides no basis for survival of the industry into the 21st Century. Major changes, critical to the pest management industry may depend on the emerging science of pesticide spray physics.

The spray application of Bt has used the conventional wisdom of chemical insecticide application. Bt is a particulate toxin, has no contact activity, and requires substantially different spray methodology from chemical systems. The successful use of Bt in pest management requires delivery of a toxic dose to the target insect in a highly specific manner. That toxic dose must be injested by the insect within a limited time frame. That time frame is determined by feeding habits of the insect and by the stability (effective presence) of Bt on the foliage within the microhabitat of the target insect. The mechanism of action of Bt, and particularly the effects of sub-lethal doses of Bt, requires that the "window of opportunity" with Bt be substantially greater than that required of most chemical insecticides. Optimum systems may require that Bt remain stable on the foliage for several weeks. Toxic levels of Bt must be provided on foliage injested during a limited feeding cycle. Substantial residual action of Bt on foliage is important.

There is no general agreement as to the relative stability of Bt (as crystals) or Bt (as spores) on foliage, in the presence of UV light, foliage bactericides, and foliage pH. The second phase of the research reported herein was concerned with the stability of purified Bt and BtI crystals and the development of formulations to stabilize these biological insecticides. Purified Bt crystals were used to provide a base-line system for the study. Purified BtI crystals were used in the study because of the speed and simplicity of its bioassay.

Experimental Data and Conclusions

In 1981, the physics of spray delivery to a spruce forest were studied in a 15-hectare plot near Searchmont, Ontario. In 1982, two 50-hectare plots near Marathon, Ontario were studied. The 1981 research involved the use of encapsulated droplets of vegetable oil as a tracer system. The 1982 research was carried out by application of encapsulated droplets of Bt as a tracer system. During the 1981 test, wind velocity and turbulence was close to zero. In the 1981 test the wind velocity was 13-17 Km/hr. The combined data confirm that spray droplets larger than the range of 100 microns have substantially zero probability of delivery to the microhabitat of the spruce budworm. The delivery optimum (as a function of mass and number) is in the range of 15-50 microns. In general, typical Bt sprays used by present field application methods are not more than 5% efficient in delivery to the spruce budworm. To the extent that commercial Bt sprays are unstable on the foliage, the "window of opportunity" for spruce budworm control by Bt is very limited.

The data in Tables I and II, when combined with the physics of particulate distribution in sprays, and the feeding habits of spruce budworms, provide a basis for prediction that present field methods for spruce budworm control by Bt will continue to be marginal until brought into conformity with applicable physical principles. The quantitative analytical data available from the 1981 and 1982 research are not different from analytical data available from the fluorescent particle spray droplet tracer system (1969), the dry liquids tracer system (1978), the reversibly soluble pigment tracer system (1978), the fluorescent pigment system (1978-79), and by other research in spray

physics which has been publsihed. In contrast, no quantitative data exist to support the widespread, widely accepted conventional wisdom that pesticide spray droplets of "any" size can fall into foliage, and can penetrate to target microhabitats.

TABLE 1
1982 SIZE RANGE AND DROPLET VOLUME DISTRIBUTION: FOLIAR DATA

Drop size range (um)	Average drop size (um)	Frequency distribution %	Cumulative frequency distribution %
2 - 5	3.5	0.00	0.00
6 - 10	8.0	0.33	0.33
11 - 20	15.5	7.81	8.14
21 - 30	25.5	23.51	31.65
31 - 40	35.5	24.48	56.13
41 - 50	45.5	13.83	69.96
51 - 75	63.0	9.00	78.96
76 - 100	88.0	15.15	94.11
101 - 125	105.5	5.89	100.00

VMD = 38u

TABLE 2
1982 SIZE RANGE AND DROPLET NUMBER DISTRIBUTION FOLIAR DATA

Drop size range (um)	Average drop size (um)	Frequency distribution %	Cumulative frequency distribution %
2 - 5	3.5	3.22	3.22
6 - 10	8.0	14.17	17.39
11 - 20	15.5	46.56	63.94
21 - 30	25.5	23.76	87.70
31 - 40	35.5	8.18	95.88
41 - 50	45.5	2.38	98.26
51 - 75	63.0	0.90	99.16
76 - 100	88.0	0.71	99.87
101 - 125	105.5	0.13	100.00

NMD = 13u
Dmax = 110u
Dmin = 3u

The stabilization and formulation studies were approached on the basis of use of purified crystal preparations. The crystals were isolated by renografin gradients, followed by suspension in deionized water. The fluorescence studies were made with crystals solubilized with dithiothreitol. Quartz cuvettes were used to allow study of the absorption and fluorescence emission of the solubilized preparations. In order to determine the effect of a standardized (hard) UV source, crystals were suspended in a quartz cuvette and irradiated for specific periods of time. Aliquots of the suspension were then solubilized and the fluorescence emission obtained. Other aliquots of the crystals were used to determine biological activity. The biological activity studies were limited to BtI because of the ease and speed of the bioassay procedure. At present we are starting bioassay work with cabbage looper (T. ni), and with cotton boll worm (H. zea). The fluorescence data obtained were not different with Bt (HD-2) crystals and BtI crystals. The fluorescence spectra correspond to protein tryptophan (class B proteins) in which the tryptophan exists in a non-polar environment.

Under the experimental conditions used, BtI and Bt cyrstals are photolytically unstable and their tryptophan fluorescence decreases relative to the time of irradiation. In the case of BtI, that decrease in tryptophan fluorescence is directly related to decrease in biological activity. If tryptophan fluorescence can be correlated with biological activity, then it could be a basis for analytical quality control, by spectroscopy. The tryptophan absorption spectra show no significant differences before and after irradiation.

Encapsulated formulations of BtI crystals with UV-Chek® (Ferro Chemical Co.) were prepared and subjected to irradiation using the same UV source under standardized conditions. Under these conditions, unprotected BtI crystals lost all biological activity after 6 hours. At present we have found no loss in biological activity of encapsulated, protected, samples irradiated continuously over a period of 20 days. In control studies, all adjuvant components of the formulation were shown to have no adverse effect on the mosquito larvae.

This research was supported by the Canusa Program of the U.S. Forest Service under grant 23-786.

SPRAY SWATH PATTERNS OF SMALL AIRCRAFT AND VERTICAL DISTRIBUTION OF MICROBIAL SPRAY DEPOSITS

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Introduction

Each year in Northeastern United States over 500,000 acres of oak forests are aerially sprayed to prevent massive defoliation by the gypsy moth. In Pennsylvania alone 400,000 acres were proposed for treatment in 1983 with commercial preparation of Bacillus thuringiensis (Bt).

Preparations of Bt must be consumed by the pest insect to be effective, and cannot be absorbed through the insect's integument like most of the organic insecticides. Therefore, it is important that the Bt spray deposit be distributed evenly over the feeding surface and in sufficient quantity to be lethal. Consequently, the more efficient and precise the application

technique is, the greater chance that the target site will collect an amount that will be efficacious.

Aircraft calibration and lateral spray deposit distribution determinations are generally considered routine activities prior to aerial spray operations, but such results are usually omitted from the literature. Consequently, comparisons between different aircraft is difficult. In addition, the actual relationship between the spray droplet characteristics and their biological activity has vitually been unexplored with microbial pesticides, including those used against the gypsy moth.

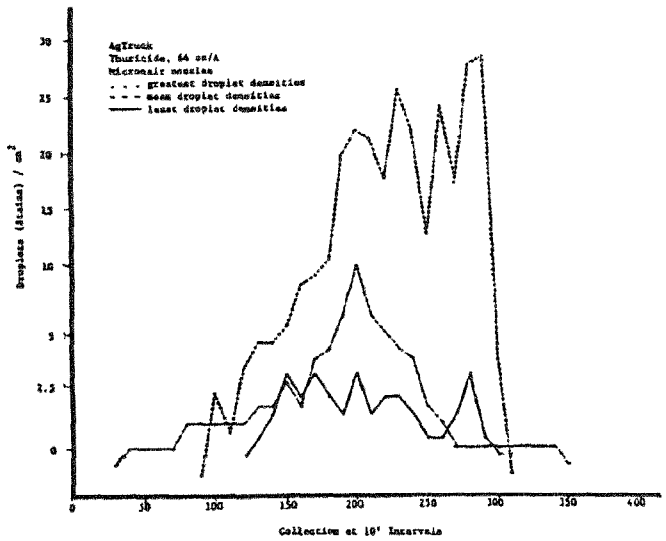
Swath Widths

The term, swath width, is generally defined as "the area (span) in which the amount of spray expressed in various ways equals or exceeds a specified amount thought to produce the requisite effectiveness." In these trials "lateral distribution width" (LDW) was used to mean the detectable spray that was deposited over a surface at various points along a 90° lateral direction on each side of a forward path of the application system.

In 1983 swath width trials were conducted using a Cessna AgTruck®. The aircraft's speed was 120 mph. The nozzle systems used in these trials were the Mini-Micronair (AU5000) and the flat fan, stainless steel No. 8004. Two commercial preparations of Bt, Thuricide 48LV® and Dipel 6L®, were used at the rate of 12 BIU. These formulations were applied at the rate of 64 fl. oz./A. The aircraft was calibrated for a 75' swath width and the spray applied at 40 PSI pressure. White 4 x 5" Kromekote® cards, placed on 2' wire stakes, were spaced at 10' intervals and used to collect the spray emission. The aircraft was then flown 50' over the collection line and into the wind. Most of the LDW applications were made in the early morning hours and when no wind could be detected.

The LDW resulting from the Bt formulations dispersed by the Micronair AU5000 nozzles ranged from 90 to 270 feet with a mean of 188 feet. In a few trials low force cross winds were present and did skew the droplet distribution slightly.

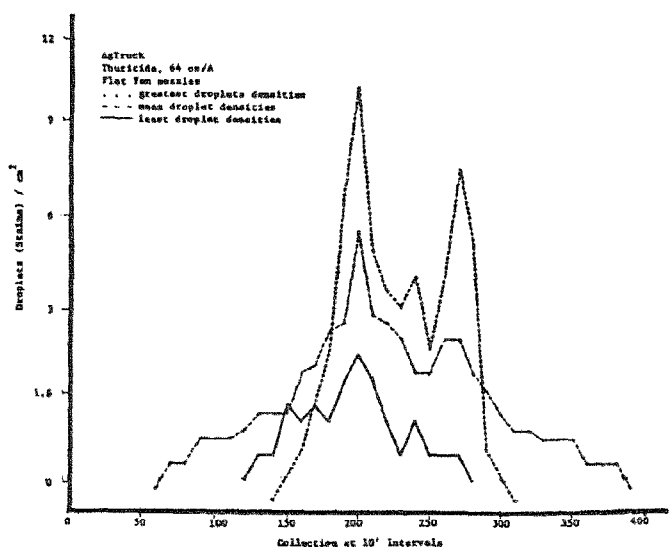
The following figure illustrates the droplet (stains) density spray patterns over the 400' detection line:



Three curves are presented, one showing the greatest droplet density, another the mean droplet density of 12 Micronair trials, and another the LDW curve depicting the trial having the least droplet densities.

The mean number of droplets/cm^2 for the total LDW of the various trials varied from 0.5 to 12.5 with an overall average of 3.5 droplets/cm^2. Peak droplet densities ranged from 28 to 3 droplets/cm^2. In the 24 trials, including Thuricide and Dipel, there was considerable variation in the LDW and/or swath width with regards to droplet density.

The following figure illustrates the droplet density (stains) spray patterns over the 400' detection line for the flat fan No. 8004 nozzles:



These spray droplet patterns show the greatest droplet densities/cm^2, the mean droplet density of the 12 flat fan trials and the LDW curve depicting the trial resulting in the least droplet densities.

The total LDW for Bt dispersed by the flat fan nozzles in a single flight pass ranged from 160 to 400 feet. For all flat fan trials the overall LDW mean was 255 feet. In general, meterological conditions were stable, but wind speeds were detected in some trials ranging from 7 to 10 knots/hour. The mean number of droplets/cm^2 for the total LDW of the formulations ranged from 0.4 to 4.3 with an overall average of 1.8/cm^2. Some collection sites obtained droplet density means of 10 droplets/cm^2, while others were much lower.

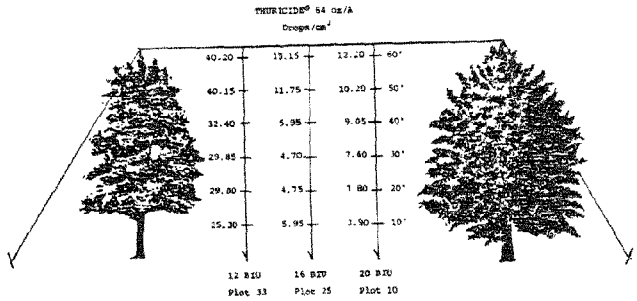
When the replicated trials or flight passes were compared considerable variation was found in droplet density and spray deposited in the LDW patterns. With such variation between trials, it would seem that an applicator would have a problem in selecting an LDW pattern that would more accurately characterize the aircraft type and be used in its calibration. Although some of the prescribed techniques for determining aircraft-spray swath widths or LDW are suitable, there remains the need to develop standard techniques that will provide a more accurate estimation of these parameters for each aircraft type. The following questions still remain unanswered: In calibrating an aircraft should it always be flown into the wind and only when such conditions prevail, or should the swath width determinations be undertaken in "0" wind conditions? What elevations should be used for the swath determinations? Should multiple flight paths be used in addition to a single flight path?

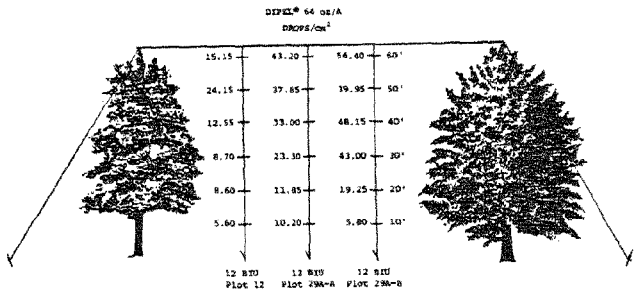
The overall average volume mean diameters (VMD) of the Micronair was 186 µm for Thuricide and 165 µm for Dipel. For the flat fan nozzles, the overall average VMD was 304 µm for Thuricide and 262 µm for Dipel.

Aerial Evaluations

To evaluate spray deposits at various elevations, a series of vertical lines were constructed and secured to a horizontal aerial line. The horizontal line was attached to the canopy tops of two oak trees. Using a cork-paper clip attachment system, collection surfaces could be positioned at various intervals along the upright aerial line, usually at 10' intervals. These aerials were placed in open spaces in oak forests being treated by air with Bt. With this system an evaluation of the physical droplet characteristics including VMD, density, and spray mass per unit area could be made.

The following figures illustrate the droplet densities that were collected at various heights in an oak forest being treated with Thuricide and Dipel at 64 fl. oz./A by air:





When spray droplets were collected at various elevations and their densities compared, the 60' elevation collected at least 3 times more droplets than the 10'. In the case of the Thuricide treatment, the VMD's ranged from 286 to 590 µm. In most trials the bottom card had the largest VMD of 50 µm. These preliminary investigations clearly indicated that additional research is needed to better understand droplet distribution in a free-fall system and within the oak canopy.

GENERAL DISCUSSION

D. SCHMIDT: Having listened to the previous speakers talk about the problems of pesticide aplication, I have a question. "Given the information available and not what you would like to have, and knowing the equipment that the operator has, can you give an estimate of just how effective his spray program will be?"

M. PELLETIER: I really can't answer that question at the operational level -- many times we just cross our fingers.

D. SCHMIDT: I understand your problem as an operator, but I thought that some of our other speakers who have talked about the information they have and what they would like to have would want to know if their operation was going to be a success.

W. SMIRNOFF: Just one comment, your explanation is valid if you study chemicals which are a solution, but with a suspension such as Bt it is not the same. The number of spores per droplet can vary from one to several hundred thousand.

P. FAST: On the contrary, we have laboratory data to show the exact number, or very close to the exact number, of spores and crystals in a droplet.

DISCUSSION PANEL: FIELD TEST DESIGN & DATA ANALYSIS

Panel Members: Daniel M. Schmitt, Chairman; William E. Waters; B. Leo Cadogan; Gerald S. Walton

Panel Chairman:

I think it is time to open the panel. On my left respectively are Bill Waters, Professor of Entomology and Forestry, University of California, Berkeley; Gerald S. Walton, Biometrician, Northeastern Forest Experiment Station, Hamden; and Leo Cadogan, Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario. My name is Dan Schmitt, Program Manager, Northeastern Forest Experiment Station, U.S.F.S., Broomall, Pa.

I am going to ask each of the panelists to discuss field test design and data analysis for pesticide applications in the context of three general areas: (1) field and pilot tests, (2) current approaches to experimental tests, and (3) suggestions and possibilities for improving experimental tests. Bill?

Bill Waters:

There seems to be a general understanding as to the role of field testing, but not as to its purpose. The purpose should be to find the most efficient combination of treatment variables, i.e. formulation, concentration, application rate, atomization, and timing, that will consistently provide a specified degree of control of the target insect and/or foliage protection under operational conditions. The question is not which treatment or treatment combinations perform best, but which, if any, will achieve the desired results. Comparisons are of course part of the process of evaluation, but they are not the purpose of field testing itself.

In order to achieve the purpose of field testing we need to have performance criteria, which should be stated in advance. These criteria are the explicit bases for evaluation. The best way to state criteria is in terms of what remains following treatment, not in how much a population is reduced nor how much defoliation has been reduced. For the insect this means number of survivors, not percentage killed; for foliage, the amount remaining not the amount lost. If we are truly managing pests, we must to be able to make decisions on alternative treatments in terms of probable outcomes over a time span meaningful to the Forest Manager. The models that are being developed for this purpose therefore deal with insect survival densities and amounts of foliage produced over time with and without treatments.

Once we are clear about the purpose of field testing, we can consider the distinction between field experiments and pilot or operational tests. Both come under the umbrella of field testing. Field experiments simply involve experimental combinations of the treatment variables applied under field conditions, with the understanding that the insect is present at measurable densities. Practically, this means a range of population levels causing sufficient defoliation to be considered for control. Field experiments also imply that the equipment employed (unless the manner in which it is employed is itself a treatment variable) is representative of conventional operations.

Everyone is eager to go from the laboratory to the field. However a basic rule is that field experiments should be initiated only when the treatment variables have been reduced to a number that can be reasonably encompassed in an efficient experimental design. Generally, these variables are best combined in factorial designs, but a relatively large number of units are required for even a simple complete experiment. For example, a single replication of a factorial experiment with two levels each of four variables requires 32 plots. In many cases, incomplete designs are necessary. To acquire all of the information needed a programmed sequence of experiments must be planned.

Pilot tests are operational field tests of the treatment found to be optimal by field experiments. The purpose is to determine how well the treatment performs under fully operational conditions. When field experiments fail to distinguish an optimum treatment, there may be justification in subjecting several to a pilot scale operation. In such cases, special care should be taken to acquire the information necessary for objective evaluation, with adequate data taken to assure that comparisons can be made in terms of specified performance criteria. One need only consider the tremendous cost of pilot scale tests to appreciate the importance of sound progression through all developmental steps to the pilot scale test, and the need for adequate data from the test itself.

Currently we seem to design, often simplistically, both field experiments and pilot tests and then compromise them because of 'realities'. I have had much experience with such negotiations and compromise and, in my opinion, they have in many cases killed us, i.e. time and again we have obtained inconclusive results. It behooves all of us to start with basic concepts, objectives, appropriate experimental designs, and adequate sampling, and stick to them.

A related problem affecting the current difficulties in interpreting the results of field tests with B.t. is differences in expectations regarding outcomes--expectations of what constitutes satisfactory performance with regard to suppression of the insect and foliage protection-- and, in fact, of opinion as to differences in expectation of which objective is the most important, or whether there is an optimal balance. If there is not agreement on

what we expect, how the devil can we design an experiment or pilot test to get specific information to evaluate performance? Because we don't have a clear understanding on what constitutes satisfactory performance nor when it is optimal to move from the laboratory to the field, we have a continued history of what I would call premature field experiments.

In listening to the previous speakers, I was struck by the fact that people essentially referring to the same range of data, were supplying quite different interpretations. Obviously, there can be little developmental progress if the same data mean different things to different people.

For the spruce budworm there is a scientific basis from population studies for specifying minimal performance or even better, what the outcome will be. There is a basis in spruce budworm research for saying whether 5% survival is or is not satisfactory, for example. Better, it is possible to state, given x number of insects on a 45 cm branch after a control operation, what the number is likely to be the next season. It is the neglect of population information, and unrealistic faith in efficacy couched in percent population reduction, percent foliage reduction, etc. that has caused public agencies to lose credibility and, in the case of the US Forest Service, to find itself in court. Managers simply do not understand, after being told that an operation on their land resulted in a certain percent population reduction or foliage protection classified as successful -- why, the following season they must spray again.

The only other comment I want to make on future improvement applies to sampling. Our basis for evaluating field tests is the data we gather. The small data sets usually planned for, and subsequent compromises, have left us with a very small data base on which to make evaluations. Our current procedures in this respect are not only scientifically unsound, but irresponsible in view of the terrible price we have paid in credibility. I will make one suggestion regarding population sampling. Since the objective is to determine whether or not the surviving population is at a level specified for a satisfactory control operation, sequential sampling is the most efficient procedure.

Chairman:

Thank you, Bill. Jerry?

Jerry Walton:

I think the main concern in a field experiment is that the objectives be very clear and well defined. Certainly it would not be useful to restrict research objectives nor should the same data on populations, foliation, etc. be collected for every objective. I see the field experiment as a method of answering very specific questions and not as an occasion to collect miscellaneous information.

Nor do I believe that field experiments should be necessarily duplicate operations, e.g. with respect to gallonage and dosage. It may be the purpose of the experiment to establish an optimum, in which case it is efficient to determine the end points for the parameter in question. Whether or not these end points are realistic from an operational standpoint is irrelevant.

Once specific questions, i.e. objectives, have been formulated the place to start is with an adequate design. Constraints on time, space, and funding may make compromises unavoidable. But one must constantly ask if a compromise is really necessary. The availability of suitable test areas is often a problem-- a limiting problem. In such situations some adjustments in ideal design may be necessary. One possibility is to use smaller spray blocks. But if the blocks are too small, the results will not be transferable to operating conditions. So there has to be some happy medium. Knowing costs and other constraints, one can proceed toward the optimal design.

Having mentioned costs, it should be clearly understood that information costs money; you are going to pay for what you get. Also, if you don't get the information, you lose everything. But we often do things that are costly and do not provide useful information. For example, placing deposit collectors next to trees with the idea that the droplet record on the collector can be correlated, by tree, with population reduction or foliage saved. The difficulty here is that population mortality on a single tree is so largely unknown that you cannot establish a correlation that is practically significant. And yet a great deal has been spent on this sort of thing.

There is a tendency also to expand objectives, a feeling that collecting additional information for another objective is a more useful way to allocate resources than intensifying the sampling to collect data relevant to the specific objectives of the field experiment. The result is a waste of sampling effort. I think the Core test[1] is a prime example. You go out with very marginal sampling efforts and many sub-objectives on the assumption that things are going to go right -- and they never do. Any time anybody says, 'well, as long as we are out there we may as well measure ...', don't. It is a waste of time better spent on the collection of relevant data.

Conforming to conventional approaches to field experiments is another insidious means of wasting resources. For example, we tend to use the same post-spray sampling time sequence, irrespective of what insecticide, microbial or chemical, is used, or what species the target insect is. I think a lot of our problems stem from traditional

approaches with little thought to the problem and experimental conditions at hand.

There is another kind of mind set that affects data analyses and interpretation of field tests. I call it the DDT syndrome, the feeling that at least one of the treatments must produce results equivalent to what DDT would have. Saying it, or on paper, this is nonsense. We don't do research this way. But why then is there wide spread resistance to adjusting post-spray population data for natural mortality? It is also traditional to consider a field experiment a success if, using sophisticated statistical testing methods, you can detect whether or not you sprayed anything. That is all "statistical significance" means, and it is hardly a worthy criterion for success.

Do we need to measure foliage protection? I don't think that is the way to ask the question. Better, given the objectives of the experiment, does foliage protection tell us anything? If, for example, the size of the surviving population after treatment compared to pre-treatment size is the issue, foliage information is not relevant. If the rate of mortality is the issue (i.e. how soon the insects die), then the level of foliage protection is an indication of how effective the treatment was. In brief we should clearly think through the purpose of a field experiment, frame the questions to be answered specifically, design efficiently, and collect data, and enough data, to answer the questions, and collect no other data.

Before discussing improvements, I'd like to comment on performance as a general objective of field experiments. My general attitude towards a field test is that it is to derive information. It is my feeling that trying to reach goals that will make the user happy has interfered with doing just that. Interim specfic objectives should be interposed as part of a planned program of testing leading to optimal performance, rather than performance as an objective per se.

Another point, which struck me especially at this meeting, is that operations people normally make incremental adjustments in spray and insecticide parameters to improve efficacy. They may well be ahead of research in appraising the effects of such changes, though without the analysis. So maybe the area in which research can help most would be evaluation of the radical and innovative things that people in the field can't justify.

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[1]Refers to a microbial field test reported in "Spruce budworm core B.t. test -- 1980: combined summary. USDA Forest Service RES. PA NE-506." The chairman of this panel suggested the general approach and imposed funding contraints. Experimental design and sampling methods were the product of an ad hoc committee.

I do not like the current use of controls -- check plots. There is something suspect about their selection, about the way control populations behave. Perhaps it would be better to establish a standard treatment as a basis for evaluating experimental ones.

Again, depending on the objectives, you may not need controls. If the objective is to compare two treatments, the control in the traditional concept does not provide relevant information. Finally, although new statistical methods and approaches to experimental design and data analysis are coming on line all the time, there is nothing in statistical theory per se that is going to make the entomologist particularly confortable in his field experimental work.

Chairman:

Thank you Jerry. Leo.

Leo Cadagon:

There are misunderstandings about field tests, especially field tests involving the use of microbials. Microbials must satisfy biological or natural laws; however when encompassed as part of a spray, their application is governed by certain physical laws. Predicting the behavior of biological entities is very much more hazardous, because of their biological properties, than of abiotic entities which conform to chemistry and physics.

The Canadian Forestry Service has an experimental protocol intended to lead to the registration of the pesticide and all that that implies. The protocol starts in the laboratory. Laboratory investigations treat toxicology, LD-50 assays, half life of the material, formulation tests, analyses of stability, determination of physical and chemical parameters affecting delivery as a spray, etc. In general laboratory investigations are intended to identify problems at an early stage and resolve them. There is no reason, for example, to go to the field and find out 'it didn't stick'.

The next step is single tree application experiments. Here information is gathered on deposit characteristics, more information on stability under more realistic conditions. We then proceed to small scale trials. These are thorough experimental trials. The intention is not just to evaluate potency but to acquire information leading to improvements in the material or its application. We then proceed to semi-operational trials on about 300-400 hectares. These are often laid out or supervised by forest or forest pest managers but data collection and the rigor of analysis approximate the level of small scale field tests.

If all goes well we will have identified an insecticide, its appropriate formulation for the delivery systems which are likely to be used, and performance information over a range of probable conditions. We are now ready to conduct large plot operational trials.

I mentioned earlier that microbials, particularly B.t., have unique properties some of which are very desirable. Consequently there is a temptation to go into the field early to see how it works, bypassing much of the protocol described. If the product fails, one can argue that you should not have gone to the field. But a field season has been wasted and often field tests provide data which suggest that a single factor caused the failure and the manager can fix it. So the product does not go back to the laboratory; the manager "fixes" the problem and the product is field tested again the next year. In this way an error-prone, insensitive, cyclical adjustment process is introduced. In fact microbials are one of the few products where, years after registration, exploratory research that should probably have been conducted prior to registration is still being conducted.

Regarding current methodology I presume Dan (the chairman) was asking for opinions on the B.t. Core tests. Now, conducting research in a natural forest is a difficult task. Conducting research with microbials is doubly difficult. I presume the core tests were initiated to deal with inconsistencies. Although the concept (multi-location field tests, each with the same experimental design) is a good one, I am not sure it can deal with inconsistencies in microbials.

Classical agricultural designs cannot always be adapted to natural forests, although they may be suitable for plantation R&D. Designs for forest research must consider the many constraints that are evident in natural forests. One of the most restrictive for formulation and application R&D is the availability of adequate sites -- those with suitable insect populations, accessibility, and which are conducive to safe aircraft operations. The design must take into account logistical requirements, be realistic, and not carved in stone.

However the original design should still be optimal. If absolutely necessary, compromise! For example I do not think you can find enough areas in Ontario to conduct a truly randomized design involving more than two variables. Last, but not least, you have to recognize the "Chancellor of the Exchequer". We can always design the experiments we like, but the money might not be there.

Finally, after listening to the discussions of the past two days, I feel that perceived difficulties with products, formulations, and delivery systems, might be really design problems. It should be recognized that design includes specifications for implementation, including sampling. We need to honestly look at the procedures we employ in relation to design.

Regarding improvements in current methods, I think first of all we have to tackle attitude. Researchers in microbials are perceived to be promoting, not testing, microbials.

As mentioned there is room for improvement in design. Whatever the outcome of the core test, to the extent it was a cooperative effort, it was a start in the right direction. I think all the actors need to get together very early and settle design problems. Positive controls in the sense of a standard treatment are worth looking at, but they add expense. Actually I have no problem with check plots provided they are representative of the area and the population. Sampling is a problem, but it is one we can deal with.

Analyses and interpretation of microbial field tests are difficult. Anyone who has seen Ed Kettela's presentation ("Review of problems with efficacy of B.T.K. in spruce budworm control operations in the Maritime Provinces of Canada" in this Proceedings) will appreciate what I am talking about. For example if A, with 98% defoliation is significantly different than B, with 96%, practically what does this mean? Probably nothing. We have an obligation to carefully and objectively interpret data and not to take refuge behind a barricade of statistics and meaningless statements of "significance". Thank you.

Chairman:

Alright, we will now entertain questions and comments. Wladimir?

Dr. Wladimir Smirnoff. Dr. Waters commented that field tests should be representative of operational conditions. But if the product or formulation provided for experimentation is under or over strength interpretation of the results is going to be very very difficult.

I'd also like to comment on Leo's remarks on registration. Because a product is registered people spray big territories with it without paying much attention to the label and also there can be a problem of the type I just mentioned. Sometimes believing in the registration without regard to the label or professional research advice can lead to big mistakes.

People also don't appreciate true costs compared to out of pocket costs. For example in the name of economy some applicators prefer to increase the dosage, and volume rather than perform calibration tests, and so artificially increase the cost by 50%. Others try products which worked on 6th instar larvae which is ridiculous since defoliation is already completed.

I agree with Dr. Waters that we must have standard criteria for evaluating pesticide applications. Currently criteria fluctuate from operation to operation, and it is impossible to fairly evaluate performance.

Next, combining B.t. with some chemical is unacceptable since it has never been studied.

The dye Erioacid red, for example, has been added at the rate of two kilo/100 gal. regardless of the effect on the formulation. No further comment.

Chairman:

Thank you, Wladimir. I believe you were endorsing some of the panels' comments. I think the panel tangentially addressed the matter of information expected from field vs pilot tests. Does anyone from the audience care to comment. Jim Bean?

James Bean:

Listening to the discussion, I am sure Bill (Waters) went back to probably 1962 because he is saying some of the things he said then. And it is just as apropos today. There is a definite need to start at the beginning and get good basic information. I agree we have often gone to field tests too soon.

John Cunningham:

I never really thought of two particular categories of tests. Viruses are rather a peculiar type of biological insecticide and it is probably necessary to take them to the field at a fairly early stage to determine if you will get a virus epizootic. In other words you want to see if there is a cummulative effect.

You can get great results in the laboratory, but in the field there may be a success or a total failure. The forest includes a range of factors that cannot be evaluated in the laboratory. John Podgewaite may want to comment on this as he is working with Gypsy Moth virus. So I feel for potential virus candidates, the only thing to do is to take them out and try them in the field at a fairly early stage. The alternative is to use viruses as B.t. -- in which case they become fairly expensive stomach poisons. We really can't go this route with viruses.

Another factor, not mentioned so far, affecting field tests is the health of the target population. Now older populations of budworm become heavily infected with microsporidia. There is extremely little work done on the differences in population quality and how such differences affect insecticide applications, although Wladimir has done some research on this subject in relation to B.t.

I conducted some small laboratory tests and find high mortality following NPV infection, if the larvae are previously infected with microsporidia or B.t. On the negative side virus yield from such insects is very much reduced.

Another factor that crops up in the field is fungus epizootics. They may affect your check plots or treated plots. The only way to check this possibility is to examine your population samples microscopically in the laboratory.

Chairman:

Thank you John. You did raise an issue -- population quality -- that needs to be more thoroughly explored. And, of course, there is another, host quality. The panelists and the comments from the audience have stressed the inadequacy of information collected. And here I will raise a question. It is customary to collect information on current defoliation. In the case of microbials, in a research sense, is this the information we want? Anybody care to comment?

Paul Fast:

I will. If you plot some population measure, larvae per bud, or buds per larvae, against defoliation, you will get the following kind of relation (illustrates). The point is you can measure the variance about the regression or correlation and get a darn good idea of what effect your measured population is having on defoliation. We find that larvae/bud instead of larvae per branch provides a narrower variance.

Now once your experiment is finished you can plot the location of your test plot on the graph. You can then see by inspection whether it falls within the confidence limits of the graph and determine whether you got the desired effect.

This is more expensive because it means doing three or four or five checks at different population levels in order to get the curve, but it is much higher resolution than using one or two or several check plots to compare differences in a number of parameters between treated and check plots. In brief the resolution of the evaluation technique we currently use is not good enough to detect the kinds of differences we are interested in.

Chairman:

Thank you Paul. Wladimir, you wanted to add something?

Wladimir Smirnoff:

Yes. Based on my figures of foliage protection obtained over many years in permanent plots with careful measurement of larvae/bud, larvae/branch, dead larvae, new buds, etc. before and after treatment, I can say that it is possible to project foliage protection in terms of tons/hectare/year.

Chairman:

Thank you Wladimir. Now I'd like to add a thought regarding lab tests, field test, pilot tests. The idea, which has been roundly trashed everytime I mentioned it, was that the lab tests should provide all the information on toxicology, stability, resistance to water, etc. and the purpose of the field test then was to essentially

raise the question of why the field test did not come out like the laboratory test.

It sounds like a fairly ridiculous idea, but it occurred to me that if such a research protocol had been in place, the need for the kind of research that Charles Wiesner, Paul Fast, and others are doing now, would have become evident much, much sooner.

Dr. Waters:

I don't think that is a legitimate or relevant question. Admittedly, laboratory research is the foundation for the field experiments, but the questions to be raised and the hypotheses to be tested in the field experiments are of a different sort, having to do with operating characteristics of the material. The information base for the two kinds of research is not the same. The lab tests lead to the field tests, but one doesn't test the results of one against the other.

Chairman:

I won't pursue this matter any more, but will turn to a subject first mentioned by Dr. Waters -- the need for programmed experiments. He pointed out that experiments could be conducted over time. That is a missing ingredient in field testing -- a total design in which time is one of the variables and in which one set of factorials would be put in one year and the total experiment might require three years.

Imants Millers:

The problem is that, in B.t. in particular, the product changes faster than you conduct the research. For example of what value were the core tests? Current products in use are completely different than the tested ones.

Oswald Morris:

I have heard a lot of rather wild statements this afternoon and so will make one of my own. First, I do not think there is any such thing as a failure or a negative result in research. Research is a continuum. Negative results are merely steps in a positive direction.

Carol S. Glenister:

I am a private contract researcher working with Gypsy Moth. People concentrate on evaluating levels of defoliation when they do not know what the volume of leaf tissue is in the field. Assume you have a population, one population, and it will consume 100 kg. of leaves. If the volume available is different in the plots -- and you don't know it -- the percent defoliation will be different. Add different treatments and different populations on the plots, it is easy to see that the current expression of the defoliation is meaningless.

Dr. Waters:

This is just one of the problems in expressing results in terms of defoliation. There are other problems with using defoliation as a measure of treatment effectiveness. The use of check plots for this purpose is particularly questionable. These problems can be avoided by getting sample counts of known precision and using them to relate to populations in treated plots.

Carol Glenister:

In the lab you have known mortalities and known population. In the field you essentially don't know mortalities and you think you can go from percent defoliation to residual population. But you can't unless you know what the volume is that is being consumed.

Now there are models available in which foliage weight can be estimated from tree diameter. You can still use visual percent defoliation estimates but you would have to convert it back to volume consumed in the stand. You can then better estimate what happened following treatment to your population and then compare percent mortality in the field with lab mortality.

John Cunningham:

One thing we need to remember in expressing results, like "foliage protected," is the client. In gypsy moth the client is usually the home owner; in budworm, the forest manager, etc. Each insect affects a different client, so you need to express results that are meaningful in the context of the client's problem.

Leo Cadogan:

Yes, but the researcher's clients also differ at the experimental stage and the field stage. In research I think our clients are our colleagues or peers, and it is very difficult to say to them, "it didn't work" or, "it was fairly good." That is fine for the homeowner, but not for persons who expect a more definitive statement of results.

Dan Kucera:

Getting back to the availibility of virus suitable for field and pilot testing, cooperators who provide such areas are increasingly concerned about environmental impacts. I think in the future we are going to have to be very careful in telling the land manager what we plan to do, how we are going to do it, what we hope for in our results; and we must assure the land manager that we are going to take all the precautions we can. Without this we are going to be very restricted in what we can actually test.

(At this point the panel ended)

BIOASSAY OF FORMULATIONS OF *BACILLUS THURINGIENSIS* FOR USE IN FORESTRY: PANEL DISCUSSION OF THE ROLE OF THE BIOASSAY IN STANDARDIZING FORMULATIONS OF *B. THURINGIENSIS*[1/]

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The panel discussed various aspects of *Bacillus thuringiensis* formulations and fermentations and concluded that the only means at present of standardizing these formulations or discovering more potent strains of the *Bacillus* is through carefully controlled bioassays.

Introduction -- Dulmage

The basic problem in the use of *Bacillus thuringiensis* (*B.t.*) for the control of either the gypsy moth, *Lymantria dispar*, or the spruce budworm, *Choristoneura fumiferana*, is not whether it can work -- it can, as I think that we have seen from the papers that we have heard during these meetings -- but whether it can work reliably. And difficult though it may be to achieve, "reliability" means that we must assure the user that the amount of activity present in a *B.t.* formulation is what the label says it is -- each and every time. This means that the quantity of delta-endotoxin in a formulation (i.e., the potency of a formulation) must always be the same, and that the remainder of the formulation will be assembled in such a way that the efficacy of the formulation will repeat from application to application.

Potency and efficacy depend on each other -- how much delta-endotoxin a formulation contains and how effectively a formulation performs are, and always will be, interdependent. If we restrict ourselves to a specific number of IU's/mg or ml in a series of formulations, the effectiveness of the formulations will then depend on the different qualities of the formulation ingredients. This concept frequently gets lost in our consideration of *B.t.* (or, for that matter, any microbial insecticide). Most people are able to accept the concept that changes in composition of the carriers in chemical formulations can make formulations containing the same quantities of active chemical ingredients (say a synthetic pyrethroid) more or less effective in field applications. In the case of chemical insecticides, we realize that, to define a formulation, we need to know both the level of active ingredients and the recommended application rates of formulations of chemical insecticides like the synthetic pyrethroids. We accept this and agree that both are needed on the label. The same applies to a *B.t.* formulation. Just as in the case of a chemical insecticide, we also need to know both the level of active ingredients (IU/mg or ml) of the delta-endotoxins produced by *B.t.*, and we need to know the recommended application rates of the *B.t.* formulations. Here too, both are needed on the label. I don't know why, but what seems clear and well understood and accepted in the case of chemical-based insecticides, is not clear, understood, or accepted in the case of *B.t.* formulations. In the case of *B. thuringiensis*, if formulations produced by two companies contain the same levels of delta-endotoxin as measured in bioassays, but have different effectiveness in the field so that the recommended application rates are different, then it is frequently claimed that the bioassays that determined the IU's/mg or ml of the *B.t.* put into the formulation must be wrong. It is then concluded that biological materials such as *B.t.* cannot be standardized.

This conclusion is not new -- it arose in the early days of the commercial production of *B.t.* Because of it, companies and scientists predicated much of their work on the belief that "there is no reliable bioassay of *B.t.*" As a result, each company producing *B.t.* developed its own method of standardizing its product -- methods ranging from spore counts, to microscopic crystal counts, to bioassays against this insect species or that, to, I sometimes used to think, assessing which planet was in which sign of the zodiac at the time the formulation was prepared. This was wrong then -- and it is wrong now. Formulations of *B.t.* can be standardized, and once the effectiveness of a given composition of carriers and IU's has been determined, formulations can be made reproducible by assaying the quantity of delta-endotoxin contained in the formulation and keeping it constant. The user can rely on the IU's determined in a properly designed bioassay. The design of the bioassay -- and this will be covered in detail by Dr. Beegle, may have to be adapted to the situation -- certainly, Dr. Beegle and industry in a recent -- and I think outstanding -- cooperative study have greatly improved our bioassay procedures and our understanding of these procedures. Thanks to their work bioassays of even the newer flowable formulations are reliable, the IU's expressed on the label are accurate, and the efficacy of the product can be trusted. We have suffered in the past from the practice of each company designing its own bioassays and procedures. This led to confusion (and really to chaos) to the user, and,

[1/] Revised and edited proceedings of a panel discussion held in conjunction with the meeting of CANUSA, Windsor Locks, CT, April 12, 1984.

as the user tried to follow IU's determined in different ways, he lost faith, not only in the formulation that he was using, but in B.t. itself.

"Trust" is not meant to imply that any one company might intentionally market an inferior product, disguising it with faulty assays. But, as will be seen, improper design of bioassays can lead to misleading potency determinations. There has to be some agreement between producers, the USDA (ARS and FS), and the government regulatory agencies as to what we are measuring and how we should measure it. We must not let the competitive urge lead us to forget how important the standardization of bioassay procedures between producers is. This point should be reiterated: Let each company go its own way and put its own IU's on its labels, and we are going to so confuse the user that his trust in B.t. will be destroyed.

Fortunately, the producers of B.t. realize this, and industry and the USDA, under the leadership of Dr. Beegle, has been exploring improved ways to standardize B.t. taking into account the various problems that have arisen in the standardization process, and Dr. Beegle will now discuss these problems and what is being done to coordinate bioassays.

Problems in Standardization -- Beegle

Formulations of B.t. are standardized in bioassays against a selected test insect. In these assays, the test insect is exposed to diet containing variable levels of the B.t. formulation and, after a suitable period of incubation, the percentage kill for each level is recorded, and the LC_{50} of the formulation is determined. The LC_{50} of the test formulation and the LC_{50} of a standard reference material tested at the same time against the same population of insects are compared. Since the IU/mg of the standard is known, the potency of the formulation can be determined by the following formula:

Potency, IU/mg, test sample =

$$\frac{LC_{50}\ \text{Standard} \times \text{Potency Standard, IU/mg}}{LC_{50}\ \text{Sample}}$$

In 1971, representatives of the three commercial producers of B. thuringiensis in the United States and the USDA met in Brownsville, Texas, and agreed in principle to a standardized bioassay procedure using larvae of the cabbage looper, Trichoplusia ni, to standardize the potencies of their respective products (Dulmage et al. 1971). Because of the adoption by industry in 1970 of a much more potent strain of B. thuringiensis, which was of a different serotype than earlier B.t.'s, the Pesticide Regulation Division of the U.S. Environmental Protection Agency requested that a standard be produced and adopted which was the same serotype as that being commercially produced (subsp. kurstaki). This was accomplished in 1972, when HD-1-S-1971 was adopted as the Primary U.S. Reference Standard with an assigned potency of 18,000 IU//mg (Dulmage 1973). Recently, the supply of HD-1-S-1971 became exhausted, and a new standard, labeled HD-1-S-1980, has been prepared and assigned a potency of 16,000 IU/mg after comparison with HD-1-S-1971 and E-61. Today all preparations of B. thuringiensis produced in the U.S. for use against lepidopterous larvae are standardized against either HD-1-S-1980, or an internal HD-1 standard which was initially standardized against HD-1-S-1971.

In 1980, a problem emerged in the standardization of lepidopterous-active B. thuringiensis preparations. The Brownsville USDA laboratory was contracted by the U.S. Forest Service to bioassay the tank mixes of the flowable B. thuringiensis products, Thuricide and Dipel, used in the CANUSA B. thuringiensis Core Tests. The potencies determined by Brownsville, using neonate T. ni larvae and diet containing antibiotic, were only 40-60% of the label values. Both commercial products were standardized using 4-day-old T. ni larvae and diet without antibiotic. In 1981, research was undertaken by the USDA and the Upjohn Co. to determine the reason for the difference. In these studies two preparations of B.t. from the Upjohn Co. (SOK W and SOK L) and two formulations from Abbott Laboratories (Dipel WP) were tested. The results are shown in Table 1. In the case of the SOK wettable powder (W) and liquid (L), it is clear that the use of 4-day-old larvae in the bioassay results in higher determined potencies than when neonate larvae are used. In the case of the Dipel wettable powders (WP), one of them (0452BJ) was larval age sensitive and the other (F-1980) was not. In my experience that is typical of Dipel products -- some are larval age sensitive and others are not.

In addition, the USDA and the Forest Service set up a blind test where differing amounts of viable HD-1-S-1980 were added to Sandoz and Abbott flowable carriers. These samples, along with flowable Dipel and Thuricide samples were coded and sent out for assay to the USDA laboratories in Brownsville, Texas, and Columbia, Missouri, and to Abbott and Sandoz. The results of these assays are presented in Table 2. Except for two values (2870 for Thuricide, and 16,100 for Dipel, the results in Table 2 for the commercial flowables substantiates the results observed in Table 1 concerning the commercial flowables: the use of 4-day-old larvae in the bioassay resulted in significantly higher potencies than when neonate larvae are used. It was shown by Beegle et al. (1981) that 1-day-old T. ni larvae respond the same as neonate larvae in B. thuringiensis bioassays. It is noteworthy that larval age did not significantly affect the potencies of the samples where HD-1-S-1980 was incorporated into the commercial flowable carriers. This indicates that the observed larval-age-difference effect on determined potencies is due to differences in the bacteria rather than in the formulation. In fact, Yamamoto (pers. comm.) has cautioned that anyone who uses the HD-1 isolate

Table 1. Effect of Larval Age and Antibiotic on Potency Determinations of Commercial *Bacillus thuringiensis* Products.[a]

Preparation	*Trichoplusia ni* Larval Age	Antibiotic in Diet?	USDA Brownsville No. Assay	USDA Brownsville $\bar{X}$ IU/mg or $1X10^3$/ml ± SD	USDA Brownsville % CV of Assays	Upjohn No. Assays	Upjohn $\bar{X}$ IU/mg or $1X10^3$/ml ± SD	Upjohn % CV of Assays
SOK W 747 JF	neonate	yes	3	13,600 ± 814	6	5	11,600 ±1910	16
SOK W 747 JF	neonate	no	3	15,400 ±1510	10	6	13,400 ±2040	15
SOK W 747 JF	4-day-old	yes	3	27,700 ±5300	19		-	
SOK W 747 JF	4-day-old	no	4	30,100 ±5370	18	7	36,300 ±6930	19
SOK L 645 JB	neonate	yes	6	3,640 ± 692	19	8	2,950 ± 514	17
SOK L 645 JB	neonate	no	3	3,640 ± 369	10	4	3,700 ± 534	14
SOK L 645 JB	4-day-old	yes	3	6,310 ± 936	15		-	
SOK L 645 JB	4-day-old	no	5	7,850 ±1310	17	6	9,420 ±1260	13
Dipel WP 04523BJ	neonate	yes		-		9	12,300 ±1670	14
Dipel WP 04523BJ	neonate	no		-		2	12,100 ±1310	11
Dipel WP 04523BJ	4-day-old	no		-		3	24,300 ±1580	6
Dipel WP F-1980	neonate	yes	3	14,800 ±2280	15		-	
Dipel WP F-1980	neonate	no	4	16,300 ± 594	4		-	
Dipel WP F-1980	4-day-old	yes	3	12,000 ±2490	20		-	
Dipel WP F-1980	4-day-old	no	4	11,500 ± 675	6		-	

[a] Taken from Beegle et al. (in press).

Table 2. Results of Blind USDA-Industry Bioassay Test.[a]

Laboratory	Larval Age	No. Assays	Range of CVs	IU/mg 01-S 1980	02-S 1980	03 Thuricide	04-A 1980	05 Dipel	06-A 1980
USDA-Brownsville	neonate	18-20	15-18	1700	2610	1290	4170	5630	5620
USDA-Columbia	1-day-old	3- 4	-	1660	2010	1380	4210	16,100	6620
Sandoz	4-day-old	-	9-19	1960	2760	3970	3310	8110	4520
Abbott	neonate	6-14	5-15	2340	2870	2870	3180	3720	3480
Boyce Thompson Calculated	-	-	-	1720	2570	3430	3810	8800	5800

[a] Lewis, F.B., Report on Blind *BT* Sample Bioassays, USDA-Forest Service, New Hamden, CT, 1982.

to study the genetics or chemistry of *B. thuringiensis* should keep in mind that there are differences in the plasmid and P-1 HPLC patterns among cultures labeled as HD-1.

Because of the discovery that the use of a standard does not correct for differences in assay procedures in all cases, the USDA and the U.S. producers and/or marketers of *B. thuringiensis* products held a meeting in 1982 and decided that we needed a new standardized lepidopterous *B. thuringiensis* bioassay that everybody would follow. The following summarizes the decisions reached at that meeting:

1. Rearing

   Origin of *T. ni* colony, rearing diet, rearing conditions, and rearing techniques and procedures will not be standardized.

2. Bioassay

   a. Diet - Brownsville diet without antibiotics or KOH.
   b. Diluent - deionized or distilled water.
   c. Sample homogenization - sonicate in bath sonicator for 5 min. at sonicator loading of 3 ml (container size)/watt.
   d. Number of dilutions - enough for a minimum of five on-curve dosage mortality points, at least two above and two below LC_{50}.
   e. Type of dilutions - 3:4 (75%).
   f. Diet incorporation - 1:10 with 55°C diet, mix for 2 min.
   g. Number of insects/dose - minimum 25.
   h. Age of larvae - 4-days-old at 30°C.
   i. Holding (infesting) density - multiply or singly.
   j. Holding time and temperature - 4 days at 30°C.

3. Potency Determinations

   a. Each preparation must be bioassayed at least three times over at least two days (three days preferable).

b. A mean potency value for a preparation should consist of at least three potency values from valid assays whose group coefficient of variation is less than 20%.

c. Valid assays are defined as those with a minimum of five on-curve points, with at least two above and two below the LC_{50}, significant regressions, significant F values, and 95% confidence values of less than a 3X range. Assays with atypical slopes, significantly high X^2s, erratic points, or 95% confidence value ranges greater 2X, but less than 3X, should be discriminated against if necessary, but not be considered invalid.

Table 3 is presented to establish the validity of the determined potency of 16,000 IU/mg for the new U.S. B. thuringiensis HD-1 standard. E-61, which had an assigned potency of 1,000 IU/mg; HD-1-S-1971, which had an assigned potency of 18,000 IU/mg; and HD-1-S-1980 were concurrently bioassayed by four companies and USDA-Brownsville. On the basis of over 200 bioassays, it was determined that HD-1-S-1980 had a potency of 16,000 IU/mg. We have known for years that HD-1-S-1971 was not homologous with "normal" HD-1's in its activity towards larvae of Heliothis virescens. Since HD-1-S-1971 is a little over twice as active toward H. virescens larvae than most HD-1 preparations, the Tn/Hv ratio (the ratio will be discussed in the next section, but represents the comparison of potencies determined against T. ni and those determined against H. virescens) of most HD-1 preparations is a little over 2. HD-1-S-1980 appears to be homologous in this respect to most HD-1 preparations, as the mean Tn/Hv ratio of 87 HD-1 preparations assayed against this standard at Brownsville was 1.04.

Reproducibility and the Activity Ratio -- Dulmage

One of the most important and fascinating aspects of the delta-endotoxins produced by B. thuringiensis is that different subspecies -- and, indeed, isolates -- of this Bacillus species, kill different insects or differ in their relative activities against them. Dr. Beegle has discussed the relative differences found between different isolates of subsp. kurstaki in their activities against T. ni and H. virescens. This can lead to two legitimate concerns: (1) Will the same endotoxin be produced in repeat fermentations, or will the type of endotoxin vary? (2) Can we use one insect species in our bioassays -- and does that have to be the target insect? All this, of course, leads to the label and what influence all this has on what is put on the label.

These are important questions and pose serious problems. Table 4 illustrates the differences between subspecies and isolates. The table shows the spectra of insecticidal activity of a series of different B.t.'s produced by subsp. alesti, kurstaki (k-1), kurstaki (k-73),

Table 3. Standardization of HD-1-S-1980.

Sample	Standard	IU±SD	CV	No. Assays
		Abbott Laboratories		
E-61	HD-1-S-1971	853± 150	17	14
E-61	HD-1-S-1980	827± 145	17	14
HD-1-S-1971	E-61	21,800±4390	20	14
HD-1-S-1971	HD-1-S-1980	17,500±1560	9	14
HD-1-S-1980	E-61	19,900±3640	18	14
HD-1-S-1980	HD-1-S-1971	16,000±2770	17	64
		Biochem Products		
E-61	HD-1-S-1971	758± 191	25	9
E-61	HD-1-S-1980	683± 342	50	9
HD-1-S-1971	E-61	24,600±4600	19	9
HD-1-S-1971	HD-1-S-1980	16,000±4090	26	9
HD-1-S-1980	E-61	25,700±6180	24	9
HD-1-S-1980	HD-1-S-1971	19,000±4390	23	9
		Sandoz, Inc.		
TS[a]	E-61	21,500±2040	4	13
HD-1-S-1971	E-61	16,600±5820	15	8
HD-1-S-1971	TS	15,900±5760	17	12
HD-1-S-1980	E-61	14,200±2440	7	7
HD-1-S-1980	TS	14,900±3230	11	36
HD-1-S-1980	HD-1-S-1971	14,700±4030	11	6
		Upjohn Co.		
E-61	HD-1-S-1971	1,135± 381	33	3
E-61	HD-1-S-1980	1,319± 260	20	3
HD-1-S-1971	E-61	17,000±4950	29	3
HD-1-S-1971	HD-1-S-1980	18,000±3592	20	8
HD-1-S-1980	E-61	12,400±2120	18	3
HD-1-S-1980	HD-1-S-1971	16,500±3070	19	8
		USDA Brownsville		
E-61	HD-1-S-1980	1,280± 247	19	32
HD-1-S-1971	HD-1-S-1980	21,300±4170	20	42
HD-1-S-1980	E-61	12,900±2570	20	30
HD-1-S-1980	HD-1-S-1971	15,700±1990	13	23
		All Sources Combined: Nonweighted		
E-61		980± 260	27	84
HD-1-S-1971		18,700±3070	16	119
HD-1-S-1980		16,500±3800	23	209
		All Sources Combined: Weighted[b]		
E-61		1,010± 392	39	84
HD-1-1971		19,600±4360	22	119
HD-1-S-1980		16,000±3139	20	209

[a] Sandoz internal standard.
[b] Weighted as to number of assays.

galleriae, and aizawai. As the table shows, there is a wide difference between them in their activities towards lepidoptera and in their relative activities against different insect species when they are active against them. (One variety, not included in this table, is not even active against lepidopterous insects, although it is extremely active against mosquitoes and aquatic black flies.) With this proliferation of different spectra, the problem of standardization seems almost insurmountable. Industry, Dr. Beegle, and myself all agree on the desirability of using T. ni as the assay insect of choice for the present commercial delta-endotoxins active against lepidopterous insects. Yet, as the table shows, the existence of delta-endotoxins with different spectra of insecticidal activities makes it perfectly possible that the potency of delta-endotoxins produced by two isolates of B.t. may be the same when measured against T. ni but may differ considerably when evaluated against L. dispar. However, if the same type endotoxin is produced in replicate fermentations of each of these isolates of B. thuringiensis, then our problem merely parallels the case with different formulations of chemical insecticides, where the same quantities of a chemical in different formulations may differ widely in their effectiveness in control of a target insect. In the case of the chemicals, the differences arise from differences in their formulation. Microbial formulations may vary for this reason too, but with microbials, the differences may also reflect differences in the chemical structure of the delta-endotoxins produced by different isolates, i.e., the basic activity of the endotoxin. With our present knowledge, we do not know what these differences are -- remember, we cannot weigh or identify in a quantitative chemical assay the delta-endotoxins that we are producing -- we only measure their activities: i.e., what insects they kill and how well they kill them.

Table 4. Spectra of Activities of Selected Subspecies of Bacillus thuringiensis.

Culture No.	Variety	Crystal Type	Potency - IU/mg Measured vs.: T. ni	H. vir.	H. cunea	B. mori
HD-1	kurstaki	k-1	39800	15400	47200	50500
HD-73	kurstaki	k-73	23100	34500	12800	Inact
HD-263	kurstaki	k-1	39600	54600	18100	18400
HD-70	alesti	ale	Inact	Inact	47900	34000
HD-160	galleriae	gal	5920	894	23800	4100
HD-275	aizawai	aiz	8110	969	19500	44400

To illustrate the problem, let us create a hypothetical case of two formulations of B.t., each containing, say, 10,000 IU/mg of activity as measured against T. ni. However, one is several times more active against L. dispar than the other. How do we standardize formulations of each for use against this insect? Must we measure each and every formulation only against every target insect that we desire to kill? Such a path will lead to chaos and confusion -- and very likely to the need of a multiplicity of labels for each B.t. formulation. Fortunately, there seems to be an alternative.

Potencies of formulations of B.t. delta-endotoxins are determined relative to a selected reference standard, as Dr. Beegle has discussed. If the formulation being tested contains the identical endotoxin to the standard, then it will not matter which insect species is used in the bioassay. However, if the test endotoxin and the standard endotoxin are not homologous -- i.e., if they differ in their spectra of activity -- then the choice of insect will have a profound influence on the IU's determined. For example, a formulation of subsp. alesti would be considered inactive if bioassayed against T. ni, but would be considered very active if assayed against the webworm, Hyphantria cunea. In a less extreme example, a formulation of subsp. kurstaki (k-73) might have a potency of 20,000 IU/mg if measured against T. ni, but have a potency of 40,000 IU/mg if measured against H. virescens. By constructing a fraction from these data, we can derive an "Activity Ratio." An activity ratio of a formulation is simply a straight-forward comparison of the activity (in IU's) against two different insect species. Thus in our example here, the Tn/Hv activity ratio is simply determined by dividing the activity determined against T. ni by the activity found against H. virescens, as shown in the following equation:

$$\text{Tn/Hv Activity Ratio} = \frac{20{,}000 \text{ IU/mg}}{40{,}000 \text{ IU/mg}} = 0.500$$

The ratio will, of course, vary with the endotoxin being tested and with the insect species being used. The value of the ratio thus becomes an accurate mathematical definition of a portion of the spectrum of activity of a delta-endotoxin.

Encouragingly, our data so far leads to the conclusion that, while the spectra of activity of the delta-endotoxins produced by different isolates may differ widely, the spectra of activity of the delta-endotoxins produced under different fermentation conditions by a single isolate of B.t. are reproducible. This is shown for four formulations in Table 5. If this is true -- and we believe that it is -- then, if we know the relative activities of an endotoxin against T. ni and the gypsy moth, we can assay against the cabbage looper and reliably predict the potency of the endotoxin against the gypsy moth. Once we are satisfied that this is so, then the "active ingredients" of B.t. on a label of a forestry formulation can be safely listed as IU/mg vs. T. ni and represent how much B.t. was put into the formulation, and the efficacy of the formulation can be expressed in pints or pounds/acre. Activity and efficacy are being presented on the label in the same way as a chemical -- and, once we have gotten used to this, it should be no more of a communication than with chemicals.

Table 5. Reproducibility of Activity Ratios.

Culture No.	Number of Formulations	Avg. Tn/Hv Ratio	Coefficient of Variation
HD-1	60	2.14	0.25
HD-241	26	0.63	0.24
HD-244	26	1.54	0.23
HD-263	136	0.44	0.29

Thus it is my view that an assay insect does not need to be the target insect, but need only be a convenient, easy-to-rear-and-use insect which is susceptible to the delta-endotoxin in the product. The cabbage looper seems to fit these characteristics. However, it may very well happen that someone will discover a new B.t. that is very effective against L. dispar that may not be active enough against T. ni for this insect to serve as an assay tool. In such a case, of course, another insect species may be needed. However, at present no such B.t. is known, and T. ni should be satisfactory for these assays. The label should then read in IU's as determined against this insect. The differences in efficacy that may occur should be shown in the recommended application rates, not as different IU's. It has been suggested that one could include on the label a Tn/Ld Activity Ratio as a parenthetical addition. However, this may be confusing and of little value to the user. Thus it is our belief that the bioassay against T. ni should remain the key for standardizing B.t. in forestry formulations.

Miscellaneous Aspects of Evaluating New Isolates of B.t. -- Dubois

In a paper that I presented earlier in this meeting, I discussed some of our research seeking new isolates of B.t. with increased activity over HD-1 against L. dispar. Drs. Dulmage and Beegle have just discussed their work with bioassays and activity ratios, particularly as they relate to T. ni and H. virescens. In this portion of our panel program, I would like to discuss how some of their observations relate to our experience with L. dispar.

Tn/Hv Ratios

A few years ago, our FS laboratories at Hamden, CT, joined in an International Screening Program to survey the spectra of insecticidal activities of about 350 isolates of B. thuringiensis. The results of this survey were very complex and will not be discussed here (for more details, see Dulmage et al. 1981). One of the observations that was reported was that the Tn/Hv activity ratios of formulations derived from isolates of subsp. kurstaki and which contained the k-1-type crystal were about 2.0. Formulations of this subspecies which contained the k-73-type had Tn/Hv ratios of about 0.40. The difference in the two types seemed to lie in the activity of k-73 formulations against H. virescens. Simply, the k-73 endotoxin was more active against H. virescens than was the k-1 toxin. The parallel to L. dispar was interesting. When we examined the more potent isolates of B. thuringiensis, we found that these formulations had Tn/Hv ratios of about 2.0, belonged to subsp. kurstaki, and contained the k-1 crystals. Interestingly, those isolates of subsp. kurstaki that produced k-73-type crystals were not as active vs. L. dispar. These generalities should not be taken to mean that all isolates of a given subspecies will have the same potency. Some formulations will be very active, some will have little or no activity, and the activities of others will lie somewhere in between.

Reproducibility of the Response of L. dispar to B.t.

The primary need for an accurate B.t. bioassay is, of course, a supply of uniform, healthy, bioassay insects. Even with such an insect, however, the assay will not be reliable if the response of the insect to B.t. formulation changes according to the source of the insect. To see if this were a serious problem in assays with L. dispar, we have compared the response of a series of sources of L. dispar to the two standard B.t. formulations, HD-1-S-1971 and HD-1-S-1980. The cultures of L. dispar studied were diverse and included the New Jersey strains in our laboratory and the F_{18} through F_{24} generations of our laboratory colony. The responses of L. dispar to these standards were evaluated by the LC_{50}'s determined for the standard against the various insect cultures. The results of these assays are summarized in Table 6. The responses were very uniform, and what variations were observed seemed to be due to the diet, not to any change in the response of the insects. I think that we have reasonable assurance that the source of the L. dispar does not affect the response to B.t. in our bioassays.

These tests were all run with laboratory-reared insects. They leave open a very important question -- How do the responses of laboratory insects compare with wild insects? -- i.e., do we need to maintain a laboratory colony generation after generation, or could we collect wild egg masses in the field each year and use these for our bioassays? The study is not yet completed, but we have examined the responses of insects reared from egg masses of wild sources of L. dispar collected in Connecticut, New Hampshire, New York, Massachusetts, and Pennsylvania. We found some minor differences in the responses of the different insect strains (which might very well have been due to diet again), but overall, the LC_{50}'s determined for the standard formulations did not vary much with insects of different origin. This is not to say that wild insects are the insect of choice -- one must consider the risk of results being distorted by the presence of unrecognized disease in the wild culture.

Table 6. Slopes and LC_{50} of the *Bacillus thuringiensis* Standards, HD-1-S-1971 and HD-1-S-1980, Bioassayed Against the New Jersey F_{18} to F_{24} Generations of *Lymantria dispar* L. Continuously Reared on Artificial Diet.

Fermentation	No. of Bioassays	Slope	C.V.	LC_{50} (ng/ml)	C.V.
		HD-1-S-1971 (18,000 IU/mg)			
F_{18} (1979-1980)	4	4.25	0.16	7.82a[a/]	0.09
F_{19} (1980)	4	4.54	0.20	5.83ab	0.27
F_{21} (1980)	28	4.72	0.20	5.70ab	0.09
F_{22} (1981)	5	4.86	0.27	7.18ab	0.38
		HD-1-S-1980 (16,000 IU/mg)			
F_{22} (1981)	24	4.62	0.21	5.33ab	0.33
F_{23} (1982)	29	4.87	0.21	5.01ab	0.25
F_{24} (1983)	13	5.09	0.16	5.24ab	0.25

[a/] Numbers followed by the same letter are not significantly different from each other at the 5% level (Scheffe's method for all possible contrasts).

Table 7. LC_{50} of Bioassays of Several Sources of F_1 Generations of Instar II *Lymantria dispar* L. fed on Fresh and on Old Diet.

		LC_{50} (µg/ml of diet) on Dates:					
Larval Source	Diet	2/9	3/13	3/14	3/24	3/28	3/31
New Jersey	fresh	6.9			5.9	5.0	6.8
F_{26}	old		17.8	13.0			
Cockaponset, CT	fresh	12.4			---	---	8.3
F_1	old		13.6	21.6			
Storrs, CT	fresh	7.9			7.9	6.9	---
F_1	old		15.3	27.3			
New Hampshire	fresh	8.4			6.3	6.4	---
F_1	old		13.1	16.0			
Manhpac, MA	fresh	8.3			6.9	7.4	---
F_1	old		15.7	20.6			
Pennsylvania	fresh	10.6			6.7	7.9	
F_1	old		9.2	34.6			

Influence of Diet on the LC_{50}

The composition of the diet can affect the observed LC_{50}'s of *B.t.* formulations. This was discovered early in our program. We began then, and still do today, to buy a premixed insect diet from a single supplier. Over a long period of time, we amassed considerable experience in just where the LC_{50}'s of our standard formulations and test samples would be. We recently received a new shipment of diet that was mistakenly contaminated with antibiotics. When we attempted to use this diet, we found that the LC_{50}'s of both the standard and the test samples were very high -- it appeared that our materials had lost activity. When we used antibiotic-free diet that had been stored frozen for 18 months, the LC_{50}'s were again very high, indicating a loss of activity of the *B. thuringiensis* preparations, an increased resistance of the insect, or an incompatability to the diet. Meanwhile, we received a new batch of diet, and the results of our assays dropped, returning to the "normal" range (Table 7). Thus the cause of the erratic LC_{50}'s lay, not in the insect, but in the diet.

Influence of Formulation on the Bioassay

Earlier, Dr. Beegle described some of the problems that they encountered with bioassays of flowable formulations and the influence of insect age and the presence or absence of antibiotic in the diet. We have had similar problems. During the field work last year in Maine, we used drums of Thuricide 32LV and SAN 415 32LV. We sent samples to Donald Hostetter in Columbia, Missouri, for him to assay by his surface contamination technique against *T. ni* (different from the usual *T. ni* assays), while we assayed duplicate samples against *L. dispar*. The assays obtained were very similar to each other and to the results obtained the previous year at Brownsville against *T. ni*.

This led us to believe that the causative mechanism for the discrepancies observed in the bioassays against *L. dispar* was the changes in the diet and larval age in the various assays. However, the composition or structure of the delta-endotoxins in the various *B.t.* isolates may also play a role. This observation may be reinforced by the assay results obtained with two

experimental flowable concentrates prepared by Sandoz for a comparative study of HD-1 and NRD-12 (HD-945). When the concentrates of the two strains were bioassayed against T. ni, both were reported by Sandoz to contain 32 BIU/gal, but by Dr. Hostetter's assays against T. ni were found to be only 14 BIU/gal -- a discrepancy vs. the label potency similar to that found in our previous assays of commercial concentrates. However, when these two flowables were bioassayed against L. dispar, the HD-1 concentrate assayed 15 BIU/gal (similar to the assays vs. T. ni), while the NRD-12 concentrate assayed 42 BIU/gal (nearly 3 times higher than the HD-1 concentrate with an "R-value" of 2.3). Similar differential assays were found in the tank mixes prepared from these concentrates: Assays against the HD-1 mix were essentially the same regardless of which insect was used in the assay; NRD-12 again assayed nearly 3 times higher against L. dispar (R-value = 2.9) (Table 8). Thus the two materials were similar when measured against T. ni, but different when measured against L. dispar -- further evidence of the importance of Dulmage's warning about the necessity for monitoring activity ratios in these studies.

Table 8. Differences Between the Potency Estimates of Thuricide 32LV (HD-1) and SAN 415 32LV (NRD-12) and their Diluted Tank Mixes[a] when using Lymantria dispar and Trichoplusia ni.[b]

Source of Concentrate	Potency Estimates in BIU/gal: Lymantria dispar	R[c]	Trichoplusia ni	R
Concentrate: labeled 32 BIU/gal				
Thuricide 32LV	18.1		13.7	
		2.3		1.1
SAN 415 32LV	42.2		15.3	
Tank mixes: calculated 24 BIU/gal				
Thuricide 32LV	5.4		9.5	
		2.9		1.2
SAN 415 32LV	15.8		11.4	

a/ Samples were from the spruce budworm spray test in Maine, 1983. Tank mixes are from the concentrates diluted with water.

b/ T. ni assays done by D. Hostetter, USDA, ARS, Columbia, MO.

c/ $R = \frac{LC_{50}\ NRD\text{-}12}{\text{Potency HD 1}}$

Summary

The results of the work discussed in these papers, combined with the results previously reported in the literature, show very strong evidence that, at our present stage of knowledge, it is essential that we rely on a bioassay as our only means of standardizing products formulated from B. thuringiensis fermentations. The results also indicate the need for a standard in the evaluation of these bioassays, and finally, show the value of activity ratios as means of discriminating between the delta-endotoxins produced by the various isolates of B. thuringiensis. The reproducibility of these activity ratios, in particular the relative activities of L. dispar vs. T. ni, confirms that one insect species (e.g., T. ni) can be used to standardize a formulation against another insect species (e.g., L. dispar) with confidence that the relative activities will extrapolate between insect species.

Joint Statement by Panel Members -- Dulmage, Beegle, Dubois

We find no fault with the use of T. ni as an assay insect for standardizing commercial formulations of B. thuringiensis (although care must be taken to develop a proper protocol for these assays, as shown by Beegle in his talk), and, since its use is so widely accepted, we recommend this insect for these assays. We also recommend the use of HD-1-S-1980 as a standard in these assays. We disagree with the use of spore counts as a substitute for these bioassays, since we see no correlation at all between spore counts and insecticidal activity. The spore count may serve a purpose in following the immediate dispersal of a spray in test application, but not in following the potency or persistence of the B. thuringiensis formulations.

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Cockaponset, CT	fresh	12.4			---	---	8.3
F_1	old		13.6	21.6			
Storrs, CT	fresh	7.9			7.9	6.9	---
F_1	old		15.3	27.3			
New Hampshire	fresh	8.4			6.3	6.4	---
F_1	old		13.1	16.0			
Mashpee, MA	fresh	8.3			6.9	7.4	---
F_1	old		15.7	20.6			
Pennsylvania	fresh	10.6			6.7	7.9	
F_1	old		9.2	34.6			

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Summary

Peter W. Orr, Staff Director, Forest Pest Management, USDA Forest Service, Broomall, PA

The theme running through all sessions of the symposium was that B.t. is an effective and reliable material for use in management of the gypsy moth and spruce budworm. This new status is the result of recent developments in the use of increased dosages, finer droplet deposition, ULV application rates, and competitive costs.

The symposium reaffirmed the outcome of several recent conferences on microbials by identifying two major areas in which continued international cooperative research and development is needed; (1) molecular biology and modification to develop more virulent strains of the pathogens; and, (2) more reliable formulation, application, and deposition technology.

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