Proceedings

Integrating Cultural Tactics into the Management of Bark Beetle and Reforestation Pests[1]

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PREFACE

This proceedings results from a conference held at the Monastery of Vallombrosa, near Florence, Italy, on September 1st - 3rd 1996. The meeting, held under the guidelines of the International Union of Forestry Research Organizations, gathered three IUFRO Working Parties: S7.03.03 (Insects Affecting Reforestation), S7.03.05 (Integrated Control of Scolytid Bark Beetles) and S7.03.07 (Population Dynamics of Forest Insects). Forty-nine participants, from 15 countries, presented 33 papers and 8 posters. These figures understate the contributors' input; considerable sharing of expertise and enthusiasm also occurred between the sessions, and links for present or future co-operation were renewed or established.

The organizers wish to express their gratitude to the Italian colleagues who helped to organize locally the meeting: Prof. Riziero Tiberi and Dr. Franco Cerchiarini (Istituto di Patologia e Zoologia Forestale e Agraria, Universita degli Studi, Firenze), Dr. Andrea Battisti and Mr. Massimo Faccoli (Istituto di Entomologia Agraria, Universita' degli Studi, Padova).

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Tomicus piniperda in North America: An Integrated Response to a New Exotic Scolytid

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ABSTRACT The pine shoot beetle [*Tomicus piniperda* (L.); Coleoptera: Scolytidae] was first discovered in North America in Ohio in 1992. As of 1 October 1996, it was found in 186 counties in 8 US states (Illinois, Indiana, Maryland, Michigan, New York, Ohio, Pennsylvania, and West Virginia) and in 17 counties in 1 Canadian province (Ontario). A federal quarantine imposed in November 1992 regulates movement of pine (*Pinus*) trees and logs from infested to uninfested areas. This paper summarizes information on the discovery of *T. piniperda* in North America, early survey efforts, recent interception history, development and changes in the federal quarantine, research efforts, development of a national compliance management program, and current efforts to evaluate the clerid *Thanasimus formicarius* (L.) for possible release as a classical biological control agent in the US.

KEY WORDS *Tomicus piniperda, Scolytidae, Thanasimus formicarius, Cleridae, Pinus,* exotic insect, biological control, regulatory entomology, quarantine.

THE PINE SHOOT beetle [*Tomicus piniperda* (L.); Coleoptera: Scolytidae] was discovered in Ohio in July 1992. As of October 1996, established populations of this beetle have been found in eight states in the United States and in one Canadian province (Table 1, Fig. 1). This exotic scolytid likely arrived in the Great Lakes region on one or more cargo ships that carried infested crating or dunnage (wood braces). DNA analyses suggest that there were at least two separate introductions: the first in Ohio and a more recent introduction in Illinois (Carter et al. 1996).

The Great Lakes region of North America is a highly suitable location for an insect such as *T. piniperda* (Niemela and Mattson 1996), given the region's temperate climate and widespread occurrence of pine (*Pinus* spp.) forests, Christmas tree plantations, and nurseries. Considering only the Christmas tree industry, over 10 million trees are cut and sold annually in the Great Lakes region, and of these about 75% are Scotch pine (*Pinus sylvestris* L.) (Dr. Melvin R. Koelling, Michigan State University, pers. comm.). Pine Christmas tree plantations are especially suitable for a univoltine, early spring-flying bark beetle like *T. piniperda*, because breeding material (e.g., pine stumps and slash) is created each autumn and there is ready supply of shoots for maturation feeding.

Considering the potential damage that this bark beetle could cause, the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) imposed a quarantine to regulate the movement of pine from regulated (infested) counties to unregulated (uninfested) counties within the US (USDA APHIS 1992). This paper discusses the history of *T. piniperda* in North America, including its discovery, survey, interception history, quarantine issues, research

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programs, development of a national compliance management program, and classical biological control efforts.

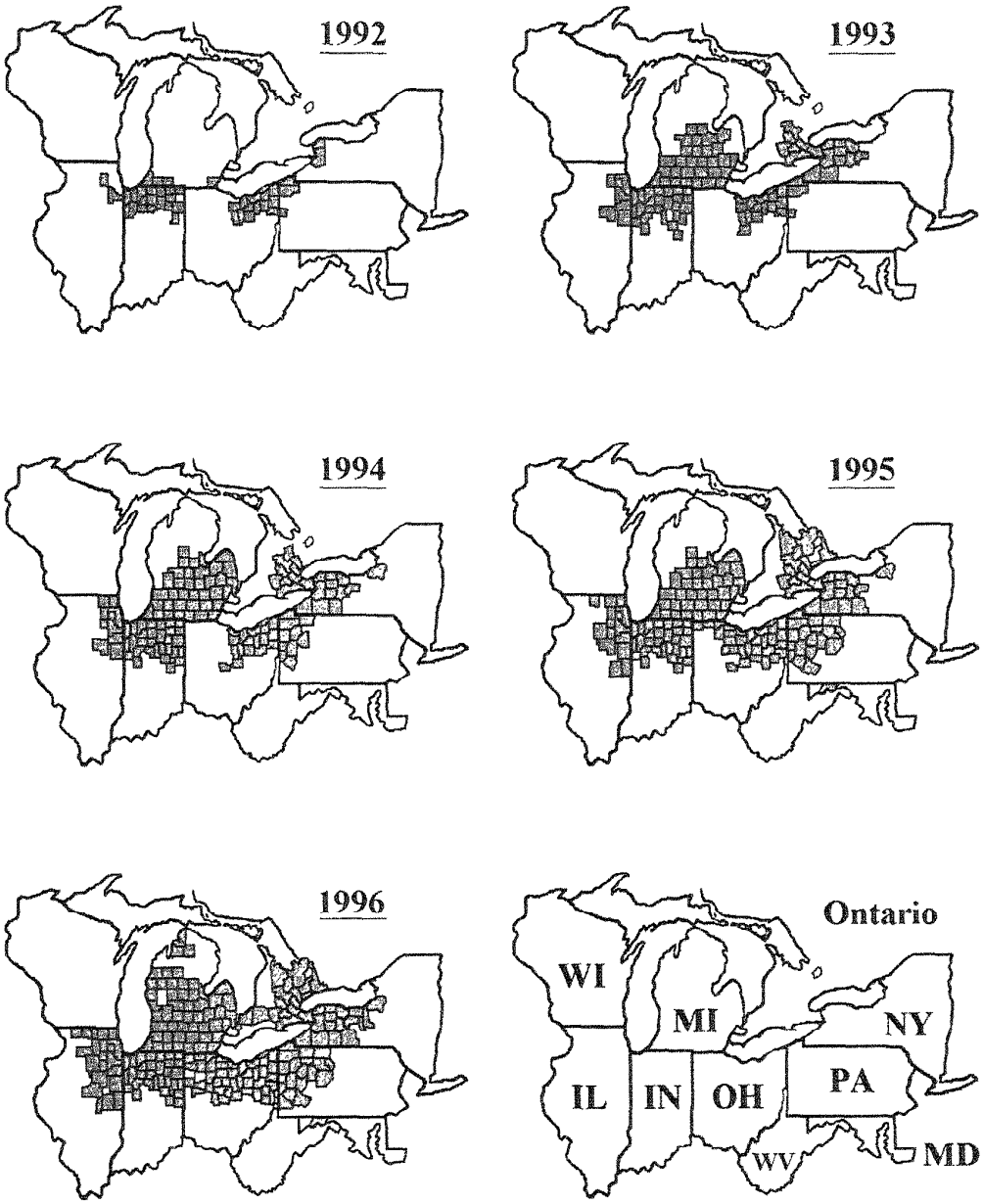

Fig. 1. Known distribution of *T. piniperda*-infested counties in North America from 1992 through 1 October 1996 (Source: USDA APHIS and Agriculture Canada). State abbreviations are: IL = Illinois, IN = Indiana, MD = Maryland, MI = Michigan, NY = New York, OH = Ohio, PA = Pennsylvania, WI = Wisconsin, and WV = West Virginia.

Table 1. Historical information for the discovery of *T. piniperda* in North America, including for each state or province the year when *T. piniperda* was first found, and the cumulative number of quarantined counties by year.

State or Province	Year of initial discovery	Cumulative number of positive counties by year				
		1992	1993	1994	1995	1996
Illinois	1992	2	7	10	15	21
Indiana	1992	18	26	31	31	32
Michigan	1992	4	30	37	37	52
Ohio	1992	14	16	18	31	43
New York	1992	2	10	12	13	16
Pennsylvania	1992	3	3	10	18	19
Maryland	1995	0	0	0	1	1
West Virginia	1995	0	0	0	1	2
US county totals		43	92	118	147	186
Ontario	1993		7	10	14	17

Initial Discovery and Survey Efforts

Specimens of an unknown scolytid were collected in a Christmas tree plantation in Lorain County, Ohio, near the port city of Cleveland on 1 July 1992. These beetles were delivered to Dr. David Nielsen for identification at Ohio State University. Not recognizing the beetles, he mailed the specimens to Dr. Stephen Wood, Brigham Young University, on 9 July 1992. Dr. Wood identified the beetles as *Tomicus piniperda* (L.) in a letter dated 16 July 1992. After receiving the letter, Dr. Nielsen informed the APHIS office in Ohio on 22 July 1992, which in turn notified its headquarters office near Washington, DC, that same day. Additional specimens of *T. piniperda* were collected from the original site and one additional site in Lorain County on 23 July 1992. Likewise, on 23 July 1992 in Washington, APHIS established a "New Pest Advisory Committee" to evaluate the potential pest status of this insect and options for quarantine and control. On 24 July 1992, USDA APHIS electronically notified regulatory and extension agencies throughout the US about the presence of *T. piniperda* in Ohio.

A great flurry of activity soon followed this announcement. Dozens of Ohio Christmas tree plantations and nurseries were inspected. Several training sessions were held in Ohio for APHIS inspectors and forest health specialists from nearby states to see the beetle and its associated damage. Regulatory personnel began to inspect Christmas trees and nurseries in surrounding states, and within one month of the initial USDA announcement, five new states reported finding *T. piniperda*: Indiana on 4 August, Pennsylvania on 13 August, Michigan on 14 August, New York on 20 August, and Illinois on 21 August 1992.

The fact that *T. piniperda* was found in six states during a 4-week period in 1992 strongly suggests that this scolytid had been in the US for several years before its discovery. One likely

reason for the presumed delay in discovering *T. piniperda* is that it causes shoot damage that appears similar to that caused by several native insects and diseases, and thus the shoot damage associated with *T. piniperda* caused no special alarm. The current Great Lakes populations of *T. piniperda* probably became established in the 1980s given that scolytids surveys in Indiana (Deyrup 1981, Deyrup and Atkinson 1987) and Michigan (Dr. Lawrence R. Kirkendall, University of Bergen, pers. comm.) during the late 1970s and early 1980s did not detect *T. piniperda*. The earliest record of *T. piniperda* in a private insect collection from the Great Lakes region is a single adult collected in 1991 by Ronald Priest from a shoot on eastern white pine, *Pinus strobus* L., in Ingham County, Michigan; this beetle was first identified in 1993.

By the end of 1992, *T. piniperda* had been found in 43 counties in the 6 states of Illinois, Indiana, Michigan, Ohio, Pennsylvania, and New York (Table 1, Fig. 1). Surveys during 1993 and 1994 found several new infested counties but no new infested states. In April 1995, two new US states were added: Maryland and West Virginia. The first infested counties in Canada were found in the province of Ontario in 1993. As of 1 October 1996, *T. piniperda* was known to occur in 186 counties in 8 US states and in 17 counties in 1 Canadian province (Table 1, Fig. 1). When explaining the rapid increase in the number of infested US counties, it is important to consider both natural spread by dispersing adults as well as more intensive surveying by regulatory officials.

Most surveys for *T. piniperda* have been conducted in late summer or early fall (August-October) and have focused on locating damaged shoots. In more recent years, surveys have also been aimed at the initial spring flight of parent adults, using trap logs and traps baited with alpha-pinene. The largest survey effort that used trap logs was conducted by the state of Michigan during the years 1994-1996 in which all unregulated counties in Michigan's lower peninsula were trapped; over 300 trapping locations were monitored in 1994 (Haack and Lawrence 1994).

Table 2. Number of *T. piniperda* interceptions on wood products (e.g., dunnage, crates, and pallets) at US ports of entry during 1985-1995 by country of origin. (Source: USDA APHIS)

Interceptions		Interceptions		Interceptions	
No.	Country	No.	Country	No.	Country.
28	France	7	Europe*	1	Finland
19	United Kingdom	3	Netherlands	1	Greece
13	Italy	3	Russia	1	Hong Kong
12	Spain	2	Japan	1	Portugal
8	Belgium	2	Unknown	1	Sweden
8	Germany	1	China	1	Switzerland

*These seven interceptions were on cargo that originated in Europe, but no individual country could be identified.

Table 3. The 10 most commonly intercepted scolytids on wood products (e.g., dunnage, crates, and pallets) at US ports of entry during 1985-1995, including number of interceptions, number of countries of origin, and the five countries accountable for shipping the most infested material, in decreasing order (Source: USDA APHIS)

Insect species	No of inter-ceptions	No. of countries of origin	Top five countries of origin
Pityogenes chalcographus	409	24	Germany, Italy, Belgium, Russia, Spain
Orthotomicus erosus	337	18	Spain, Italy, China, Portugal, France
Hylurgops palliatus	229	18	Germany Belgium United Kingdom Italy France
Ips typographus	182	19	Italy, Germany, Russia, Belgium, France
Hylurgus ligniperda	151	12	Italy, Portugal, Spain, Chile, France
Ips sexdentatus	125	10	Italy, Spain, France, Belgium, Portugal
Tomicus piniperda	112	16	France, United Kingdom, Italy, Spain, Belgium
Hylastes ater	40	8	Spain, Germany, United Kingdom, France, Italy
Polygraphus poligraphus	33	7	Italy, Germany, Belgium, Russia, France
Pityogenes bistridentatus	30	6	Spain, Italy, France, Israel, United Kingdom

Recent Interception History

It is not surprising that *T. piniperda* became established in North America, considering that it is commonly intercepted at US ports of entry on wood products such as dunnage, crates, and pallets. For example, during the 11-year period 1985-1995, *T. piniperda* was intercepted 112 times on cargo arriving from at least 16 different countries (Table 2). Overall, *T. piniperda* was the seventh most commonly intercepted scolytid on wood products at US ports during 1985-1995 (Table 3). *Tomicus piniperda* has been intercepted at ports throughout the US, from San Francisco, California, in the west to Brooklyn, New York, in the east, and from Miami, Florida, in the south to Duluth, Minnesota, in the north. The top five US ports for *T. piniperda* interceptions are, in decreasing order: Toledo, Ohio; New Orleans, Louisiana; Houston, Texas; Detroit, Michigan; and Miami, Florida. Overall, more than 40% of all *T. piniperda* interceptions in the US since 1985 have been at port cities on the Great Lakes. Hopefully the recent regulatory changes dealing with the import of logs, lumber, pallets, and other solid wood packing material will reduce the number of interceptions (USDA APHIS 1995a, 1995b).

Quarantine History

On 19 November 1992 a federal quarantine was imposed by APHIS on the movement of pine material from regulated (infested) counties to unregulated (uninfested) counties within the US (USDA APHIS 1992). The original quarantine affected movement of pine, spruce (*Picea*), fir (*Abies*), and larch (*Larix*) logs or lumber with bark attached, pine Christmas trees, and pine nursery stock. Regulated articles could be moved to areas outside the quarantine zone, but only after certain conditions were met (see below).

To many pine producers within the infested region of the US, especially within the Christmas tree industry, the federal quarantine came as a relief because it established one set of rules for the entire country. Up to that time, each state was able to declare its own separate quarantine, using its own set of conditions. For example, before the federal quarantine, seven individual US states had imposed their own state quarantines: Florida, Georgia, Kansas, Louisiana, North Carolina, Oregon, and West Virginia. The first state quarantine was imposed by North Carolina on 28 September 1992. The seven state quarantines ranged from being relatively flexible (e.g., Florida and North Carolina) in which regulated articles could be imported after passing a visual inspection and being declared free of *T. piniperda*, to relatively restrictive (e.g., Kansas) in which no regulated articles were allowed at all.

Since the original federal quarantine was imposed in November 1992, it has undergone several modifications (see below). These changes were based on new research findings from studies conducted in the US and on expert opinion from two European forest entomologists -- Dr. Alf Bakke of Norway and Dr. Bo Langstrom of Sweden -- who were consulted by USDA APHIS

Research Efforts

A large research effort on *T. piniperda* has been underway in the US since 1992. Research funding has come largely from APHIS and USDA Forest Service, but many other state, private, and professional organizations have contributed time and funding. These studies have involved various aspects of *T. piniperda* biology and management. Although most studies have not yet been formally published, early results have always been made available to APHIS so that the federal quarantine could be modified as needed. The bulk of the university research within the US has been conducted at Michigan State University, Ohio State University, Purdue University, State University of New York-Syracuse, University of Georgia, and University of Wisconsin-Madison. Some of the refereed publications on *T. piniperda* in the US have dealt with timing of initial spring flight (Haack and Lawrence 1995b), influence of pine felling date on colonization by *T. piniperda* and native *Ips* bark beetles (Haack and Lawrence 1995a), shoot feeding in native pines (Lawrence and Haack 1995, Sadof et al. 1994), foliar chemical control (McCullough and Smitley 1995), and DNA analysis of various *T. piniperda* populations (Carter et al. 1996). Many other papers have now been submitted or are in preparation.

Development of a National Compliance Program

After the 1992 federal quarantine was published, many state, federal, and industrial organizations began to seek changes in the quarantine that would ease the regulatory restrictions on the affected pine industries but still minimize the risk of accidental spread of *T. piniperda* to uninfested regions of the US. The three major industries affected by the quarantine include the forest, Christmas tree, and nursery industries.

The forest industry. As originally written in November 1992 (USDA APHIS 1992), the federal quarantine affected movement of logs and lumber with bark from all species of pine, spruce, fir, and larch. Moreover, the original ruling required that all regulated logs and lumber be either debarked or fumigated with methyl bromide before being transported to unregulated areas. Neither of these regulations were acceptable to the industry, so transport of logs from regulated to unregulated areas basically stopped. However, in 1993, two important changes were made to the federal quarantine. The first change exempted spruce, fir, and larch logs from regulation, because it

was felt that these species were not primary hosts for *T. piniperda* reproduction and overwintering. The second change allowed for free movement of pine logs and lumber with bark if these trees were felled during July through October and if all branches and foliage were removed and left at the harvest site. This change was made because *T. piniperda* primarily feeds in shoots during these 4 months. Therefore, if all branches and foliage were left behind there would be relatively little risk of transporting *T. piniperda* to uninfested areas.

Although the forest industry favored the above changes, it requested additional easing of the regulations that affected log transport during November through June. As a result, meetings were held in both Michigan and Washington, DC, to explore possible modifications. Although much progress was made, no formal changes have yet been published in the Federal Register as of October 1996. Nevertheless, one possible scenario for moving logs with bark from regulated to unregulated areas within the US is as follows:

July 1 to September 30. Newly harvested pine logs (with no attached foliage) can be moved freely.

October 1 to February 14. Newly harvested pine logs can be moved to an approved facility (e.g., sawmill) if all slabs or bark from these logs are treated or destroyed before February 14 or within 4 weeks of felling.

February 15 to June 30. Newly harvested pine logs can be moved to an approved facility if all slabs or bark from these logs are treated or destroyed within 4 weeks of felling.

Record Keeping. Facility managers must keep records that pertain to the origin, date of felling, and date of milling for all pine logs that they receive from regulated areas. Similarly, managers must record the date on which all slabs or bark are treated or destroyed. Managers must allow regulatory officials to inspect their facilities and their records at any time.

The Christmas tree and nursery industries. In the original 1992 federal quarantine, Christmas tree managers were given the option either to have a certain number of trees inspected in each field scheduled for harvest or to subject the trees to an approved cold treatment or methyl bromide treatment before moving them to unregulated area. Under the inspection option for Christmas trees, evidence of a single *T. piniperda* beetle or a single attacked shoot was sufficient to cause rejection of all trees from that particular field. Because of the added costs of the cold or fumigation treatments and their potential harm to the foliage, almost all managers chose the inspection option. In both the Christmas tree and nursery industries, inspections were usually conducted a few weeks before shipping. Such timing was especially worrisome to Christmas tree managers. Many Christmas tree producers sign contracts with buyers during June and July, but their trees were not inspected usually until October. Therefore, even a single damaged shoot could result in serious economic losses for large wholesale Christmas tree producers. Overall, no major changes have been made to the original federal quarantine that pertain specifically to the Christmas tree industry.

As for the nursery industry, the original 1992 quarantine did not allow pine nursery stock to be moved to unregulated areas unless it was less than 24 inches (60 cm) tall (USDA APHIS 1992). However, in 1993, the quarantine was changed to allow pine nursery stock from regulated areas to move freely if it was either (1) less than 36 inches (90 cm) tall or less than 1 inch (2.5 cm) in stem diameter at the ground line, (2) subjected to 100% inspection and found free of *T. piniperda*, or (3) subjected to an approved cold treatment. Under the inspection option, infested trees could be rejected on an individual basis.

Given the above situation, it is understandable that the Christmas tree and nursery industries continued to request further modifications to the federal quarantine. In response to these requests, a research program to develop a national *T. piniperda* compliance management program was initiated in 1994. After a series of experiments, field trials, meetings, and approval by the National Plant Board this program was released for public comment in August 1996 (USDA APHIS 1996). This document is also available on the internet (http://www.ceris.purdue.edu:80/napis/pests/psb/index.html). Program guidelines will be modified as new information becomes available. Briefly, the major change made by this program is to allow growers in regulated areas to ship their trees to unregulated areas without inspection by regulatory agents if they agree to and follow a set of management guidelines. The rationale for this program is that if all pest management steps are followed, then the resulting *T. piniperda* population will be very low or undetectable. Therefore, shipping these trees will pose only a minimal risk of introducing *T. piniperda* into new areas.

Although there are several differences between the programs developed for the Christmas tree and nursery industries, they have many components in common (USDA APHIS 1996). For example, both programs will require (1) sanitation of all potential brood material by a specified date in spring, (2) monitoring of spring adult populations with the use of trap logs or traps baited with alpha-pinene, (3) chemical applications to foliage, trunks, or stumps when specified conditions are met, (4) monitoring trees for evidence of *T. piniperda* shoot feeding, and (5) record keeping of all pertinent information. The dates specified for field activities such as putting out traps or trap logs in spring will vary by state. In addition, managers must allow regulatory officials to inspect their trees and records at any time. To help this new program succeed, university extension specialists and state regulatory officials will (1) train growers in monitoring techniques and identifying beetles, (2) establish compliance agreements with producers and monitor their fields and records, and (3) establish a computerized master list of all cooperating producers that will be available on the internet. Educational materials and programs will be provided to producers, shippers, and buyers of these regulated products (McCullough and Sadof 1996). This program should be operational in 1997.

Biological Control Efforts

Classical biological control is being considered as part of the overall regional suppression program for *T. piniperda* in the US (USDA APHIS 1996). The main objective is to establish biocontrol agents that will reduce *T. piniperda* populations in forested areas of the US where active pest suppression using cultural and chemical controls is not practical or economical. In most years in the Great Lakes region, *T. piniperda* initiates host colonization 4-8 weeks before any competition from native pine-infesting bark beetles and their associated natural enemies (Haack and Lawrence 1995a, 1995b). As a result, entomologists looked to Europe and Asia for natural enemies that were better synchronized with the early spring flight of *T. piniperda*. After reading the literature and consulting with several European and Asian forest entomologists, there was general consensus that the clerid *Thanasimus formicarius* (L.) was the best candidate because (1) its spring flight closely matches that of *T. piniperda*, and (2) it causes high levels of *T. piniperda* mortality. In addition, species of rhizophagid beetles in the genus *Rhizophagus* were considered to be the next most important mortality agents of *T. piniperda*.

A cooperative effort was initiated in 1995 among three USDA agencies -- APHIS, Forest Service, and Agricultural Research Service (ARS) -- to evaluate *T. formicarius* for possible release

in the US. Briefly, under the direction of Dr. Richard Dysart at the ARS European Biological Control Laboratory in Montpellier, France, several thousand *T. formicarius* adults were collected in 1995 and 1996 in France and shipped to Dr. Larry Ertle at the ARS quarantine facility in Newark, Delaware. These adults or their progeny were later shipped to Dr. David Prokrym at the APHIS biological control laboratory in Niles, Michigan, where various rearing techniques were developed. In 1996, several hundred *T. formicarius* eggs were shipped to the Forest Service laboratory on the Michigan State University campus to study potential non-target impacts of *T. formicarius* on the native clerid *Thanasimus dubius* (F.).

In September 1996, state officials from the *T. piniperda*-infested states who are responsible for approving the release of exotic natural enemies within their states' borders voted to postpone any release of *T. formicarius* until 1998 at the earliest. The main reasons for this vote were (1) *T. piniperda* has not yet caused significant levels of damage in the US to justify the release of an exotic predator, and (2) there is insufficient information available on the potential non-target impacts from such a release. Concern was raised that because *T. formicarius* is a "general predator" its release could destabilize the normal balance between native conifer-infesting bark beetles and their natural enemies, resulting in greater outbreak frequency of native bark beetles. In addition, some felt that *T. formicarius* could competitively displace certain native natural enemies and thereby upset native biodiversity. Perhaps in 1998, after more information has accumulated on the damage caused by *T. piniperda* in forested areas of the US and on the degree of non-target impacts to be expected, *T. formicarius* will be reconsidered for release.

Summary

The repercussions of a newly introduced exotic organism cannot be easily predicted. Their impact can range from no apparent change to dramatically altered forest ecosystems. In North America, although *T. piniperda* has not caused any widespread negative growth impact on native forests so far, federal quarantines have been implemented in both Canada and the US to slow the spread of this beetle. These quarantines have greatly altered normal trading relations and have had major economic impacts in the affected pine industries. Both national and international trade has been affected by the arrival of *T. piniperda*, especially among the countries of Canada, Mexico, and the US. The national *T. piniperda* compliance management program is a bold step towards reducing the regulatory burden on the affected pine industries while still minimizing the risk of spreading *T. piniperda* to uninfested areas. This program will serve as a model for dealing with future exotic pests that will undoubtedly arrive on our shores.

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Preliminary Investigations On The Relationships Between Phloem Phenolic Content Of Scots Pine And Maturation Feeding Of The Pine Weevil *Hylobius Abietis*

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ABSTRACT The phloem phenolic content of 5 Scots pine clones was analysed in the branches of 3 trees per clone. Five emerging beetles were encaged in one branch per tree and the damage were estimated 2 weeks later by measuring the area of removed bark. Phloem phenol content was analysed in another branch of the same trees. Clones were clearly separated from each other by their phenol content, but not by weevil damage. However, a significant negative correlation was found between the concentration of an acetophenone glycoside and weevil damage.

KEY WORDS *Hylobius abietis, Pinus sylvestris,* soluble phenols, maturation feeding, clonal variability

THE PINE WEEVIL *Hylobius abietis* L. causes heavy damage to young seedlings in coniferous plantations throughout Europe (Coutin 1984; Langstrom 1985; Ward 1988; Heritage et al. 1989; Sundkvist 1994). During adult maturation, it feeds on the bark of small branches and shoots, thus leading to the death of the attacked seedlings. All conifer species can be attacked, but almost nothing is known about the intra-specific variability of tree's susceptibility to attacks, especially in relation to host's secondary metabolites (Selander & Kalo 1979). The present paper reports a preliminary assay aiming at determining if phloem phenolic content of Scots pine branches can be related to resistance to pine weevil feeding.

Materials and Methods

The experiment was carried out with ramets of 5 Scots pine clones. The trees, which were grafted in 1982, originated from the natural provenance of Taborz (Poland) and were cultivated in the nursery of INRA (Cadouin, Dordogne, France). The beetles were obtained from Scots pine brood logs attacked in the field (Forest of Orléans, France) in May-June the year before the experiment and stored at 5°C from August until the beginning of the experiment. The logs were then placed at 25°C and emerging beetles were collected.

In early June 1996; 3 ramets per clone were submitted to weevil feeding. Five beetles were encaged with the distal 50 cm of one branch per ramet. Two weeks later, the encaged branches were cut from the trees, the weevils were removed, and the percentage area of consumed bark was measured on the main axis and the lateral axes. The same day, one 15 cm long sample of branch was cut from the main axis of a neighbouring branch in each

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ramet, freezed in the field in liquid nitrogen, then freeze-dried in the lab, and stored until analysis.

For analyses, after removing the bark, the phloem was taken from each branch sample and ground. After washing the powder with pentane to remove resinous compounds (Alcubilla, 1970), soluble phenols were extracted at 4°C by 80% methanol containing 25 mg/l of gallic acid as internal standard, according to a method previously described (Lieutier et al., 1996). Analysis was performed by reverse-phase HPLC (Waters 600 E, photodiode array detector 991, column 250x4 mm with a C18 grafted silica phase). The detailed methods of analysis and the elution gradient have already been described (Lieutier et al., 1996). Results were read at 310 nm, except for (+)-catechin (280 nm), corrected using the internal standard, calibrated using pure compounds and expressed in nM/g of freeze-dried powder.

Phenolic compounds were characterized by UV spectra and by refering to the previous analyses carried out in the same tissue (Lieutier et al., 1996).

Multivariate analysis (principal component analysis = PCA) was used to check the existence of groups of variables (compound concentrations) explaining the phenolic between-clone variability. Beetle feeding parameters were added as supplementary variables, and trees were projected in the axes defined by the PCA.

ANOVA, followed by a Tukey's test, was used to compare between-clone concentrations of particular compounds. Correlations were calculated by linear and non linear regression.

Results

Between-clone variability of phloem phenolic content. The phenolic compounds observed were a paracoumaric acid ester, an acetophenone glycoside, taxifolin glucoside, taxifolin, catechin, and 5 other undetermined compounds. Their characteristics are presented in Table 1.

Table 1. Chromatographic and spectral characteristics of the phloem phenolic compounds.

Compounds	Retention time (min)	U.V. spectra		
		Maxima (nm)	Minima (nm)	Shoulder (nm)
Peak 1[a]	21	280	255	310
para-cou.	22	310	252	290
catechin	24	278	255-300	
Peak 2[a]	28	280	258	
acetophenone glycoside	30	277-306	252-297	
Peak 3[a]	37	280	260	310
Peak 4[a]	42	270	252	305
Peak 5[a]	56	280	260-300	
taxifolin glucoside	57	285	257	330
taxifolin	58	285	257	330

a = Unidentified compound. Retention times at 310 nm, except for catechin (280 nm). para-cou = paracoumaric acid ester.

Axes 1, 2 and 3 of the PCA explained 35 %, 26 % and 11 % of the variability, respectively (Fig. 1). Axis 1 was characterized by a group (group F1) of 6 closely and positively correlated variables, P2, P3, P5, taxifolin glucoside, taxifolin and catechin (Table 2). Axis 2 was characterized by P1, paracoumaric acid ester and P4. Axis 3 was correlated with acetophenone glycoside only.

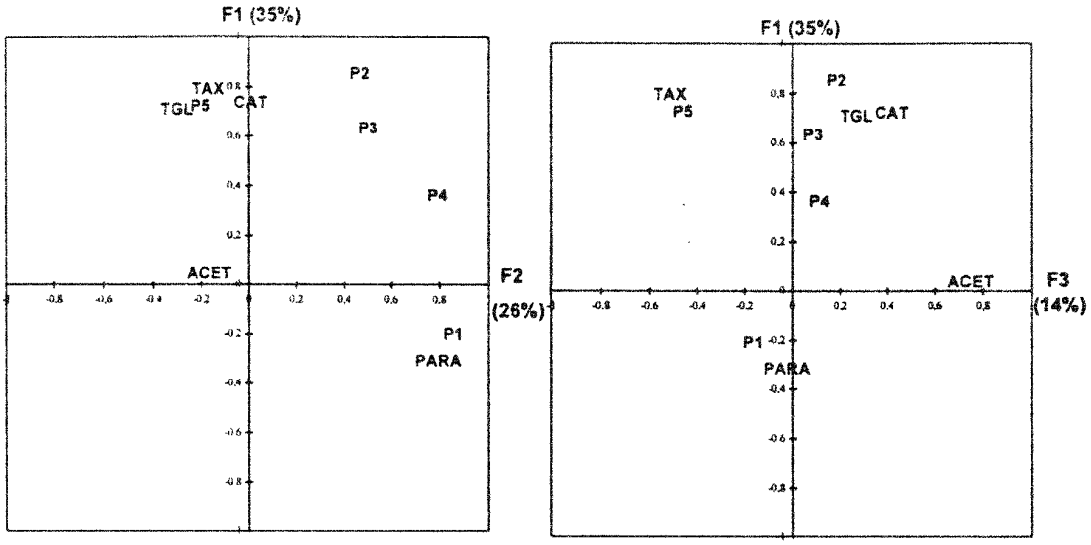

Fig. 1. Principal Component Analysis (PCA) between phenolic variables. P1 = peak 1; PARA = paracoumaric acid ester; P2 = peak 2; ACET = acetophenone glycoside; P3 = peak 3; P4 = peak 4; P5 = peak 5; TGL = taxifolin glucoside; TAX = taxifolin; CAT = catechin.

Table 2. Factor pattern of the Principal Component Analysis: correlations (r values) between variables and axes.

Compounds	Factor 1	Factor 2	Factor 3
Peak 1[a]	-0.20520	**0.86422**	-0.18041
para-cou.	-0.32333	**0.77642**	-0.02156
catechin	**0.70546**	-0.00985	0.40044
Peak 2[a]	**0.84197**	0.44966	0.17470
acetophenone glycoside	0.02383	-0.20167	**0.74410**
Peak 3[a]	**0.62727**	0.49451	0.06893
Peak 4[a]	0.36247	**0.78476**	0.10349
Peak 5[a]	**0.72351**	-0.22099	-0.47556
taxifolin glucoside	**0.69937**	-0.31251	0.26279
taxifolin	**0.76151**	-0.20312	-0.54072

a = Unidentified compound. Retention times at 310 nm, except for catechin (280 nm). Abbreviations: see Table 1.

Projection of the trees in these axes, according to their coordinates (Fig. 2), showed that clones were well separated with regards to axis 1 and axis 3. No real separation was observed along axis 2. Clones 278 and 306 were opposed to clones 312 and 316 along axis 1 and clone 278 to clone 312 along axis 3.

ANOVA and Tukey's test, for each phenolic compound tested individually, gave significant differences between clones for catechin, taxifolin glycoside, acetophenone glycoside, P2, P3 and P4 (Table 3).

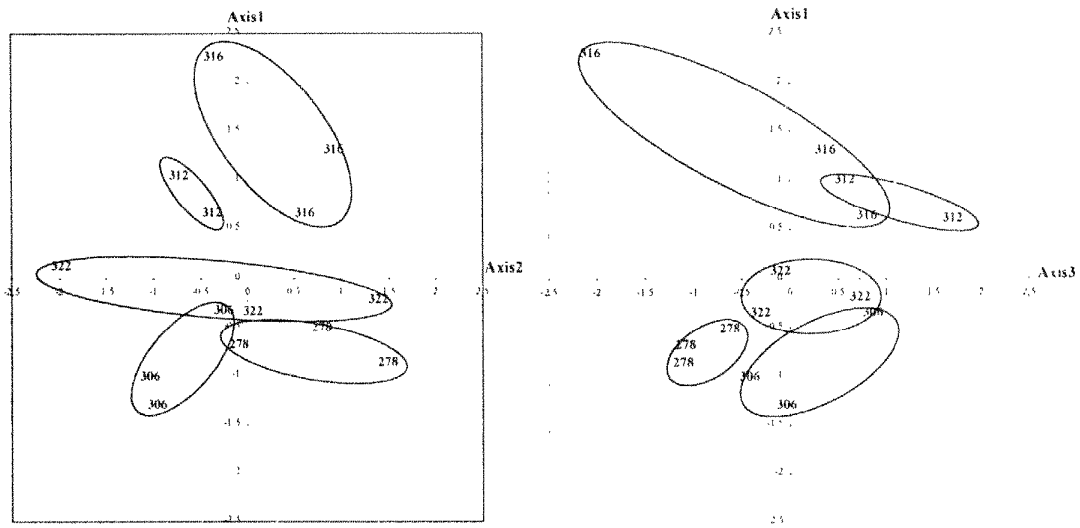

Fig. 2. Projection of trees in the axes defined by the multivariate analysis (PCA) between phenolic variables. Numbers refer to clones and trees.

Table 3. ANOVA between clones for different phenolic compounds tested individually.

Compounds	Clone separation					P values
correlated with Axis 1						
catechin	316(a)	322(a)	312(a)	306(b)	278(b)	0.0006
Peak 2	316(a)	312(a)	278(b)	322(b)	306(b)	0.0017
Peak 3	316(a)	278(ab)	312(ab)	306(b)	322(b)	0.1309
taxifolin glucoside	312(a)	316(b)	322(bc)	306(c)	278(c)	0.0001
correlated with Axis 2						
Peak 4	316(a)	322(ab)	278(ab)	312(ab)	306(b)	0.1252
correlated with axis 3						
acetophenone glycoside	312(a)	306(ab)	322(abc)	316(bc)	278(c)	0.0382

Only the significant results are presented. Abbreviations: see Table 1. Clones with the same letter did not significantly differ regarding compound concentration.

Between clone variability of weevil feeding. No differences among clones was observed concerning weevil feeding, in the main and the lateral axes (Fig. 3). Variability among trees inside a clone looked equal to or higher than clone variability.

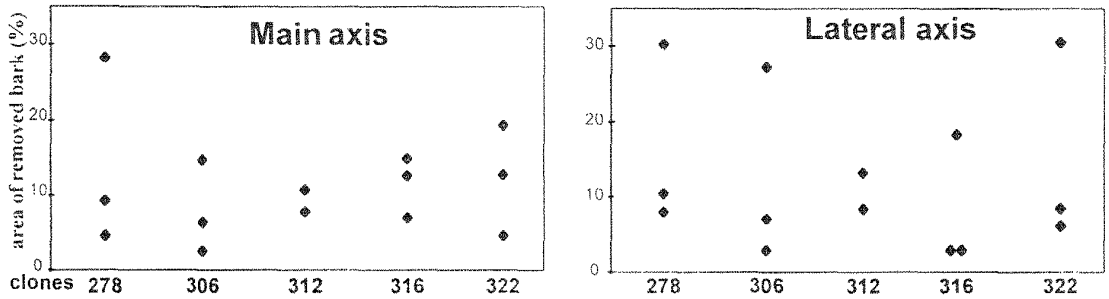

Fig. 3. Percentage area of bark removed by the weevils in the main and lateral axes, for the different clones. Each point represents one tree.

However, when weevil feeding parameters are added as supplementary variables in the axes defined by the above PCA, a slight opposition is observed between the feeding variables on the one hand and the phenolic variables correlated with axes 1 or 3 on the other (Fig. 4). Feeding parameters were not correlated with group F1 but weevil feeding in the main axis of the branches was significantly and negatively correlated with acetophenone glycoside in this axis when considering clones as elementary data (Table 4).

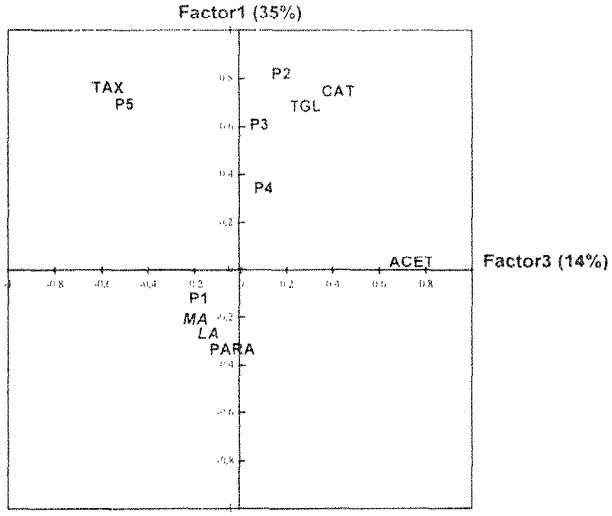

Fig. 4. Position of the weevil feeding parameters in the axes defined by the multivariate analysis (PCA) between phenolic variables. MA = weevil feeding on the main axis of the branches; LA = weevil feeding on the lateral axes. Other abbreviations: see Fig. 1.

Table 4. Correlations (r values) between, on the one hand, the feeding variables measured in the main and the lateral axes of the branches and, on the other hand, the groups of phenolic variables characterizing axes 1 and 3 of the PCA, when considering either trees or clones as elementary data.

	Group F1 (Axis 1) (P2, P3, P5, TGL., TAX., CAT.)		Axis 3 (Acetophenone glycoside.)	
	Trees	Clones	Trees	Clones
Main axis	-0.15 (P=0.60)	-0.77 (P=0.13)	-0.50 (P=0.07)	**-0.88*** (P=0.05)
Lateral axis	-0.26 (P=0.38)	-0.24 (P=0.69)	-0.42 (P=0.13)	-0.48 (P=0.41)

Abbreviations: see Fig. 1.

Discussion

Scots pine clones were well separated from each other by their phloem phenolic content in the main axis of the branches. Within clone variability was thus higher than within tree variability. In these conditions, the lack of within clone differences regarding weevil feeding does not allow us to conclude to a relationship between weevil feeding and constitutive phenolic content of the phloem. However, the existence of a significant negative correlation between acetophenone glycoside and weevil feeding in the main axis of the branches would lead to opposite conclusions.

The very small scale of the assay is probably responsible for these contradictory results. Before being allowed to conclude in one direction or another, an extended study with more trees and clones is necessary.

Acknowledgment

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Integrated bark beetle control: experiences and problems in Northern Germany

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ABSTRACT The integrated system of bark beetle control, including the use of traps since 1983 broadly practised mainly against *Ips typographus*, is described with particular reference to it's effective proper implementation. When correctly applied and under ordinary conditions (no competition by large windthrow or by an active attack simultaneously at hundreds of spruce next to a trap line) the complete control system is able to reduce infestation of living spruce by 70-100% compared to tree mortality where the system is runned without traps.

Recently, timber harvesting by the "Harvester" (a bark crushing harvesting machine) proved to be a good means to avoid successful breeding of *Ips typographus* and *Pityogenes chalcographus* in trunks stored undebarked in the forest. Thinning of younger stands - which leaves all material on the spot - should be conducted in plots separated from each other by a sufficient extent of space (>0.5 km) or of time (1 year) to prevent spreading of infestation by one bark beetle generation to the next throughout the whole of the forest.

Two problems remain unsolved: in very hot summers *Ips typographus* prefers the cool inner parts of stands for breeding instead of the stand margins. Inside the stands, it is very difficult to detect new attacks timely enough to stop spreading of infestation by sanitary felling.

Additionally, pheromone traps cannot be employed because the security distance of 10-12 m between trap and nearest spruce cannot be kept. To shorten the security distance, (s)-verbenon in different dosages has been tested: it was repellent only in traps but on living trees it was (slightly) attractive. To improve the efficiency of traps, monoterpenes ([-]-alpha-pinen and [+]-limonen) have been added to the commercial pheromone dispenser (Pheroprax^{R}), but with no difference to traps baited with Pheroprax^{R} alone. Poisoned heaps of thin spruce wood (tops, boughs etc.) containing one pheromone dispenser each proved to be very effective trapping devices, demanding only 6m of security distances, even with dry wood. No side effects of the alpha-cypermethrin contaminated beetles on nearby living birds could be proved.

KEY WORDS bark beetles, integrated control, pheromone traps, host volatiles, repellents, cultural tactics, trap wood heaps

IN GERMAN FORESTRY the spruce engraver *Ips typographus*, is the economically most important insect always found in all norway spruce forests. For hundreds of years, sanitary felling has been the basic control strategy. Trap trees were recommended first by H.J.v.Uslar at the end of the 18th century after a long lasting series of bark beetle outbreaks which devastated 30,000 ha of natural spruce forests in the Harz mountains in south-eastern Lower Saxony (Kremser 1982). In those times the biology of *Ips typographus* was widely unknown and scientists as well as practical foresters gave very contradictory recommendations how to deal with the "small black worm".

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We now know much more about bark beetle biology but still the recommendations on control measures differ significantly not only throughout Europe but also throughout Germany.

The integrated system of bark beetle control in northern Germany

This system, broadly practised since 1983 but published only in german forestry-journals and leaflets, shall be briefly explained here; it consists of:

1. Frequent searching for new infestations, in order to allow a timely sanitary felling.

2. Sanitary felling of newly infested trees. The success of the entire control strategy depends mainly on whether the very first infested trees of a certain spot are felled soon enough to interrupt the initial pheromone mediated „chain reaction" of infestation from tree to tree - i.e. before the beetles can have an economically significant impact on resources.

3. Additional mass trapping of bark beetles either by trap trees (or trap wood heaps, see below) or by pheromone traps (black slot trap; Niemeyer et al.1983).

According to our experiences, pheromone traps should be employed only where bark beetle infestations occurred in the current or previous year - not in uninfested stands! To avoid trap induced infestations of nearby standing trees, traps baited for *Ips typographus* must maintain a safe distance from the next spruce of about 12 m, in no case more than 15m or less than 10m. Along the margin of a stand or of a big gap inside a stand, the traps must form a line with distances from trap to trap of about 30 m (less when the population level is high, more when it is low - but 50m at maximum).

I must emphasize that no bark beetle trapping system is able to reduce the population density of e.g. *Ips typographus* over a large area or a long time. Its mode of action is to lure at short range (maximum 50m) a sufficient portion of the beetles searching for material to breed in away from the endangered neighbouring trees and collecting them in the traps. The remaining population of the beetles at this spot is then not numerous enough to overcome the resistance of normal vigorous spruce by mass attack.

Field experiments clearly showed that the complete control system with traps (if well applied!) reduced the successful infestation of living spruce by 70-100% compared to tree mortality of systems without traps (Dimitri et al. 1992; Busch et al. 1992, Niemeyer et al. 1994, 1995).

There are also situations when employment of trap chains is not reasonable:

- where fresh windthrown stems in high numbers compete with the traps;
- when all man- and machine-power is needed to prevent the infestation of living trees from rapid spreading all over the forest. This was the case in the Harz National Park in early summer 1996 after 2 previous years with extremely hot summers and no sufficient countermeasures. In one area of about 60 ha thousands of spruce were infested within a few weeks. Positioning of trap lines was postponed to the end of the sanitary felling activities.

Thinning techniques supporting the integrated control system

During the last 2 years special thinning techniques have been integrated into the control system with good results:

1. Using a "Harvester": When thinning in spruce stands of medium diameter (up to 25 cm), the trunks normally are stored within that forest undebarked for some months, and constitute a steady source of *Ips typographus* and *Pityogenes chalcographus* to infest adjacent spruce stands. However, processsing the timber by means of that machine called "Harvester" proved to be a 100% effective prevention against successful breeding of both of these harmful insects. The reason is the crushing (squeezing) of the bark while the stem is pressed through the toothed conveying rolls; this procedure makes the bark of norway spruce unsuitable for the above mentioned harmful species but highly attractive for a competitor, the strictly secondary and therefore harmless bark beetle *Hylurgops palliatus*.

2. Separating subsequent thinnings: In stands of non-marketable spruce with small diameters, the thinned material remains after being felled on the spot. In practice, normally one thinned plot borders on the next and one thinning activity follows the next within a short time. Thus, each of the subsequent bark beetle generations gets ample breeding material and the infestation rate increases year by year throughout the district. It is simple to avoid this, but the practice obviously needs recommendations like the following: Separate each thinning plot from the next one by either space (0.5 km in minimum) or time (1 year).

Infestations inside stands: a problem unsolved

In very hot summers, the spruce engraver prefers the (cooler) inner parts of stands for breeding instead of the stand margins which are normally its habitat. Inside the stands, it is very difficult to detect bark beetle attacks early enough, and normally not possible to employ pheromone traps because safe distances between traps and neighboring spruce can seldom be kept.

Trap wood heaps: Although environmentally undesirable, when urgently needed, we construct small poisoned heaps of slash (e.g. tops and boughs), about 1m high and 1-2m wide, containing one pheromone dispenser each. This works very well in terms of reduction of nearby infestations, demanding only a 6m distance from uninfested trees. In 1995, field experiments with 106 trap wood heaps at different places revealed a 100% reduction of lethally infested trees.

Thus, such trap wood heaps can be used in small gaps of about 12m diameter which are not too rare in older spruce stands. However, so far we have not found a sufficient explanation as to why a safe distance between the traps and uninfested trees is so much shorter than with black slot traps. Even trap wood consisting of dry material (without bark, cut in the previous year) was able to reduce infestations of living spruce in their vicinity up to 100%, provided, the pyrethroid dosage was high enough (2g of Cypermethrin per liter spray liquid resp. 0.3 g alpha-Cypermethrin). However, an experiment with an alpha-cypermethrin dosage of only 0.15 g per liter spray liquid and the trap wood heaps underlaid with plastic sheets showed that fresh wood should be much better than dry:

Table 1: Numbers of dead *I. typographus* under trap wood heaps with fresh (TWF) or dry wood (TWD), both types in pairwise arrangement (pairs as columns)

TWF:	1319	520	914	847	1611	1986	total: **7197**
TWD:	242	60	143	559	393	964	total: **2361** (32.8%)

Wilcoxon matched pairs signed rank test: R^ = 0; R (6;0.05)=0; significance: 5%

This doesn't necessarily mean that fewer beetles are lured to the dry trap wood, for bark beetles on fresh wood spend a longer time there and try to bore into the bark; so they get more pesticide exposure and drop off earlier than bark beetles on dry wood. A much larger proportion of these latter fly away from the trap wood over a short distance but cannot start breeding anymore.

A remark on possible environmental impacts of contaminated bark beetles may be of interest: checks of 20 bird houses set at a distance of only 8-10 m to trap wood heaps did not reveal any influence on the development of the nestlings (tits, flycatchers, sparrows) compared with another set of 20 bird houses more than 0.5 km apart from poisoned trap wood.

Adding host volatiles to pheromones: Following findings of Reddemann (1993), we tested (-)-alpha-Pinen plus (+)-Limonen in PE-dispensers added to Pheroprax^R (identical with the natural aggregation pheromone of *Ips typographus*) resp. Chalcoprax^R (for *Pityogenes chalcographus*) in black slot traps to improve trapping efficiency and perhaps thereby to shorten the safe distance at which traps can be placed with reference to uninfested trees. Our results have been published recently in Anzeiger für Schädlingskunde, Pflanzenschutz, Umweltschutz (Niemeyer and Watzek 1996), remarks here are limited to some short conclusions: In 3 experiments, with 30 repetitions, no monoterpen-induced enhancement of *typographus*-catches was found; the same was true with *P. chalcographus* (2 experiments with 18 repetitions). The differences in catches between traps with and without the additional host volatiles, were extremely low: between 1 and 6%. These differences were not significant (Wilcoxon matched pairs signed rank test).

Repellents to protect spruce near traps: (S)-Verbenon seems to be a repellent, according to different authors (Ryker and Yandell 1983, Schlyter et al. 1987, Baader and Vité 1990, Kohnle et al. 1992, Kostyk et al. 1993). Indeed we also found a 98% reduction of *typographus*-catches when Pheroprax^R -traps were compared with those additionally baited with verbenone in polyethylene bags (Niemeyer et al. 1995). But since 1990 we have tried in vain to achieve a repellent effect of verbenone on living trees with release rates of 2.5g resp.57g /tree/day (Rumpf 1990, Greeb 1992).

In 1993 a higher release rate - namely 93mg/tree/day - and another type of dispenser were tested in the vicinity of an uncontrolled *Ips typographus*-outbreak area in the Harz mountains (Niemeyer et al. 1995): 6 test locations were set up, each with one Pheroprax^R-baited slot trap and with one verbenone-treated and one untreated living spruce, both of them at about 6m distance from the trap. At each of the 4 directions on the treated tree one PE-tube was suspended at a height of 3.8m. Each tube was 3.3m long, with 6 compartments holding

10ml of verbenone each, i.e. 240ml of verbenone per tree. The results were in direct opposition to what we expected, (Table 2.)

Verbenone-trees were attacked as soon as 2 days after treatment, untreated spruce not before 11 days after the beginning of the experiment. Verbenone-trees received 111 boring holes in total, untreated trees 3. The maximum height of attacks corresponds very well with the height of the verbenone-dispensers, and there was not even the slightest repellent effect next to the dispensers.

Table 2: *Ips typographus* - Starting date of boring and boring holes at verbenone-treated (V) and untreated control trees (C); number of caught *I.typographus* per trap (at 6m distance)

Rep.	Starting Date		Number		Max. height (V)	Mean distance from PE-disp.	Beetles
	V	C	V	C	m	cm	caught
1	5/2	-	16	0	2.88	3.28	756
2	5/2	-	4	0	3.10	2.25	1.101
3	5/2	-	7	0	2.85	5.43	737
4	5/2	5/11	22	2	3.80	4.16	1.615
5	5/2	-	38	0	3.50	3.47	1.143
6	5/9	5/11	24	1	3.30	6.90	1.747

This total lack of any repellent activity of verbenone on living spruce does not depend on photoisomerization of verbenone, converted to chrysanthenone, as described by Kostyk et al. (1993), as is shown by the following field test data (again obtained in the Harz mountains):

Table 3: Number of *I. typographus* caught in traps baited with Pheroprax^R (P) or P + verbenone after different durations of light exposure of verbenone

Exposure of verbenone	Duration of light-exposure to verbenone			
	1 hour	1 day	1 week	1 month
in full sun	35	39	31	61
sheltered in slot trap	67	40	45	44
not exposed	19	31	3	6
Pheroprax^R only (no verbenone)	1.635	1.639	1.492	1.239

Thus, verbenone released from a polyethylene-bag dispenser within a slot trap baited with Pheroprax^R reduced the response of *Ips typographus* at the (in trap trials) well known level of about 95-98%, regardless of the duration of the preceding light exposure within the

scope of 1 hour to 1 month. That means, photoisomerization cannot be accepted as explanation for the failure of verbenone to repel *Ips typographus* from living spruce.

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Phenolic and Resistance of Scots Pine to Bark Beetles

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ABSTRACT The response of Scots pine, *Pinus sylvestria,* via phenolic content to aggressions was investigated in clones susceptible and resistant to mass inoculation of *Leptographium wingfieldii*, a fungus associated with *Tomicus piniperda*. Modifications in phloem phenolic composition was studied during a period of 60 days after inoculation with fungi or sterile malt agar. In all resistant clones, pinosylvin, its monomethylether, pinocembrin and taxifolin were synthesized in the reaction zone after both fungal and sterile inoculations. In the susceptible clones, these compounds were neosynthesized only after fungal inoculation. Taxifolin glucoside was more abundant in the resistant than in the susceptible clones, both after inoculations and in unwounded phloem. Effects of these compounds on *L. wingfieldii* were assayed in liquid medium in microwells at different concentrations. All neosynthesized compounds except taxifolin inhibited fungal growth but they had no apparent effect on *T. piniperda*. These results are discussed regarding tree phenolic defense strategies to attacks by bark beetles.

KEY WORDS Scots Pine, phenolic compounds, resistance, markers, phloem induced reaction, *Tomicus piniperda*, *Leptographium wingfieldii.*

THE PHLOEM INDUCED reaction plays a basic role in conifer resistance to attacks by bark beetles and their associated fungi. It takes place around each point of attack, and important chemical modifications occur. In cases of limited numbers of attacks, it stops the attacking agents, but above a certain density of attacks, it becomes ineffective and the bark beetle population can successfully established. This threshold of attack density above which a tree is overcome is a measure of tree resistance and depends on the physiological status of the tree. Another relative estimation of the tree resistance can be obtained by replacing bark-beetles attacks with artificial mass inoculations of fungi isolated from beetles, thus leading to the determination of a threshold of inoculum density.

Previous experiments on Scots pine have showed that phenolic composition varies considerably after fungal inoculation. In the unwounded phloem of Scots pine, phenolics are mainly flavonoids [(+)-catechin and taxifolin glucoside], p-coumaric aci ester and acetophenone glycoside. In the wounded phloem (reaction zone), four compounds appear: two flavonoids (Taxifolin and Pinocembrin) and two stilbenes (Pinosylvin and its monomethylether).

This paper is a synthesis of several experiments on the interaction between Scots pine, *Tomicus piniperda* and its associated fungus *Leptographium wingfieldii.* The role of phloem phenolics in the efficiency of the induced reaction in stopping attacking agents (*T. piniperda* and *L. wingfieldii*) and in Scots pine resistance was investigated in two steps.

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species groups. Sibling species exist in both the European and the American groups. In recent years Cane et al. (1990) and Cognato et al. (1995) have started to work on the phylogenetic relationship of the American *grandicollis* group which is comprised of seven species that have five pairs of spines on the elytral declivity. Allozymes and random amplified polymorphic DNA (RAPDs) revealed congruence in the parsimonious analysis and the grouping paralleled morphological and behavioural similarities. Stauffer et al. (1997) investigated the European *Ips* species by sequencing a region of the mitochondrial (mt) DNA and screening several isozymes. Extensive nucleotide divergence was found, suggesting a long divergence time for the species and the dendrogram suggested four speciation events regarding the host.

In this study a molecular technique to determine the species of the European genus *Ips* by using PCR and agarose gel electrophoresis is presented. A non-coding region showed variability in length among the species, suggesting this region to be a good marker for identification of *Ips* sibling species.

Methods

The parental generations of the seven *Ips* species were sampled from felled trees during the spring flight season in 1994. Only adult beetles were collected. Province and town for each site is given: *I. typographus* (Tyrol, Ehrwald), *I. amitinus* (Lower Austria, Gutenbrunn, Austria) and *I. duplicatus* (Morawia; Ostrau) were collected from *Picea abies*, *I. cembrae* (Styria, Kindberg) from *Larix decidua*, *I. mannsfeldi* (Lower Austria, Gänserndorf) from *Pinus nigra*, and *I. acuminatus* (Lower Austria, Retz) and *I. sexdentatus* (Lower Austria, Gänserndorf) from *Pinus sylvestris*.

Beetles were preserved in ethanol and DNA was extracted from head and thorax of individual specimens by using the protocol of Juan et al. (1995). A fragment was polymerised by using primers UEA 9 and UEA10 developed by Lunt et al. (1996). The PCR procedure is described in Stauffer et al. (1997). The products were screened on a high resolution agarose gel (2%). In order to distinguish between *I. typographus* and *I. cembrae*, a restriction enzyme (*Dra*I) was used. DNA purification and sequencing reaction of the single stranded DNA followed the procedures of Cooper and Hewitt (1993).

Results

The primers were used to polymerise a DNA product of about 300bp length. The PCR reaction was done for at least 6 individuals, for each species and the products were screened on agarose gels. *Ips mannsfeldi* showed a fragment of the size of 362bp. *Ips duplicatus* and *I. acuminatus* had a fragment of 307 bp and *I. sexdentatus* of 304bp. *Ips amitinus* had 319bp, *I. cembrae* 327bp and *I. typographus* 332bp. The restriction enzyme was used to differentiate between *I. typographus* and *I. cembrae*. *DraI* cut the fragment of *I. typographus* in a piece of 285bp and a fragment of 47bp. *Ips cembrae* had no restriction site for *Dra*I.

Table 1 presents the sequences responsible for this length variability. The region corresponds to a non-transcribed region between COI and the $tRNA_{Leu}$ gene. Among four *Ips* species considerable difference in length was found. The length varied among 10, 18, 23

and 57 for *I. amitinus*, *I. cembrae*, *I. typographus* and *I. mannsfeldi* respectively. *Ips sexdentatus*, *I. acuminatus* and *I. duplicatus* had no intergenic region. Stauffer et al. (1997) reported that the stop codon of the COI gene varied between a single nucleotide (T for *I. duplicatus*, *I. acuminatus* and *I. sexdentatus*) and three nucleotides (TAA for *I. typographus* and *I. amitinus*, or TAG for *I. mannsfeldi)*. Further an insertion deletion (INDEL) before the stop codon in *I. mannsfeldi* and *I. sexdentatus* was found.

Table 1. Non transcribed region between COI and $tRNA_{LEU}$ of four species of *Ips*

I. amitinus	AATAAAACCT
I. cembrae	CCATAAAAGA AAAAACCT
I. typographus	ATTTAAATAA AGAAATTTTC TTT
I. mannsfeldi	AAAATAAAAA TTAAGAATTA AGTTTAAATA AAGAATATTA TTATTCGTTA TATTCAT

Discussion

In recent years mt DNA sequence data have been used in coleopteran species to investigate problems in systematics (Funk et al. 1995; Emerson and Wallis 1996), conservation biology (Vogler et al. 1993) and colonisation (Juan et al. 1995). An important and often neglected feature in this science is the identification of appropriate markers which should be polymorphic, however should not be too polymorphic to intrigue the results.

Cytochrome oxidase (CO) provided useful information for inter- as well as intra-specific insect studies. The mt genome of honeybees is characterised by the presence of a long intergenic sequence located between the COI and COII genes. The length of this sequence varied between and within species and several length categories were characterised up to 650bp. High AT bias, stability profile, hairpin and coverleaf putative secondary structures suggest that this non-coding intergenic region might contain an origin of replication (Cornuet et al. 1991; Crozier et al. 1989).

Here a region between COI and tRNALEU in the genus *Ips* showed variability in length. The intra-specific variation was studied from the seven species (between 6 and 9 specimens) and no variation was found. In *I. typographus* about 124 specimens were screened during a phylogeographic study (Stauffer, Lakatos and Hewitt unpubl. data). No variation was found among these specimens.

The AT bias in the intergenic non-transcribed region was about 20% higher than in the COI gene in *I. mannsfeldi*. Also secondary structures of the putative ancestor sequence could be demonstrated in the black pine beetle.

The difference in length was long enough, to screen the differences on agarose gels, thus, avoiding the expensive and laborious procedure of sequencing. In the case of the two sibling species, *I. cembrae* and *I. typographus,* a blunt end restriction site (TTT/AAA) made the distinction easier.

Although in the scolytids the basic alpha taxonomy of the North American and European bark beetles is known, species groups, the phylogeny and other basic systematic questions are still unresolved (Bright, 1992). Bright (1992) mentions the lack of qualified future scolytid systematics. It would be helpful to have a genetic key book with which species can be

determined. Such a key book would be absolute and applicable to all laboratories. The methods available for obtaining discrete markers, are the sequencing of mt DNA or also the genomic DNA (e.g. microsatellites). There are several groups attempting this approach. Therefore it is possible that in the near future more markers like the intergenic non-coding region between COI and COII will be available.

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inoculation with *L. wingfieldii* : three were susceptible and three others were resistant. One tree of each clone was inoculated with *L. wingfieldii* and with sterile malt agar. Samples of inoculated phloem were taken 3, 7, 14, 30, 60 days later. At day 0 (the day of inoculation) and day 30, samples of unwounded phloem were also taken from each tree. Monophenols were analysed by HPLC.

Mechanism of the induced phenolic response. Changes in phenolic composition of the phloem after wounding and after wounding plus infection by *L. wingfieldii* showed that tree's reaction was not specific to the attacking agents. However, this reaction was modulated by the presence of the fungus. Two steps could be distinguished during reaction development:

1 - The first was an accumulation of compounds lacking in unwounded phloem (taxifolin, pinocembrin, pinosylvin and its monomethylether). 2 - The second, depended on the presence of the fungus, and was a stabilization of the concentration of these compounds after 7 to 14 days.

Because pinosylvin, its monomethylether, and pinocembrin and their precursors were completely absent in the unwounded phloem, the accumulation of these compounds, after both a sterile and a fungal attack, very likely resulted from neosynthesis by the tree. The stabilization of the concentration of the new compounds during the second step could result from the metabolization on the synthesized compounds by the fungus (pinocembrin, pinosylvin and its monomethylether). The appearance of these three compounds could thus result from the induction of a new metabolic pathway in Scots pine phloem. The cinnamate CoA ligase and stilbene synthase could be induced or activated during the induced reaction. The appearance of taxifolin after inoculation, could result from the activity of chalcone synthase, or could be due to the hydrolysis of taxifolin glucoside by the tree itself or by the fungus.

Differences between trees. The resistant and the susceptible trees were compared with respect to their phenolic composition. In both unwounded and wounded phloem, the resistant trees differed from the susceptible ones by higher concentrations of taxifolin glucoside. After fungal inoculation, four phenolic compounds appeared in all types of trees but later and/or more slowly in the susceptible trees than in the resistant ones. After wounding alone, the four compounds appeared but only in resistant trees. We thus hypothesize that susceptible trees differ from resistant ones by the inhibition of the cinnamate CoA ligase in a normal situation which can be removed in the presence of fungi and by a slower ability for neosynthesis after fungal attack.

In vitro bioassays

Fungal bioassays. Tests were performed in microwells. Phenolics and fungal spores were added in the liquid medium in sterile conditions. Fungal growth was measured with a spectrophotometer by measuring the difference between the absorbance of the fourth day of culture and day zero (beginning of the experiment). Effect of the fungus on phenolics was measured by extracting the compounds from the medium at the end of experiment and by analyzing the extracts with HPLC. *In vitro* bioassays showed that pinosylvin, its monomethylether, and pinocembrin inhibited fungal growth; that taxifolin had no effect; and (+)-catechin stimulated fungal growth. The fungus was able to metabolize pinosylvin, its monomethylether and pinocembrin, but it did not metabolize (+)-catechin and taxifolin.

Thus, corroborating the hypothesis concerning its role in the stabilization of these compounds during the second phase of the reaction.

Insect bioassays. Tests were performed in a rearing medium largely composed of ground phloem and agar. Phenolic compounds were added to the surfusion medium at 50°C. The effect of the phenolics on *T. piniperda* was measured by medium consumption and beetle mortality after 6 days. The phenolics present in rearing medium were re-extracted at the beginning of the experiment and at the end of the experiment, and were analyzed with HPLC.

The phenolic compounds tested in this experiment (catechin, taxifolin, pinosylvin, its monomethylether and pinocembrin) did not have a visible effect on the consumption of artificial medium by *T. piniperda*. However, analysis after re-extraction showed that the phenols were damaged in the medium.

Conclusions

The variations in the concentrations of phenolic compounds and their effect on the aggressors lead us to consider that phenolic compounds play an important role in Scots pine resistance to bark beetles and their associated fungi. The results of *in vitro* bioassays are in agreement with the tree's response mechanism by suggesting that the second step of this mechanism could effectively result from metabolization of the pinosylvin, its monomethylether and pinocembrin by the fungus. However, during their metabolization, these compounds would continue to be synthesized by the tree and they would thus be able to stop the growth of *L. wingfieldii*. Indicators of resistance can be proposed. They are pinosylvin, its monomethylther, pinocembrin, taxifolin glucoside and taxifolin.

Technology Transfer of Treatments using Verbenone for the Suppression of Southern Pine Beetle Infestations

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ABSTRACT A three phase technology transfer program is currently underway to present potential users with the operational methods developed for suppressing southern pine beetle infestations with verbenone. The program is designed to ensure that the tactic is properly applied and to solicit feedback on the ease and viability of the methods. Three technology transfer sessions have been conducted to date, and the initial results indicate the application methods and directions for use are satisfactory. The major concerns voiced by foresters were treatment cost and efficacy. The results underscore the necessity of providing effective training in treatment application once verbenone is registered for use by the Environmental Protection Agency.

KEY WORDS southern pine beetle, verbenone, technology transfer, pheromones

THE SOUTHERN PINE beetle (SPB), *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), is the most destructive forest insect pest in the southern United States (Payne 1980). Methods for suppressing infestations have evolved over the past century. Early techniques involved salvage of infested trees, burning infested material, injection of chemicals into the sap of standing trees, and exposure of felled trees to solar radiation (St. George and Beal 1929, Craighead and St.George 1938). In the 1940s these tactics gave way to pesticide applications on felled trees, and the objective of control programs was the elimination of epidemics (Billings 1980). By 1970 it had become clear that pesticides were instead adversely affecting natural enemies of SPB (Williamson and Vité 1971), and epidemics continued to occur, so the emphasis shifted to integrated control. Forest managers utilized silvicultural practices to reduce SPB hazard, and implemented direct control measures to suppress infestations and reduce losses. Four suppression methods were commonly used and authorized by the Final Environmental Impact Statement for the Suppression of the Southern Pine Beetle: cut and remove, cut and leave, cut and hand spray, and pile and burn (USDA 1987).

While the approved direct control methods have proven efficacious for suppressing individual SPB infestations (Redmond and Nettleton 1990, Billings 1995), they all involve tree felling and therefore cannot be implemented in certain areas. Also, cut and remove and cut and leave include felling a buffer of uninfested trees. Researchers began searching for methods which did not require tree felling or sacrificing uninfested timber. Vité (1971) suggested the use of semiochemicals for the control of SPB, and techniques involving

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attractants and inhibitors have since been tested. The most promising tactic tested thus far has been deploying verbenone around the active front of an infestation (spot) to prevent continued spot expansion.

Verbenone is a pheromone produced primarily by male SPB. At high concentrations it inhibits aggregation of both male and female SPB and prevents overcolonization of individual hosts (Rudinsky 1973). In the summer, SPB are generally aggregated within infestations. Emerging and reemerging beetles are attracted by aggregation pheromones to trees currently under attack, and males release verbenone and other inhibitory pheromones. As concentrations of these inhibitory pheromones increase, beetles are deterred from these trees and instead attack nearby uninfested pines. By placing synthetic verbenone on uninfested trees at the head of a SPB infestation, it was theorized that spot expansion would be disrupted, emerging and reemerging SPB would disperse, and the spot would become inactive. After continued field testing and refinement of application methods (Payne and Billings 1989, Salom et al. 1992, Payne et al. 1992, Billings et al. 1995), standardized and effective operational techniques for two treatment techniques, verbenone plus felling and verbenone only, were developed (Salom et al. 1997a). These treatments will suppress SPB infestations within specified size categories.

Three steps are necessary to put operational techniques for suppressing SPB infestations using verbenone into practice:

1. Further refinement of the methods to potentially increase efficacy and ease of application while reducing costs
2. Final registration of verbenone from the U.S. Environmental Protection Agency (EPA)
3. Technology transfer to train potential users on the correct application of this tactic.

The focus of this paper is on the process of technology transfer.

Technology Transfer

The USDA Forest Service, Texas Forest Service, University of Georgia, and Virginia Tech have developed the operational methods and are working cooperatively to transfer this emerging SPB suppression tactic to southern foresters. The technology transfer program consists of three phases:

Phase 1. An initial survey of potential users

Phase 2. Preliminary, informal technology transfer sessions with small groups before final product registration

Phase 3. Formal technology transfer sessions with potential user groups or licensed applicators after product registration is complete.

The initial survey was designed to gauge the depth of knowledge of SPB among potential users and to help design the technology transfer protocol and materials necessary to effectively train foresters. The results from the survey (Salom et al.1997b) were used to plan phase 2.

Even though they precede EPA registration of verbenone, the phase 2 technology transfer sessions are important for several reasons. First, the operational methods of applying verbenone we developed may be effective, but they may not gain acceptance if foresters are hesitant to use them due to cost, complexity, time constraints, manpower required, or other considerations. These initial sessions will assess how users regard the tactic and suggest if

and what refinements are necessary before EPA registration is complete. The feedback will help us identify problem areas and focus our future technology development . Second, the sessions will indicate the potential usage pattern of the product. This information will be beneficial to the company pursuing registration, Phero Tech Inc. (Delta, British Columbia), in developing marketing strategy, and to the EPA when considering exposure risks during the registration process. These sessions also serve to familiarize foresters with verbenone. The initial survey included questions on treating infestations with semiochemicals, but did not specifically mention verbenone, so these sessions will help ensure name recognition when verbenone becomes available for use. Finally, the feedback from these sessions will aid us in further developing the training materials and presentation techniques for phase 3.

After verbenone registration is obtained, the phase 3 technology transfer sessions will be designed to make foresters aware that verbenone is now available as a treatment option, and will teach them how and when to effectively apply the treatment. We want to insure that the product is properly applied so that treatment failures are minimized and foresters develop and maintain a high level of confidence in verbenone as a SPB suppression tactic.

Phase 2 Technology Transfer Sessions
Methods

The preliminary technology transfer sessions consisted of classroom instruction and a field demonstration, the preferred training methods indicated by foresters (Salom et al. 1997). The classroom instruction emphasized four areas: SPB biology, SPB population dynamics including the role of pheromones, history and results of verbenone technology development projects, and the current methods of verbenone application for SPB infestation suppression. When discussing SPB biology, we emphasized distinguishing SPB adults and infestations from those of *Ips* beetles, determining if fresh attacks are present and if the spot has the potential to expand, and how to determine if a tree has been vacated by SPB. These determinations are critical for effective application of the treatments, and all were areas where the survey indicated increased knowledge was needed.

The effects of verbenone on SPB behavior were stressed in the segment on SPB population dynamics. We explained how SPB uses semiochemicals to coordinate mass attacks on pines and initiate spot expansion. This discussion was designed to provide the logic for using verbenone to suppress SPB infestations. This led into a description of the history of technology development projects testing verbenone. We described the evolution of the elution device to the white pouch with sponge currently in use, and the development of standardized operational procedures detailed in Salom et al. (1997a).

Finally, we gave each participant a copy of the use directions we have written for inclusion on the verbenone label, and went through the treatment protocol step by step. We described how to use the tables to determine the number of pouches required to treat the infestation and the number of pouches per tree. We also prepared and distributed single page, simplified instruction sheets which are designed for field use.

In the field session, we first examined a spot we had previously treated with verbenone. This allowed us to illustrate how the tactic should be applied, and to demonstrate how to monitor treatment success. We then visited an untreated infestation and allowed the participants to analyze the spot, determine the number of verbenone pouches needed,

designate the trees to be treated, and apply the treatment. The trainees practiced using the "Hundle" hammer, a verbenone application tool which allows the user to nail the pouch to the tree at a height of 4-5 meters. Questions were welcomed and addressed throughout the classroom and field sessions.

At the close of the field session, a training evaluation form was distributed to all attendees. The evaluation was designed to elicit how and to what extent the tactic might be used, and what factors were most important in determining its use pattern. We also included questions asking the participants to assess the practicality of the application methods and the effectiveness of the training session.

Results And Discussion

Three phase 2 sessions for USDA Forest Service personnel have been conducted to date. Ten people from the Sabine National Forest in Texas attended the first session, fourteen people from the Davy Crockett and Angelina National Forests in Texas were at the second session, and the third session was given to 37 people at the Ouachita National Forest in Arkansas. We had hoped to conduct sessions throughout the south in 1996, but the collapse of SPB populations did not allow us to schedule more training. The field sessions are vital to demonstrating the application procedures and allowing hands-on experience, so we plan to hold more training sessions when SPB infestations are available.

The attendees at the first three sessions all responded positively to the verbenone treatment procedures as described in Salom et al. (1997a). Every evaluation stated the elution devices were satisfactory, the tables designed for calculating number of pouches per tree and infestation were easy to use, and overall the application methods were practical. The comments we received during the field sessions indicated that verbenone applications would not be too time-consuming. Suggested refinements for applying the treatments included modifying the "Hundall" hammer so that it could accept nails on both ends, and producing pouches of various size so that only one pouch would be used per tree.

Comments from the first training session suggested that a more detailed explanation of treatment monitoring was needed, so we emphasized monitoring procedures in the subsequent sessions. We stressed that the full 6 week monitoring period is often necessary, and the treatment goal, particularly for the verbenone only treatment, is not to prevent attack on every treated tree, but rather to disrupt spot expansion and protect some of the green trees which would have been felled in other treatments. This point was particularly pertinent at the Arkansas demonstration session, where many of the treated trees had SPB attacks. Of the attacked trees, many only exhibited a few pitch tubes near the verbenone pouches, with no evidence of attack on the rest of the bole. We explained that these trees may not be successfully attacked, and the infestation might still go inactive, so two more weeks of monitoring (six weeks total) were needed. If the beetles had advanced beyond the treated buffer, or if fresh attacks were present at that time, then retreatment using verbenone or another suppression tactic should be considered. Monitoring two weeks later revealed that no new trees had been attacked.

A majority of the responses indicated that foresters would use both treatments on less than 25 percent of SPB infestations. They would usually recommend using verbenone in place of cut and leave or cut and hand spray. Foresters also responded that they favor using

the treatments more in sensitive areas such as wilderness or streamside zones than in non-sensitive areas.

When asked to select factors which would be very important when deciding whether to use verbenone to treat a particular spot, and also to designate the most important factor, infestation size and preventing additional tree loss received the most mentions, and infestation size and treatment cost were rated as the most important factors. From their questions during the training, it was apparent that the most important concerns of the foresters about using verbenone in general were treatment efficacy and cost. Though we presented results from our field trials which demonstrate the treatments are effective when applied correctly, it appeared that positive results from personal applications or observations would be necessary to gain the foresters' complete confidence in the tactic. They also expressed reservations about the cost of the treatment, particularly when compared to costs of current suppression techniques.

The feedback from these initial sessions illustrate the importance of designing and providing effective technology transfer of this tactic. Foresters must be trained to use the tactic only within the infestation size parameters established for each treatment, and to apply the verbenone correctly. Comprehensive training should lead to acceptable treatment results, consumer confidence, and increased treatment usage. We may also need to develop and test methods which would allow larger infestations to be treated. The concerns over treatment costs indicate we need more data on benefit/costs of the tactic. We need to demonstrate that the value of the trees protected by verbenone which would have been felled in a buffer strip when using other treatments is greater than treatment costs. We should also search for ways to reduce product costs.

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Girdling of crownless Norway spruce snags: a measure against *Ips typographus* ?

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ABSTRACT In August 1992 a hailstorm destroyed 73 ha of forest on the north face of Rigi Mountain in Central Switzerland. Many spruces, though not thrown by the wind, had their crowns broken off. As the trees stood on steep slopes, the forest service decided to leave the snags standing as a protection against rockfalls and other natural hazards. This raised the question as to whether this procedure would enhance the propagation of bark beetles, in particular *Ips typographus*. An experiment was conducted to investigate whether girdling these fresh stumps would accelerate their dessication and thus more rapidly render them unattractive to bark beetles. Two measurements of wood moisture showed that neither girdling nor the height at which the crown broke off influenced the dessication of snags. The only significant factor was the diameter of the stem: thin stumps dried out more rapidly than thick ones. In this study, *Ips typographus* invaded only broken crowns lying on the ground, and it was possible to remove these in time to prevent the beetles from spreading. One year after the hailstorm all the snags had dried out without attack by *Ips typographus*.

KEY WORDS forest protection, storm damage, Norway spruce, girdling, bark beetles.

IN THE PAST few years forests in Switzerland have more frequently suffered storm damage as a result of extremely high winds. The storm "Vivian", which struck many parts of Europe on 27/28 February 1990 brought the worst gale ever recorded in Switzerland. It caused the loss of 4.9 million cubic meters of wood, about one year's regular cut for the whole country (Holenstein 1994).

Until now, it has been the usual practice to clear damaged areas of forest completely for regeneration or for reasons of forest protection, in particular to prevent secondary damage by bark beetles. These measures, however, do not cover their own costs and worsen the already difficult financial situation of forest owners.

On 21 August 1992 a hailstorm destroyed a large part of the forest on the north face of Rigi Mountain in Central Switzerland belonging to the Swiss Federal Railways. Over an area of 73 hectares, 28 000 cubic metres of wood, of which Norway spruce (*Picea abies*) comprised 25%, were broken off. As part of salvage operations, the upper parts of the stems which had been broken off were removed while the crownless stumps were left standing. The aim was to maintain protection against rockfalls, snow movement, mud slides and erosion. There was a simultaneous need to avoid the use of standing stumps as a haven for the reproduction and spread of harmful forest insects, in particular *Ips typographus* (Kläy and Mani 1993). This raised the question as to whether girdling these snags would accelerate their drying out and thus more rapidly render them unattractive to forest pests.

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Over large parts of the areas damaged by the storm of February 1990 it was observed that spruce stems lying on sunny sites with total destruction frequently dried out before the population of *Ips typographus* had time to expand and consequently remained free from attack. On shady sites, in contrast, stems lying on the ground but still having a certain amount of contact with their roots were still being invaded one or even two years after the storm (Forster 1993). In order to accelerate their drying out, such stems were cut free of the stump.

The effects of girdling standing trees have been investigated in several studies, but these deal only with trees with intact crowns and are mostly concerned with finding economical methods of eliminating competitors.

Materials And Methods

As no reports on experiences with the girdling of standing crownless trees and the course of their drying out could be found, the forest service of the Swiss Federal Railways, in cooperation with the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, decided to conduct a case study to investigate the effects of girdling snags (Feiger et al. 1996).

In this study, conducted on three sites with total damage, 100 spruce snags were girdled and 100 were left ungirdled, and beetle attack and the course of dessication of the two groups were compared. The experiment was designedly focused on *Ips typographus* , because records from areas damaged by "Vivian" in 1990 showed that the most extensive secondary damage was caused by attacks by this bark beetle in damaged neighbouring stands (Forster 1993).

On three sites all the spruce snags, which totalled 200, were numbered and mapped after the storm. Diameter at breast height, height of the break in the stem and any particular features such as splits were recorded for each snag. At the beginning of May 1993 half of the snags were girdled three times above the base of the stem, cuts about 3 cm deep being made with a chain saw.

In May 1993 a lath sample was taken from every snag on the study sites and its moisture content determined in the laboratory (DIN 52'183. 1977). In September 1993 the process was repeated in order to determine the extent to which the stems had dried out.

During the summer of 1993 and in the spring of 1994 all snags were examined for insect attack. The adjacent area with damage was also checked. Further, the population of *Ips typographus* was monitored by means of pheromone traps.

Results

In May and June 1993, broken wood still lying on the ground displayed attack by *Ips typographus*. It was however possible to process and remove it before the new generation of beetles took wing. In contrast, none of the snags, whether girdled or not, evidenced attack.

In the autumn and winter of 1993/94 some standing but damaged trees outside the experimental plots still exhibited signs of attack, though all these trees still had some green branches.

Ips typographus did not appear on the stumps of the experimental plots. Both girdled and ungirdled spruce stumps were however frequently attacked by the following beetles causing secondary damage:

- *Hylurgops palliatus* (*Scolytidae*)
- *Polygraphus poligraphus* (*Scolytidae*)
- *Dryocoetes autographus* (*Scolytidae*)
- *Trypodendron lineatum* (*Scolytidae*)
- *Rhagium inquisitor* (*Cerambycidae*)
- *Hylecoetus dermestoides* (*Lymexylonidae*)

Free-standing snags were in general less heavily attacked by these beetles species than those scattered through the inner part of the remaining stands. The bark of heavily attacked snags began to peel off as early as July 1993. Such snags rapidly lost any attraction for *Ips typographus*.

Further checks in June 1994 , almost two years after the storm, showed that there were no longer any snags attractive to *Ips typographus*: the stems were already too dry.

The wood moisture measurements taken in spring 1993 showed a very wide range: minimum 30%, maximum 148%, scatter 16%. By autumn of that year the wood moisture of the snags had declined: minimum 16%, maximum 73%, scatter 12%.

Girdling did not influence the course of drying out of the snags. There were no differences between the wood moisture of girdled and that of ungirdled snags. Neither was there any evidence that the height of the breakage had any influence. On the other hand, in the spring of 1993 thick snags showed a higher wood moisture than thin ones. That was the only detectable difference. By the autumn of 1993, 15 months after the storm, this difference had disappeared. The thick snags must have dried out somewhat more slowly or later than the thin ones.

Because the snags were not attacked by *Ips typographus* at all and there was hardly any attack on the trees left standing within the stand, it is not possible to draw any definite conclusions as to the time frame of the insect's ability to breed or the probability of attack on the snags.

Discussion

In the case investigated, *Ips typographus* attacked only broken crowns lying on the ground, and it was possible to remove these in time to prevent the beetle from spreading. One year after the hailstorm all the snags left standing had dried out to such an extent that they were no longer attractive to dangerous forest pests, so that here it proved worthwhile to leave the snags standing: they maintained their protective function to a large degree, and there was enough manpower to remove wood already infested or any that might have attracted the pests.

The snags will continue to fulfil their function as a protection against rockfall, erosion and to a certain degree against snow movement until the forest has grown up again on the damaged areas. Further, the large amount of deadwood left undisturbed meets the needs of nature conservation. Even in protection forests, economics need not conflict with ecology.

Because attack by bark beetles and the development of their populations depend on numerous factors, such as the initial population, the weather and available breeding sites, it is not possible to make any generalized recommendations for the treatment of areas with storm damage.

Storm damage often poses complex problems for forest owners, involving work capacity, opening-up, marketing, forest protection, financing of operations, safety at work and many other factors, and therefore require in turn equally complex solutions tailored to meet each individual case. These may include leaving wood assortments likely only to show deficits standing or lying. Under today's conditions (state funding, nature conservation), indiscriminate processing and clearing at any price is becoming increasingly questionable. In every case of damage it is worthwhile first to analyse the situation in detail, then to estimate possible hazards, and finally to work out a refined concept of operations.

If it is decided to leave snags standing, it is unnecessary to girdle fresh stumps, as this does not accelerate their drying out. It is certainly possible that if *Ips typographus* undergoes a population outbreak even stumps may be invaded. Therefore it is essential to maintain observations for at least two years after storm damage occurs. Any infestation should be detected as early as possible and combative measures taken before a new generation is ready to take wing.

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Changes in the occurrence of bark beetles on Norway spruce in a forest decline area in the Sudety Mountains in Poland

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ABSTRACT From 1988 to 1994 investigations on the occurrence of the bark beetles were carried out in the Western Sudety, Poland, where about 15 thousand hectares of Norway spruce stands died as a result of outbreaks of *Zeiraphera diniana* and bark beetles in 1977-86. During this period changes in the frequency and dominance of bark beetle species were found. *Pityogenes chalcographus* increased in dominance while the diversity of the total bark beetle complex on Norway spruce and their parasitoids declined. Possible causes of this phenomenon, such as the characteristic of trees and stands and their limited resistance, consequences of outbreaks of other insect species, and weakening of trees, are discussed.

KEY WORDS *Picea abies*, bark beetles, forest decline, Sudety Mts.

THE FORESTS IN the Sudety Mts. in Poland have exhibited a decline in vitality during the last 20 years. Composed mainly of Norway spruce, artificially planted at the beginning of the century from seedlings of foreign or unknown origin, these forests have been adversely affected over a long time by abiotic factors, such as snow, wind and air pollution. From 1977-83 Norway spruce in this area has been repeatedly defoliated by *Zeiraphera diniana* Gn. (Lepidoptera: Tortricidae). As a result of this defoliation and an outbreak of bark beetles in 1981-86, nearly 15 thousand hectares of trees died (Grodzki 1994). These outbreak conditions and mass trapping of bark beetle populations (Grodzki 1995), resulted in some changes in insect populations, described in this paper.

The aim of the study was the description of:

- qualitative and quantitative characteristics of the bark beetle populations in weakened stands,
- changes in characteristics compared with other Norway spruce stands in Polish mountains,
- role of selected factors influencing bark beetle populations.

Materials and Methods

Investigations were carried out in 1992-94 in Norway spruce stands when stand age varied from 45 to 124 years. In 12 experimental plots located at various altitudes and expositions, trees were numbered and defoliation levels were ranked using 5 classes. All trees infested by bark beetles in the late spring - early summer and in the late summer - early autumn, were felled and the infested bark was analysed in 4 half-meter sections: 1 - basal

Pages 105-111 in J.C. Grégoire, A.M. Liebhold, F.M. Stephen, K.R. Day, and S.M. Salom, editors. 1997. Proceedings: Integrating cultural tactics into the management of bark beetle and reforestation pests. USDA Forest Service General Technical Report NE-236.

(0.5-1.0 m above the ground level), II - between the ground level and the crown base, III - in the crown base and IV - in the middle of the crown. The occurrence of all species infesting the bark was recorded and the infestation density, (i e. the mean number of the mating chambers [m.ch.] per dm^2), of the three most important species was estimated. Tree samples were also reared in the laboratory, to confirm the bark beetle composition and to determine the species of natural enemies. Comparative observations and analyses were carried out in Pieniny and Bieszczady National Parks, in the Carpathians. During the study period, 147 infested trees were analysed in the Sudety Mts.; additionally 68 samples were reared in the laboratory. For the comparative observations in the Pieniny and Bieszczady, 2 and 4 trees respectively were used together with 18 laboratory rearings.

Results

Species composition and frequency. During the study period, 71 species of insects were found. The most abundant were Coleoptera with 53 species from 15 families. There were 18 species from 6 families of Hymenoptera, and only 1 species of Diptera was found. Among Coleoptera, the most abundant were bark and wood boring families: Scolytidae with 21 species and Cerambycidae with 10 species. Species diversity of the group of bark and wood-boring insects, found in the Sudety Mts., was relatively low in comparison with other mountain regions in Poland. The presence of *Pissodes harcyniae* and the absence of Monochamus and Acanthocinus in the Sudety Mts. was an interesting phenomenon, indicating the differences in the quality of the breeding material between the Carpathian and Sudety's Norway spruce stands.

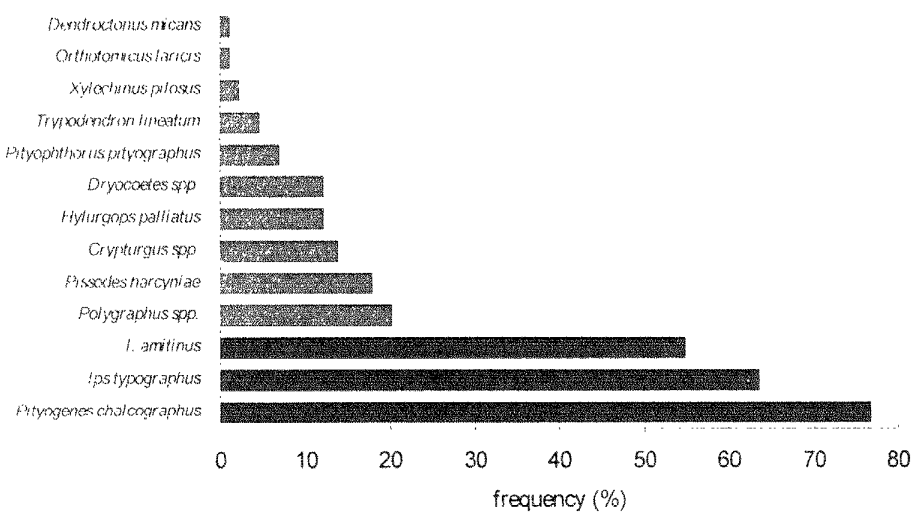

Fig. 1. Frequency of insect species on experimental plots in the Sudety Mts.

The most common species was *Pityogenes chalcographus*, found on 77% of sample trees; slightly less frequents were *Ips typographus* (64%) and *I. amitinus* (55%). In the most cases, infestation by these three species was the main cause of trees deaths. Insects belonging

to the genus *Polygraphus* and the weevil *P. harcyniae* played a similar but less important role. Insects from the genera *Dryocoetes*, *Hylurgops* and *Trypodendron* were found on dying or dead trees. Other bark beetle species, like *Pityophthorus pityographus* or *Xylechinus pilosus* were rather scarce and played a negligible role (fig. 1).

The frequency of the most important species in tree sections was related to the different preferences of each insect (fig. 2). The frequency of *I. typographus* was the highest in the section I and decreased in higher tree sections; while in the case of *I. amitinus* the opposite tendency was observed. The frequency of *P. chalcographus* was less varied; the concentration of the species was the highest in sections II and III, but even in section I, this insect was found on 29% trees.

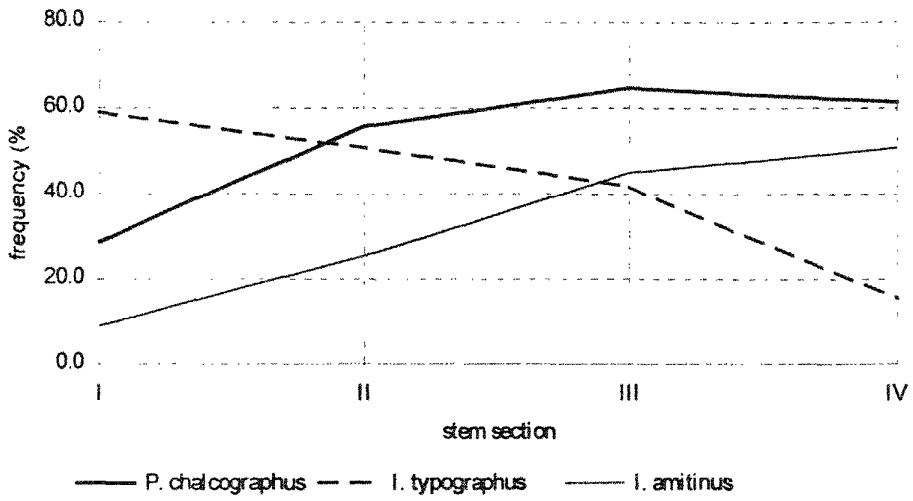

Fig. 2. Frequency of the most important bark beetle species in tree sections.

Infestation density of the most important species. The infestation density was analysed on 122 infested trees, for 3 insect species: *I. typographus*, *I. amitinus* and *P. chalcographus*. The highest mean density (1.78 m.ch./dm² with a range of 0.02-7.07) was reached by *P. chalcographus* : this density is much higher than reported by Ossowska (1990) from the Carpathians (1.0 m.ch./dm²). The infestation density and its range for *I. typographus* and *I. amitinus* was much lower, reaching 0.54 (0.01-2.25) and 0.31 (0.02-0.97) respectively. The infestation density of the species in the tree sections was related to their frequency; for example, *P. chalcographus* was less abundant in the section I (max. 7.73 m.ch./dm²) than in sections III and IV. (9.99 m.ch./dm²).

Table 1. Infestation density of 3 bark beetle species related to its co-occurrence. (Tukey HSD test).

Factor (occurrence of):		Infestation density (m.ch./dm²) for:		
		I. typographus	*I. amitinus*	*P. chalcographus*
I. typographus	present	---	**0.80**	1.80
	absent	---	**0.26** ***	1.64
I. amitinus	present	**0.18**	---	**0.74**
	absent	**0.49** ***	---	**2.70** ***
P. chalcographus	present	0.38	**0.31**	---
	absent	0.30	**0.03** ***	---

***p<0.0001

The presence of each of the three species, significantly influenced the density of the other species except for the pair *I. typographus* - with *P. chalcographus* (Table 1). In most cases, the presence of one of the species inhibited the infestation density of the other species, except the species *P. chalcographus* and *I. amitinus*. This positive association was probably related to the similar bark thickness preferences of these two species.

Infestation density was related to elevation and aspect of the stand. Densities were higher at elevation > 800 m (ANOVA indicated significant effect of elevation on *I. typographus* [F=15.4***] and *P. chalcographus* [F=45.8***] densities. The highest densities of *I. typographus* were found on northern slopes (F=3.5*), and *P. chalcographus* was most abundant on western slopes (F=8.6***).

Insect communities and its role. Two main insect communities were present on living trees:
I. *P. chalcographus* + *I. typographus* + *I. amitinus* attacking living, weakened trees in groups, on stand edges and in forest gaps. This is the most important community from the phytosanitary point of view, but these species are difficult to control because of the dominance of *P. chalcographus*.
II. *Pissodes harcyniae* + *Polygraphus* sp. + (species from the community I), attacking individual trees inside more humid, dense parts of the stands. Local abundance of this community is related to favourable stand conditions.

The species composition of these communities is relatively poor in comparison with other parts of Polish mountains, even in the neighbourhood of the study area (Capecki 1978, Konca 1991).

Natural enemies. Among the natural enemies, the parasitoids from Chalcidoidea, and Braconidae were the most frequent and of major importance (Table 2). The three species, *Tomicobia seitneri*, *Dinotiscus eupterus* and *Rhopalicus tutela* (Pteromalidae) [polyphagous parasitoids attacking various bark beetle species (Balazy and Michalski 1962)] are important in the limitation of bark beetle populations composing community I. *Eubazus atricornis* (Braconidae) is a specific endoparasitoid of the genus *Pissodes* (Alauzet 1990), and its presence was related with local abundance of *P. harcyniae*. Generally the parasitoid complex

found in the study area was not diverse in comparison with the material obtained from the Carpathians. Among the predators found in the Sudety Mts. the most frequent were *Medetera signaticornis* (Diptera, Dolichopodidae), *Thanasimus formicarius* (Col.: Cleridae) and *Corticeus linearis* (Col.: Tenebrionidae) being the non-specific predators of numerous bark beetle species, including *P. chalcographus* (Balazy and Michalski 1960, Kolomijec and Bogdanova 1980, Weslien 1992).

Table 2. Main species of parasitoids and predators obtained in 71 laboratory rearings

Insect species	number of rearings	frequency (%)
Tomicobia seitneri (Ruschka)	17	24
Dinotiscus eupterus Walk.	13	18
Rhopalicus tutela (Walk.)	12	17
Eubazus atricornis Ratz.	9	13
Roptrocerus sp.	6	8
Dendrosoter middendorffii Ratz.	3	4
Eurytoma sp	3	4
Dinotiscus sp.	2	3
Ibalia leucospoides (Hoch.)	2	3
Liotryphon sp.	2	3
Medetera signaticornis Lw.	13	18

Tree and stand characteristics. Defoliation levels, estimated during the study period on the experimental plots, was extremely high: in the sample of 878 described trees, 72% were classified in classes 2-4, (i.e. > 25% defoliation). The direct effect of air pollution was not found by chemical analysis; of samples taken at the observed stands. Observed damage on the crowns and reduced increment of trees is more likely the result of poor site conditions, especially altitude and aspect: on higher and western exposures defoliation levels were higher.

82.5% of the trees infested by bark beetles belonged to class 3 (defoliation >60%) in the period just before the infestation. Growth increment declined in all stands collapsed following defoliation by *Z. diniana*, but no differences were found in growth increment between the trees infested by both beetles and non infested ones.

Discussion

Results presented here indicate that the bark beetle populations in the Sudety Mts. significantly changed over the last years. The most important change has been an increase in the frequency and infestation density of *P. chalcographus*, related to the ecological plasticity and expansiveness of this species. These changes may be a partial consequence of the bark beetle control activities namely the mass application of selective, Pheroprax baited traps (Grodzki 1995). In stands with an abundance of fresh breeding material (downed logs, logging residues - branches, tree tops) *P. chalcographus* found extremely favourable conditions for population growth. Population growth was also stimulated by the disruption

of competition patterns caused by pheromone suppression of *I. typographus*. In the study area, *P. chalcographus* was able to kill more trees and to succeed in competition with other species, mainly *I. typographus* and *I. amitinus*.

The bark beetle outbreak and later changes in insect populations are also related to the stand susceptibility and its reduced resistance (Christiansen et al. 1987). However the absence of a relation between infestation rate and tree increment was unexpected (Christiansen 1980, Nef 1994). Observations highlighted the effect of defoliation on susceptibility to bark beetle attacks (Althoff 1985, Oppermann 1985). The infestation density of *I. typographus*, and especially *P. chalcographus*, was significantly higher in stands localised on higher elevations and exposed to the west (defoliation level was also higher here). *P. chalcographus* was previously found in weakened stands (Chlodny et al. 1987), and this could explain its pattern of abundance in the study area. Generally, observed changes have rather temporary and local importance, related with the stand characteristics and favourable breeding conditions.

Conclusions

1. The major change in bark beetle populations in the study area was a reduction in diversity of bark beetle communities together with the increment of the population levels of *Pityogenes chalcographus*, dominating in the insect community and in spatial competition. Changes have temporary and local importance.
2. The reduced diversity of the natural enemies seemed to be related to the limited biodiversity of observed ecosystem, influencing its generally reduced resistance.
3. Factors causing the observed changes were both primary (origin and characteristics of stands) and secondary (insect outbreaks, breeding conditions).
4. The defoliation was a factor increasing the susceptibility of trees and stands to bark beetle attacks, but there was no relation to tree increment.
5. The main effects of the bark beetle outbreak from 1981-86 and a previously applied selective pest control strategy were mainly the disruption of competition patterns in insect communities, and the abundance of breeding material, favourable especially for *P. chalcographus*.

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Effects of integrating cultural tactics into the management of the balsam twig aphid *Mindarus abietinus* Koch (Aphididae: Homoptera) in balsam fir Christmas tree plantations.

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ABSTRACT The balsam twig aphid *Mindarus abietinus* Koch causes needle distortion and/or loss on balsam fir Christmas trees. Insecticides, herbicides and fertilizers are used in the management of the trees with little monitoring of their effects upon the aphids. A complete randomized block split-plot design incorporating whole plot treatments (19-19-19 fertilizer/mowing, 19-19-19 fertilizer/Simazine herbicide, white Dutch clover intercrop and no treatment) with subplot treatments (Imidacloprid, Diazinon, Azadirachtin and no treatment) was used to determine treatment effects upon numbers of aphids and their natural enemies. Numbers of aphids and aphid-infested shoots were low on all treatments (<12 aphids/ subplot) although fertilized treated plots had slightly higher numbers of aphids. Diazinon treated subplots had significantly lower, albeit marginally, mean numbers of aphids/subplot. All treatments and their interactions had little effect upon proportions of infested shoots and resulting undamaged shoots (≥80% undamaged). Numbers of predators were too low too detect significant effects of treatments.

KEY WORDS *Mindarus abietinus*, balsam fir, twig aphid, Christmas trees, silvicultural controls

THE BALSAM TWIG aphid *Mindarus abietinus* Koch (Homoptera: Aphididae) is a pest of balsam fir *Abies balsamea* (L.) Mill. and Fraser fir *Abies fraseri* (Pursh) Poir. in nurseries and Christmas tree plantations (Saunders 1969, Nettleton and Hain 1982, Bradbury & Osgood 1986). Aggregations of aphids feed on new shoots causing them to twist and distort which can reduce the value and prevent the sale of the trees, one to three years after attack (Saunders 1969, Martineau 1984).

In a variety of plant crop-insect herbivore systems, applications of fertilizers, insecticides and/or herbicides are known to increase levels of nitrogen in plant tissues and increase numbers of insects, particularly aphids, feeding on those tissues (reviews by Mattson 1980, White 1984). Moreover, genetic provenances of balsam fir that contain high concentrations of the monoterpene ß-phelledrane are found to be most attractive to the balsam twig aphid and thus suffer greater feeding damage (DeHayes 1980). Previous observations indicate that Christmas tree growers use various combinations of these chemicals and nursery stock with little knowledge of their combined effects on aphid numbers. They are also concerned with the economic costs, environmental and health hazards associated with insecticides and are anxious for safer and more economical alternatives. Currently, Christmas tree growers control aphids by aerial applications of Diazinon, either prior to budbreak or after detection of aphid-infested shoots (Osgood 1977, 1979, Nettleton & Hain 1982, Bradbury & Osgood 1986, Kleintjes unpublished data).

In orchard and field crop systems, covercrops and herbaceous forb conservation have been used to enhance and conserve aphidophagous predator populations (Cowgill et al. 1993,

Pages 112-121 in J.C. Grégoire, A.M. Liebhold, F.M. Stephen, K.R. Day, and S.M. Salom, editors. 1997. Proceedings: Integrating cultural tactics into the management of bark beetle and reforestation pests. USDA Forest Service General Technical Report NE-236.

Hodek 1993). In Christmas tree plantations, vegetation among trees is either mowed or treated with herbicide to reduce distortion or pathogenic infection of lower branches (growers, pers. comm.). Little is known about the effects of intercropping, mowing, or retaining vegetation among rows of trees upon insect pests or their natural enemies.

Despite the international success of utilizing cultural controls in integrated pest management, research and application for the balsam twig aphid in Christmas trees has been limited (Osgood 1977, 1979, Nettleton & Hain 1982, Bradbury & Osgood 1986). It is assumed that the competitive nature of the business, the historical reliance on chemical controls, and the variable acceptance rates of aesthetic damage have thwarted progress. As a result, the objective of this study was to examine the effect of current grower management practices (fertilizers, herbicides and insecticides) and potential cultural tactics (intercropping clover, mowing, selected use of fertilizer) upon numbers of aphids and their natural enemies.

Methods

The study was conducted on two balsam fir Christmas tree plantations, Pleasant Valley Tree Farm (PVTF)(Dunn Co.) and Swan's Balsam Tree Farm (SBTF)(Price Co.) in northwestern Wisconsin, from April to July 1996.

A complete, randomized block, split-plot design (Steel and Torrie 1980:377) incorporating whole plot treatments (fertilizer-mowing, fertilizer-herbicide, white Dutch clover intercrop, no treatment) with subplot treatments (©Provado, ©Neemix, Diazinon and no treatment) was used to determine their influence on numbers of the balsam twig aphid and their aphidophagous predators. The experimental areas consisted of four blocks (replicates) (0.21ha ea.) located within a 1km^2 area at each farm. Trees were 8-10 years old and planted at a density of 2500-2800 trees/ha. Mean ($\pm$SE) height and diameter-breast-height of trees at PVTF was 1.97$\pm$0.01m and 2.97$\pm$0.68cm and 3.47$\pm$0.17m and 2.16$\pm$0.05cm at SBTF. Blocks were located in areas that had both a history of annual balsam twig aphid infestations and previous aerial applications of Diazinon upon the appearance of aphid-infested needles. Each block was divided into four wholeplot strips (5 x 20 rows for a total of 100 trees), each randomly assigned one of four treatments with two rows of trees serving as buffers between each treatment. The whole plot treatment fertilizer-mowing consisted of a broadcast spread application of 19-19-19 fertilizer (Mixrite©, Keenan, WI.) applied at a rate of 222.3 kg/ha (late April-early May) followed by a late season mowing between rows of trees (PVTF: 15 July, SBTF: 24 June). The fertilizer-herbicide treatment included the same fertilizer application followed by a pre-budburst application of Simazine (Princep© 4L, Ciba-Geigy Inc., NC) at a rate of 3.33kg/ha in early May (PVTF, overhead sprayer; SBTF, base sprayer). The clover treatment consisted of planting white Dutch clover (*Trifolium repens*) (26.1 kg/ha) between rows of trees as soon as the ground thawed (late April-early May). Clover seed was sprinkled by hand on the soil surface and scratched 1 cm deep into the soil with a hand rake. The control plot received no treatment. Each whole plot was further subdivided into four subplots each receiving one of four treatments. Each subplot contained twenty-five trees in a 5 x 5 row grid with the central five trees receiving the treatment. Subplot treatments consisted of grower applications of: Imidacloprid, (Provado© 1.6 Flowable, Bayer Inc, Kansas City, MO.) at a rate of 0.16ml/l water, plus adjuvent Aquagene 90© (Universal Cooperatives, Inc.) at a rate of 0.31ml/l water; Diazinon AG 500 (Prentox©, Prentiss Inc., Sandersville, GA) at a rate of 1.27ml/l water, plus adjuvent Aquagene 90© at a rate of

0.31ml/l water; Azadirachtin (Neemix 4.5© botanical insecticide, W.R.Grace & Co.- Conn., MD.) at 6.3ml/l water and a no treatment control. These insecticides were used for testing because they were either commonly used by growers (Diazinon), were a new option for Christmas trees (Provado) or were a less toxic, botanical alternative (Neemix). Each treatment was applied to all sides of a treatment tree with a 15l Solo© backpack handpump fine mist sprayer. Spraying coincided with the dates aphid stem mothers reached the 3-4th instar and new growth was emerging (PVTF: 20 May and SBTF: 29 May).

Beating discs (53.4cm^2 plastic embroidary rings of black velvet) and visual counts of infested shoots (one 20cm branch/tree) were used to sample numbers of aphids and their predators on the outer midcrown of each treatment tree (5/subplot) (Kleintjes, unpublished data). Samples were taken once pre-treatment and 3 days, 1 and 3 weeks, post-treatment. Each period, beat samples were taken from a different but immediately adjacent side of the tree (clockwise rotation) with each midcrown of the tree beaten 5x to dislodge insects onto the disc. Mean numbers of aphids were calculated for each subplot each date. Visual counts were taken from permanently tagged midcrown branches to estimate the mean proportion of aphid-infested shoots, shoot size and extent of aphid damage to shoots. On the last sampling date, 3 weeks post the peak in aphid numbers, the numbers of shoots with no curling, slight curling (needles slightly twisted) and extensive curling (needles permanently curled around stem) were also counted. Preliminary sampling studies on balsam in Wisconsin and those of Bradbury and Osgood (1986) in Maine support the use of midcrown branches for sampling numbers of aphids, infested and damaged shoots.

Soil samples (15cm deep) were taken pre and post whole plot treatment with a soil core auger from each control subplot. Samples were analyzed for pH, % potassium, % phosphorus, % organic matter and total nitrogen (ppm). Leaf samples (six 5cm shoots per tree/5 trees) of new growth were collected from each control subplot and analyzed for percent total nitrogen. All analyses were conducted by the University of Wisconsin-Extension, Soil and Plant Analysis Laboratory, Madison, WI.

Split-plot analysis of variance tests were used to compare mean numbers of aphids, mean numbers of infested shoots and mean numbers of undamaged shoots among whole plot and subplot treatments (Steel and Torrie 1980). Mean numbers were log transformed and proportions received the arcsin sqrt. transformation in order to normalize data and obtain homogeneity of variances (Sokal and Rohlf 1981). Because of the block design at the whole plot level the interaction between (block *whole plot) effects was used as the correct error term to test whole plot effects (Steel and Torrie 1980:380). However, at the subplot level both the test of subplot effects and the interaction of (subplot *whole plot) effects used the error term that is the sum of the interaction of (block*subplot) effect plus the three-way interaction of (block*whole plot*subplot) (Steel and Torrie 1980:380). Numbers of aphids and infested shoots were from the sample date of highest infestation whereas data for undamaged shoots were collected post peak. An analysis of covariance(ANCOVA) was not used for post-treatment effects because numbers of aphids were too low pre-treatment, however, an ANCOVA was used to compare soil chemistry and post-treatment numbers (Sokal and Rohlf 1981). Systat©(1992) and Statistica©(1994) Software were used for analyses.

Results and Discussion

Results of the split-plot ANOVA's indicated that whole plot and subplot treatments had little effect upon numbers of aphids, aphid-infested shoots and aphid damage (Figs. 1, 2; Tables 1, 2). Treatments may have had little effect due to low numbers of aphids and aphid-infested shoots before and after treatment. At Pleasant Valley Tree Farm, less than 5 stem mothers were beaten from a total of 320 trees pre-treatment and over 90% of the shoots were undamaged. At Swan's Balsam tree Farm, less than 15 stem mothers were beaten from a total of 320 trees pre-treatment and over 78% of the shoots were undamaged. Moreover, peak numbers of aphids and aphid-infested shoots per subplot treatment at both farms were still too low (PVTF, <6 aphids; SBTF <11 aphids) to cause significant damage to shoots (over 78% shoots undamaged) (Figs. 3, 4). Cultural treatments at the whole plot level may have not proven significant due to several factors such as application methods, phenology of clover (blooming after aphid peak), variation in native vegetation among rows or timing of mowing.

Only mean number of aphids at Swan's Balsam Tree Farm significantly differed, albeit marginally, among subplot treatments with lower numbers of aphids on Diazinon treated subplots and greater numbers of aphids on the control subplots across whole plot treatments (ANOVA $F=2.7$, $df=3, 36$, $p=0.059$)(Fig. 2, Table 2). Both Nettleton and Hain (1982) and Bradbury and Osgood (1986) found insecticides (including Diazinon AG500) to cause signicant declines in numbers of aphids. Results of this study indicate that when aphid numbers are relatively low pre-budbreak, insecticides do not have a significant impact on aphids nor do they significantly reduce damage. Although application methods or timing could have been a factor for little treatment effect, the importance of monitoring can not be over-emphasized. Under non-experimental conditions these plots should not have been treated.

Of interest, particularly at Pleasant Valley Tree Farm, was that mean numbers of aphids were greater on the combination of fertilized whole plot and insecticide subplot treatments. However, numbers were neither significantly different from other whole plot treatments nor large enough to cause significant differences in damage among plots. The data suggest that higher levels of soil N influenced numbers of aphids feeding on balsam fir. Although soil chemistry differed little among treatments, it was observed that nitrogen levels increased after treatment (Tables 3,4). Most likely, fertilizer applications as well as natural biological processes associated with spring conditions increased total soil N. Since more aphids were found in fertilized areas it is suggested that growers evaluate whether annual applications of fertilizer are necessary and, if used, either avoid application in previous aphid-damaged areas or apply after the flush of new growth.

Numbers of aphidophagous predators were too low in all study areas to quantify any treatment effects or sampling methods (PVTF: 13 syrphids, 2 coccinellids on 320 sample trees; SBTF: 10 syrphids, 3 coccinellids and 3 chrysopids on 320 sample trees). Low numbers may have been a result of low prey availability or due to past use of insecticides in these areas. Studies on the use of resident vegetation or cover crops among rows of fruit trees to increase and conserve aphidophaga have produced variable results (Bugg and Waddington 1994). It is too early to conclude whether numbers of aphidophaga can be increased by either planting white clover or by mowing native vegetation after it has flowered among Christmas trees. Bugg and Waddington (1994) found that white clover was less attractive to beneficial arthropods than other annual clovers (*Trifolium* spp). On whole plot treatments of this study, white clover flowered after the aphid population had crashed although in all treatments a mix of flowering broad-leafed weeds, e.g. dandelion (*Taraxicum*

sp.) was available. On many occasions, bumblebees (Apidae) and adult hoverflies (Syrphidae) were observed foraging on aphid-infested shoots, presumably for honeydew. The syrphids may also have been laying eggs. It is unknown whether flowering plants may detract predator species which feed on nectar, pollen or honeydew from the aphid-infested shoots.

In conclusion, pre-budbreak applications of fertilizer enhanced aphid numbers while mowing and herbicide effects were insignificant (although effects may not be detected until the next growing season). The three insecticide treatments did not cause any significant difference in the proportion of shoots that remained undamaged. Results suggest that if growers monitor aphid numbers early or take in account proportions of infested shoots on marketable trees, potential damage can be predicted and wise management decisions, rather than wasteful guesses, can be made. Once curling of needles begins, insecticides do not effectively reduce aphids and their subsequent damage. Moreover, many infested shoots straighten out with maturation and if not, can be removed by annual shearing.

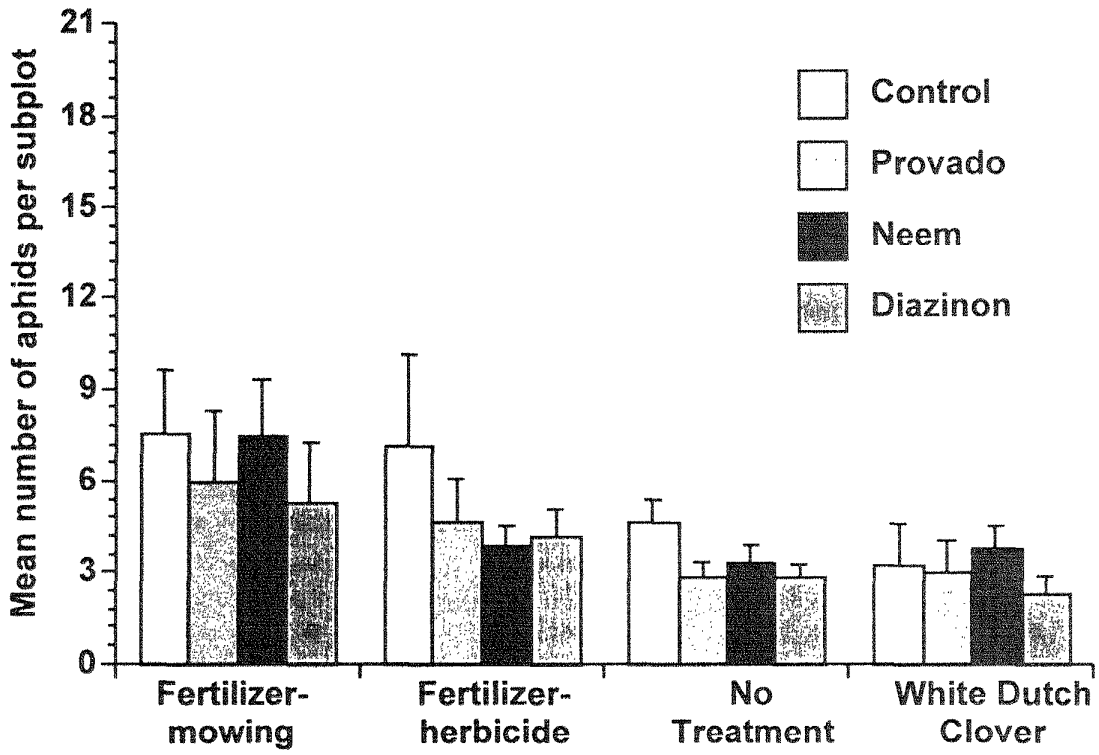

Fig. 1. Mean (+SE) number of aphids per subplot (n=16) at Pleasant Valley Tree Farm (ANOVA treatment effects; n.s. p>0.05).

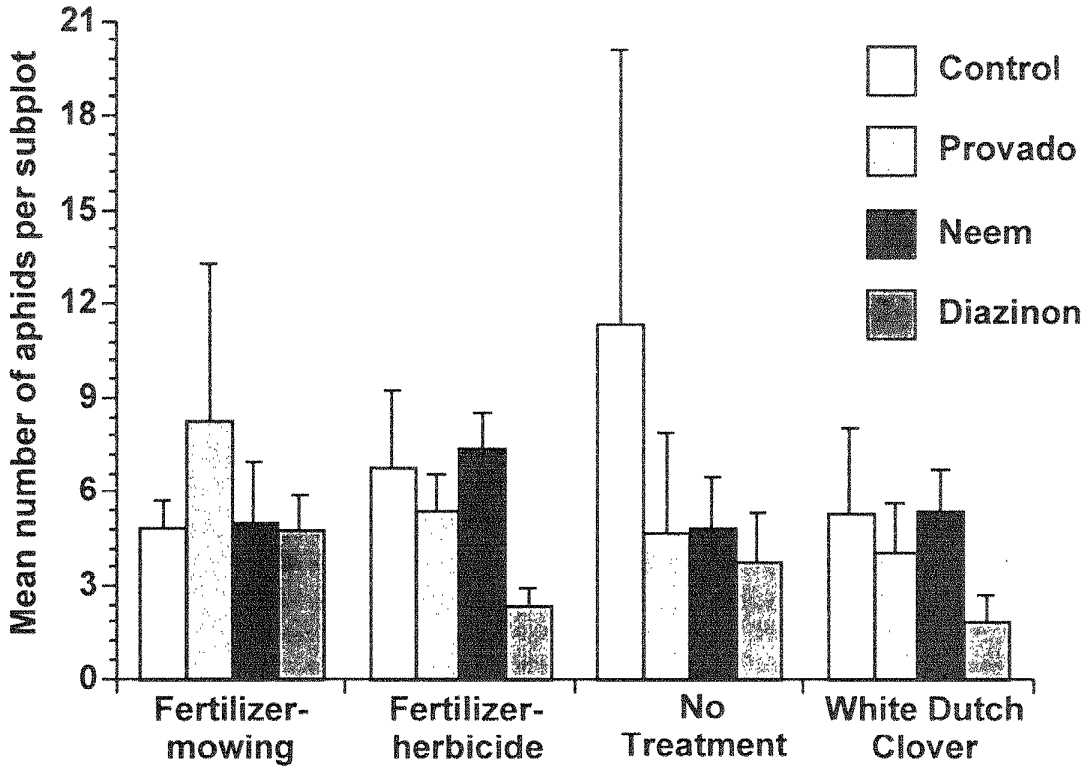

Fig. 2. Mean (+SE) number of aphids per subplot (n=16) at Swans Balsam Tree Farm (ANOVA subplot effect; F=2.71, df=3,36; p=0.059).

Table 1. Results of complete, randomized block split-plot ANOVA of whole plot and subplot effects and their interaction, Pleasant Valley Tree Farm, Dunn Co., WI.

Dependent Variable	Mean Sq. Effect	Mean Sq. Error	F	p - level
Wholeplot Effect (df=3) 1				
Aphids	1.05019	0.56980	1.843093	0.2096312
Infested Shoots	0.04874	0.04121	1.182583	0.3697808
Undamaged Shoots	0.01273	0.04968	0.25616	0.8551214
Subplot Effect (df=3) 1				
Aphids	0.30001	0.18078	1.659538	0.1929587
Infested Shoots	0.02098	0.03260	0.643495	0.5920995
Undamaged Shoots	0.01162	0.01942	0.598659	0.6200449
Wholeplot * Subplot Effect (df=12) 2				
Aphids	0.113141	0.18078	0.625849	0.8061224
Infested Shoots	0.026552	0.032604	0.814357	0.6345344
Undamaged Shoots	0.022355	0.019415	1.151444	0.3526694

1 Block* wholeplot error term: df= 9
2 Sum of the interaction of (block* subplot) effect plus the three-way interaction of (block*whole plot*subplot: df=36

Table 2. Results of complete, randomized block split-plot ANOVA of whole plot and subplot effects and their interaction, Swans Balsam Tree Farm, Price Co., WI.

Dependent Variable	Mean Sq. Effect	Mean Sq. Error	F	p - level
Wholeplot Effect(df=3)[1]				
Aphids	0.34182	0.61477	0.5560141	0.6570467
Infested Shoots	0.01176	0.03054	0.3850351	0.7664896
Undamaged Shoots	0.01637	0.06080	0.2692756	0.8460211
Subplot Effect(df=3)[1]				
Aphids*	0.76226	0.28069	2.715645	0.0590342
Infested Shoots	0.03206	0.01833	1.749181	0.1743050
Undamaged Shoots	0.00700	0.02398	0.291725	0.8310814
Wholeplot * Subplot Effect(df=12)[2]				
Aphids	0.35108	0.28069	1.250758	0.2888916
Infested Shoots	0.02471	0.01833	1.348116	0.2356897
Undamaged Shoots	0.03662	0.02398	1.527213	0.1595603

1 Block* wholeplot error term: df= 9
2 Sum of the interaction of (block* subplot) effect plus the three-way interaction of (block*whole plot*subplot: df=36

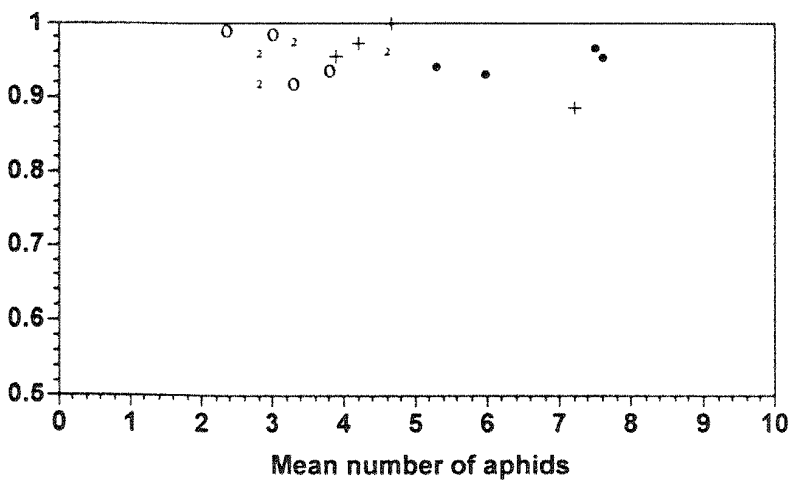

Fig. 3. Relationship between peak aphid infestation and resulting proportion of shoots undamaged within and among whole plot treatments, Pleasant Valley Tree Farm, Dunn Co. WI.

Table 3. Results of soil and leaf chemistry analyses, pre and post-treatment at Pleasant Valley Tree Farm, Dunn co., WI and Swans Balsam Tree Farm, Price Co., WI.

	pH		% O.M.		P (ppm)		K (ppm)		Total N (ppm)		% Leaf Nitrogen
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Pleasant Valley Tree Farm											
Fertilizer-Mowing	5.8a	5.9	1.7	1.8	50.0	59.0	58.8	47.5	782.8	838.8	2.9
	0.0b	*0.2*	*0.0*	*0.1*	*5.8*	*6.2*	*1.3*	*4.3*	*69.2*	*66.6*	
Fertilizer-Herbicide	5.8	5.6	1.7	1.9	80.0	79.5	62.5	51.3	731.4	864.3	3.0
	0.1	*0.2*	*0.2*	*0.2*	*13.5*	*9.0*	*2.5*	*3.1*	*111.3*	*98.2*	
Clover Intercrop	5.6	5.6	1.5	1.7	68.0	67.0	73.8	52.5	655.6	673.3	2.4
	0.1	*0.1*	*0.2*	*0.1*	*14.5*	*18.2*	*6.9*	*6.6*	*95.9*	*56.4*	
No Treatment	5.9	5.8	1.6	1.8	54.3	68.5	57.5	55.0	668.5	738.5	2.7
	0.1	*0.0*	*0.1*	*0.2*	*8.0*	*13.6*	*3.2*	*4.6*	*35.1*	*86.7*	
Swan's Balsam Tree Farm											
Fertilizer-Mowing	5.4	5.4	4.4	4.9	77.5	89.8	138.8	158.8	1684.2	1915.7	3.2
	0.1	*0.2*	*0.7*	*0.7*	*27.2*	*27.1*	*38.3*	*19.8*	*262.1*	*234.7*	
Fertilizer-Herbicide	5.4	5.4	4.9	4.8	61.5	64.3	145.0	136.3	1929.2	1938.7	3.4
	0.2	*0.2*	*0.6*	*0.3*	*13.6*	*12.8*	*31.8*	*20.3*	*211.2*	*124.2*	
Clover Intercrop	5.4	5.3	5.3	5.5	70.3	86.0	152.5	153.8	2063.6	2025.6	3.5
	0.1	*5.3*	*0.8*	*5.5*	*24.6*	*86.0*	*28.9*	*153.8*	*262.4*	*237.1*	
No Treatment	5.6	5.7	3.8	4.4	38.8	47.3	97.5	98.8	1487.0	1788.4	3.4
	0.2	*0.2*	*0.5*	*0.4*	*10.9*	*9.0*	*10.1*	*8.3*	*217.4*	*162.2*	

a=Mean of four subplots, b=SEM

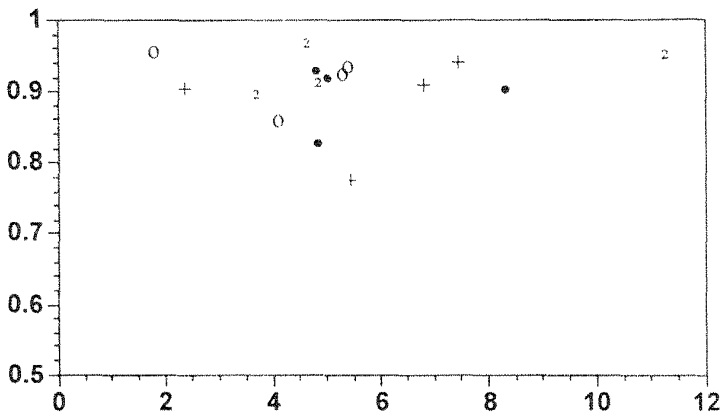

Fig.4. Relationship between peak aphid infestation and resulting proportion of shoots undamaged within and among whole plot treatments, Swans Balsam Tree Farm, Price Co.,WI.

Table 4. Results of complete, randomized block ANCOVA of treatment effects and their interaction upon soil nitrogen, Pleasant Valley Tree Farm, Dunn Co. and Swans Balsam Tree Farm, Price Co., WI.

	Pleasant Valley Tree Farm		Swan Balsam Tree Farm	
Wholeplot Effect	F	p - level	F	p - level
Block (df=3)	0.77	0.230	1.583	0.268
Treatment (df=3)	0.848	0.505	0.266	0.848
Pretreatment N (df=1)	11.41	0.010	12 139	0.008

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