Forest Health Technology Enterprise Team

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TRANSFER

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International Workshop on Biological Control of Invasive Species of Forests

Beijing, P.R. China
September 20-25, 2007

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Yun Wu and Xiao-yi Wang, Compilers

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Cover image: The Seventeen Arch Bridge on Kunming Lake at the Summer Palace, Beijing. By Yun Wu.

INTERNATIONAL WORKSHOP ON BIOLOGICAL CONTROL OF INVASIVE SPECIES OF FORESTS

September 20 - 25, 2007

Fragrant Hill Empark Hotel
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PREFACE

The first International Workshop on the Biological Control of Invasive Species of Forests was held on 20-25 September 2007 in Beijing, P. R. China. The intent of the workshop was to create a forum to improve the coordination of activities and information exchange between practitioners and researchers on issues affecting the use of natural enemies for control of invasive species of forests.

The U. S. Department of Agriculture Forest Service has developed strong professional and personal relationships over the past 15 years (1993-2007) with colleagues in China to initiate biological control programs for species invasive to both countries. Therefore, the original focus of the workshop was to summarize this cooperation, although the workshop was expanded to include the Asian-Pacific Region.

This workshop was attended by 129 delegates, including 97 from P. R. China and 32 from 14 other countries. There were 65 oral presentations and nine posters. Unique to this workshop was a session "Strategy and Technology for Monitoring and Control of Invasive Species for a Green Olympics 2008" which focused on invasive species already established in the Beijing area as well as preventing species that might arrive during and immediately following the Olympics.

An opening ceremony was presided over by: Shirong Liu, Vice President of the ChinaAcademy of Forestry. Welcome speeches were given by Lieke Zhu, Deputy Chief, China State Forestry Administration; Richard Reardon, USDA Forest Service; Yuyuan Guo, Academician of the Chinese Academy of Engineering; Patric Durst, Senior Officer, PAO Regional Office for Asia and the Pacific; K. V. Sankaran, Coordinator of the Asia-Pacific Forest Invasive Species Network; Xurong Mei, Director of the Institute of Environment and Sustainable Development in Agriculture, CAAS; and Dianmo Li, The Nature Conservancy, Beijing Program Director. Also present at the opening ceremony: Diansheng Wei, Director General of the Department of Silviculture, CSFA; Jian Wu, Senior Engineer of the Department of Agriculture, CSFA; and Jianbo Wang, Director of Forest Protection Division, Department of Silviculture, CSFA.

This workshop launched a new series of meetings that will be held every three to four years. The format of the meeting will remain small (about 200 key people) with plenty of time for discussion but will not focus on a specific geographic region.

The next meeting in this series "Classical Biological Control in Forests and Other Natural Areas" will be held on 3-7 October 2010 in Northampton, Massachusetts, USA. An international program committee is being organized by Dr. Roy Van Driesche (University of Massachusetts vandries@nre.umass.edu) to develop the workshop.

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PRESENTATIONS

Session One

General Session

Biological Invasions: Recommendations for U.S.A. Policy and Management

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The Ecological Society of America reported its evaluation of current U.S.A. national policies and practices on biological invasions in light of current scientific knowledge (Lodge *et al.* 2006). Invasions by harmful non-native species are increasing in number and area affected. Without improved strategies based on recent scientific advances and increased investments to counter invasions, harm from invasive species is likely to accelerate. Federal leadership, with the cooperation of state and local governments, is required to increase the effectiveness of prevention of invasions, detect and respond quickly to new potentially harmful invasions, control and slow the spread of existing invasions, and provide a national center to ensure that these efforts are coordinated and cost effective.

Specifically, the Ecological Society of America recommends that the federal government take the action in six areas. (1) Use new information and practices to better manage commercial and other pathways to reduce the transport and release of potentially harmful non-native species. (2) Adopt more quantitative procedures for risk analysis and apply them to every species proposed for importation into the country. (3) Use new cost effective diagnostic technologies to increase active surveillance and sharing of information about invasive species so that responses to new invasions can be rapid and effective. (4) Provide emergency funding to support rapid responses to emerging invasions. (5) Provide funding and incentives for cost effective programs to slow the spread of existing invasive species in order to protect still uninvaded ecosystems, social and industrial infrastructure, and human welfare. (6) Establish a National Center for Invasive Species Management to coordinate and lead improvements in federal, state, and international policies on invasive species.

Keywords: early detection/rapid response, federal oversight, invasive species, pathways

Restoring the Natural Balance: Biological Control of Invasive Forest Pests in the United Kingdom

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ABSTRACT

The paper described the processes that help protect the UK from potentially damaging exotic pests. Other than the natural barrier of its island status, there are a number of phytosanitary measures in place to reduce the incidence of pests and pathogens arriving and establishing in the country. However, global trade has increased in volume and speed and this has increased the incidence of new pests. Fortunately, some of these pests have been managed using classical biological control. Two sawfly species, *Cephalcia lariciphila* and *Gilpinia hercyniae*, have been reduced to negligible levels due to an introduced parasitoids and an introduced baculovirus respectively.

A more difficult problem was faced as a result of the establishment of great spruce bark beetle, *Dendroctonus micans*. An integrated management regime based on internal quarantine, selective felling and, particularly, introduction, rearing and release of the specific predator *Rhizophagus grandis* has been a great success and the bark beetle is now at low levels. The confidence in the biological control agent is so great that internal quarantine has now been dropped. Intensive studies on the biology of *R. grandis* have enabled an artificial attractant to be developed. This is now being used as a monitoring tool to assess the presence of the predator and, by implication, its specific bark beetle prey.

However, not all bark beetles are so susceptible to natural enemies and a contrast between *D. micans* and the European spruce bark beetle *Ips typographus* indicates that biological characteristics of the pest species can influence natural enemy performance considerably.

Key lessons from studies of invasive pests included:

- Pest Risk Analysis tends to be retrospective – often providing insufficient early warning
- Pathway-based strategies need to be developed, especially for plants for planting
- The best strategy is to keep pests out!
- Early detection of pioneer pest populations - methods need to be improved
- International collaboration is essential to increase knowledge transfer and data on potential pest and their natural enemies

The author would like to express his grateful thanks to Professors Xy Rumei and Yang Zhongqi for their support in enabling him to attend this important meeting.

Forest Service Threat Assessment Centers: Part of an Early Warning System for Forest Exotic Invasive Species

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Wildlands (forests and rangelands) of the Western United States are vulnerable to environmental stresses and disturbances such as fire, insect infestation, disease, invasive species, drought, and development. These stresses, alone or in combination, can have significant and long-lasting effects on ecological and socioeconomic values. Wildland managers need state-of-the art information and tools that help them anticipate and solve problems.

A new unit of the Pacific Northwest Research Station has been created to predict, detect, and assess existing and potential environmental threats to Western wildlands. Information will be developed and shared about forest threats such as invasive plants, potential insect outbreaks, the appearance of invasive insect threats, the appearance of new pathogens and other threats. The Western Wildland Environmental Threat Assessment Center (WWETAC) is in Prineville, OR, co-located with the Ochoco National Forest headquarters. A similar center in Asheville, NC, will address environmental threats in the eastern United States.

The mission of the center is to generate and integrate knowledge and information to provide credible prediction, early detection, and quantitative assessment of environmental threats in the western United States. The goal of the WWETAC is to inform policy and support the management of environmental threats to western wildlands. The objectives of WWETAC are to:

- Evaluate the effects and consequences of multiple, interacting stresses on western wildland health.
- Increase knowledge of the risks, uncertainties, and benefits of multiple environmental stresses on Western ecological conditions and socioeconomic values.
- Provide science-based decision support tools for policy formulation and land management in the Western United States.
- Provide land managers with credible predictions of potential severe disturbances in the West with sufficient warning for managers to take preventative actions.

The WWETAC is jointed funded and run by three branches of the Forest Service: the National Forest System, State and Private Forestry, and the Pacific Northwest Research Station.

Biological Control of *Mikania micrantha* Using *Puccinia spegazzinii* in India - Early Results

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ABSTRACT

Mikania (*Mikania micrantha* H.B.K.), a perennial invasive weed of neotropical origin, is widespread in a number of countries within the moist tropical zones of south and southeast Asia and the Pacific. In India, it is a big menace in natural forests, forest plantations and agricultural systems in the southwest and northeastern states. Efficacy of herbicides to control *Mikania* is short-lived. Also, herbicidal application is environmentally damaging and cannot be recommended for long-term control. Mechanical weeding is labour intensive and expensive. In this context, a rust fungus viz., *Puccinia spegazzinii*, which causes mortality of *Mikania* in its native ranges was considered for biological control of the weed. Of the several isolates of the fungus tested, one isolate from Trinidad, shown to be highly specific to *Mikania*, was imported to the quarantine facility at CABI Europe-UK for initial host specificity testing. Further host specificity tests were conducted after importing the fungus to India. Since the pathogen was proved to be highly host-specific, its release in *Mikania*-infested agricultural systems and forest plantations was permitted by the Government of India in 2005.

The rust was first released in tea gardens in Assam (NE India) in October 2005, but did not establish, due to the presence of a biotype of the weed that was partially resistant to the rust pathotype. In Kerala state (SW India), releases of the rust were made in agricultural systems and degraded moist deciduous forests in August 2006 and June-August 2007. These releases are considered successful; and disease has spread from source plants to field population of *Mikania* at all release sites and is still persisting in some of the release sites. The fungus is apparently intolerant of high atmospheric temperature and low relative humidity, so it is necessary to release a high amount of inoculum during the most favorable climatic conditions for the fungus to spread and maintain its population. This is the first instance where a fungal pathogen has been used as a biological control agent against an invasive alien plant on continental Asia.

Recent Advances in the Biological Control of Invasive Forest Pests in China

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ABSTRACT

With the brisk international business trade in recent decades, invasive pests have spread country to country and area to area. Especially forest invasive pests have caused significant destruction in forests and the environment. China is one of the countries that has suffered greatly from invasive pests. Recently, the following invasive species have caused particularly great losses and become serious problems in our forests: fall webworm (*Hyphantria cunea* Drury) (Lepidoptera: Arctiidae), red turpentine beetle (*Dendroctonus valens* LeConte) (Coleoptera: Scolytidae), pine wood nematode (*Bursaphelenchus xylophilus* Nickle) (Nematoda, Aphelenchida: Aphelenchoididae), coconut leaf beetle (*Brontispa longissima* Gestro) (Coleoptera: Chrysomelidae), pine needle scale (*Hemiberlesia pitysophila* Takagi) (Homoptera: Diaspididae), emerald ash borer (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae).

To effectively get the exotic pests under control and reach sustainable control results without pollution of the environment, the best choice is biological control by applying beneficial ecological factors, (*i.e.* natural enemies).

In recent years some biological control programs have been conducted by Chinese scientists of forest protection in the fields of classical biological control and many achievements have been attained. They are summed up below for each of the above species.

Fall webworm (*Hyphantria cunea* Drury)

This species is a serious invasive pest and has caused significant damage to forests and ornamental trees in China since its first appearance in 1979. Particularly it has spread to Beijing in recent years and threatens the successful conduct of "the Green Olympics" in Beijing in 2008. From the failed lessons of classical biological control by introductions of parasitoids from the U. S. A. and Canada that were carried out in the former USSR and Yugoslavia during the period of 1952-1965, we have focused on looking for China's native parasitoids for biological control of the pest. A search for the insect's natural enemies was conducted from 1996 to 2004 in five provinces and one municipality of China in order to select effective species for biological control. Two carabid predators (Coleoptera) and 25 parasitoid species were found, among which 23 were parasitic wasps (Hymenoptera), including five hyperparasitic species, and two tachinid flies (Diptera). The two carabids preyed on young larvae in the web, two braconid wasps parasitized larvae, 18 parasitoid species attacked the pupa and/or "larval-pupal" stages of the fall webworm. Among these parasitoids there was one genus and nine species new to science, and four species new to China which were described and published separately by Yang. The average parasitism rate in the pupae of overwintering fall webworms was 25.8 percent and 16.1 percent in the first generation (summer generation). These findings reveal that these natural enemies play an important role in the natural control of the pest. *Chouioia cunea* Yang (Hymenoptera: Eulophidae) (gen. & sp. nov.), a gregarious pupal endo-parasitoid, was the most effective natural enemy of the fall webworm (Yang 1989). It is therefore recommended as the most promising biological control agent against the fall webworm in China (Yang *et al.* 2007). Through the effort of successive studies for 21 years, the effective pupa parasitoid, *Chouioia cunea* Yang (Hymenoptera: Eulophidae) has been selected as a biological control agent. The biology, behavior (Yang and Xie 1998), ecology, female reproductive system anatomy (Yang 1995) and mass rearing of the parasitoid have subsequently been studied. Alternative hosts of *C. cunea* were tested to find potential substitute hosts for mass rearing of the parasitoid. *Antheraea pernyi* Guerin-Meneville (Lepidoptera: Saturniidae) was chosen as a surrogate host as a single pupa produced a maximum of 11,256 and an average 8,552 wasps per pupa. Meanwhile, the research on release techniques and substitute hosts for mass rearing of the parasitoid have been carried out. The excellent control results were obtained by releasing in many areas. At the same time a strain of nuclear polyhedrovirus of the fall webworm (HcNPV) with high toxicity was discovered in the pest larvae and selected. A superior artificial diet was developed for mass rearing of the fall webworm larvae first, and a large number of the pest larvae were reared with the diet, followed by inoculation with HcNPV for mass production of the virus. In this way the quality and quantity of HcNPV was guaranteed for biological control of the pest larvae. An integrative biological control technique has been developed, (*i.e.* spraying HcNPV in the larval stage and releasing the parasitoid, *C. cunea* in the pupal stage of the fall webworm respectively). By applying the technique to control two generations of the fall webworm successively, an effective and sustainable control result has been reached, and the fall webworm has been suppressed for six years in Shanghai, Dalian, Yantai and Qingdao cities (Yang *et al.* 2005). Parasitism by *C. cunea* in the areas where it was augmentatively released was usually over 80 percent and on an average 67.74 percent. Other native parasitoids, such as *Coccygomimus disparis* (Viereck), *C. parnasae* (Viereck) (Hymenoptera: Ichneumonidae) and *Exorita japonica* Townsend (Diptera: Tachnidae) caused the total average parasitism to exceed 90 percent with the maximum 96.28 percent in the release areas. These successful releases indicate that an introduced pest species can also be controlled by mass-rearing and release of native parasitoids in the country of introduction. The biological control technique is safe for the environment, attaining excellent control result without influencing the environment and biodiversity because the biological control agents are original ecological factors in forest ecosystems. It has been used in Beijing for control of the fall webworm to ensure that the goal of the "Green Olympics" is realized in 2008. (Wei *et al.* 2003, Yang *et al.* 2006, Yang and Zhang 2007).

Red turpentine beetle (*Dendroctonus valens* LeConte)

The red turpentine beetle (RTB), *Dendroctonus valens* LeConte (Coleoptera: Scolytidae), is a common borer of conifers in North America. It was accidentally introduced to China in the 1990s from the New World and spread rapidly to Shanxi, Henan, Hebei and Shaanxi provinces in recent years. In 1999, the pest infested over 0.5 million hectares of forests of *Pinus tabuliformis* Carr. and other pine species and killed more than six million trees. It was found in Beijing in 2005. This invasive bark beetle has become a serious threat to China's forests.

The State Forestry Administration of China and the local governments have carried out a series of control measures against the pest after its outbreaks, including suppression of the population using insecticides, trapping RTB adults with kairomone lures, and many others management strategies. These efforts helped to suppress its spread and the damage. However, because it has a large population living and attacking host trees under the bark and over 80 percent individuals going underground to feed on roots, the practices could not get ideal control results. Natural enemies are important factors in pest suppression in general. According to the classical theory of biological control, introducing natural enemies of an exotic pest to the newly invaded areas is a basic measure. However, no very effective natural enemies of *D. valens* were found in its native distribution area of North America until now. Belgium, France, the United Kindom and Georgia, achieved some considerable success by using a predator, *Rhizophagus grandis* (Coleoptera: Rhizophagidae) against *D. micans*. RTB has similar biological features to *D. micans*, so the entomologists at the Research Institute of Forest Ecology, Environment and Protection, Chinese Avademy of Forestry, have carried out research to introduce the predator from Belgium for biological control of the invasive pest in China.

These studies included observations on the biological features of RTB, the role of the pest occurring in a year, the species and the features of natural enemies in local regions in China, studies on the introduction of the predator for biological control, etc.

A series of achievements have resulted from using *R. grandis* against RTB:

1) After six years studying release techniques, it was determined that the larva and adult could be released with the same control results.

2) Mass rearing techniques of the predator have been developed, including artificial diet, substitute preys, egg-laying stimulator etc. We have tested many times and finally selected three antagonistic microorganisms to treat the sand in the pupation box. Thus, over 92 percent of mass-reared mature larvae of *R. grandis* safely pupated and emerged as adults. In this way we overcame the key problem in mass rearing the predator to ensure a large number could be produced for biological control.

3) The studies on the relationship of *R. grandis* and RTB both in the laboratory and in forest stands were carried out, and a series of good results have been obtained. *R. grandis* adults easily find their way to enter the RTB galleries by themselves through the holes chewed out for aeration by RTB adult females. An adult of the predator feeds 0.72 egg and 0.22 fist instar larva in a day and its one larva could eat 25.88 mg of RTB larva body weight which is equivalent to a whole body of one third instar larva of RTB. Four pairs of adults of the predator and their offspring could reduce 48.4 percent individuals of RTB numbers with 182.5 larvae which is about second instar larvae in a gallery of RTB on an average within duration of 30-34 days.

4) The attraction responses of different kairomone lures were determined with a wind-tunnel the first time. The main results were similar to results from other research,(i.e, (S)-(–)-β-pinene; (+)-α-pinene and (S)-(+)-3-carene are active components to RTB adults and the responses are similar among them). On the other hand, it was found that turpentine of *Pinus tabuliformis* could attract RTB adults, too. It could be used as an economical lure to monitor RTB population dynamics.

5) Besides determining the general characteristics of RTB, we found that RTB can overwinter in *P. tabuliformis* stumps in some regions in China. This new finding can help determine better pest management strategies in the future.

6) The native natural enemies of RTB in China were investigated and a total of 18 species of predators and parasitoids were found, some of which were described both in characters and biology.

7) The cold-hardinesses of RTB and its predator, *R. grandis* were determined. The results showed that

R. grandis could overwinter safely in the release areas in northern China. This information can be used in forecasting the potential distribution areas of RTB and *R. grandis* as well.

8) The integrated management system is proposed by us during the present study. It includes biological control measures using *R. grandis* against RTB, protecting the native natural enemies, and monitoring of the RTB population dynamics by kairomone lure. By extending the biological control techniques, we are confident that RTB can be controlled in China.

9) By releasing the predator *R. grandis* in Shanxi, Henan, Shaanxi and Hebei provinces with the total control area 3334 ha., RTB was brought under control in the experimental forest stands. The predator has successively established in those released forests. Not one dead tree has been found in the infestation forests during the two years after the release. It shows that the biological control technique is successful and has bright prospects in sustainable control of the invasive bark beetle.

Pine wood nematode (*Bursaphelenchus xylophilus* Nickle) and its vector pine sawyer beetle (*Monochamus alternatus* Hope)

Pine wilt disease is caused by the pine wood nematode, *B. xylophilus* Nickle. Since its first discovery in 1982 at Nanjing City, it has spread to 12 provinces (and/or municipalities) with 113 counties in China. Currently, it is the most destructive forest pest in China. It is commonly understood that to control the disease the first step is to manipulate its vector, the pine sawyer beetle, *Monochamus alternatus.* Thus, a series of studies has been conducted for control of the cerambycid by its natural enemies.

B. xylophilus is the number one pest in China's forests. The pine sawyer beetle, *Monochamus alternatus,* is the most important vector of the nematode. The key control technique of *B. xylophilus* is to suppress the population of *M. alternatus*. Therefore, we studied the technique of biological control of pine sawyer. This dissertation summarized the research of the program. It contains two parts: the biology, behavior and control technique of the cerambycid which previously conducted by others, has been described, and the research including investigations on insect natural enemies, biology of the cerambycid, and biological control technique using the natural enemies carried out by the present author, are introduced. The research results obtained are as follows:

1. The technique by which the adult *Dastarcus helophoroides*, a primary parasitoid of *M. alternatus*, was brought to mass-produce eggs developed successfully within one year. The dormancy of oviposition of the parasitoid was broken artificially by different measures. One female produced 6,550 eggs on average in a year. Thus, the biggest problem for mass-rearing the important parasitoid was solved, so that large numbers of *D. helophoroides* could be reared first for biological control of *M. alternatus*.

2. A very good substitute host for mass-rearing, *D. helophoroides* was selected through our studies, and an artificial diet was developed for its adult as well. The technique for mass rearing of *D. helophoroides* was shown successfully by the substitute host and the diet. To date, over 560,000 adults and eggs of the parasitoid have been produced and released in the infested forests of Guangdong, Zhejiang provinces.

3. The results of biological control by the parasitoid *Scleroderma sichuanensis* Xiao (Hymenoptera: Bethylidae) was found to be better than that by another bethylid parasitoid *S. guani* Xiao et Wu, which had been used for control of *M. alternatus* previously, according to our field and laboratory experiments in many areas of China.

4. A biological control technique was developed, whereby releasing S. *sichuanensis* during the second instar larval stage of *M. alternatus*, and releasing the adults and/or eggs of *D. helophoroides* in the thirdand/or fourth instar larval stage of the cerambycid, good control results has been achieved. The parasitism rates of *S. sichuanensis* and *D. helophoroides* were 25.45 and 54 percent respectively in the late year check.

5. The integrated techniques of introducing the insect natural enemies of *M. alternatus* were developed in our studies. Meanwhile, it is important to protect the native natural enemies in the local forests. Thus, we sum up the biological control measures including protection of original insect natural enemies and release of mass reared parasitoids to increase the beneficial insect populations, *i.e.* covering the cut logs of trees killed by the nematode with metal mesh allows the parasitoids and predators to exit after emergence, but keeps the adults of *M. alternatus* inside.

6. A new predator of *M. alternatus* was found for the first time in China, *Cryptalaus berus* (Coleoptera: Elateridae), attacking over 28 individuals during its larval stage. Another two new predators of *M. alternatus* were found in China, the gold-ringed elaterid and the black elaterid (needs to be identified). The former species can consume over 24 host larvae, and the latter consumed over 27 middle-aged host larvae in their larval stages respectively. They were very aggressive and entered into many galleries of *M. alternatus* larvae to find a host for consumption. They have a high potential as biological control agents against the pest. They have great potential as predatory natural enemies for use in the biological control of *M. alternatus*.

7. Use of a nonwoven fabric sheet with fungal spores of *Beauveria* to control *M. alternatus*. By wrapping the sheet on the trunks of infested trees the cerambycid adults are infected by the fungus when they emerge and eventually will be killed. This technique has been used in Anhui, Zhejiang and Yunnan provinces to control *M. alternatus* (Wang *et al.* 2003).

Coconut leaf beetle *Brontispa longissima* (Gestro)

The coconut leaf beetle, *Brontispa longissima* (Gestro) was introduced into China in June 2002 and has since become the number one pest in southern China (Hainan, Guangdong provinces). The worst affected palm tree areas are in Hainan Province. The pest larvae and adults feed on the palm's furled fronds, and can cause the infested tree's death. The insect pest now threatens the smallholder coconut industry and the nursery trade in ornamental palms, as well as the nation's only tropical tourism industry in Hainan Province. In order to control this insect pest, entomologists in the Environment and Plant Protection Institute, Chinese Academy of Tropical Agriculture Sciences, have carried out biological control studies of the pest. Two parasitoids were imported in 2004 and studies of their biology, ecology, mass rearing and release techniques, tracking techniques, control effects assessment and effects of insecticides on parasitoids were carried out. An effect on controlled dispersal of the pest has been determined. The main results are as follows:

1. Two parasitoids were imported and established successfully, a larval parasitoid *Asecodes hispinarum* Bouček from Vietnam and a pupal parasitoid *Tetrastichus brontispae* Ferrière, from Taiwan (both parasitoids belong to Eulophidae (Hymenoptera: Chalcidoidea)) and passed the safety assessment of State General Administration of Quality Supervision, Inspection and Quarantine of the People 's Republic of China in 2004 (Lu *et al.* 2005).

2. The temperature and relative humidity have significant effects on the parasitism and development of *A. hispinataum* and *T. brontispae*, while the photoperiod has little influence. When temperature and

humidity increased in the certain scope, they are growing, (*e.g.* parasitism rate, emergence rate). It was shown that temperature 20–26° C and RH 60 percent to 90percent were the most suitable for *A. hispinataum* rearing and propagation, while temperature 24°–28°C and RH 65 percent–95 percent were most suitable for development of *T. brontispae*.

3) The longevity of adult wasps of *A. hispinarum* and *T. brontispae* was obviously extended by feeding sucrose, honey and glucose. However, the effects of the nutrition on oviposition, development duration and sex ratio were not significantly different from the control group.

4) The natural host was successfully applied to rearing coconut leaf beetles. The product ability was about 50,000 coconut leaf beetles per day. The artificial diet was studied in-depth and 3-5 instars were successfully reared with it. Furthermore, a large number of *A. hispinarum* and *T. brontispae* can be produced.

5) A simple device for the parasitoid's release in the field was designed and simple applied techniques for the parasitoid's release were developed. In addition, after the parasitoids were released, the tracking techniques were studied in-depth and formed.

6) Based on the results of functional responses, interference effects, index of population control, population dynamics, investigations in winter and summer were also studied. The system of sustainable control of the coconut leaf beetle has been developed.

7) Toxicities of insecticides to the parasitoids were evaluated, and an effect of different insecticides on the parasitoids were confirmed. These would conduce to the coordination by biological control and chemical control in the process of sustainable control of the pest.

8) There were four parasitoids rearing rooms located at the Environment and Plant Protection Institute, Chinese Academy of Tropical Agriculture Sciences, Hainan Forestry Research Institute and Danzhou Forestry Pest Control and Quarantine Station, respectively. The production ability was about 200,000 individuals of *T. brontispae* and 600,000 individuals of *A. hispinarum* per day. Furthermore, the techniques of applying *A. hispinarum* and *T. brontispae* to control coconut leaf beetle were demonstrated and extended. To date, there have been 100 million *A. hispinarum* and 30 million *T. brontispae* released in Hainan Province. All of the palm plants damaged by coconut leaf beetle grew new leaves after the parasitoids were released.

9) In Hainan, technical training for reasonable control of the coconut leaf beetle has been carried out. There were 3,000 trainers present and 4,000 small copies of coconut leaf beetle control technology were distributed to coconut farmers. Thus, the biological control techniques were extended and its sustainable management will be realized.

Pine needle scale (Hemiberlesia pitysophila Takagi)

The scale belongs to the family Diaspididae (Homoptera). Japanese coccids scholar S. Takagi found it in Taiwan for the first time in 1956. Kawai confirmed that it was also distributed in Okinawa, Japan in 1980. The pest first entered China in the early 1980s in Guangdong Province, and has since become widespread causing serious damage to Masson pine (*Pinus massoniana*) stands. Infested forests amounted to 1.4249 million ha. in 2004. The total occurrence in forests is 544,400 ha. with severe damage 70,300 ha. The pest now occurs in most areas of Guangdong Province, and has spread to Fujian, Jiangxi, Guangxi provinces in China.

Entomologists in the Forest Pests and Diseases Control and Quarantine Station of Guangdong Province, and Fujian Agriculture and Forestry University *et al.* studied the pest control techniques since its invasion. A list of achieved results follows:

In China, pine needle scale host trees are *Pinus massoniana* Lamb., *P. ellottii* Engeim, *P. thunbergii* Parl., *P. taedal*, *P. carbaea* Morelet, in which *P. massoniana* was the most seriously damaged. *P. Massoniana* is native and the predominant cultivated tree species in southern China, it thus caused great losses both to the economy and environment. Saplings to 30-year old trees of Masson pine are damaged by contiguous wither until they are finally killed. In Guangdong Province, the yearly growth capacity of infested Masson pine was reduced by 2.7 m^3 per ha. on an average, and the pine resin production per year was reduced 900 kg per ha. The heavily infested trees may wither in three to five years and most of the infested Masson pines will eventually be killed.

A parasitoid, *Coccobius azumai* Tachikawa (Hymenoptera: Aphelinidae), is a predominant natural enemy of the scale. It was introduced from Okinawa, Japan to Guangdong Province in 1986-1989. The original parasitism rate in Japan was 25.6 percent. After introduction the parasitoid was release-supported from its first introduced Masson pine stands in Guangdong in 1990. A significant effect on the scale population was found after its release. The parasitism rate of the female coccids reached 20-30 percent and the population density of the female coccid was controlled to 0.3-0.6 individual per needle leaf, and it was effectively controlled. However, because of the high host specificity of *C. azumai* for its development and the unusually dry climate in summer time, its population dropped significantly in the original release area after 1991 and was difficult to find in 2001. Subsequently the population of the coccid increased and its damage again appeared.

For these reasons, a large-scale survey to search for the native parasitoid of the pine needle scale was carried out in Guangdong in 2004. An interesting phenomenon was observed that although the population density of the coccid was over the middle level in some infested forests where the pest has existed for ten years, but the status of Masson pine was healthy. By detailed investigation, *Encarsia amicula* Viggiani & Ren and *Aphytis* sp. (Hymenoptera: Aphelinidae) were found in pine stands (four stands in the province with a total 60 ha.). Their population and parasitism rates were very high. The study of the biology of *E. amicula* and *Aphytis* sp. showed that they are facultative parasitoids and can parasitize *Abgrallaspis cyanophylli*, *Aonidiella aurantii*, and *Chrysomphalus aonidum* which are all local coccids in Masson pine forests in Guangdong. According to the survey and study, the natural population number of *E. amicula* and *Aphytis* sp. in the above four stands was 125-278 individual per kg of the Masson pine twigs. The parasitism rate in the coccid female was 25.3–33.3 percent, and the two parasitoids could develop and reproduce in a single year, maintaining a certain number in the forest. Their peak period of adult populations is from March to May and a lesser peak period occurs in October to November in Guangdong Province.

The four Masson pine stands with high populations of native parasitoids were used as the parasitoids resource in 2004-2005 by collecting the twigs which have both the coccid and the parasitoids and releasing at the infested forests. In 2006 the old parasitoids resources and the released areas in 2004 were used as the new natural enemy resources. The artificial-helped transfer and spread technique were confirmed effective for the parasitoids dispersing. First the pine twigs with more than 100 parasitoids/one kg of pine twigs were collected and transferred for a long-distance to the infested areas to scatter. A release site was set in every 13.3 ha, hanging the twigs (about 10 kg) in three pine tree trunks which are linked together with more than 900 individuals of the parasitoids. After the release for 9-12 months it was found that dispersal distance of the parasitoids was about 200m, and 12 months after release the population and parasitism rate were 153/kg twigs and 16.8 percent respectively. After 24 months they were 299/kg twigs, 24.8 percent respectively with a maximum 572/kg twigs and parasitism rate 36.3 percent. From 2004 to 2006, the total release areas were 48,800 ha. in Guangdong Province. A significant control result was obtained, and the disaster mitigated. The damage areas of the coccid with moderate and severe levels in 2006 were reduced by 37.6 percent and 44.5 percent, compared with 2004.

Emerald ash borer (*Agrilus planipennis* Fairmaire)

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: *Buprestidae*), is native to eastern Asia, including Japan, Korea, China and the Russian Far East. EAB larval feeding damages ash trees (Oleaceae, *Fraxinus* spp.) by forming zigzag galleries in the phloem and outer sapwood. During heavy infestations, individual galleries coalesce, girdling trunk and branches and eventually killing entire trees. EAB mainly attacks *F. mandshurica* Rupr. and *F. rhychophylla* Hance. in northeastern China (Liaoning, Jilin, and Heilongjiang provinces) and *F. velutina* Torr. in northern China (Tianjin Municipality, Shandong, and Hebei provinces). Historically, EAB in China has been considered a minor pest, but our early investigations found that in some locations, such as the seacoast forest-belt of Tianjin, nearly 80 percent of the ash trees were infested, with a mortality rate at 35 percent In 2002, EAB was discovered attacking ash trees in southeastern Michigan in the U. S. A. and has since spread to adjacent areas of Windsor, Canada, northwestern Ohio (Lucas and Defiance counties), and northeastern Indiana. In those areas, EAB is devastating both ornamental and forest trees (McCullough 2002, Plant Health Division 2005). The concealed *A. planipennis* larvae are difficult to detect until the foliage wilts, dead twigs become noticeable, or adult emergence holes are noted. This delay in detection challenges control efforts with conventional pesticides. The clear threat to ash in North America and China spurred the formation of a Sino-American program to study the potential for biological control of *A. planipennis*.

As an exotic invasive pest of the U.S.A, classical biological control comes into consideration. A joint cooperative program for EAB biological control, including survey of natural enemies of emerald ash borer in China was initiated in 2003 with an aim to screen potential biological control agents against the pest in both the U.S. and China by the USDA Forest Service, APHIS and the Chinese Academy of Forestry, includes surveys of insect natural enemies, mass rearing and release of important biological control agents, evaluating control results, etc.

The following parasitoids were discovered during the natural enemy investigation:

1. *Spathius agrili* Yang, sp. nov. (Hymenoptera: Braconidae) is a gregarious ecto-parasite of EAB larva (Yang *et al.* 2005).

2. *Tetrastichus planipennisi* Yang, sp. nov. (Hymenoptera: Eulophiae) is gregarious endo-parasite of EAB larva) (Yang *et al.* 2006).

3. *Scleroderma pupariae* Yang et Yao, sp. nov. (Hymenoptera: Bethylidae) is gregarious parasite on EAB prepupa and/or pupa (Yang and Yao 2007)

4. *Deuteroxorides orientalis* Uchida (Hymenoptera: Ichneumonidae) is a solitary parasitoid of EAB mature larva)

5. A species of Platygasteridae (Hymenoptera) is an egg parasitoid of EAB.

6. *Oobius agrili* Zhang et Huang, sp. nov. (Hymenoptera: Encyrtidae) is a solitary parthenogenetic egg parasitoid of EAB.

7. *Beauveria bassiana* is a special variety of fungus found on EAB larva.

During the study it was found that the parasitoid *Spathius agrili* Yang is predominant on EAB in northern China (Hebei, Tianjin) in the ash stands of *Fraxinus velutina* and *F. chinensis* and *F. rhynchophylla*. And *Tetrastichus planipennisi* Yang is predominant on EAB in the ash stands of *F. manschurica* in northeast China (Liaoning, Jilin and Heilongjiang), and *Oobius agrili* is only found in Changchun, JilinProvince to date.

The first reported parasitoid reared from EAB is *S. agrili* Yang. Life history observations of *S. agrili* in the field and laboratory indicate it is a gregarious idiobiont ecto-parasitoid and has up to four generations per year. Parasitism rates in the field ranged from 30 to 90 percent, with one to 35 *S. agrili* eggs associated with a single host. From a host larva 1-18 adult wasps (average of 8.4) were reared. Based on laboratory rearings, the emerging adult female to male ratio is 3:1. We studied the differences of emergence date between overwintered *S. agrili* and its host, parasitism rates at different periods, relationships between parasitism rates and host densities, and relationships between ovipositions of braconid wasp and body sizes of host larvae using methods of regular surveys in forests and observations in the laboratory. Results revealed that the emergence of *S. agrili* was more than one month later than that of its host, suggesting good synchrony between parasitoid emergence and host availability. The overwintered *S. agrili* emerged from mid June to mid August with the peak in July in 2003, lasting from late May till late July with the peak during late June to early July in 2004. While the EAB emergence period ranged in mid-late May in 2003, and in mid April through mid May in 2004 (in laboratory). The emergence date of the parasitoid asynchronously inoculated with the optimum developmental instars of the earliest host larvae, which was the result of long-term co-evolution between the two species. It was also suggested that the parasitoid *S. agrili* could be a specialized natural enemy of EAB. The natural parasitism rates gradually increased in the field, on the whole, at different periods. The body sizes of host larvae, *i.e.* larval instar, affected the decision of the parasitoid *S. agrili* to lay eggs or not. Under natural conditions, this parasitoid usually oviposited only on those host larvae with a prontum and body width more than 1.5 mm, and a body length in excess of 12 mm, *i.e.* the thirdor fourth instar larvae, in the natural conditions. Egg deposition by female wasps showed no significant difference between the thirdand fourth instar host larvae. These findings would consequently contribute to the further successfully biological control of the trunk borer.

Effects of host larvae sizes on the parasitoid oviposition, offspring sex allocation, and wasp adult body length were investigated. Results showed that egg production of parasitoid *Spathius agrili* Yang (Hymenoptera: Braconidae) has a significantly positive linear relation with the larval gallery width of host emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: *Buprestidae*). As the increase of host larvae sizes, oviposition of the wasp increased too. The number of eggs deposited on hosts was clearly and positively related to the host larvae pronotum width, body length, and body width. Percentages of male wasp offspring from the relatively larger hosts were significantly lower than those from small hosts. The male proportions in the offspring developed from those hosts whose gallery widths were less than 3 mm were as high as more than 40 percent. While male percentages of progeny development from larger host larvae were even less than 20 percent, male percentages of the offspring braconid were negatively related to host larvae sizes. More parasitoid progeny successfully developed from large host larvae, and their survival (≈80 percent) improved at a certain extent comparing to those from small host larvae (≈70 percent), but the differences were not significant. Statistical analysis suggested that survival of braconid offspring were remarkably positively related to the gallery width of host larvae. There were significant differences among the body length of offspring wasp adults which were developed from different sizes of host larvae, especially the female wasps were more clearly. The body length of offspring wasp adults from parasitized host larvae whose gallery's width were over 4 mm increased by 7-8 percent and 20 percent comparing to those from hosts whose gallery width were 3.1-4 mm and under 3 mm, respectively. Furthermore, the female body sizes were usually longer than male in the parasitoid offspring developed from the same sizes of host larvae.

To date, we have studied the parasitoid's biology, mass rearing and release techniques, as well as host specificity both in China and U. S. A.. In the summer of 2007, *S. agrili* has been approved by US APHIS for field release, and the first group of *S. agrili* was released in the infested ash forests of Michigan. It has a bright future to control the severe pest in U. S. A..

Tetrastichus planipennisi Yang is a gregarious endoparasitoid of EAB larvae, producing 56-92 individuals from a single host with parasitism rates ranging from 32 to 65 percent . A koinobiont, it allows parasitized EAB larvae to continue development. The parasitoid larvae inside the EAB larva did not kill their host until reaching the late mature larval stage (fourth instar), completely consuming all of the host body contents. The host larval integument became translucent and the mature *T. planipennisi* maggot-like larvae (3-4 mm) were clearly visible. Wasp larvae only at this time exited their host membranous integument, and soon pupate adjacent to it, which is typically near the end of the gallery. It took approximately 15 days for adult wasps to eclose. The newly emerged wasp chewed a hole through the bark to exit the host gallery, with other emerging wasps in the brood often using the same hole. We estimate that at least four generations are produced a year in northeastern China, with the last generation overwintering as mature larvae inside their host's gallery. The sex ratio of the emerging adults was skewed towards females 2.5:1. The longevity of the parasitoid female adult was about 15 days and male 13 days at room temperature (25°C) during our rearing.

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Import and Application of Two Parasitoids of the Coconut Leaf Beetle, *Brontispa longissima* (Gestro)

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ABSTRACT

The coconut leaf Beetle, *Brontispa longissima* (Gestro) is one of the most important insect pests of palm plants in South East Asia and the Pacific. It is also the second category quarantines risk insect pest in China. The larvae and adults feed on the furled leaves, to the point of causing plant death. The coconut leaf beetle was first discovered in Haikou City in June 2002, and shortly afterwards in Sanya City. Now, it has become the most serious threat to Hainan's smallholder coconut industry, the nursery trade in ornamental palms, as well as the nation's only tropical tourism industry. In order to control this insect pest, we imported two parasitoids in 2004 and had in-depth studies of their biology, ecology, rearing and release techniques, tracking techniques, control effects assessment and effects of insecticides on parasitoids release. These studies had a great effect on controlling the dispersal of the coconut leaf beetle. The important results are as follows:

1. We imported and established two parasitoids: the larval parasitoid *Asecodes hispinarum* Bouček from Vietnam and the pupal parasitoid *Brontispa longissima* (Gestro) from Taiwan, and passed the safety assessment of State General Administration of Quality Supervision, Inspection and Quarantine of the People 's Republic of China in 2004.

2. The temperature and relative humidity had significant effects on the parasitism and development of *A. hispinataum* and *T. brontispae*, while the photoperiod had little influence. With the increase in temperature and humidity, the parasitism rate, emergence rate, number of wasps per mummy and the intrinsic rate of natural increase (rm) of *A. hispinataum* and *T. brontispae* increased, while their development duration decreased. Temperatures of 20–26^0 and 60–90 percent RH were the most suitable for *A. hispinataum* rearing and propagation, while temperatures of 24^{0}C–28^{0}C and 65–95 percent RH were most suitable for *T. brontispae*.

3. The longevity of *A. hispinarum* and *T. brontispae* was obviously extended by feeding sucrose, honey and glucose, but the effects of all nutrition sources on ovipostion, development duration and sex ratio were not significantly different from the control group. In addition, the female's longevity and survival rate with parasitic behaviors decreased.

4. Natural host was successfully applied to rearing coconut leaf beetles. The product ability was about 50,000 coconut leaf beetles per day. Now, artificial feed was studied in-depth and 3-5 instars were successfully reared. Furthermore, large numbers of *A. hispinarum* and *T. brontispae* were able to be

produced. These previously mentioned techniques were very useful in controlling the occurrence of and damage by the coconut leaf beetle.

5. A simple device for parasitoids release in the field was designed and a very easy parasitoid release technique was developed. In addition, after the parasitoids were released, tracking techniques were formulated and studied in-depth.

6. Based on the results of functional responses, interference effects, index of population control, population dynamics, investigations in winter and summer, etc., a system of continual coconut leaf beetle control was developed.

7. Toxicity of insecticides to the parasitoids were evaluated and the effects of different insecticides on the parasitoid releases were confirmed. These are conducive to the coordination of biological control and chemical control in the process of continual coconut leaf beetle control.

8. There were four parasitoids rearing rooms founded at Environment and Plant Protection Institute, Coconut Research Institute, Chinese Academy of Tropical Agriculture Sciences, Hainan Forestry Research Institute and Danzhou Forestry Pest Control and Quarantine Station, respectively. The product ability was about 200,000 *T. brontispae* and 600,000 *A. hispinarum* per day. Furthermore, the techniques of applying *A. hispinarum* and *T. brontispae* to control the coconut leaf beetle were demonstrated and extended. To date, there were 100,000,000 *A. hispinarum* and 30,000,000 *T. brontispae* released in Hainan. All of the palm plants damaged by the coconut leaf beetle grew new leaves after the parasitoids release.

9. In Hainan, technical training on how to control the coconut leaf beetle were in good stage. There were 3,000 trainers and 4,000 small copies of the coconut leaf beetle control technology were distributed to coconut farmers. This training had a good effect on continual coconut leaf beetle control.

Keywords: *Brontispa longissima* (Gestro), *Asecodes hispinarum* Bouček, *Tetrastichus brontispae* Ferrière, import, application

Alien Forest Insects Have Not Found Finnish Forests, Yet

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ABSTRACT

Virtually all ecosystem types are affected by invasive species and they pose one of the biggest threats to biodiversity worldwide. It is likely that changing climate, increasing trade, tourism and transportation of wood will increase the risk of invasive alien species problems associated with forest trees. So far, Finland is one of the few countries in the world where not a single alien forest insect species has been observed. The main alien forest pest species threatening Finnish forests are the pine wood nematode (*Bursaphelenchus xylophilus*), the Dutch elm disease spread by the elm bark beetles of the genus *Scolytus*, and the Siberian moth (*Dendrolimus* spp.). Lack of invasive alien forest insects is mainly due to harsh climate, high level of forest hygiene, low number of tree species, legislation and good luck.

Keywords: forest pests, boreal forests, *Bursaphelenchus xylophilus*, *Ophiostoma ulmi*, *Scolytus*, *Dendrolimus superans*.

INTRODUCTION

Biological invasions of alien species coupled with environmental changes are a major threat to biodiversity. Intentional movement of plant and animal species around the world has been characteristic for human activity for a long time, but in addition to the intentional transport of species, also unintentional introductions of species may occur. Many of the invasive alien species do best in human-disturbed environments, mainly affecting species composition, but some can invade undisturbed natural habitats. Though exotic phytophagous insects are invading forest ecosystems worldwide, number of terrestrial introduced alien species has remained relatively low in Finland (Nummi, 2001). At present, Finland is one of the few countries in the world where not a single alien forest insect species has been observed.

Characteristics of the Finnish Forests

Finland belongs to the boreal forest zone in northern Europe. The land area of the country totals approximately 30 million hectares, of which 86 percent is classified as forest land. Since the 1960s, the forest area has increased by 1.6 million ha as a result of afforestation of agricultural lands and peatlands, as well as intensive forest improvement efforts (Peltola and Ihalainen, 2005). With these figures, Finland is the most heavily forested country in Europe.

In spite of large forest areas, there are very few economically important tree species in Finland. Almost half of the growing stock, now amounting to more than 2000 million cubic meters, consists of Scots pine (*Pinus sylvestris* L.). The proportion of Norway spruce (*Picea abies* (L.) H. Karst) is 33 percent, leaving 19 percent for birches (mainly *Betula pendula* Roth and *B. pubescens* Ehrh.) and other broadleaved trees. In addition, there are some exotic tree species, of which the lodgepole pine (*Pinus contorta* Douglas ex Loudon) is economically most important. It was introduced from North America to Finland in 1910. Large-scaled introductions took place in 1970s, and the total area now covered by this exotic pine is approximately 10,000 ha. In Sweden, the total area of the lodgepole pine exceeds 600,000 ha (Lindelöw and Björkman, 2001).

International trade in plants facilitates the introduction of species that are able to attack local native trees in natural forests. Finland ranks third after Japan and China with respect to the volume of imported wood which makes the probability of alien species attacks high in international comparison. From the early 1990s, the imported wood volumes have more than doubled. At present, almost one-fourth of the wood consumption of the Finnish forest industries is accounted for by imported wood. Imported wood mainly originates from Russia (80 percent) (Peltola and Ihalainen, 2005).

In Finland, the three main tree species can form both pure and mixed stands. There is a hypothesis that mixed stands are characterized by lower insect herbivory than pure stands, but the relationship is a complex one and still poorly understood. Also the density of trees and age structure can influence the likelihood of the pest attacks (Battisti, 2006). The tree species structure has remained relatively stable over considerable long periods in Finland. At present, one-fourth of the forest area is mixed forest (Peltola and Ihalainen, 2005).

Potential Invasive Forest Pests

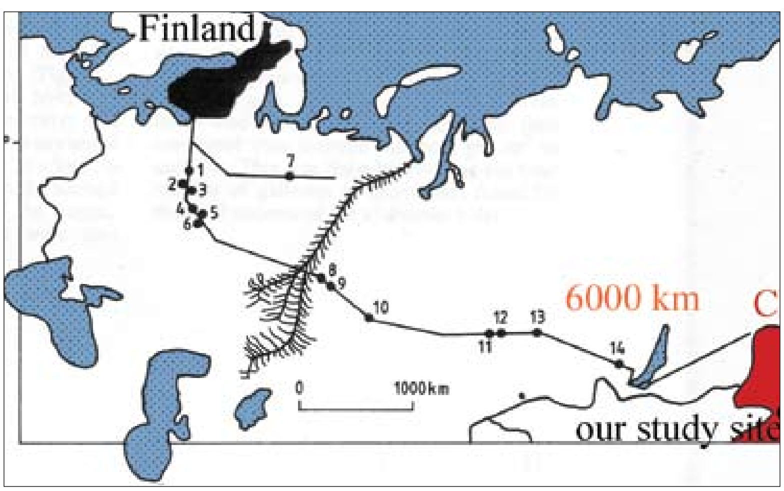

Figure 1. Railway from Siberia to Finland. Samples taken from the railway wagons with coniferous pulpwood (numbered sites) have shown that considerable numbers of insects are conveyed alive on timber. Redrawn from Siitonen 1990. Black dot indicates the recent study site.

Increased timber transportation especially from Russia involves a risk of introducing new, exotic forest pests to Finland. For instance, 17 million cubic meters of raw timber were transported by rail from Siberia in 2006. The transport distance has often been thoU. S. A.nds of kilometers reaching close to the border of China. Samples taken from the railway wagons with coniferous pulpwood have shown that considerable numbers, both species and individuals, of tree-living beetles are conveyed alive on timber (Fig. 1). More than twenty species of bark beetles (Scolytidae) and about twenty other phloem

feeding insect species were found in

1990. Also three species new to Finland, two of them known as major pests of larch in Siberia, were found (Siitonen, 1990). It is, of course, difficult to predict what will be the first invasive alien forest pest species in Finland. There are many candidates, of which the pinewood nematode, elm bark beetles and Siberian moths are examples.

Pinewood Nematode

The pinewood nematode (*Bursaphelenchus xylophilus* (Steiner & Buhrer 1934) Nickle *et al.* 1970) now occurs in Japan, China, Korea, Portugal, the United States and Canada (Fig. 2). It is believed to have originated in North America. Due to long-term evolution with its indigenous conifer tree species the nematode does not cause damage to these hosts in North America. However, the nematode has caused considerable mortality in Asia where the trees have not adapted to the parasite. The spread of the nematode between countries and continents results from international trading and other human activities. Within forests, the nematode is known to be vectored by sawyer beetles (*Monochamus* spp.) The pinewood nematode has been under surveillance by Finnish plant health officials since 1984. The risk of the nematode spreading to Finland in coniferous wood packing material from countries known to have the nematode is very real. Out of hundreds inspected lots of coniferous packing wood, 3 percent have contained living pinewood nematodes. Though Finnish pine forests would meet the nematode's biological requirements, no successful establishment has taken place so far. However, the discovery of the pine wood nematode in Portugal in 1999 has confirmed the estimate that the nematode also could cause extensive forest damage in Europe.

Dutch Elm Disease Spread by the Elm Bark Beetles

Dutch elm disease is the most destructive disease of elm trees (*Ulmus* spp.) in the northern hemisphere (Anderson and Holliday, 2003). The caU. S. A.l pathogen of the disease is the fungus *Ophiostoma ulmi* (Buisman), and since the 1960s also its more virulent strain *Ophiostoma novo-ulmi* (Brasier) (McLeod *et al.*, 2005). A wide range of beetle species, mainly in the genus *Scolytus* (Coleopera, Scolytidae), have been identified as potential vectors of the disease (Webber, 2004). Two most common vector species in Europe are *Scolytus scolytus* (Fabricius) and *Scolytus multistriatus* (Marsham) (Fig. 2).

In Europe, *O. novo-ulmi* has been spreading towards the Nordic countries reaching Denmark in 1955 (Viiri, 1998; Jørum and Hvass, 2003). In 1979 it was recorded in Scania in southern Sweden (Anderbrant and Schlyter, 1987; Oheimb and Brunet, 2007) and later on in Stockholm and Uppsala (Viiri, 1998). The most important vector has been *Scolytus laevis* Chapuis in the Nordic countries. This beetle is the most widespread *Scolytus* species in Denmark. It occurs also in Sweden and Norway (Lekander *et al.* 1977) but not in Finland.

In Finland, the Dutch elm disease has been reported from three southern seaports during 1960s: Turku (1963), Helsinki (1965) and Loviisa (1968) (Viiri, 1998). Luckily, however, *Scolytus laevis*, which is the only possible vector of the disease in Finland, has not been recorded in Finland so far. In addition, there are very few elms in Finland. Furthermore, the two elm species in Finland, *Ulmus glabra* (Huds.) and *Ulmus laevis* (Pall.) are not favored as breeding sites by *Scolytus* species (Webber, 2004). Hence, the possible permanent introduction of Dutch elm disease to Finland is possible, but rather unlikely.

Siberian Moth

The Siberian moth, *Dendrolimus superans* Butler (Lepidoptera, Lasiocampidae), is a serious pest of coniferous trees (Fig. 2). It is widely distributed in northeastern China, Siberia, Korea and Japan. The

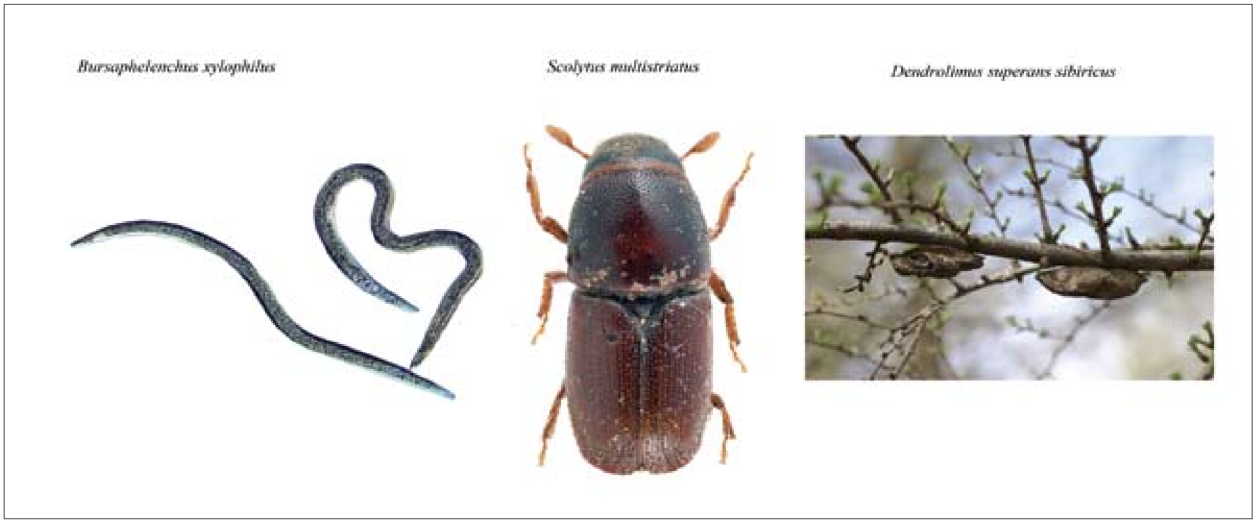

Figure 2. Potential invasive alien pests in Finnish forests. Original photo on the pinewood nematode taken by Jyrki Tomminen.

preferred host is *Larix gmelinii* (Ruprecht) (Kuzeneva, 1920), but larvae also feed on *Pinus* species, such as *Pinus koraiensis* Siebold et Zucc., *Pinus tabulaeformis* Carr., *Pinus sylvestris*, and *Picea koraiensis* Nakai (Hou, 1987).

The species status of the Siberian moth is not clear. The European Plant Protection Organisation (EPPO) treats *Dendrolimus sibiricus* (Tschetverikov) and *Dendrolimus superans sibiricus* (Butler) as one species. However, according to Mikkola (2005), there are certain differences in the male reproductive organs of the two closely related species *D. sibiricus* and *D. superans* resulting in three distinct species in Eurasia. Consequently, the Asian individuals belong to *D. superans*, the Russian individuals belong to *D. sibiricus*, and the European individuals to *Dendrolimus pini* L. (K. Mikkola, pers. comm.). More detailed investigations are needed to map the present geographical distributions of these species.

No Alien Forest Insects in Finland. Why Not?

In general, many more species have moved from Europe to the U. S. A. than vice versa. Of the more than 400 immigrant species living on trees and shrubs in North America, approximately 75 percent originate from Europe (Mattson *et al.*, 1994). In addition, there have been about six-fold more phytophagous forest insects from Europe that have successfully invaded North America than vice versa (Niemelä and Mattson, 1996). This is partly explained by the fact that there are fewer and less penetrable tri-trophic niches in Europe due to fewer and less available host plants (Mattson *et al.*, 2007). Lack of invasive alien species in Finnish forests may still sound surprising. In fact, it seems that foresters and even forest entomologists find it difficult to believe in this argument. However, Finnish forests are very carefully investigated, and researchers are very well aware of the species present in the forests.

Transportation of wood between countries increases the probability of invasive alien species. Foresters in Finland are concerned that the *D. superans* might be introduced to Finland. *D. sibiricus* already occurs in Carelian Republic, Russia, but it is not widespread in the area (Kulinich, 2005). A threat of a new forest pest is evident, since 6.9 million cubic meters of wood were transported from Russia to Finland in 2006. Though most of the wood originated from the European side of Russia, some of it, mainly larch (*Larix* spp.), was transported from the Asian side of Russia (Lilja, 2006). In addition to *D. superans*, *Ips subelongatus* Motschulsky (Coleoptera, Scolytidae), a destructive pest of *Larix* spp., was one of the species, which was found new to Finland in the timber imported by rail from Russia to Finland (Siitonen, 1990). Furthermore, *D. superans* and *I. subelongatus* coexist as pest species in the same areas in China. If *I. subelongatus* was introduced to Finland, *D. superans* could be introduced as well.

Foresters are also concerned that breeding exotic tree species may increase the risk of pest outbreaks in forests. However, several studies show that it is difficult to predict whether an introduced tree species will suffer from insect outbreaks more often than native tree species (see Dalin and Björkman, 2006). Introduction of the lodgepole pine has not brought new exotic species to Finland. Although many pests native to Finland may infest lodgepole pine, none of them seems to be destructive to the trees (Annila *et al.*, 1983).

Harsh climate may prevent invasive alien species from spreading. Finland is a northern country with a relatively long and cold winter. However, long winter and short summer do not necessarily explain the absence of alien forest pests. For instance, climatic conditions in Inner Mongolia, where *D. superans* occurs as a serious pest, are not very different from those in Finland. It is possible, that forest management strategies also affect the occurrence of defoliating forest pests. Uniform monoculture conditions with reduced diversity of the stand in many forest plantations have been assumed to favour the expansion of the moth, but the relationship is a complex one and poorly understood.

A carefully prepared legislation is one tool in the battle against invasive alien species. A Finnish law concerning the prevention of forest insect and fungal damage came into force on July 1, 1991, and the corresponding decree was established on August 1, 1991. According to these laws, it is illegal to store fresh unbarked timber so that its storing causes insect or fungal damage to surrounding forests. Therefore, timber must be either removed from cutting areas or from temporary timber stores by the dates mentioned in the law, or protected by other means (Fagerholm and Heliövaara, 2000). This legislation has increased the level of forest hygiene in the forests and partly made it more difficult for alien invasive species to invade Finnish forests. And finally, Finland has been an exceptionally lucky country so far.

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Session Two

Arthropods

Ecology of Hemlock Woolly Adelgid and its Natural Enemies in China: Implications for Biological Control of the Adelgid in the United States

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ABSTRACT

The hemlock woolly adelgid (*Adelges tsugae*) is endemic in several Asian countries, but is introduced to the eastern United States where is causing severe damage to native hemlocks. To establish biological control, a cooperative program with several institutions in both China and the United States is surveying and evaluating natural enemies of the adelgid in the provinces of Yunnan and Sichuan. Information on the phenology, biology and distribution of the adelgid and more than 100 natural enemies will be provided and implications for biological control discussed.

Biology of the Hemlock Woolly Adelgid (Hemiptera: Adelgidae) and Its Natural Enemies in Sichuan, P. R. China

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ABSTRACT

Laboratory observation and field sampling of the hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), and its natural enemies were made on hemlock in three forest types— *Tsuga chinensis* Pritz - *Picea retroflexa* Mast; *Tsuga chinensis* Pritz - *Pinus armandii* Franch; *Tsuga dumosa* Eichler - *Acer mono* Maxim - *Betula platyphylla* Suk in Kangding, Baoxing, and Danba Counties in Sichuan Province. There were three study sites in the first year, two in Kangding and one in Baoxing Counties; in the second year Baoxing County was dropped and Danba County added.

To determine the seasonality of HWA life stages, collections of HWA infested hemlock foliage were made monthly from June, 2005 to May, 2006 and examined with microscopes in the laboratory. There were two overlapping parthenogenetic generations of HWA, an overwintering (sistens) generation and a spring (progrediens) generation. The first instar crawlers of the overwintering generation were present during June and July; these settled on the new foliage and aestivated during the summer months. They began developing in September and peak oviposition was in March and April (average 67.4 eggs per female). These eggs developed into only a few winged alates (sexupara) in June and mostly progrediens adults. The latter laid eggs from mid-May through August (average 15.6 eggs per female). Development was fastest and separation between stages was more defined in the warmest site.

Additional samples of HWA infested foliage were taken during each season of the year (spring, summer, fall, winter) from each cardinal direction of the crown from the lower (1-2 meters) and upper (4-6 meters) crown (8 samples/tree). Within a tree, there was no overall significant statistical difference in HWA infestation between levels or among cardinal directions. HWA infestation was related to forest type – more severe in mixed hemlock - broadleaf hardwood forests (Zhou *et al.*., 2007).

Natural enemies of HWA were collected by beating branches of hemlock at approximately monthly intervals from 2005 to 2007 in each of the three forest types. A total of 65 species of potential HWA predators were collected belonging to Coccinellidae, Chrysopidae, and Derodontidae. Feeding tests confirmed that 28 of the predator species feed on HWA. The most abundant species in the field samples were (total # in parenthesis) — *Scymnus* (*Neopullus*) *camptodromus* Yu and Liu (498), *S.* (*N.*) *ningshanensis* Yu and Yao (450), *Oenopia signatella* (Mulsant) (548), and *Melyridae* sp. (84). The two

Scymnus species were the only predators consistently found at all sites. *Oenopia signatella* and the melyrid were abundant in the fall in the site with the highest HWA density. The *Laricobius* spp. were not abundant, with only nine adult specimens collected in the routine samples. Most of the *Laricobius* fit the description of *L. kangdingensis* Zilahi-Balogh and Jelnik, one specimen fits *L. baoxingensis* Zilahi-Balogh and Jelnik, and some specimens have characters of both species.

Analyses of the diversity of HWA natural enemies, the indices of Richness, Equitability, and Diversity are higher in hemlock forests mixed with broadleaf hardwood than in hemlock forests mixed with other conifers. The index of dominance is lower in hemlock forests mixed with broadleaf hardwood than in hemlock forests mixed with conifer. The index of Similarity indicates that HWA natural enemies among the three forest types are different in species and numbers.

Laboratory rearing of *S. camptodromus* at 14°C, with 9:15 L:D photoperiod showed that it has one generation per year with four larval instars. Females usually lay eggs singly in stem crevices. Total development time from eggs to adults was between 53 and 58 days. Egg development period lasted 12 days. The development of the first instar larva lasted six days and it could feed on eggs and adults of HWA. Development of larval instars II to IV lasted six, seven, and eight days, respectively. Prepupa development lasted three days and pupa development lasted 11 days. In the lab, adults laid eggs from December to June. Eggs laid before the end of March hatched, but eggs laid later entered diapause. Eggs laid after March hatched in December, but many of them were dead.

Laricobius spp. were reared in the laboratory at 11°C, with 9:15 (L:D) photoperiod. There was one generation per year with four larval instars. Females laid eggs singly in the HWA ovisac. Egg development lasted 15 days. The first instar larva fed only on eggs of HWA. Each of the four larval instars took five days to complete development. The mature larvae dropped to the bottom of the rearing container and were transferred to soil collected from hemlock stands that was kept at 20-30 percent soil moisture. The prepupal-pupal development period lasted 70 to 90 days. The adults began to emerge from the soil in September and began to lay eggs in November. Adults of *Laricobius* spp. collected in the field in November began to lay eggs in December.

Keywords: *Adelges tsugae*; *Tsuga*; predator; Coccinellidae; Derodontidae.

ACKNOWLEDGEMENTS

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Agroforestry and Integrated Pest Management

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ABSTRACT

The Bio-Integral Resource Center (BIRC) is an institution that does research on organic agriculture, biological control and integrated pest management (IPM). For many years we have run an active China program. During this time, we did research on Chinese biological control methods, such as the utilization of natural enemies that preyed on pests in agroforestry, and in integrated food production systems.

We studied the use of *Trichogramma* for biological control in the United States (U.S.), and also introduced 120 species of natural enemies from China to the U.S. When Asian gypsy moth, *Lymantria dispar*, became a problem in the U.S. we did research on Chinese natural enemies that would be useful in controlling this invasive species in the U.S. Among these natural enemies there were various parasitic wasps, flies and also predators. Biological control provides a lasting solution to many of the problems caused by invasive species.

During the years when the BIRC China program was active, much of Chinese agriculture was following traditional methods. Since then, pesticide use in China has been increasing following the pattern seen in the U.S. with industrialized agriculture.

Some of the lessons the BIRC learned from China are now being used in the U.S. organic agriculture. China can also learn from the U.S. about the problems with industrialized agriculture. The exchange of information has been beneficial to both countries.

Keywords: agroforestry, biological control

Studies on the Sensitivity of the Emerald Ash Borer (*Agrilus planipennis*) to Non-steroidal Ecdysone Agonists

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ABSTRACT

The emerald ash borer (EAB) is an invasive coleopteran which has become a serious pest of ash trees in some states in the U.S.A. and southwestern Ontario, Canada, where it has successfully established itself. Control of this insect is extremely difficult because of the cryptic nature of most of its life stages. There are currently no biological or chemical control products applied on a large scale to combat this pest, although many solutions are in development, such as the deployment of parasitic wasps native to Asia (*e.g.*, *Spathius agrili*) or the use of chemical insecticides by injection in high value ash trees in urban areas (*e.g.*, Imidacloprid injections) (Poland, 2007). We have initiated a study on the potential of a dibenzoylhydrazine, halofenozide, (an agonist of the molting hormone ecdysone) for EAB control. Commercially available formulations of halofenozide have shown high efficacy against a number of beetle species. Coupled with this high efficacy, the relatively narrow spectrum of insect orders it targets (Lepidoptera and Coleptera) makes halofenozide a potentially attractive compound for management programs integrating biological control agents such as parasitic wasps.

Our goal is to characterize, in EAB, the known insect target site of dibenzoylhydrazines, *i.e.*, the heterodimeric nuclear hormone receptor composed of the Ecdysone receptor (EcR) and Ultraspiracle (USP) proteins. In vitro expression of EcR/USP permits a range of assays on halofenozide, including receptor binding, activation of ecdysone responsive genes, etc., which are useful for assessing efficacy. As a first step toward that goal, we attempted to clone the genes coding for both EAB EcR and EAB USP using a polymerase chain reaction (PCR) approach.

PCR primers were designed from highly conserved regions of EcR and USP, namely from the DNA- and ligand- binding domains. Six degenerate primers were synthesized for each of the two subunits. Total RNA isolated from adult EAB and reverse transcribed into first strand cDNA was used as a template for PCR experiments. A standard PCR protocol did not result in the amplification of a product for either EcR or USP, irrespective of the primer combination used. A nested PCR strategy was then devised using various combinations of inner and outer primers. Two PCR products were generated by using this strategy with the degenerate EcR primers. However, cloning and sequencing of the products revealed that they coded for proteins unrelated EcR.

Further PCR experiments will be attempted using primers chosen from the same conserved regions, but designed using a different method (Nakagawa *et al.*, 2007). In addition, we will perform tests to assess the sensitivity of adults and larvae to a formulation of halofenozide (MACH2®, Dow Agrosciences). These tests should prove instrumental for evaluating the potential of this ecdysone agonist against EAB.

Keywords: ecdysone, halofenozide, emerald ash borer, EcR, USP.

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Parasitoids of Emerald Ash Borer (*Agrilus planipennis*) in China

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ABSTRACT

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is a bark beetle that is a serious pest of ash trees . It is difficult to detect and control as its highly hidden life history. Herein, five parasitic wasps attacking EAB recently discovered in China are introduced, including *Spathius agrili* Yang (Hymenoptera: Braconidae), *Scleroderma pupariae* Yang et Yao (Hymenoptera: Bethylidae), *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), *Deuteroxorides orientalis* Uchida (Hymenoptera: Ichneumonidae), and *Oobius agrili* Zhang et Huang (Hymenoptera: Encyrtidae).

Spathius agrili Yang is an important ectoparasitoid found on EAB larvae. This wasp has excellent biological control utilization potential to control EAB populations. The biological and ecological relationships between parasitoid and host, host specificity, mass rearing method, and host location mechanisms of the wasp have been studied in detail. This parasitoid has phototaxis and the female is more susceptible. The sex ratio is 3♀ to 1♂. Usually amphigony is the natural mode of reproduction, but the female also can oviposit without mating under special conditions. The offspring from parthenogenesis is absolutely all males. Female *Spathius* can survive a long time and its ovarioles usually contain only a few mature eggs. At 21-30^{0}C and a supplementary honey solution, *S. agrili* adults live about 1 month. Some individuals can live more than 90 days. Adult life-span increases at lower temperatures. Females usually live longer than males under the same conditions. The oviposition period ranges 8-18 days with a maximum 47 days observed. Individual *S. agrili* oviposits up to eight times, typically 1-10 eggs each time. Single female lays 6-68 eggs totally with a mean oviposition of 5.8 eggs/host. *S. agrili* can utilize hosts under bark 1–6.5 mm in depth. About 92 percent EAB larvae parasitized by *Spathius*, are distributed 1–4 mm inside the bark with the peak at 3 mm.

From late May to early June, only a few adult wasps emerge in the field, because in this period only few host (EAB) larvae can be parasitized, as most at lower instars until mid June. The emergence peak is in mid and late July. The wasp also shows a very long emergence period after overwintering, about two months even in the same clutch of cocoons. This strategy helps the offspring population survive and reproduce persistently. *Spathius agrili* larvae have five instars. *S. agrili* is about 3-4 generations, and a few two generations per year in Tianjin. The parasitoid cocoons and overwinters in the gallery of its host as a prepupa. The overwintering generation cocoons in late September to early November. Those parasitoids cocooned in this period usually do not emerge in the year, but enter into diapause

for overwintering. At the end of May to mid July of the following year, the wasps pupate and emerge in succession.

Spathius agrili adults first appear in the field in late May in Tianjin. Their population increase follows the development of the emerald ash borer larvae population. The parasitism rate of the first wasp generation is relatively low, only about 10 percent, but gradually increases and peaks with the overwintering generation, usually in excess of 40 percent.

The number of parasitoid eggs produced on each host has a significantly positive relationship to host larvae size. Number of immature *S. agrili* per larva is positively related to the host larval gallery width. And the clutch size also increases with host larval weight, indicating that females laid more eggs on large EAB larvae. The percentage of female offspring is greater when eggs are laid on large larvae. Survival of parasitoid progeny is also positively related to the width of the host gallery. The bigger adult parasitoid offspring usually have been developed on large host larvae, especially the females.

Under natural conditions in the field, all other insect species investigated have not been found to be parasitized by the wasp *Spathius agrili,* except EAB larvae. The no-choice parasitism tests show that, besides EAB larvae, *S. agrili* will also attack some other insect larvae in genera of *Agrilus*, *Buprestidae*, such as *A. mali*, *A. zanthoxylumi* and *A. inamoenus*, and the wasp progeny can develop successfully, although the parasitism rates are somewhat lower than that of EAB.

The parasitoid only attacks hidden hosts and shows no interest in the exposed EAB larvae. When the location of the host is ascertained, the female wasp slowly bores into bark with her ovipositor and then injects venom inside the host larva paralyzing it prior to oviposition. *S. agrili* locates host habitats according to the volatile secondary compounds from plants infested by hosts. During the course of host location and acceptance, the parasitoids can probe host sites underneath bark mainly by the mechanical vibrations from host feeding and movement, but not stimuli from infochemicals. The wasps do not lay eggs again on the previously parasitized hosts because the paralyzed host larvae no longer produce vibrational signals so they are undetected by *S. agrili*.

The biology, cold storage and mass production technology of *Scleroderma pupariae* Yang et Yao were also investigated. The female *Scleroderma* adults bite entrance holes in the ash bark and excavates following host frass and tracking hosts to their galleries. The female *Scleroderma* can live one month when supplied water only, and the life-spans show no difference whether supplying honey or nothing. The females live longer than males. Winged female rates are about 57 percent and 15 percent in the first and second generations, while all female offspring are wingless in the thirdto 5th generations. The sex ratio of *Scleroderma* in the first generation is about 20 females to 1 male, while in the second to 5th generations, the sex ratios range 30-50 females to 1 male. Usually only 1-2 males will occur in one clutch. The bethylid wasp has five generations a year at the maximum. Every female usually produces 3-5 clutches of eggs on shift hosts. Except EAB larvae, The *Scleroderma* can attack some buprestid larvae (*Agrilus viduus* Kerremans, *A. mali*, *A. zanthoxylumi*, *A. lewisiellus*), longhorn beetle larvae (Falderman gebleri (Falderman), *Anoplophphora glabripennis* (Motschlsky), *Stenhomalus complicates* Gressitt), and even *Tenebrio molitor* (L.), or *Chilo luteellus* (Motschulsky). The wasp has no choice on host sizes, but it lays more eggs on large hosts. This wasp can be artificially produced very easily in laboratory. One-hundred percent of female adults can survive 70 days at 8°C. About 60 percent individuals can live more than one year without food supplementation.

Tetrastichus planipennisi Yang is also a potential biological control agent which endoparasitized EAB larva in northeastern China (Jilin, Liaoning, and Heilongjiang provinces). *Deuteroxorides orientalis* Uchida

has been found in Heilongjiang Province and Tianjin, parasitizing EAB prepupa or pupa. *Oobius agrili* Zhang et Huang is an egg parasitoid of the EAB, published in 2005 by Dr. Zhang Yanzhou, which occurs in Jilin Province. The biology and application in EAB biological control needs further study.

Keywords: *Agrilus planipennis*, *Spathius agrili* Yang, *Scleroderma pupariae* Yang et Yao, host location, concealed insect pest, biological control

Biology, Damage Traits and Spatial Distribution of Emerald Ash Borer (*Agrilus planipennis* Fairmaire) in China

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ABSTRACT

Emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) (Coleoptera: Buprestidae) is a major pest of ash (*Fraxinus* spp.) trees, distributed in Heilongjiang, Jilin, Liaoning, Tianjin, Hebei, Shandong, Inner Mongolia and Xinjiang provinces in China (Wei *et al.*, 2004). Synonyms of *A. planipennis* include *A. marcopoli* Obenberger (in China), *A. marcopoli ulmi* Korosawa (in Korea and Japan), and *A. feretrius* Obenberger (in Taiwan, China) (Jendek, 1994). In the early 1960s, this pest threatened an introduced ash, *F. pennsylvanica* Marsh. var. *lanceolata* (Borkh.) Sarg, and a native ash, *F. mandshurica* Rupr., in Harbin and Shenyang; therefore, all of the introduced ash were removed from these areas (Liu, 1966; Yu, 1992). In the 1990s, EAB severely damaged ash trees that were mostly introduced from North America (*e.g.*, *F. americana* L., *F. pennsylvanica* Marsh., *F. pennsylvanica* Marsh. var. *lanceolata* (Borkh.) Sarg, and *F. velutina* (Torr.) (Liu *et al.*, 1996; Liu *et al.*, 2003; Gao *et al.*, 2004; Sun & Li, 2004; Wei *et al.*, 2004; Zhao *et al.*, 2004). EAB severely damaged the native ash, *F. mandshurica* Rupr., but it caused little damage to two widely distributed native ashes, *F. chinensis* Roxb and *F. rhynchophylla* L. in China (Yu, 1992; Wei *et al.*, 2004; Zhao *et al.*, 2004; Zhao *et al.*, 2005).

Emerald ash borer is regarded as one of the most important invasive pests in North America (Haack *et al.*, 2002; Cappaert *et al.*, 2005; Poland & McCullough, 2006; Mastro *et al.*, 2007). It was first discovered attacking ash trees in southeastern Michigan and neighboring Ontario, Canada, in 2002 (Haack *et al.*, 2002). Over 20 million ash trees have been infested in Michigan alone (Poland & McCullough, 2006). In 2007, additional infestations have been found in Illinois, Indiana, Ohio, Maryland and Virginia, primarily a result of inadvertent transport of infested ash nursery stock, firewood and logs. The potential economic, ecological and social impacts of this pest are tremendous given that ash is an important timber and landscape species throughout North America. It is estimated that there are more than 7 billion ash trees in the U. S., of which more than 700 million occur in Michigan, 280 million in Ohio, and 150 million in Indiana (Mastro *et al.*, 2007). If *A. planipennis* becomes widely established in Michigan alone, it could result in a total loss of 307 million board feet of ash saw logs and veneer with a compensatory value as high as $18.9 billion in Michigan alone (Poland & McCullough, 2006; Mastro *et al.*, 2007).

Although EAB has been recorded to be semivoltine in Heilongjiang Province (Yu, 1992), and univoltine both in Shenyang City, Liaoning Province and in Tianjin (Liu, 1966; Liu *et al.*, 1996; Gao *et al.*, 2004; Sun & Li, 2004; Wei *et al.*, 2004; Zhao *et al.*, 2004; Zhao *et al.*, 2005), it is not clear where the transitional area is located and the type of life cycle EAB has in this area. In this paper we report on the life history of EAB in different geographical areas of China and provide biological characteristics as well as damage traits and spatial distribution of EAB in that area based on 2 years of field survey combined with laboratory observations.

MATERIALS AND METHODS

1.1. Study site

Three sites were selected for this study: Harbin Experimental Forest of Northeast Forest University, Harbin, Heilongjiang Province; Jingyuetan National Forest Park, Changchun, Jilin Province; and Guangang Forest Park, Dagang District, Tianjin, China.

A 42-year-old *F. mandshurica* plantation was selected at the Harbin Experimental Forest (45°43'N, 126°37'E) at an elevation of 145 m. The plantation was 1.6 hm^2 with 0.6 canopy density. The row-spacing was 2.0 m, and the distance between trees within a row was 3.0 m. Diameter at breast height (DBH) of ash 27.4 ±6.5 cm, and height 11.6 ± 4.2 m. The soil was a medium-thick chernozem. The annual average temperature is about 4.5°C, rainfall 480 mm, sunlight 2 500 hours, and frost-free period 120- 30 days.

An 8-year-old *F. mandshurica* plantation in the Jingyuetan National Forest Park is located at 42°46'N, 125°27' E at an elevation of 220 m. The area of the plantation is 2.2 hm^2 with a south slope of 11°, 0.8 canopy density, and dark brown soil with medium thickness. The row spacing is 2.0 m, and the distance between trees within a row is 2.0 m. The average DBH of ash is 4.81±2.18 cm, and average height 3.75±0.94 m. The annual average temperature is about 4.8°C, rainfall 522-615 mm, sunlight 2 866 hours, and frostfree period approximately 160 days.

A 10-year-old *F. velutina* plantation in Guangang Forest Park is located at 38°55'N, 117°31'E at an elevation of 3 m. The area of the plantation is 2.2 hm^2. The row spacing is 1.5 m and the distance between trees within a row is 1.0 m. The average DBH of ash is 7.64±3.39 cm, and average height 5.87 ± 2.15 m. The plantation has halaquept soil with medium thickness and 2 percent-4 percent salt content. In the Dagang area, the annual average temperature is 13°C rainfall 416mm, sunlight 3 000 hours, and frost-free period is 238 days. In the study site, the infestation of EAB was serious, and the infested rate was 90 percent, and ratio of harmed trees was 45.3 percent.

1.2. Biology

The surveys of EAB life stages were conducted from April 2004 to June 2005 in Tianjin, from April 2004 to June 2006 in Changchun, Jilin Province, and from April 2004 to June 2006 in Harbin, Heilongjiang Province. Five trees attacked by EAB were sampled every 5 days in order to observe the development of each larval instar and associated damage. Five trees attacked by EAB were randomly sampled daily in order to observe pupal development and record pupation. Both lab observations and field surveys were used to observe the daily change of pupae and eggs, emergence, mating, oviposition of adults, as well as their damage traits.

The number of each larval instar and pupae surveyed and the percentage of each larval instar and pupae were calculated. EAB adults were collected from infested ash in late April in 2004 that had been placed in a sleeve cage. The numbers of male and female EAB adults that emerged from the logs were recorded daily.

A total of 45 EAB adults were captured in late April over 4 days (10 -15 adults/day) and placed in a sleeve cage (100 × 100 × 150 cm) in Guangang Forest Park. The EAB adults were fed fresh *F. mandshurica* leaves and twigs. Three *F. mandshurica* boles (length: 150 cm, diameter: 10 cm) were placed in the cage to allow mating. The time of mating was recorded, and oviposition and death were investigated every day. Eggs deposited on bark were placed in glass tubes and the changes in their development and hatch time were recorded. Ten EAB were collected during early pupation and placed in the tubes (one EAB per tube) then placed in a box with no light). The eclosion time was recorded.

1.3. Survey of damage traits

Surveys were conducted in the 10-year-old *F. velutina* plantation in Guangang Forest Park in Tianjin in July 2004 and April 2005. Five plots consisting of 50 trees each were established with four at the edges and one in the center of the plantation. One EAB-infested tree was randomly sampled per row; a total of 10 EAB trees were randomly sampled and dissected on each plot. Both trunk and branches were investigated.

1.3.1. Vertical distribution of EAB larvae in the boles

The boles were dissected and the numbers of EAB larvae recorded based on four segments of the bole measured from above the soil line:, 0-50 cm, 51-100 cm, 101-150 cm, and 151 cm and over.

1.3.2. Relationship of the attacked position of boles and their orientation

The boles were dissected and the numbers of EAB larvae recorded based on four orientations (north, south, east, and west). This survey was conducted with the vertical distribution.

1.2.3. Horizontal distribution of EAB larvae in the plantation

This survey was conducted together with the vertical distribution in July 2004. In April 2005, 50 trees with 10 on each plot were sampled and examined again using the same sample method as 2004. In two years, a total of 100 trees with 20 on each plot were sampled and the numbers of larvae were recorded.

1.4. Spatial distribution

1.4.1. Spatial distribution survey

Five plots were established in the four edges (north, south, east, west and center) in the east-west *Fraxinus velutina* forest, and 20 trees per plot were dissected, and 30 trees were dissected in the center plot, to determine the number EAB larvae per bole.

1.4.2. Vertical distribution survey of EAB larvae in the boles

100 boles were surveyed randomly, to survey the height of boles harmed by larvae. We designed 50cm is one unit, and mark off 5 segment of the bole measured from above the soil line: 0-50cm, 50-100cm,101-150cm,151-200cm and above the 200cm..

1.4.3. To determine the spatial distribution

We adopted the index of aggregation to determine the spatial distribution of EAB larvae, the method is (Zhao, 2002):

S^2 ----variance of sample
$\bar{x}$ ----average of sample

① Dispersal coefficient C, $C= S^2/ \bar{x}$

C=1 randomly distribution: C>1 aggregated distribution: C<1 uniform distribution.

②The David and Moore(1954)'s index of aggregation I, $I= S^2/ \bar{x}$

I=0 randomly distribution; I>0 aggregated distribution; I<0 uniform distribution.

③ The index of Cassie, R.M. and Kuno(1968) C_A, $C_A = S^2 - \bar{x}/\bar{x}$

$C_A=0$ randomly distribution; $C_A>0$ aggregated distribution; $C_A<0$ uniform distribution

④ The dispersal index of MorisitaI_6, $I_6 = n \sum f x_i^2 - N/\bar{x}^2$

n is the number of sample, N is the total number of insect, the xi is larval population of i sample.

$I_6=1$ randomly distribution: $I_6>1$ aggregated distribution: $I_6<1$ uniform distribution

⑤The index of aggregation by Lioyd method, it is the ratio of average congestion and average density $L= X^*/\bar{x}$, In it $X^* = \bar{x} + S^{2/}\bar{x} - 1$

L=1 randomly distribution: L>1 aggregated distribution: L<1 uniform distribution

⑥ Negative binomial distribution. $K= \bar{x}/(S^2/\bar{x})^{-1}$

K is smaller, aggregation is bigger. When $K \rightarrow \infty$, it is Poisson distribution.

1.4.4. Probability distribution

Through survey the spatial distribution of EAB larval on the boles, use negative binomial distribution to test the probability distribution, and to estimate the spatial distribution of EAB group.

1.4.5. Analyses the aggregation

The group aggregation regression λ of Blackity (1961) was adopted to determine, $\lambda \equiv r\bar{x}/2k$, in it, r is the 2k freedom $x^2_{0.5}$, k is parameter of negative binomial distribution, X is average, when $\lambda \geq 2$, the reason of aggregation is result from the habit of insect or from two factor: the habit of insect and the environment condition. When$\lambda<2$, the reason of aggregation is result only from the environment condition. 43

1.4.6. Establish the vertical distribution imitate formula of EAB

Adopt logistic curve, S curve, quadric curve, cubic curve and exponential curve to establish the vertical distribution imitate formula, and make the significant test, to ensure the best imitate formula.

This research utilized SPSS12 to make the analysis.

RESULTS

2.1. Biology

Emerald ash borer was observed to have a 1-year life cycle in Tianjin (Table 1). Pupation of the overwintering larvae lasted for approximately 31.2 ± 1.6 days (n = 45) beginning in early April, and ending in mid-June. Adult emergence began in early May, peaked in mid- to late May, declined in early June, and ended in early July. Female adults began laying eggs in mid-May and ended in mid-July. In late May, the early-instar larvae were observed to feed in the cambial region, and this damage continued until early November. From late September to early November, the last-instar larvae bored into the xylem and built pupal cells (depth: 10.7 ± 1.6 mm, length: 18.5 ± 3.0 mm, width: 5.8 ± 1.0mm, n = 33). In the pupal cell the larva doubled over with its head away from the emergence hole to prepare for overwintering. Before pupation in the following year, overwintering larvae shorten, straighten and thereby the head ends up pointed towards the emergence hole.

Table 1. Univoltine life history of emerald ash borer (Tianjin, 2004-2005).

Jan - Mar			Apr.			May			June			July			Aug.			Sept.			Oct.			Nov.			Dec.		
E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L
			☼	☼	☼	☼	☼	☼	☼	☼																			
						●	●	●	●	●	●	●																	
							○	○	○	○	○	○	○																
								◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊					
■	■	■	■	■											■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

E: The first 10 days; M: The second 10 days; L: The third 10 days. ☼ Pupa, ● Adult, ○ egg, ◊ larva, ■ overwintering larva

Emerald ash borer was observed to be semivoltine in Harbin, Heilongjiang Province (Table 2) and the majority of EAB were semivoltine in Changchun, Jilin Province. Pupation of the overwintered larvae for the second year began in late April, and ended in late June. Eclosion of EAB adults began in late May and ended in early July. The egg stage occurred from early June to late July. In mid- June, early-instar larvae bored between the phloem and the xylem and this damage continued until late September when they started overwintering in the gallery. The feeding damage of the overwintering larvae in the first year began in mid-April. In mid-August, the larva entered a pupal cell (depth: 11.06±1.19 mm, length: 16.91±2.15 mm, width: 5.80 ± 0.96 mm, n = 35), curled up with its head away from the emergence hole to prepare for overwintering. In the following year, before pupation the second-time overwintering larvae straightened up and turned its head towards the emergence hole.

Table 2. Semivoltine life history of emerald ash borer (Harbin Heilongjiang province, 2004-2006)

Jan - Mar			Apr.			May			June			July			Aug.			Sept.			Oct.			Nov.			Dec.		
E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L	E	M	L
					☼	☼	☼	☼	☼	☼	☼																		
								●	●	●	●	●																	
									○	○	○	○	○	○															
										◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊
◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊	◊												
■	■	■	■	■	■	■									■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

E: ☼ Pupa, ● Adult, ○ egg, ◊ larva, ■ overwintering larva

Adults After eclosion the EAB adult stayed in the pupal cell for 5.43 ± 1.24 days (n = 37), and then bored a D-shaped emergence hole through the bark and exited the tree. Typically, the adults exit at 12:00 h on sunny and warm days. The newly emerged EAB adult climbs upwards on the bole and vibrates its wings continuously without feeding.

In general, an EAB adult crawls for about 3 m (n = 15) before its wings totally open and it is able to fly. EAB adults are active and have strong flight capability. They were observed to move around on the leaves and branches from 8:00 h to 18:00 h when it was warm and sunny. Adults sometimes feign death.

In 2005, we investigated the timing of adult emergence. In Tianjin (1-year life cycle area) peak eclosion appeared in mid-May, 80.6 percent of total adults emerged (Table 3). The eclosion period lasted 19 days. In Harbin (semivoltine area) peak eclosion appeared in early June (51.1 percent) (Table 3). Males eclosed earlier than females, while at peak and late emergence the number of female adults was higher than that of male adults. In Tianjin (univoltine area) the sex ratio of females to males was 1:1.57 (n = 216), and in Harbin (semivoltine area) the sex ratio was 1:1.81 (n = 45). After initial flight, adults began to feed on leaves. EAB adults begin feeding on the edge of the leaves. On each occasion, the adult eats up to 1 cm^2 foliage. After feeding for 5.4±1.2 days (n = 37) the adults start mating. Mating generally takes place on the branches and leaves and peaks from 14:00-15:00 h.

One female adult can lay 70.97±14.38 eggs (n = 32). In general, a female adult slowly climbs upward on the southwestern side of a stem, finds a suitable location in the bark crevices, curves its abdomen downward, extends its ovipositor and lays eggs. In the field, one female adult laid 1.64 ± 0.83 eggs (n = 53) individually at one location, while in the laboratory one female laid a maximum of 21 eggs at one location. Longevity of the male adults was 16. 75 ± 3.32 days (n = 28), and longevity of female adults was 18.75 ± 5.79 days (n = 17).

Table 3. Number of emerald ash borer adults emerging from univoltine and semivoltine life cycles.

Location	Tianjin† (May 2005)		Harbin‡ (May - June 2004)			
Time	first-10th	11th-20th	2first-3first	2first-3first May	first-10th June	11th-20th June
Adult after eclosion	3	174	39	13	23	9
Percentage	1.39	80.56	18.06	28.89	51.11	20.0

† Sampling site: Guangang Forestry, Dagang District, Tianjin.

‡ Sampling site: Harbin Experimental Forest, Harbin.

Eggs The eggs are flat and round, with a diameter of about 1 mm. The initial color was from milky white to maize, occasionally jade-green, and all colors turned to gray after 2 days. Developmental time of eggs was 9.03±1.05 days (n = 103). Eggs deposited in direct sunlight were observed not to hatch due to dehydration.

Larvae Early larvae were achromatous and transparent, and turned to milky white after feeding. A larva was observed to bore through the bark, feed first on the phloem, and then feed on the cambium. In the cambial region, EAB larva fed in a zigzag pattern both downward (42.72 percent, n =44) and upward (57.28 percent, n = 59). Larvae mainly fed and damaged the cambium layer in naturally infested trees. The damaged area was oval-shaped with an S-shaped gallery in vigorous trees; while the damaged area and galley were irregular in stressed trees. In the areas where EAB needs two years to complete one generation, dead larvae could be seen in severely damaged and dead trees with straight horizontal or vertical galleries. In most situations, late instar larvae turn in the other direction and bore into the xylem and build pupal cells that have an opposite direction to the galleries in the cambium layer.

In Tianjin, very few EAB were observed to overwinter as second and thirdinstar larvae in their galleries between the phloem and xylem. Next year overwintering larvae continued to feed on the phloem and xylem and built pupal cells in late July or early August for overwintering. These EAB need two years to complete their life cycle, which accounted for 5-7 percent of the total EAB population in Tianjin (univoltine area) (Table 4).

In Tianjin, EAB with a 1-year life cycle bored into the xylem and built pupal cells starting in late September, peaking in mid-October, and ending in early November. It took approximately 100 days from the time larvae were found in the phloem in early June until they started building pupal cells in late September. Late-hatched larvae were still in early development and stopped feeding when the tree stopped growing. These EAB need two years to complete one generation. This result indicated that EAB larvae have a slow development.

In Heilongjiang Province, although the majority of the EAB population had a semivoltine life history, a few EAB were univoltine accounting for 8 percent of the total EAB population in Harbin (Table 4). The univoltine EAB larvae hatched earliest and built pupal cells in mid- or late October; while semivoltine EAB larvae built pupal cells in mid-August of the second year of development. The frass in the gallery of univoltine EAB larvae was the same color and fresh; while the frass in the gallery of semivoltine EAB larvae had two distinctive colors, fresh (light brown) and old (dark brown). This phenomenon may be used to determine the type of life cycle of the larvae in the galleries.

In Changchun, Jilin Province, the majority of EAB were semivoltine, and the rest were univoltine. The adults were observed from May to July. During the surveys in April, 2004 to 2006, every larval instar was observed in the galleries. From August to October, the last-instar larva was observed to bore into the xylem and build pupal cells. These 2-year life cycled larvae overwintered in the galleries, then pupated and emerged. In Changchun, EAB were also observed to damage the small branches (<2 cm in diameter) of *F. mandshurica*. However, the larvae were smaller and they were unable to bore into the xylem and build pupal cells. Thus they were unable to complete their life cycle. No pupal cells or exit holes were found on these small branches.

Table 4. Overwintering larvae of emerald ash borer

Location	Time	Number of over-wintering larvae in galleries	Number of overwintering larvae in pupal cells	Number of pupal cells for univoltine	Number of pupal cells for semivoltine	Total	Percentage
Tianjin	April 2004	7	93	/	/	100	7
Tianjin	April 2005	5	95	/	/	100	5
Harbin	April 2006	/	/	2	23	25	8

Note: Parasitized larvae are not included.

Pupae EAB pupae are white exarate, 12.51±1.19 mm (n = 53) in length, and about 4 mm in width. Compound eyes began to change color 10 days after pupation. Mouthparts turned to black 15 days after pupation. Eclosion occurred 30 days after pupation (Table 5). Pupation was from early April to mid-June in Tianjin.

Table 5. Field and lab observation of emerald ash borer (EAB) pupation

Description	Collection	Eye turns color	Wing bud occurs	Black ordinate	Mouthpart turns black	EAB turns black	EAB turns green hard	Wing becomes	Eclosion
Date†	7th Apr.	17th Apr.	18th Apr.	19th Apr.	20th Apr.	25th Apr.	27th Apr.	30th Apr.	6th May

Description	Collection	Eye turns color	Wing bud occurs	Black ordinate	Mouthpart turns black	EAB turns black	EAB turns green hard	Wing becomes	Eclosion
Date‡	9th Apr.	17th-18th Apr.	18th-20th Apr.	19th-27th Apr.	20th-2thirdApr.	25th-30th Apr.	28th Apr.-6th May	30th Apr.-8th May	6th-15th May

†Field survery of EAB pupation in Tianjin. Five pupae were collected and observed daily. Date the changes began.

‡Lab observation of EAB pupation in Tianjin. Observed daily and the date recorded from the changes began to all adults emerged. The number of pupae observed is 30.

2.2. Damage traits

Vertical distribution of larvae in boles EAB larvae mainly damaged the 51-150 cm region of the boles in which the number of larvae accounted for 76.73 percent of the total larvae (0-50 cm: 9.48 percent; 151 cm and above: 13.79 percent, n=116).

Relationship of the attacked positions and the degree of smoothness of bark Smooth bark at the attacked position accounted for 12.74 percent of the total bark, while coarse bark accounted for 87.26 percent (n = 102).

Relationship of attacked positions of boles and their orientation The attacked positions on the south side of the boles accounted for 43.40 percent of total attacked positions, while the attacked positions on the other three sides (north:21.70 percent, east: 16.98 percent, and west: 17.92 percent, n = 106) accounted for about 20 percent each.

Horizontal distribution of EAB in the plantation EAB density in the southern and western sampling plots in the plantation accounted for 51.82 percent (n = 301) of the total population surveyed, while the central sampling plot accounted for 13.62 percent (n = 301) of the total population. EAB caused more severe damage to the ash at the edges of the plantation than in the center.

2.3. Spatial distribution

2.3.1. Determine the index of aggregation

We adopted six indices to determine the spatial distribution of EAB, the result is in table 6, the result of six indices all indicate that the spatial distribution of EAB in boles is aggregated distribution.

Table 6 EAB's index of aggregation

Sample	Average	Variance	Dispersal Index	Cassie Kuno	David Moore	Morisita	Lioyd	Negative binomial	Blackity
N	/X	S^2	C	C_A	I	$I_б$	M	K	λ
110	3.00	355.909	115.181	36.952	114.181	37.226	37.952	0.027	7.286
			C >1	**C_A>0**	**I >0**	**$I_б$ >I**	**M >1**	**K >0**	**λ >2**

2.3.2. Test the probability distribution

For the aggregation distribution, if the ratio of variance and average is about 1, then it is about Poisson distribution; if $S^2 < \bar{X}$, then the sample comes from binomial distribution; if $S^2 > \bar{X}$, then the sample comes from negative binomial distribution (Bliss 1953)[9]. Through analysis the data of EAB's distribution, S2 = 355.909 >$\bar{X}$ = 3.09, this indicate that these data come from negative binomial distrtibution. To test the negative binomial distribution, the real data is x^2 = 25.613<x^2 0.05 = 82.53, the test results prove that the distribution of EAB is negative binomial distribution.

2.3.2. Analysis of aggregation

We adopted group aggregation regression λ of Blackity(1961) to determine, $\lambda = r\bar{x}/2k$=7.8022, when λ ≥2, the reason of aggregation is result from the habit of insect or from two factor: the habit of insect and the environment condition. The result of survey indicate that all the trees grow bad, more bad of the tree grow, more serious harmed by insect. In contrast, the health or grow good trees rarely harmed by EAB. EAB have some topotaxis for a weakened tree, so we can ensure that the reason of aggregation is result from two factors: the habit of insect and the difference of trees.

2.3.4. Imitate the vertical distribution curve formula of EAB larvae on trunk

Through numerous surveys, we analyzed the relationship between the number of EAB larvae and the height of the bole. The results indicate that larvae primarily distribute between 50 to 150 cm of the boles. We adopted respectively logistic curve, S curve, quadric curve, cubic curve and exponential curve to imitate. The result in figure 3, from figure 3 we can see, through test the significant of imitate curve, the best is cubic curve, the formula is $y = -5.1429 + 17.9437X + 21.6623X^2 - 14.545X^3$. In the 95 percent confidence interval, its relative coefficient $R^2 = 0.915$, the significance F is 0.013<0.05, so this formula is credible (Table 7).

Table 7. Imitate formula and its significant test.

Curve	Formula	R^2	P
Exponential	$Y = 5.6041e^{0.4182x}$	0.066	0.593
Logistic	Y = 13.0756 + 5.3778 1n X	0.167	0.315
S	$Y = 1/(2.916 - 0.5313e^{-x}$	0.392	0.097
Quadratic	$Y = -16.393 + 64.7619X - 27.429X^2$	0.864	0.007
*Cubic**	$Y = -5.1429 + 17.9437X + 21.6623X^2 - 14.545X^2$	*0.915*	*0.013*

DISCUSSION

Life histories of EAB in Changchun, Jilin Province include semivoltine and univoltine, and some in between. This area is the transitional zone of EAB life history from semivoltine to univoltine. This is the first report of EAB life history in this transitional area. This research confirmed the previous research that EAB requires 2 years to complete one generation in Harbin, Heilongjiang Province (Yu, 1992), and 1 year in Tianjin and Shenyang, Liaoning Province (Liu, 1966; Liu *et al.*, 1996; Gao *et al.*, 2004; Sun & Li, 2004; Wei *et al.*, 2004; Zhao *et al.*, 2004; Zhao *et al.*, 2005). In North America, EAB generally has a 1-year life cycle in southern Michigan but could require 2 years to complete a generation in colder regions (Mastro *et al.*, 2007).

In branches < 2 cm in diameter and EAB are small and unable to enter the xylem, while they are successful on larger branches. One possible explanation for this phenomenon is that the small branches are not able to provide sufficient nutrition for the development of EAB. An alternative explanation is that the branches are physically too thin to build pupal cells. In Tianjin, EAB does not damage trunk and branches smaller than 3 cm in diameter (*e.g.*, Yang, ZQ, 2004, pers. com.). The difference could be due to different tree species or climatic factors, and needs further investigation.

Emerald ash borer prefers abundant sunlight for mating and oviposition. The 51-150 cm region of the boles has more bark crevices compared to the regions of 150 cm and above, which benefits oviposition, egg protection, and is suitable for feeding by early instar larvae. Smooth bark was less likely to be selected by females for oviposition, as it is not suitable for egg protection and hatching. Thus breeding ash with smooth bark can be a method of resistance by reducing the number of EAB host trees.

The survey in Tianjin shows that the developmental rate of EAB larvae is slow compared to that in previous results, and it takes about 100 days from larvae boring into the xylem to complete the pupal cell. However, previous results indicate that in this stage larvae develop rapidly (45 days) (Liu *et al.*, 1996; Zhao *et al.*, 2005). The results in this study suggest that the larvae with rapid development are 5 percent-7 percent of the total EAB population, which need 2 years to complete their life cycle. The frost-free period in certain areas may be used to explain the biological characteristics of EAB (*e.g.*, longevity, life cycle) in the same area. It takes at least 150 days for EAB to complete one generation. In Harbin the frost-free period is 120-130 days which is less than the 150-day requirement, so EAB is semivoltine in Harbin. However, in Changchun, the frost-free period is 160 days, so EAB produces a mixed population of semivoltine and univoltine. In Shenyang, the frost-free period is 183 days, and likewise, in Tianjin, the frost-free period is 210 days, so EAB produces univoltine populations both in Shenyang and in Tianjin. All Buprestidae overwinter in the larval stage. They basically have a 1-year life cycle and no two-generation per year life cycle; in cold northern areas, Buprestidae needs two or three years to complete one generation (Yu, 1992). The results in this paper support the previous research and indicate a slow development in EAB larval stage.

Six methods were adopted to determine the spatial distribution of EAB, the results indicate that it is aggregation distribution, its basic element is individual group, its rule of dispersal is aggregation dispersal, this is same to the observe result in field. The result of aggregation reason analysis indicates that the main reasons to form this spatial distribution are its flight ability and its oviposition habit. EAB has a strong flight ability, and mostly of the eggs are deposited in cracks in the bark or under the bark, although most eggs are single, but they are relatively concentrate, the adult female can deposit eggs on another tree, they have a topotaxis of weakened tree, this characters must lead to aggregation distribution of EAB larval.

Through significant test of vertical distribution imitate curve, the best imitate curve is cubic curve, the formula is $y = -5.1429 + 17.9437X + 21.6623X^2 - 14.545X^3$, and in the 95 percent confidence interval, its relative coefficient $R^2 = 0.915$, F is $0.013<0.05$, so this formula is credible.

The early research about EAB indicates that the age of the most seriously harmed trees is concentrated on ten year old trees (Liu, 1966; Xiao, 1992). The object of the research in this paper is EAB larvae which are 1-year life cycle, and aim at research its spatial and vertical distribution in a ten year forest. To be the important content of group ecology, confirm the spatial distribution pattern and vertical distribution curve it is convenient to find out the distribution rule of EAB (1-year life cycle) larval in forest, and then we can enhance the control efficiency. At same time we also strengthen the research of EAB 2-year life cycle's, spatial distribution pattern and vertical distribution rule, this has more significance in practice.

Some Advances in Research on Asian Longhorned Beetle (*Anoplophora glabripennis*) in P. R. China

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ABSTRACT

1. Extended distribution area of Asian Longhorned Beetle (ALB), especially in Xinjiang

It was realized about ten years ago that ALB (*Anoplophora glabripennis*) occurs in most provinces of China except Tibet, Qinghai, Xinjiang, Hainan and Taiwan. However, according to an authoritative paper of Prof. Wu published in 1998, ALB is not so widely distributed as reported previously.

The first establishment of ALB was found in Xinjiang in 2002. The common host-tree species are *Acer negundo*, *Salix* spp., *Populus nigra* var. *thevestina*, *Populus alba* var. *pyramidalis*, *Populus euphratica*, *Elaeagnus angustifolia*, etc. Experiment results show that ALB has one generation each year in Xinjiang.

2.0 ALB invaded natural/secondary forest in China

Ten years ago in China, ALB was only a typical pest of urban forests and other artificial plantations consisting of poplar and willow. Reportedly, ALB had been found in the natural/secondary forest of Daqing Mountain, Inner Mongolia seven years ago, but there is no direct evidence, so we made a special survey/investigation of ALB invasion in natural/secondary forest in China in recent years. The first direct discovery of ALB invasion in the natural/secondary forest is very important for protection of a large area of the natural/secondary forest in China.

2.1 ALB is a very important threat to the natural forest of *Populus euphratica* in Xinjiang

ALB can successfully complete its life cycle in *P. euphratica*. Natural *P. euphratica* forests along the Talimu River in Xinjiang are under high risk of ALB infestation. Resistance sequence (from resistant to sensitive) to ALB among main tree species in artificial plantations in Xinjiang are *Morus alba*, *Elaeagnus angustifolia, P. euphratica*, *Ulmus densa*, *P. alba* var. *pyramidalis*, *Ulmus pumila*, *P. nigra* var. *thevestina, Acer negundo*, *Salix matsudana*, and *Salix matsudana* f. *umbraculifera.*

Generally, the bark thickness of mature *P. euphratica* is about 3–5cm and the thickest 8–10cm. According to our experiment, it can been confirmed that ALB can invade where the bark thickness is less than 7 mm.

2.2 ALB invaded natural secondary forest about 10 years ago in Xinlongshan, Gansu, mainly damage *Betula platyphylla* and *Salix* spp.

Natural suppression of ALB by woodpeckers in birch is very effective, with a pecked rate of ALB reached 91 percent.

3. Do ALB adults have " Memory Effects" in selecting host tree species?

ALB have a very broad range of host tree species belonging to different degrees in resistance and many families in taxonomy in China. Whether ALB adults emerged from different resistant levels of tree species have the "memory effects" in recognizing and selecting original/larval host trees?

3.1 "Memory effects" mensurated in the lab.

Beetles for the experiment were newly emerged ALB adult (1–3d old) collected from the following tree species : *Populus nigra* var. *thevestina* (PN), *P. alba* var. *pyramidalis* (PA), *P. simonii* × *P. pyramidalis* cv 'pera 8277' (PS), *Salix matsudana* f. l*obato-glandulosa* (SM).

According to the results of taxis response of ALB adults to volatiles emanating from their larval host twigs, it was concluded that with clear air as control, ALB adults emerging from four host tree species (PN, PA, PS and SM) showed respectively significant taxis response to volatiles emanating from the twigs of its natal host tree species. With the ashleaf maples as the control, ALB adults emerging from four other host tree species (PN, PA, PS and SM) did not obviously respond to volatiles emanating from the twigs of its natal host tree species respectively.

3.2 "Memory effects" mensurated in the field

The five tree species selected for the experiment were *Acer negundo* (AN), PN, PA, PS and SM mentioned above. Beetle sources are the same as the taxis response experiment above.

Results show that inhabiting, feeding, mating and ovipositing of emergent ALB adults from five species (PN, PA, PS, AN and SM) planted in cages are different respectively from the five tree species above. In the field, as to inhabiting, feeding, mating and ovipositing, the beetles emerging from five different tree species showed a significant preference to the asheaf maple.

3.3 Comparsion of cellulase activities of ALB adults emerged from 4 host-tree species.

Results follow: The sequence of 3 kinds of cellulase activities of ALB adults emerging from four host-tree species(PN, PA, PS and SM) are the same: Cx-cellulase > ß-glycosidase> C_1-cellulase. There is no distinct difference in Cx-cellulase activity in both male and female beetles emerging from four tree species, but the Cx-cellulase activity in males is evidently higher than females when they emerged from same host-tree species. No distinct difference in C_1-cellulase or ß-glycosidase activity among beetles (either in male or female) emerging from different host-tree species, even from the same host-tree species.

3.4 Comparison of Cx-cellulase Isozymes of ALB Adults Come from Different Hosts

The main results show no obvious difference in strip type and transferred distance of Cx-cellulase isozymes in male or female beetles emerged from four host-tree species (PN, PA, PS and SM).

Hemlock Woolly Adelgid Population Distribution: Role of Environmental Factors in Relation to the Study of Its Natural Enemies in Yunnan Province

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ABSTRACT

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae), is native to China but is not considered a pest there. Although innocuous, it is widespread on the three species of hemlock which grow in Yunnan (*Tsuga dumosa*, *T. forrestii*, and *T. chinensis*). As part of an investigation of natural enemies of HWA in China for the potential biological control agents of HWA in eastern North America, we examined the level of infestation of HWA within the crown of the hemlock tree, between different sites, and during different seasons.

HWA was surveyed from August 2005 to December 2006 in three sites in Lijiang Prefecture, Yunnan Province. Two sites were north of the city of Lijiang on the northeastern slope of Yulong Snow Mountain. Although these sites were separated by only 5 km, one of the sites had *T. dumosa* while the other site, which was drier, had *T. forrestii*. The third site was southwest of Lijiang at the southern end of Laojun Mountain; it had *T. dumosa* and was the wettest site. At each site, 10 trees at least five meter tall were selected. Each tree was sampled six times (August, November, March, April, May, and December). On each sample date, a total of eight branches were cut from each cardinal direction (north, east, south, west) of the lower crown (<2.5 meters) and the upper crown (4-6 meters). A total of 50 terminal shoots were examined on each branch with the aid of a hand magnifier and the presence or absence of HWA was recorded (= percent twigs infested).

There were more twigs infested with HWA (32 percent) on the north side of the crown than on the west (18 percent) and east sides (21 percent). The south side had 28 percent twigs infested, which was not significantly different than the north side of the crown. Seasonally, the highest percentage of twigs infested was in May, which coincided with the onset of the new HWA generation. Overall, HWA infestation was more severe in the lower crown (26 percent) than higher in the crown (23 percent), but in the May samples the percentage of twigs infested in the lower canopy was higher than in the upper canopy (33.6 percent vs. 40.1 percent, respectively). There were dramatic differences in infestation rate between the three sites— the driest site had the lowest infestation rate about 10 percent, and was about the same at each sampling date. The percentage of twigs infested at the other two sites was about the same at each sampling date except for May, when the wettest site had 60 percent of the

twigs infested and the intermediate site had only 40 percent twigs infested with HWA. A correlation analysis across the three sites indicated that the slope orientation, average annual relative humidity, and average annual rainfall were positively correlated with the infestation rate of HWA, while temperature, altitude, slope degree, canopy closure, and tree height were not significant statistically. Since the goal of our research on HWA in China is to document the most effective natural enemies, sampling the lower canopy would sample the area of the crown where HWA is denser and undergoes the biggest numerical change seasonally. This larger change in HWA density may reflect that natural enemies are also more abundant in the lower crown. Thus, it is both practical and efficacious to sample natural enemies only from the lower crown.

Keywords: *Adelges tsugae*, *Tsuga dumosa*, *Tsuga forrestii*, infestation rate, environment, sampling

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Studies on *Dastarcus helophoroides* (Fairmaire), A Natural Enemy of Longhorned Beetles

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ABSTRACT

Dastarcus helophoroides (Fairmaire) (Coleoptera: Bothrideridae) is an important natural enemy of longhorned beetles (Coleoptera: Cerambycidae), such as *Anoplophora glabripennis* Motschulsky, *Monochamus alternatus* Hope, *Massicus raddei* (Blessig), and other beetles in the family Cerambycidae. Chemical cues involved in host location by *D. helophoroides* were investigated in a Y-tube olfactometer. Responses of *D. helophoroides* adults toward different odor sources from their host larvae (*M. raddei*) and host tree (*Quercus mongolicus* Fisch. ex. Turcz.) were tested in both single and dual choice tests. Wood with host larval tunnels (WLT) and frass of host larvae (FL) were found to be major sources of attractive volatiles from the plant-host complex. Volatile chemicals emitted from WLT and FL were collected on Porapak Q and analyzed by gas chromatography - mass spectrometry (GC-MS). Several volatile compounds identified from WLT and FL, including ß-pinene, Δ-pinene, Δ-3-carene, limonene and ß-myrcene, were hypothesized to be behaviorally active components. Responses of *D. helophoroides* toward these compounds were tested in the same Y-tube olfactometer. The result showed that *D. helophoroides* adults were attracted to (R)-(+)-limonene, with or without the background odor from uninfested fresh wood. It suggests that (R)-(+)-limonene is an important kairomone for this parasitic beetle.

Based on these studies, we investigated whether there were different biotypes among *D. helophoroides* populations collected from different host beetles in different geographic locations. Results showed that three different *D. helophoroides* populations displayed different olfactory responses to larval frass from different longhorned beetle species. The first laboratory colony of *D. helophoroides* parasitized larvae and pupae of *A. glabripennis* in the trunk of *Salix babylonica* Linn., which were collected in 2002 in Xi'an City (Latitude: 34°15', longitude: 108°50' and the altitude: 450 m), Shaanxi Province, China. The second colony parasitized larvae and pupae of *M. alternatus* in the trunk or branches of *Pinus Massoniana* Lamb., which were collected in 2003 in Guangzhou City (Latitude: 23°80', longitude: 113°17' and the altitude: 120 m), Guangdong Province, China. The third colony originated from *D. helophoroides* which parasitized larvae and pupae of *M. raddei* in the trunk of *Quercus mongolicus*, which were collected in 2002 in Meihekou City (Latitude: 42°10', longitude: 125°30' and the altitude:

500 m), Jilin Province, China. There were no differences in morphological characteristics between the three populations tested. Nevertheless, all populations were significantly attracted to the larval frass of their original host beetles. Parasitism rates of different *D. helophoroides* populations also varied when supplied with host larvae of the same longhorned beetle species. These results indicate that three tested *D. helophoroides* populations were different at host-related behavioral levels. Therefore, the population of *D. helophoroides* from different geographical locations must be taken into consideration when implementing biological control programs for different species of longhorned beetle.

Keywords: natural enemies, host seeking, tritrophic interaction, wood borer, population differences, *Massicus raddei*, *Anoplophora glabripennis*, *Monochamus alternatus*, Y-tube olfactometer, *Quercus mongolicus*, olfactory response

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Analysis of the Biological Control Program for *Oracella acuta* in the P. R. China

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ABSTRACT

A cooperative program for the biological control of the mealybug *Oracella acuta* in China was conducted from 1995-2004. Parasitized females were collected from the native range of the mealybug in the southeastern United States and shipped to China. The parasitoids were used for mass-rearing and in releases. A summary of project results will be presented and discussed.

The Benefits and Costs of a Quarantine to Prevent the Spread of *Tomicus piniperda* in the Eastern United States

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This paper looks at the biology of *Tomicus piniperda,* an invasive forest insect that has been introduced into the Lake States and the potential for this insect to spread to the southern pine states. A quarantine has been in place for the past 12 years preventing the importation of nursey stock and Christmas trees.

The Brown-marmorated Stink Bug, *Halyomorpha halys* (Heteroptera: Pentatomidae) in P. R. China

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ABSTRACT

The brown-marmorated stink bug, *Halyomorpha halys* (Stål), is native to East Asia and was recently found to be established in North America. The present paper gives a review of research done on *H. halys* in China, which also includes some of our findings. Presently about 45 plants are found to be associated with *H. halys* in China. Its damage to orchards has increased as less chemical insecticides are used in some ecological apple orchards. It produces one or two generations a year, and a partial second generation appeared in North China. Bagging apples one week earlier than usual and capture of the adult bugs in the fall and early spring could provide sufficient control in apple orchards. Other control methods are also discussed.

Keywords: *Halyomorpha halys,* host plant, apple orchard, control strategy

The brown-marmorated stink bug, *Halyomorpha halys* (Stål), is native to East Asia, distributed in China (excluding Xinjiang and Qinghai), Japan, Korea, Vietnam, and Myanmar (Li, 1985; Wang and Liu, 2005). It was reported from Allentown, Pennsylvania, in the United States in early October, 2001 (Hoebeke and Carter, 2003). It appears to have entered a phase of rapid expansion, and will rapidly spread not only from the original infestation but also from more isolated populations. The stink bug overwinters as an adult, and will often enter homes and other buildings in the fall when seeking sheltered sites. It was this behavior that caused residents or visitors to complain about the invader. However, it is also a polyphagous pest. Host plants include, but are not limited to, various fruit trees, vegetables, and row crops, including soybeans. This paper gives a review of research done on *H. halys* in China, including some of our findings.

HOST PLANTS IN CHINA

H. halys is a polyphagous plant feeder. There is no complete list of host plant species for it in China. We try to make a list from our observations and the literature. Some of the Chinese authors used *Halyomorpha picus* in their publications and in fact it referred to *H. halys*.
A total of 31 plant species have been listed in the literature: *Phaseolus* spp., *Camellia sinensis*, *Glycine max*, *Syringa* spp., *Citrus reticulate*, *Lycium barbarum*, *Malus* spp., *Pyrus* spp., *Prunus domestica*, *Triticum aestivum*, *Gossypium hirsutum*, *Paulownia* spp., *Malus pumila*, *Vitis vinifera*, *Crataegus pinnatifida*, *Punica granatum*, *Diospyros kaki*, *Prunus* spp., *Beta vulgaris*, *Ficus carica*, *Firmiana*

platanifolia, *Prunus armenica*, *Nicotiana alata*, *Robinia pseudoacacia*, *Prunus pseudocerasus*, *Brassia* spp., *Camellia oleifera*, *Ulmus pumila*, *Cinnamomum camphora*, *Populus tomentoo*, and *Ziziphus jujube*. In addition, we observed its nymph or adult on another 14 plant species: *Wisteria sinensis*, *Althaea rosea*, *Solanum nigrum*, *Dendranthema morifolium*, *Helianthus annuus*, *Humulus scandens*, *Zea mays*, *Euonymus japonicus*, *Vitex negundo*, *Sophora japonica* f. *pendula*, *Rhus typhina*, *Platycladus orientalis*, *Cayratia japonica*, and *Artemisia argyi*. To date a total of 45 plants were found to be associated with *H. halys* (Hsiao, 1977; Shen, 1993; Zhang *et al.*, 1993; Song & Wang, 1993; Yang & Cao, 2002; Cai, 2003; Zheng & Chen, 2002).

The bug likes to feed on twigs of peach and pear, but apple and walnut. However, it likes to feed on the young fruit of peach, pear and apple. It occasionally preys on caterpillars in the orchard (Li *et al.*, 1996).

DAMAGE TO ORCHARDS AND OTHERS

Early season feeding of the stinkbug result in dimples or depressed areas on mature fruits, which lower their market value. The damage to peach was from 15.36 – 64.37 percent and to pear was 18.23 percent – 38.9 percent (Chu *et al.*, 1997). On average, each nymph and adult can damage 11.5 and 8.67 dates (common jujube) a day respectively (Song and Wang, 1993). Our survey in an ecological apple orchard indicated that its damage to apple was 25.7 percent in 2006 (Zhang *et al.*, 2007).

There is no definite record of its damage to ornamental trees. However, some articles reported it as a vector of *Paulownia* witch's broom, a phytoplasma disease of this tree (Sun *et al.*, 1999). It has been reported that the rate of the pathogen transmission to *Paulownia* seedlings by third and fourth instar nymphs of reared pathogen acquisition was 61.7 percent and 41.4 percent, which were much higher than that by nymphs of natural pathogen acquisition (16 percent). For adults, the transmission rate was 14.1 percent, higher than that of natural pathogen acquisition (4.5 percent).

H. halys overwinter as adults that exhibit behavior similar to Asian ladybird beetle, *Harmonia axyridis*, and can congregate on houses in late fall and eventually move indoors. Once inside they can become a nuisance and emit an offensive odor if crushed. In the fall, the adults stopped on visitors in Fragrant Hill Park, west of Beijing, and many of the bugs entered hotel rooms.

LIFE HISTORY

H. halys was reported to have one or two generations a year in North China (Feng, 1990; Qin, 1990) and later studies found it to occur one to two generations a year (Li *et al.*, 1996), or a partial second generation. Both first and second generation's adults will overwinter. Usually the bugs begin to enter houses in late August peaking in mid-October. At the end of October, the bugs all come to the overwintering location. However, a few bugs were observed in late November, 2007. If the eggs were laid later than early June, the new generation of bugs will not lay eggs. The adults can disperse up to 2 km in distance (Zhang *et al.*, 1993). Female bugs can mate a maximum of five times. Female can lay at most five egg masses with a total of 109 eggs. The egg mass is in the form of triangle with 28 (56.25 percent), 27 (31.25 percent), 26 or 14 eggs. Eggs lasted about six days. First instar nymphs stayed around the egg mass without feeding for 5.86 days, and second to fifth instar lasted 12.69, 9.76, 10.39 or 10.46 days respectively (Chu *et al.*, 1997). Based on the observation of 30 females, parthenogenesis occurs in 53 percent females. Their egg hatch rate is 12.47 percent (Chu *et al.*, 1997).

NATURAL ENEMIES AND CONTROL METHODS

From the literature, natural enemies include: *Trissolcus flavipes* Thomon with an egg parasitism rate of up to 63.3 percent (found in pear orchard) (Chu *et al.*, 1997) and *Telenomus mitsukurii* (Ashmead) with an egg parasitsm rate up to 84.7 percent (found in pear orchard) (Zhang *et al.*, 1993). Yang reported four egg parasitoids were recorded from Beijing: *Trissolcus halyomorphae* Yang, *Anastatus* sp., *Acroclosoides* sp. and *Telenomus* sp. (Yang, Z.Q., Nov. 2006, pers. comm.). We found *Telenomus* sp. and *Anastatus* sp. in an apple orchard. The latter one is easy to mass rear by using eggs of the Chinese Oak Silkmoth (*Antheraea pernyi*) and it is also easy to store. Li and Li (2004) studied the effect of temperature on the development of *T. halyomorphae*.

We used the following methods to control the damage to apple caused by *H. halys*: 1) to bag apples one week earlier than normal time; 2) to capture the adult bugs in fall and early spring; and 3) to protect orchard biodiversity and enhance natural control by using parasitoids. We collected a large number of adult bugs in the fall in 2006 and the early spring in 2007. There was much lower damage to apple in 2007 than in 2006 in our experimental orchard. It was hard to find the bugs, though some damaged apples could still be found in the orchard. We are satisfied with this result.

DISCUSSION

H. halys may become a serious pest in the ecological orchards in which reduced or no insecticides are used. The orchard we surveyed was in the second year without application of chemical insecticides and a lot of bugs entered the orchard, causing economic damage to apples. Reduction of the number of the bugs entering the apple orchards is an important strategy for control. The stink bug prefers some plants, including *Paulownia* spp. and *Ziziphus jujube*. It is likely to be practical to take some treatments on those plants.

Overwintering aggregation of *H. halys* provides an opportunity to reduce its population density. We collected *H. halys* in fall or early spring. The overwintering aggregation of *H. halys* is guided by a chemical or tactile interaction among individuals, at least within a narrow range (Masatoshi *et al.*, 2006). This suggests that a certain pheromone may exist in *H. halys*. Aldrich *et al.* (2007) attracted the stink bug with methyl 2,4,6-decatrienoates. This kind of chemical may be used to control *H. halys* population.

Natural enemies could provide another opportunity to reduce the population of *H. halys*, particularly for the surrounding environments. Some parasitoids are easy to mass rearing. However, the parasite rate after release needs to be investigated.

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Studies and Utilization of Insect Natural Enemies of Longhorn Beetle, *Massicus radei* (Blessig)

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ABSTRACT

Massicus radei (Bleesig) is a pest causing serious injury to *Quercus* spp. In order to control this longhorn beetle, studies and utilization of insect natural enemies have been undertaken to study biological characteristics, and mass rearing and releasing techniques. The results are as follows:

1) The primary natural enemies are the ecological type of *Dastarcus helophoroides* (Fairmaire) which host is *M. radei*, *Zaommoencyrtus raddeii* Yang and *Euurobracon* sp.

2) The biology characteristics of the ecological type of *Dastarcus helophoroides* was well-studied.

3) It is nearly impossible to distinguish live male and female adults of *D. helophoroides* by external morphological characteristics. It has caused great difficulty in mass rearing and releasing for biological control pairings. To solve this problem, analyses of some main external morphological characteristics that probably have different features, such as the end angle of anal plate (as well as length and width of the anal plate), the length and width of wing and its trigonal end zone, have been carried out, according to the careful observation for the morphological characteristics of many male and female adults. Results show that making use of the above characteristics can simply distinguish live male and female adults without injuring them. It can be used in utilizing the natural enemy to control longhorn beetles.

4. The technology of mass rearing and releasing of *D. helophoroides* has been well-studied.

Keywords: *Dastarcus helophoroides*, biology characteristics, sexualization, mass rearing and releasing

The Role of Parasitic Wasps in Biological Control of Exotic Harmful Scale Insects

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ABSTRACT

The occurrence, injury, parasitoids and biological control of four exotic harmful scale insects, *Matsucoccus matsumurae*, *Oracella acuta, Hemiberlesia pitysophila,* and *Pseudaulacaspis pentagona*.

Keywords: invasive pests, scale insects, parasitic wasps, biological control, natural enemies

Scale insects are important tree pests, and as covered with scale or wax, they are difficult to kill with insecticide. Parasitic wasps (parasitoids) have been used successfully to control scale insects, and are very important in natural pest control.

As international trade increases, especially in the last 20 years, many exotic organisms have been introduced into Chines ecosystems, resulting in huge disasters. This leads to more harmful exotic organisms invading into Chinese local ecosystem, and resulting in huge disasters. There are at least 4 species of harmful exotic forest scale insects in China, *Matsucoccus matsumurae* Kuwana, *Oracella acuta* (Lobdell) Ferris, *Hemiberlesia pitysophila* Takagi, and *Pseudaulacaspis pentagona* (Tagioni-Tozzetti) (Li, J. *et al.* 2005).

1. Exotic scale insect damage

1.1 *Matsucoccus matsumurae* originally found in Japan and in China, it was first found in Lushuenkou, Liaonin, China in 1942 and also found in Lao Shan, Shandong in 1950 (Li, J. *et al.*, 2005). The scale insects were found later in Jilin, Jiangsu, Zhejiang, Shanghai, Anhui provinces, causing damage on *Pinus massoniana*, *P. thunbergii*, *P. densiflora*, *P. tabulaeformis*, *P. densiflora* cv.*umbraculifera*, *P. densiflora* var. *pendula*, *P. taiwanensis, P. massoniana* ×*P. thunbergii,* and *P. luchuensis*. *M. matsumurae* is the most serious pest on *Pinus* branches injuring both mature and trees less than 4 years of age. Serious damage occurred on 3-15 years old Pinus trees. The second-instar nymphs of the scale insects causes injury on the shaded side of the branches, causing warping sagging and defoliation. Branches wilt and bark becomes thick and hard. Secondary pests are attracted and invade the tree, which eventually dies.

1.2 *Oracella acuta* were originally found in southeast America. It spreads rapidly. After invading Guangdong in 1988, it caused serious damage. Today it mainly occurs in Guangdong and Guangxi. It's hosts are *Pinus taeda, P. elliottii, P. echinata, P. palustris, P. virginiana*, *P. clausa* var. *immuginata, P. thunbergii, P. caribaea and P. massoniana.* Overwintering nymphs of *Oracella acuta* suck juices from the bases of old needles causing massive loss of sap. Needle turn brown and drop off. In serious infestations, 70 percent to 80 percent of needles will drop off. From spring to summer season, large quantity of females and nymphs feed on young shoots, excrete wax covering branches, causing short branches and short leaves. In serious cases, young shoots become curved and dead, fruit are small, affecting the quality and quantity of the seeds.

1.3 *Hemiberlesia pitysophila* was collected in 1965, it was then found in Sakishima Islands, Okinawa Islands Japan, and Taiwan of China, and suggested as new species in 1969. It was found in Zhuhai near Macco of the Chinese continent in 1982, after investigation; it was found to occur in Macco and Hongkong for several years and caused serious damage. The hosts of it are *Pinus massoniana, P. thunbergii, P. elliottii, Pinus taeda, P. caribaea, P. luchuensis, P. latteri*. The female insects mainly suck plant juices in the bases of old needles, new growth including young shoots, fruits, young needles; while the male scale insects live on the upper surface of the needles. The damaged needles become dark and decay, upper leaves become yellowish, then turn brown and drop off, serious damage that can cause the whole tree to die.

1.4 *Pseudaulacaspis pentagona* was originally found in Russia, It occurs in Liaoning, Inner Mongolia, Hebei, Henan, Shanxi. There are more than 100 host species, including *Morus alba, Prunus persica, P. salicina, P. armeniaca, P. mume, P. pseudocerasus, Pyrus* spp., *Malus pumila, Vitis vinifera, Diospyros kaki, Juglans regia. M. alba, P. persica* suffered the most serious damage. The female adults and nymphs live on host's branches. In severe infestations the scale insects completely cover the branches as well as fruits. Injured plants show weak growth, delayed development, and death.

2. The utilization of local and exotic natural enemies

2.1 The natural enemy of *Matsucoccus matsumurae*

M. matsumurae occurs in 7 provinces. there were some research associated with its natural enemies in these areas. The scale insects have been heavily injured in Zhejiang province since the1970s. Much research has been focused on the scale insects. Thirteen families and 31 species of natural enemies on *M. matsumurae* have been found, but all of them are predators. Parasitoids have not been found (Cai, X.M., 1999, also see table 1). While parasitoids are the most important natural enemies, their role cannot be compared to the predators.

2.2 The natural enemies of *Hemiberlesia pitysophila*

There is some research on parasitoids of *H. pitysophila*, but the species of the parasitoids was often misidentified as those parasitoids are very small, usually about 1 mm. Wang, Z.H.(2005) discussed 3 parasitoids of *H. pitysophila*, *Encarsia amicula, E. citrina,* and *Coccobius azumaia.* According Wang's analysis of the parasitism efficiency, *C. azumai*, introduced from Okinawa, Japan has better control effects on the *H. pitysophila* population in forests than the other two local parasitoids, since *C. azumai* can directly attack *H. pitysophila.*

Table 1. Species of predatory enemies of *Matsucoccus matsumurae*

Natural enemies	Prey status
Coccinellidae	
Harmonia axyridis (Pallas)	Elderly nymph, female adult, egg
Exochomus mongol Barovsky	Elderly nymph, female adult, egg
Harmonia obscurosignata (Liu)	Female adult, eld nymph, egg
Calvia chinensis (Mulsant)	Elderly nymph, female adult, egg
Sticholotis punctatus Crotch	Female adult, eld nymph, egg
Chilocorus kuwanae Silvestri	Elderly nymph, female adult, egg
Propylaea japonica (Thunberg)	Elderly nymph, female adult, egg
Coelophora saucia Mulsant	Elderly nymph, female adult, egg
Rodolia limbata Motschulsky	Elderly nymph, female adult, egg
Lemnia biplagiata (Swartz)	Elderly nymph, female adult, egg
Formicidae	
Irridomyrmex anceps Roger	Elderly nymph, female adult, male pupae
Polyrhachis dives Smith	Elderly nymph, female adult, male pupae
Pormica f usca japonica Motschulsky	Elderly nymph, female adult, male pupae
Anthocoridae	
Elatophilus nipponensis Hiura	egg, female adult, young nymph
Dufouriella ater (Dufour)	egg, female adult, young nymph
Reduviidae	
Velinus nodipes (Uhler)	Female adult
Inocellidae	
Inocellie sp.	young nymph
Hemerobiidae	
Hemerobius humuli Linnaeus	nymph, female adult, male pupae, egg
Hemerovius lacunaris Navas	nymph, female adult, male pupae, egg
Sympherobius weisong Yang	nymph, female adult, male pupae, egg
Chrysopidae	
Chrysopa septempunctata Wesmael	nymph, female adult, male pupae, egg
Chrysopa kulingensis Navas	nymph, female adult, male pupae, egg
Chrysopa formosa Brauer	nymph, female adult, male pupae, egg
Cociomyiidae	
Oligotrophus sp.	egg

Natural enemies	Prey status
Phlaeothripidae	
Karnyothrips f lavipes (Jones)	egg, nymph, female adult
Syrphidae	
Syrphus serarius Wiedemann	nymph, female adult
Anystidae	
Anystis sp.	egg, young nymph
Anystis baccarum L.	egg, young nymph
Oxyopidae	
Oxopes seriatus (L.) Koch	female adult, male pupa
Lycosidae	
Lycosa coelestis (L.) Koch	female adult

(Cai, X.M., 1999)

2.3 The natural enemies of *Oracella acuta*

Chinese entomologists have done much research on parasitoids since *O. acuta* was introduced. They systematically investigated the natural enemies of *O. acuta* in newly invaded areas of Guangdong in 1993–1994, but they did not find parasitoids. The predators found are of certain control effects but were not able to control the mealybug population. Therefore, the introduction of natural enemies from abroad to control *O. acuta* was suggested. Two encyrtids *Acerophagus coccois* and *Zarhopalus debarri* were introduced from the country of origin (Pan, Z.P., 2002). To date, this project has experienced good results, but these two parasitoids have not established colonies in forests yet since the introduced parasitoids were not seasoned with the climate in Guangdong. In 1997, several natural enemies were found in pine forests, which were the earliest site *O. acuta* invaded. An encyrtid, *Anagyrus dactylopii* was the most advantageous species, with a higher parasitism rate and stable population in parts of the forest, it was speculated that the parasitoid spread from local orchards (Ren, H. *et al.*, 2000). Today the parasitoid trend is population growth and the parasitism rate is rising. It is possible for it to become an important natural enemy to control *O. acuta* by artificial reproduction and transfer.

Table 2. The natural enemies of *Oracella acuta* in Guangdong

Natural enemies	Natural enemies
Bothrocalvia alboli neata (Schoenber)	*Anagyrus dactylopii* (Howard)
Calvia chinensis (Mulsant)	*Aeurodothrips faciaperris*
Cryptolaemus mont rouzieri Mulsant	*Chrysopa septempunctata* Wesmea
Harmonia yedoensis (Takizawa)	*Tenodera sinensis* Saussure
Keiscymnus taiwanenisis Yang et Wu	*Agelena difficilis* Fox
Pharoscymnus taoi Sasaji	*Angalis faceanum*
Propylaea japonica Thunberg	*Carrhotus xanthogramma* (Latreille)
Scymnus ovimaculatus Sassaji	*Cyclosa monticola* Boes1et Str1
Synonycha grandis (Thunberg)	*Erigonidium graminicola* (Sundvall)

Natural enemies	Natural enemies
Menochilus sexmaculata (Fabricius)	*Pardosa pseudoannulata* (Boes1et Str1)
Brumoides lineatus Weise	

(Pan, Z. P. *et al.*.2002)

2.4 The natural enemies of *Pseudaulacaspis pentagona*

Pan, Mengxiang *et al.* 1990 and Zhu, Wenfang et. al 2006 investigated the natural enemies of *P. pentagona*. The results showed 14 species of parasitoids on *P. pentagona*, belong to Aphelinidae, Encyrtidae, Tetrastichidae. Of all the parasitoids of *P. pentagona*, *Encarsia berlesei* is the most advantageous species with a population quantity is 80 percent of all the natural enemies complex. Field collection and laboratory research show *E. berlesei*'s natural parasitism as 30–70 percent. It is considered to be a promising natural enemy.

3. The problems worth focusing on in biological control of exotic harmful scale insects. As international trade increases, the risk of exotic harmful organism's invasion continues to increase, people should be aware of the risk in the long term, and keep the technologies reserved. Scale insects are small insects, inactive most of the time, not easily found, and are those species easy to succeed in invasion of all exotic harmful organisms. Upon successful invasion, they can cause heavy damage to forests. When chemical control is ineffective, biological control of scale insects is especially important. Therefore, we should focus on the recognition and utilization technologies of parasitoids on exotic scale insects and keep the technologies in reserve.

Table 3. The natural enemies of *Pseudaulacaspis pentagona*

Natural enemies	Note
Encarsia berlesei Howard	
Aphytis proclia Walker	
Aphytis sp.	
Archenomus orientalis Silvestr	
Marietta carnesi Howard	hyperparasitic
Azotus perspeciosus Girault	
Epitetracnemus comis Noyes et Ren	
Thomsonisca amathus Walker	
Physcus sp.	
Pteroptrix sp.	
Apterencyrtus microphagus Mayr	=*Zaomma lambinus*
Quaylea sp.	misidentified
Aprostocetus sp.	misidentified
Pteroptrix chinensis How	

(Zhu, W. F. *et al.* 2006)

3.1 With exotic harmful scale insects, if there are no effective parasitoids domestically, we should introduce the effective parasitoids from the country of origin, to fill the ecological niche vacancy. Some scale insects are special ones, like *M. matsumurae* without parasitoids whether domestic or abroad (Tachikawa 1981), then we have to find another way or try to utilize its predators.

3.2 Sufficient research on local parasitoids and utilize them
Chemical control of *O. acuta* in forests has not been effective. The introduced parasitoids from country of origin had certain progress, but were not easily to established as a stable population. *Anagyrus dactylopii*, the local parasitoid of *O. acuta* has been associated with higher parasitism, forming a stable population in *Oracella acuta* infested areas, and the population density and parasitism trend to rise. It has the possibility to become an important natural enemy of *O. acuta*. Therefore, local parasitoids should receive further study and utilization.

As to local parasitoids on scale insects, we have collected 295 species from all over China, which include Encyrtidae: 63 genera, 188 species; Aphelinidae: 12 genera, 92 species; Eulophidae: 2 genera, 5 species: Pteromalidae: 4 genera 5 species, Signiphoridae: 2 genera, 4 species; and Eupelmidae: 1 genus, 1 species (Xu 2004). These local parasitoids can parasitize over 300 species of scale insects, including exotic harmful scale insects. These parasitoids can also be exported overseas for biological control of scale insects.

3.3 The parasitoids on scale insects are important factors of biological control. There are many successful examples abroad (Greathead, 1986), but in reality introducing those parasitoids to our country is not simple like directly transporting them in. Some parasitoids are monoparasitic, some are polyparasitic with many other hosts. Some parasitoids are hyperparasites, for example *Marietta carnesi*, which benefits harmful scale insects, or *Zaomma lambinus*, which can parasitize nine species of diaspidids including *Pseudaulacaspis pentagona*, and can also parasitize other parasitoids such as *Microterys* spp. Parasitoids have ecological requirements for their survival and establishment, therefore it is necessary to conduct studies on the biology and ecology of parasitoids, sufficient evaluation and argumentation before introduction of parasitoids.

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Research on Using Local Parasitic Chalcid of Coccids to Control Exotic *Hemiberlesia pitysophila* Takagi (Homoptera: Diaspididae)

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ABSTRACT

Hemiberlesia pitysophila Takagi belongs to Diaspididae, Hemiberlesia. Japanese coccids scholar *S. Takagi* found it in Taiwan for the first time in 1956. Kawai confirmed that it occurred in Okinawa and Xiandao in 1980. It caused widespread and serious damage after it was introduced to China in the early 1980s in Guangdong Province. The distribution area amounted to 1.4249 million hectares in 2004, the distribution area is 544,400 hectares and the serious damaged area is 70,300 hectares. It occurred in a large area of Guangdong Province, as well as some areas of Fujian, Jiangxi, Guangxi, and other provinces (regions) in China.

The hosts of *H. pitysophila* Takagi in China are *Pinus massoniana* Lamb, *P. ellottii* Engeim, *P. thunbergii* Parl, *P. taedal*, and *P. carbaea* Morelet. *P. massoniana* Lamb is the most seriously damaged from saplings to two to 30 years old all exhibited contiguous wither. In Okinawa, the major host tree is *P. luchuensis* Mayr. In Guangdong, when the pest was first discovered, the growth capacity per year of *P. massoniana* Lamb was reduced by 2.7m^3 per hectare on average, pine resin production per hectare was reduced by 900kg. The more heavily damaged trees may wither in three to five years.

Coccobius azumai Tachikawa which is a natural parasite enemy of *H. pitysophila* was introduced from Okinawa, Japan to Guangdong Province in 1986-1989. The origin parasitic rate in Japan was 25.6 percent. The natural multiplication source of the parasite settlements were used to released it in a large area in Guangdong in 1990. And there is a significant effect, the parasitic rate of the female coccids reached 20-30 percent and the population density of the female coccids was controlled at 0.3-0.6 per needle leaf, and the coccids were effectively controlled. However, because the specialty of *C. azumai* Tachikawa in reproductive and development and the unusually dry climate and other reasons, its population dropped significantly in the original release area after 1991 and disappeared in 2001. Then the population of *H. pitysophila* Takagi took an upward trend.

In a large-scale survey to search and study the pest's parasite in Guangdong Province in 2004, we found that population density of *H. pitysophila* Takagi was over the middle level in a few forests where the pests have existed for ten years ,but the status of *P. massoniana* Lamb was very healthy. *Encarsia amicul* and *Aphytis* sp. were found from such a few pine forest (four areas in the province were found, covering an area of about 60 hectares). Its population and parasite rate were very high. *E. amicul*, *Aphytis* sp. and *C. azumai* Tachikawa all belong to Hymenoptera, Cholcidoidea, Aphelinidae

but they belong to different genera. *C. azumai* is an obligatory parasite, *E. amicul* and *Aphytis* sp. are non-obligatory parasites (*Abgrallaspis cyanophylli, Aonidiella aurantii, Chrysomphalus aonidum* which are all local coccids in Guangdong). According to the survey, the natural population density of *E. amicul* and *Aphytis* sp. in the four areas is 125-278/ kg leaves. The female coccids' parasite rate was as high as 25.3-33.3 percent. According to the study, two species of chalcids can grow and reproduce in one year and maintain a certain number in the forest. Their peak population period is from March to May and a smaller peak comes in Ocotober-November.

Four areas of local natural parasite chalcid resources were used in 2004-2005 in Guangdong Province. In 2006 the multiplied chalcid resource reproduced in the controlled in 2004 were also used. A method was adapted to transfer the chalcids (pine branches having more than 100 chalcids/kg twigs were collected and transferred for a long-distance to the control area to release). A release site was selected in every 13.3 hectares, and more than 10kg branches with more than 900 chalcids were hanged in every release spot. The branches were divided into three groups and hanged on three adjacent pine tree. More than 300 chalcids were released on every tree. Nine to twelve months after the release the natural spread distance is about 200m. Twelve, twenty-four months after the release the population and parasite rates were an average of 153/kg twigs, 299/kg twigs and 16.8 percent, 24.8 percent, a maximum of 572/ kg branches, 36.3 percent. The total area where the chalcids were released amounts to 48,800 hectares from 2004 to 2006. Disaster mitigation was obtained, the pest's moderate and severe damage area ratio in 2006 was reduced by 37.6 percent and 44.5 percent compared to 2004.

SESSION THREE

PLANTS

Biological Control of Mile-A-Minute Weed, *Persicaria perfoliata* (Polygonaceae) in the Eastern U.S.A.

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ABSTRACT

Mile-a-minute weed, *Persicaria perfoliata* (L.) H. Gross (formerly *Polygonum perfoliatum* L.), is an alien invasive weed from Asia that infests natural areas in the mid-Atlantic region of the U. S. A., where it can form dense monocultures. A biological control program was initiated by the U. S. Department of Agriculture, Forest Service in 1996. Over 100 insect species were identified on mile-a-minute weed in China, including several that appeared to have a narrow host-range (Ding *et al.*, 2004). One of these, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), was tested on plant species in China and in quarantine in Delaware in the U. S., and found to be extremely host-specific (Colpetzer *et al.*, 2004). This insect was approved by U. S. Department of Agriculture, Animal and Plant Health Inspection Service for release in the U.S. in 2004.

Eggs of *R. latipes* are laid on *P. perfoliata* plants. Larvae feed internally in plant stems, then drop to the ground and pupate in the soil. Adults emerge and feed on mile-a-minute weed leaves and terminals, and go through three to four generations between May and September (E. Lake, unpublished data). The weevils have been reared at the New Jersey Department of Agriculture Phillip Alampi Beneficial Insect Laboratory since 2004, and by 2007 more than 60,000 weevils had been reared and released.

Dispersal and population increase was monitored at three replicated release sites in southeastern Pennsylvania in 2005 through 2007. All three sites showed substantial increases in weevil numbers (E. Lake, unpublished data). Seed cluster production was significantly reduced at two of the three sites, and the average number of seeds per cluster was lower at all three release sites in 2007 compared to control sites without weevils. Weevils dispersed beyond the release sites, up to two miles in two years.

The impact of the weevil on *P. perfoliata* seed production was assessed on single plants in replicated field cages in 2006, with two levels of weevil numbers (5 or 20 per cage) added at two different times (26 May or 23 June), and plants in cages with no weevils included as a control. Seed production was almost completely suppressed between late July and mid-September in the cages with early application of 20 weevils per cage; however, all plants produced numerous seeds in October, resulting in no significant difference by treatment in total seed production over the season. In this experiment the caged plants were unusually large and robust, because of minimal competition with other plants. A similar experiment was conducted beginning in May 2007, with 0, 10, 20, or 40 weevils per cage and with more natural levels of plant competition. Plant mortality by the end of July 2007 was 12.5 percent with no weevils, 50 percent with 10 weevils, 62.5 percent with 20 weevils, and 75 percent with 40 weevils.

While the program is still in its early stages, indications are that *R. latipes* can successfully suppress mile-a-minute weed populations in the U. S.

Keywords: Mile-a-minute weed, *Persicaria perfoliata*, *Rhinoncomimus latipes*, weevil.

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Mile-a-Minute Weed and its Biological Control Using Plant Pathogens

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ABSTRACT

Mile-a-minute weed (*Persicaria perfoliata*) is an invasive plant introduced from Asia in the 1930s and subsequently spread to eleven states in the United States. Surveys for natural enemies in the eastern U.S. found no effective biological control agents.

Surveys for fungal associates of mile-a-minute weed were conducted in China from 1997 to 2002. More than 50 samples of mile-a-minute weed were collected from 13 locations in six provinces in northeastern, northern central and southwestern China. Over 300 fungal isolates were obtained from these samples. In laboratory tests, 44.5 percent of the fungal isolates had no infection, 13.1 percent intermediate, and 42.4 percent high infection on detached leaves.

All selected pathogenic fungal isolates with high infection ratings from Guizhou and Shandong provinces were *Alternaria* spp. while those collected from Henan and Liaoning Provinces were more diversified, including *Colletotrichum* spp., *Fusarium* spp., *Phyllosticta* sp., *Ascochyta* sp., *Sphaeropsis* sp., *Rhizoctonia* sp., and *Alternaria* spp.

Screening Insects in China for Biological Control of Mile-a-minute Weed and Japanese Knotweed in the U.S.A.

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ABSTRACT

Mile-a-minute weed, *Persicaria perfoliata* L. (Polygonaceae), is an annual or perennial herb that is native to India, China, Korea, Japan, Bangladesh, and the Philippines. It has invaded eight states in the northeastern U.S.A. To screen insect biological control agents, field surveys were conducted in China in 1996-2001. About 111 arthropods were discovered from the plant (Ding *et al.*, 2004). A geometrid moth, *Timandra griseata* Petersen (Lepidoptera: Geometridiae), was first introduced from China to a quarantine facility in the U.S. but host range tests showed it had a broad host range in Polygonaceae. The Asian weevil, *Rhinoncomimus latipes* Korotyaev (Coleoptera: Curculionidae), was considered a promising biological control agent as it is a host-specific insect (Colpetzer *et al.*, 2004). It was released in the U.S. in 2004. The impact of this agent on mile-a-minute weed is under evaluation.

Japanese knotweed, *Fallopia japonica*, is an important invasive plant in North America and Europe (CABI, 2007). In its native range in China, this species is widely distributed in more than 10 provinces, from central to southern areas. An Asian leaf beetle, *Gallerucida bifasciata* (Coleoptera: Chrysomelidae), was previously recorded as an important natural enemy attacking this plant in China, with an ambiguous host range. We examined the beetle's host specificity through a set of choice and no-choice tests in the laboratory and field in 2006-07 (Wang *et al.*, 2008). We found *G. bifasciata* larvae were able to complete development on only seven out of 87 plant species in larval development tests, while adults fed and oviposited on only 10 plants (*i. e. Persicaria perfoliata*, *Polygonum multiflorum*, *Polygonum chinense*, *Polygonum runcinatum*, *Fagopyrum esculentum*, *Fagopyrum dibotrys*, *Fagopyrum tataricum*, *Rumex acetosa*, and *Oxalis corymbos*a) in no-choice tests. Multiple choice tests showed that adults strongly preferred *Fallopia japonica*, *Persicaria perfoliata* and *Polygonum multiflorum* over all other plants tested. Open field tests and field surveys further revealed that these three species were in its ecological host range. The impact of temperature on egg and larval development indicated that the optimal temperature was about 25^{o}C. The results suggest that *G. bifasciata* may be a potential promising agent for control of both Japanese knotweed and mile-a-minute weed. The beetle may adapt to the climate in the northern United States and Europe, although additional host specificity tests and risk assessment should be completed.

To screen more potential biological control agents, we conducted extensive field surveys in seven provinces in China, including Hubei, Henan, Hunan, Guizhou, Yunnan, Fujian and Guangdong Provinces, between June 2006 and May 2007. Among the herbivores we found during the surveys, twospecies were considered to have promising potential as biological control agents, based on their damage on the plant, field populations and literature-recorded host ranges. They are the leaf-rolling weevil, *Euops splendida* (Coleoptera: Attelabidae), and the stem borer, *Ostrinia* sp. (Lepidoptera: Pyrilidae). Our work indicates that potential promising biological control agents against Japanese knotweed exist in China and more detailed study should be conducted in the next few years to further screen and evaluate these insects.

Keywords: *Persicaria perfoliata*, *Fallopia japonica*, biological control, host range test

Potential Distribution of *Eucryptorrhynchus brandti* (Harold) in the United States Based on CLIMEX

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ABSTRACT

"Compare location" option of the CLIMEX model was used to predict the potential distribution of *Eucryptorrhynchus brandti* (Harold), a natural enemy of *Ailanthus altissima* (Mill.) Swingle, in the United States. Results demonstrated that 58 locations in 17 states, most of which were located in the western United States, were suitable for survival of *E. brandti*. The potential distribution of *E. brandti* was smaller than the distribution of *A. altissima* in the United States.

INTRODUCTION

A. altissima (Mill) Swingle, is widely distributed in China, grows in acid, neutral and limestone rich soils. *A. altissima* is a fast-growing native species in northern China and the Huaibei area of Anhui Province. Additionally, *A. altissima* has a strong resistance to dust and sulfur dioxide (Ge 2000; Ni 2004). Furthermore, *A. altissima* is used to prevent erosion in landscaping as ornamental and roadside trees (Zhang, 2001; Zhang *et al.* 2001).

A. altissima has been found in 41 states and considered to be an alien invasive tree in the United States. It is capable of prolific root and stump sprouting as well as producing a generous amount of seed (Miller 1990). If allowed to become established, *A. altissima* will create a pure stand, with little opportunity for other plant species. *A. altissima* has also been found to produce allelopathic compounds in its bark and leaflets that are toxic to numerous woody and herbaceous species (Miller 1990; Heisey 1996).

Eucryptorrhynchus brandti (Harold) (Coleptera: Curculionidae) is a forest pest in China, feeds on *A.altissima* and its variaties. Therefore, *E. brandti* is considered to be a potential natural enemy of *A. altissima* (Zheng *et al.* 2000).

Before introducing a biological control agent to a new habitat, it is necessary to analyze its potential suitable area. Climate is considered to be a determining factor. CLIMEX is a dynamic model that is used to infer species' responses to climate from observations of the geographical distribution and seasonal abundance. The potential distribution of *E. brandti* in the United States is predicted based on the experimental data and related material.

MATERIALS AND METHODS

Introduction to CLIMEX

The biological modeling program, CLIMEX version 1.1, was used to predict the potential distribution of *E. brandti*. The model assumes that most animal and plant populations experience a season which is favourable for population growth and one that is unfavourable and may jeopardize its persistence in a given area. (Maywald and Sutherst, 1991). Ecoclimatic Index(EI) with values between 100 and 0 gives an overall measure of the potential of a given location to support a permanent population of a plant, and is calculated by subtracting the climatic stresses (Cold (CS), Hot (HS), Wet (WS) and Dry (DS) stresses) from the Growth Index (Sutherst *et al.* 1999). 100 is condisered to be the ideal climate for optimal survival of the species, while 0 indicates survival is not possible. Values below 10 indicate the climate is generally unsuitable for the target species, though survival may still be possible in protected microhabitats (Rachel and Bryce, 1996).The program contains data from 2031 meteorological stations around the world, including 85 meteorological data in China and 173 in the United States.

Distribution of *Eucryptorrhynchus brandti* (Harold) and biological data

The known distribution of *E. brandti* in China including Ningxia autonomous region, Shanghai municipity, Gansu, Heilongjiang, Liaoning, Beijing, Hebei, Shandong, Shanxi, Jiangsu, Anhui, Sichuan, Hubei, Shanxi and Henan province (Ge 2000; Zhang *et al.* 2001).

Biology and experimental data

Adult weevils overwinter in the upper 1-2 cm of soil, while larvae overwinter in the tunnels in the branches and trunk. *E. brandti* has one generation in Yinchuan, Ningxia autonoumous region and Laizhou, Shandong province (Zhang *et al.* 2001; Qin *et al.* 1999). The experiment showed that 20-32^{o} was the optimal temperature ranges for *E. brandti* growth. 36^{0} was the heat limit to *E. brandti* growth. Developmental threshold and effective accumulated temperature for the whole generation of *E. brandti* was 6.72^{o} and 1352.8 day degree, respectively. Low temperature experiment showed lowest temperature that adult could endure was -17^{o}. Eggs could hatch when moisture between 41 percent and 92 percent. Egg hatch rate was influenced after dipping in water for 10 minutes, but after dipping in water for 48h, a small number of eggs still could hatch. During winter observation, adult weevils overwintered in dry soil. Furthermore, field observation showed that long-time rainfall greatly decreased the weevil population.

Predicting method

The experimental data was used as initial parameters, then parameters were adjusted to make predicted areas match the actual known range as much as possible. Then final parameters were used to predict the potential distribution in theUnited States.

Results and analysis

Seventeen parameters were used in this study. Growth parameters including temperature (DV) and soil moisture (SM). For temperature, DV0 and DV3 define the cold and heat limits to species growth. DV1 and DV2 describe the optimal temperature ranges for growth. Furthermore, developmental threshold temperature and effevtive accumulated temperature were aslo used in this study. The same process defines soil moisture values. Stress parameters defined in this study include cold, heat, dry and wet stress.

The stress parameters was composed by two components, 1) the threshold beyond which stress occurs (defined by the limiting parameter threshold values), and 2) the rate at which stress occurs.

Predicting parameters

Table 1. CLIMEX parameters used for predicting potential distribution areas of *Eucryptorrhychus brandti* (Harold) in the United States.

CLIMEX	Initial parameters	Final parameters
DV0 (limiting low temp.)	7	7
DV1 (low optimal temp.)	20	20
DV2 (Upper optimal temp.)	32	32
DV3 (Limiting high temp.)	36	36
PDD (Minimum day-degree)	1350	1350
SM0 (Limiting low moisture)	0.05	0.05
SM1 (Lower optimal moisture)	0.1	0.3
SM2 (Upper optimal moisture)	0.3	0.7
SM3 (Limiting high moisture)	0.9	0.9
TTCS (Cold temp. threshold)	-17	-17
THCS (cold stress temp. rate)	0.0005	0.0003
TTHS (Heat temp. threshold)	36	36
THHS (Heat stress temp. rate)	0.0005	0.0005
SMDS (Dry threshold)	0.3	0.1
HDS (Dry stress rate)	0.0005	0.0001
SMWS (Wet threshold)	0.5	0.5
HWS (Wet stress rate)	0.0005	0.005

Mapping the distribution of Eucryptorrhynchus brandti (Harold) in China

Ecoclimatic index (EI) values larger than 10 were considered to be suitable for *E. brandti* growth. *E. brandti* were known to occur in 15 provinces, mucipalities and autonomous regions in China. For the known distribution of *E. brandti*, the parameters used in Table 1 predicted that 13 provinces were suitable for the growth of *E. brandti*, with fitting degree around 0.87. Some provinces, including Jilin, Tianjin, Qinqhai, Xinjiang and Inner Mongolia, where no weevils were known to occur, also had EI values larger than 10. Jinlin and Tianjin were among the known distributed belt of *E. brandti*, including Heilongjiang, Liaoning, Hebei and Beijing, so it is possible for *E. brandti* to survive. *E. brandti* was quarantine pest in supplementary of forestry quarantine pest of Qinghai province, so Qinghai was suitable for *E. brandti* growth. *A. altissima* was known to be planted in the early 1980s in Tibet, but no studies reported that *E. brandti* had spread to this area. The history of *A. altissima* in Inner Mongolia was long, but no study had ever showed *E. brandti* occurred in this area.

Potential distribution of *Eucryptorrhynchus brandti* (Harold) in the United States

The CLIMEX module produced by this study predicts *E. brandti* growth can occur in the western half of the United States (Fig. 2). A total of 58 locations in 17 states are suitable for growth of *E. brandti* in the United States (Table 2).

Table 2. Predictable results for *Eucryptorrhynchus brandti* (Harold) in the United States (number in brackets represents the EI values).

States	EI ≥	EI <10
Arizona	Flagstaff (17)	Tucson (4), Phoenix (3), Winslow (2)
California	San Diego (26), Los Angeles (20), Santa Maria (17), San Jose (14), Fresno (13), Stockton (10)	Bakersfield (7), Red Bluff (5), Sacramento (4), Bishop (2), San Francisco (2)
Colorado	Pueblo (13), Colorado Springs (19), Denver (15)	Grand Junction (4)
Idaho	Lewiston (12)	Pocatello (9), Boise (8)
Iowa	Sioux City (41)	Des Moines (8)
Kansas	Concordia (45), Wichita (44), Topeka (43), Dodge City (38), Goodland (27)	
Minnesota	Minneapolis (20)	
Montana	MilesCity (17), Billings (17), Havre (17) , Glasgow (16), Great Falls (16), Helena (13), Missoula (11)	
Nebraska	Lincoln(46), Norfolk(43), Omaha(43), Grand Island (35), North Platte (30)	
Nevada		Winnemucca (3), Ely (2), Reno (2), Las Vegas (1)
New Mexico	Roswell (19), Santa Fe (19)	
North Dakota	Ellendale (30), Fargo (30), Bismark (24), Williston (21)	
Oklahoma	Oklahoma City (45), Tulsa (3)	
Oregon		Pendleton (9), Baker (7), Burns (3)
South Dakota	Sioux Falls (39), Huron (28), Pierre (27), Rapid City(23)	
Texas	Brownsville (71), Abilene (47), Austin(46), Amarillo (45), Wichita Falls (42), Waco (32), Lubbock (30), Dallas (11), El Paso (6)	
Utah	Salt Lake City (11)	Modena (6), Milford (2)
South Carolina		Columbia (2)

Washington	Walla Walla (11), Spokane (6), Yakima (2)	
Wyoming	Cheyenne (17), Sheridan (14), Casper (12)	Lander (9)

DISCUSSION

It is necessary to predict the potential distribution of a new species before introducing it to a new habitat. CLIMEX is a dynamic simulation system, which is considered to have high accurancy and application value in predicting the potential distribution of species and has been widely used worldwide(Marywald 1991).

The potential distribution predicted by CLIMEX, which occurred to the north or along the Yangtse River, was close to the actual distribution of *E. brandti*. The distribution of *E. brandti* was smaller than the actual distribution of *A.altissima*, which could be explained as follows: 1) the weak mobility of *E. brandti*, the long distance spread of was greatly dependent on the transportation of *A. altissima*, for example, damage of *A. altissima* in Yinchuan, Ningxia was caused by weevils accompanied by transportation of *E. brandti* (Zhang *et al.* 2001). This might be one reason why there were no weevils in Xinjiang and Inner Mongolia; 2) influence of rainfall, field investigation showed that rainfall, especially heavy rain or lengthy periods of rain greatly influenced the weevil population, which might restrict its distribution in Southeast regions, such as Guangdong province; and 3) no study had ever reported it might be another reason.

The potential distribution of *E. brandti* located in the western half of the United States predicted by CLIMEX, where climate was suitable for the growth of *E. brandti*, but for the difference between the United States and China on terrain condition, climate characteristics. Furthermore, CLIMEX only takes into consideration the influence of climate on the distribution of *E. brandti*, other non-biological factors such as soil type, geographic characteristics, physical geographical barrier (including desert, ocean, mountain) vegetation type and interrelation of sepcies (including the relationship between human beings), biological factors and historical reasons were not taken into consideration. Therefore, there is no doubt that there are limitations to the results we get. In recent years, (Davis *et al.* 1998) criticized this method because it contained climate factors, but neglected other non-climate factors. It was still considered to be a useful method to predict potential distribution using climate factors from its known areas (Song *et al.* 2004).

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Potential Biocontrol of *Ailanthus altissima* with *Verticillium*

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ABSTRACT

Ailanthus altissima is an invasive tree species within most of the U.S.A., and is aggressively controlled along highways using chemical herbicides. We recently observed thousands of *A. altissima* trees wilting and dying within a Pennsylvania state forest where no herbicides had been applied. Isolation of the pathogen from symptomatic trees, inoculation of potted seedlings in a greenhouse, and inoculation of canopy trees in the forest revealed that the pathogen to be the soil-borne wilt fungus *Verticillium*, a potential biological control agent for *A. altissima*.

Potential for Biological Control of Kudzu, the "Weed that Ate the South"

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ABSTRACT

Kudzu, *Pueraria montana* var. *lobata* (Willd.) Maesen & S. Almeida, was brought to the U. S. from Asia in 1876, and was widely planted in the 1940s in the southeastern U.S., primarily for erosion control. However, the plant subsequently proved unmanageable and invasive. In 1999, the U. S. Department of Agriculture Forest Service initiated a cooperative biological control program with China. A systematic survey revealed more than 100 insect species feeding on kudzu in China, some of which appeared to be quite host specific (Sun *et al.*, 2006).

Two of these species, *Gonioctena tredecimmaculata* (Jacoby) (Coleoptera: Chrysomelidae) and *Ornatalcides* (Mesalcidodes) *trifidus* (Pascoe) (Coleoptera: Curculionidae), were studied in quarantine in the U. S. (Frye *et al.*, 2007). Adults of *G. tredecimmaculata* were ovoviviparous and reproduced throughout the summer, producing offspring that had an obligate adult diapause. In no-choice tests, adult and larval *G. tredecimmaculata* rejected most of the plant species tested, but consumed foliage and completed their life cycle on soybean (Glycine max [L.] Merr.) and on a native woodland plant, hog-peanut (*Amphicarpaea bracteata* [L.] Fernald), which are in the same subtribe as kudzu (Glycininae). Insects showed similar responses to field- and greenhouse-grown soybean and kudzu foliage, despite measurable differences in leaf traits: field-grown foliage of both plants had greater leaf toughness, higher total carbon content, higher trichome density, and lower water content than greenhouse foliage. *O. trifidus* adults also rejected most of the plants tested, but fed on and severely damaged potted soybean and hog-peanut plants in addition to kudzu. Further tests in China are needed to determine whether these species will accept non-target host plants under open-field conditions.

Keywords: kudzu, *Pueraria montana* var. *lobata,* biological control, *Gonioctena tredecimmaculata, Ornatalcides* (Mesalcidodes) *trifidus*

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rDNA-ITS Sequence Analysis of the Imitation Rust Pathogens (*Synchytrium*) on *Pueraria* spp.

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ABSTRACT

Four different DNA extraction methods are used to purify genomic DNA from the sori isolated from *Pueraria lobata* (Willd.) Ohwi., *P. thomsonii* Benth., and *P. montana* (Lour.) Merr. The modified protocol using benzyl chloride is the most efficient method to extract genomic DNA. rDNA-ITS can be successfully amplified directly from a single sporangium by PCR. The ITS sequences of the isolates from *P. lobata* and *P. thomsonii* are 856bp and 907bp with 92 percent sequence similarity respectively. The ITS sequences of two isolates (SPM-1 and SPM-2) from *P. montana* are 1148 and 1147 bp, with 54 percent and 55 percent sequence similarity to the isolates from *P. lobata* and *P. thomsonii* respectively. We proposed that the isolates from *P. lobata* and *P. thomsonii* are different varieties of the same species and the isolates from *P. montana* are another species of *Synchytrium*, or the isolates from *P. lobata* and *P. thomsonii* are two closely related species and the isolates from *P. montana* is a more distantly related species.

Keywords: *Synchytrium puerariae*, *Pueraria lobata*, *Pueraria thomsonii*, *Pueraria montana*

Biocontrol of Weeds in Native Hawaiian Forests

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ABSTRACT

A key mission of the USDA Forest Service's Institute of Pacific Islands Forestry is to develop insect agents for classical biological control of forest weeds in Hawaii and other Pacific Islands, with particular emphasis on weeds that invade and transform native forest ecosystems. Currently targeted weeds include *Psidium cattleianum* (Myrtaceae) and *Miconia calvescens* (Melastomataceae). These and other recent target species have been mostly of neotropical origin, however many important weeds of Pacific Islands originated in Asia. We have begun collaborations in China with an initial focus on a species native to the Himalayan region, *Rubus ellipticus* (Rosaceae).

Biology and Host Specificity of *Puccinia spegazzinii*, a Biocontrol Agent for *Mikania micrantha*

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ABSTRACT

Biology and specificity of *Puccinia spegazznii*, a potential biological control agent of the invasive weed, *Mikania micrantha* were investigated under greenhouse quarantine. The fungus displayed similar characteristics in this case as with reports from other counties. The rust pathogen is an autoecious obligate parasite that completes its entire life cycle on *M. micrantha* by teliospores and basidiospores. The results obtained from this research are similar to previous reports. *P. spegazzinii* infected vegetative organs of the host plant, particularly leaves and petioles. Plant parts affected by the pathogen became chlorotic 4-5 days after inoculation. Twelve to fifteen days later, yellow telia appeared on the back of the leaf. The infected leaves died and/or defoliated. Finally, the whole plant died. Teliospores were yellow to brown in color without dormancy. Teliospores germinated and produced basidiospores, re-infecting the host plant under conditions of high humidity.

Seventy-two species of plants belonging to 29 families and 62 genera were chosen for host range testing. They were divided into 12 groups in the testing program. There was *M. micrantha* in each group as CK plant. Three replicates were selected. Method of inoculation of the pathogen was referenced to sequence and regulation of CABI. Results of the testing showed that in response to inoculation of the pathogen, a chlorotic spot appeared on *Asparagus cochinchinensis*, *Eupatorium adenophorum*, *Elephantopus scaber* and *Helianthus annuus*. However, no mycelium or haustorium were found. The pathogen did not threaten the plants. It is recognized that *Puccinia spegazzinii* infected *Mikania micrantha* and *M. cordata* in China.

Some Considerations on Nonindigenous Plants in China

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ABSTRACT

Nonindigenous species invasion has become a world wide problem. From the 1990s, our country began paying attention to alien species. To date, while most of the research has focused on comparatively harmful species, less study has been carried out on the exotic species which are not harmful or are in their latent period. Because there are no unified standards to understand the connotation of nonindigenous species and invasive species, opinions vary on how many nonindigenous species and how many invasive species exist in China. In this paper, the nine standards brought forward by Presten and utilized to determine which species are nonindigenous species and which ones are native species are introduced. The inhibiting and supplanting mechanisms of those nonindigenous species on native species through competition for sun light, water and nutrition are also included. The ecological influences of those introduced species on native species is also discussed. The integrated control techniques were put forward to control and get rid of those invasive nonindigenous species.

Keywords: nonindigenous plants, non-native species, evaluation on invasive capacity, ecological influence, control

Analysis of the Allelopathic Effects of Yellow-top Weed (*Flaveria bidentis*) on Germination and Growth of Wheat

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ABSTRACT

Yellow-top weed (YTW), *Flaveria bidentis*, is a new invasive plant in northern China and a serious pest affecting agricultural crops, especially wheat. It presents a great threat to biodiversity in habitats of the infested areas in China. In the present study, the pot soil experiment and beaker-blotting paper bioassay were used to analyze the allelopathic effect of the weed. Results from the experiments showed that YTW rhizospheric soils significantly inhibited seed germination and later seedling growth of wheat ($P<0.05$). The ethanol extract from YTW rhizospheric soil and the aqueous extract from the aerial part of YTW plants also inhibited seed germination and seedling growth of wheat significantly ($P<0.05$). These results suggested that allelopathy could be the major mechanism of YTW's high invasiveness and rapid establishment of populations in new habitats.

Keywords: yellow-top weed, *Flaveria bidentis,* allelopathy, inhibitive rhizospheric soil, extracts, wheat germination and growth.

INTRODUCTION

Yellow-top weed (YTW), originated from South America, is a new invasive plant species in northern China and is now also widespread over the states of Alabama, Florida, Georgia, Massachusetts in the United States, and Central America. It is now also widely distributed in Europe, Africa and West Indies (Gao *et al.*, 2004). Since *F. bidentis* was first recorded in China in 2001, it is now widely distributed inmore than 47 cities in Hebei Province and Tianjin City. Its distribution is quickly expanding in China. This alien invasive weed often occurs on disturbed land and in areas along river valleys in northern China. A mature plant can produce as many as 100,000 seeds each year. It can easily establish monocultures over large areas and compete with populations of native plant species for space, water and nutrient resources in the soil. Previous studies demonstrated that the chloroform extracts of *F. bidentis* also had antibacterial activities on two strains of *Staphylococcus aureus* and the methanol extracts can significantly suppress *Sitophilus oryzae* (Bardón *et al.*, 2007; Broussalis *et al.*, 1999). An assessment of its primary invasiveness has suggested that it is an exotic weed of high risk and a potential threat to biodiversity in its infested ecosystems.

Allelopathy, as defined by Molish (1973), is the chemical interaction between plant species, including stimulatory and inhibitory influences. Recent studies showed that allelopathic interactions might contribute more to the success of invasive plant species than previously thought (Bais *et al.*, 2003;

Fitter, 2003). Accordingly, allelopathy has been suggested as a mechanism for the successfulness of invasive plants for population establishment in their new habitats. The accumulation of allelochemicals in soil environment can occur mainly through release of water soluble leachates from leaves and roots, exudation from roots of the plants. Allelochemicals can accumulate in soil in concentrations sufficient to affect their neighbor plants. Study of the allelopathic effect of alien invasive weeds may help to understand invasion mechanisms and in developing strategies to prevent and control the spread of those alien invasive weeds. The purpose of this paper is to document the allelopathic effect of YTW on wheat and to identify the mechanism of its high invasiveness.

MATERIALS AND METHODS

YTW, its rhizospheric soils, and weed-free soils were collected from Monancun village of Hebei Province in September 2006. The rhizospheric soils were collected by pulling YTW plants from the soil and shaking soil off from the roots. Wheat (*Triticum aestivum* L.), a crop widely cultivated in northern China, was selected as target species for allelopathic tests (the variety: Zhongmai number one) and its seeds were obtained from the Crop Research Institute, Chinese Academy of Agricultural Sciences.

Pot Soil Experiment

Ten wheat seeds were uniformly sown in each of the six pots (9 cm in diameter) containing yellow-top rhizospheric soil, and weed-free soil as control. All pots were placed in a growth chamber at 20-25°C, lighting 16h/day (18,000 lx with luminous bar lights) and 75 percent relative humidity. The pots were randomly arranged and each treatment was replicated three times. Pots were watered and checked every day till plants were harvested for data recording.

Beaker Blotting-paper Experiment

Preparation of ethanol extract of yellow-top infested rhizospheric soils: The soils used in this experiment were air-dried and sieved (2 mm mesh) to remove debris and root tissues. 150 g of the soil was mixed with 450 ml of ethanol and was extracted for 24 h at 20°C on a rotating shaker (120 rmp). The filtrate was concentrated in vacuscope. The concentrated matter was resuspended with 30 ml of distilled water and fractions of the suspension were diluted to relative extract concentrations of 1.0, 2.0, 3.0, 4.0, 5.0 g soil per ml of water for different treatments.

Preparation of aqueous extracts of YTW tissue: The aerial parts of YTW plants were air-dried and ground into fine powder. 0, 0.1, 0.2, 0.3, 0.4 mg of the plant powder were placed into individual glass beakers respectively, 10ml of distilled water was added into each beaker to soak the powdered tissues. The beakers were shaken for 24 h at room temperatures on a rotating shaker (120 rpm). The extraction liquid in each beaker was filtered through double layers of muslin cloth. Then extract concentrations of filtrates were relatively expressed as 0, 0.01, 0.02, 0.03, 0.04 g/ml.

Wheat plant bioassay: Growth inhibition effects of the ethanol extract from the rhizospheric soils and the aqueous extracts from YTW tissues were evaluated through bioassays against wheat. The wheat seeds were surface-sterilized with 0.5 percent NaClO and different lots of sterilized seeds were treated with the solutions of the above extracts. One lot of seeds was treated with distilled water only as control. For each treatment, 10 seeds were placed on three layers of wet blotting-papers in a glass beaker and three beakers were sown as three replicates.

Data Recording and Statistic Analysis of Data

In the pot soil experiment, wheat germination (emergence) rate was recorded everyday and seedlings were harvested after three weeks to measure the length and weight of roots and seedlings. In the beaker blotting-paper experiments, germination rate of wheat seeds in each treatment, length and weight of the roots and seedlings in each beaker were recorded three days after the treatment. Pictures were taken at the same time.

The mean values of individual treatments were calculated. The means between different treatments in each of the experiments were compared statistically by Duncan's new multiple range test using the SPSS package (version 12.0).

RESULTS

Results from the pot soil experiment showed that the germination and growth of wheat were strongly inhibited by the yellow-top weed rhizospheric soils. It indicated that *F. bidentis* infested soil was toxic or inhibitive to wheat growth. The relative inhibition rates of germination and seedling growth reached 55 percent and 58 percent respectively, compared with those in the weed-free control soil. In addition, the emergence of seedlings was delayed by five days in the treatment of the rhizospheric soil. As a result, there appeared to be some inhibitants or phytotoxins released by yellow-top plants into the rhizospheric soils. The presence of these inhibitants or phytotoxins in the soil was undoubtedly involved in the inhibition effect of the weed on wheat growth. While after germination, the growth rate of wheat treated with the rhizospheric soils was much slower than that of the wheat growing in the yellow-top free soils.

Effect of Extracts from Yellow-top Plants and Soils on Wheat

The ethanol extract of yellow-top rhizospheric soil inhibited the growth of wheat roots, sprouts, and fresh weight of roots and seedlings ($F_{5,12}=3.58$, $P<0.05$; $F_{5,12}=4.11$, $P<0.05$; $F_{5,12}=8.26$, $P<0.05$; $F_{5,12}=9.19$, $P<0.05$) significantly. The inhibition rate on wheat seedlings increased as concentrations of the extract was increased. The inhibition rate was much higher on the growth of seedlings than that on the roots. The highest inhibition rate was 15.6 percent on the length of roots and 35.85 percent on the length of seedlings at the concentration of 5 g/ml.

The aqueous extracts of yellow-top plants inhibited the growth of wheat roots, seedlings and fresh weight of roots and seedlings ($F_{4,10}=75.8$, $P<0.05$; $F_{4,10}=46.0$, $P<0.05$; $F_{4,10}=89.2$, $P<0.05$; $F_{4,10}=73.7$, $P<0.05$) significantly. The inhibition effect increased as the concentration of the weed extracts was higher. The aqueous extract from *F. bidentis* not only suppressed the growth of the roots, but also affected the morphology of the roots and the seedlings. The number of hairy roots of treated wheat was reduced remarkably; the color of the top section of the roots was changed into brownish; and the above-ground parts of the seedlings became yellowish.

CONCLUSION AND DISCUSSION

The widespread distribution of YTW has received much attention of the researchers in China. In a new ecosystem, due to a lack of natural enemies, *F. bidentis* can spread quickly. It has been speculated that allelopathy may play an important role on the invasion and dominance of *F. bidentis*. The experiments in this study were designed to evaluate the allelopathic potential of *F. bidentis* and the results showed that its rhizospheric soil and aerial part extracts inhibited the germination and growth of wheat. Indeed, results from the experiments on *F. bidentis* also revealed that this invasive plant could release chemical compound which strongly suppressed the growth of wheat. So further investigations are needed to isolate, purify and identify the key allelochemicals, and to examine the phytotoxicity of these wheat-inhibitive compounds. These investigations are on going and the results will be reported and discussed elsewhere.

In the present study, extracts from the weed rhizospheric soils and dried weed plants were obtained and tested, but that from living plants was not assayed and compared because the living plants were not available during the time of the experimentation. However, the allelopathic effect of living plants at different growth stages are required to be evaluated because this may help understanding the dynamical changes of the allelopathy of the weed during the growing seasons.

YTW has a strong allelopathic effect on wheat, and perhaps on other native species, through exudation of inhibitive or toxic compounds to the environment (*e.g.*, soils). This is probably the major mechanism for its invasion and quick establishment of populations in the new habitats or ecosystems.

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Alien Invasive Weeds and their Management in China

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ABSTRACT

Surveys were carried out in 15 provinces of China and the numbers of alien invasive weeds in different provinces (from 22 to 114 species) were recorded. The total recorded alien invasive weeds in China were 188 species, but this number is estimated to reach about 300 when census observations in all the provinces are completed. The most important 23 alien invasive weeds in China are listed in this paper. A large proportion of the alien invasive weeds in this country originated in America and Africa and some were from Europe. Many species arrived in China accidentally, through packing materials, hay, animal fodder, or on the ballast of ships, while others were purposely introduced as ornamentals, forage, or medicinals. The alien invasive weeds in China, depending on the species, cause great damage or economic loss of cultivated crops, forests, or biodiversity in ecosystems. Many alien invasive weeds share the same characteristics, such as rapid growth in a variety of conditions, abundant seed production, highly successful seed dispersal, germination, and colonization, the ability to propagate vegetatively, a lack of pests or pathogens acting as natural controls, the ability to out-compete native species, and a high cost to remove and control. The methods of alien invasive weed management used in China include legal quarantine and prevention, mechanical and chemical methods, biological control and utilizing beneficial aspects of some alien invasive weeds. With respect to biological control of using plant pathogens, the authors have obtained promising fungal biological control agents for use against each of the four most important and widespread alien invasive weeds in China: alligatorweed (Alternanthera philoxeroides), crofton weed (Eupatoriium adenophorum), water hyacinth (Eichhornia crassipes) and shamrock (Oxalis crymbosa). The achievements of these biological control studies are briefly outlined in this paper.

Key words: alien invasive weeds, provincial surveys, integrated control, biological control with pathogens, mycotoxins.

The most troublesome weeds are alien species that have invaded China from other regions of the world. By comparison, fewer and less aggressive weeds are native species. The distinction between alien plants and native species is not always clear, and it is not easy to measure the impact of non-native or alien weeds on the native vegetation. The alien or non-native weeds are usually regarded as those that have been brought to China by human activities since the beginning of the nineteenth century, while native plant species are those that originated in China or were introduced by various means before the last century. The term alien invasive species can be seen more and more frequently in scientific literature in recent years in China, indicating that the economic and ecological significance of the alien invasive organisms have been recognized and the studies of these species are given increasingly greater attention by biologists, ecologists, plant protection experts and workers in other related scientific fields.

ALIEN INVASIVE WEED SPECIES IN CHINA

A number of surveys of alien invasive plants in many provinces have been conducted as baseline research in China and some results from the reported surveys are presented in Table 1 which lists the number of alien invasive species for each province surveyed. The references in Table 1 provide the names of the alien invasive weeds that were recorded in the provincial surveys. Xu, Qiang & Han *et al.* (2004) summarized the reports before the year 2004 and they concluded with a list of 188 species of alien invasive plants in China. However, the number may be more than 250 species if we take into account the surveys made after the year 2004 and will reach nearly 300 species when the remaining 15 provinces in China are surveyed. Further investigations are required to provide more reliable baseline data for alien invasive plant species, especially in provinces where the surveys have not been conducted.

TABLE 1.
Alien Invasive Weed Species in Different Provinces of China

Province	Number of Species	Number of Genera	Number of Families	Reference
Beijing	66	46	18	Che, 2004
Henan	30	26	13	Tian, Li & Xu *et al.*, 2005
Anhui	55	44	23	Chen, Shu & Zhang, 2004
Jilin	38	-	-	He, Wang, Sheng *et al.*, 2004
Liaoning	51	35	17	Qi & Xu, 2006
Shandong	65	46	22	Yi, Li & Qiang, 2005
Shanxi	41	35	19	Shi, Xie & Wang, 2006
Sichuan	>50	-	-	Zhou, Zhang & Chen, 2007
Chongqing	46	39	21	Shi & Tian, 2004
Guizhou	>30	28	13	Tu, 2002
Yunnan	75	-	23	Xu & Lu, 2006
Guangxi	114	80	36	Xie, Wang & Tan, 2007
Hubei	65	41	24	Liu & Qing, 2004
Shanghai	22	-	-	Qin, Yu & Jiang *et al.*, 2007
Inner Mongolia	22	-	-	Hars, Jin & Shuya, 2007
Total	188	62	38	Xu, Qiang & Han *et al.*, 2004

Among the recorded alien invasive weeds, some species are economically and ecologically much more important and invasive than others. The most widespread and damaging alien invasive weed species are listed in Table 2, together with their supposed origin and distribution in China.

TABLE 2.
Twenty-two Most Important Alien Invasive Weeds in China*

Scientific name	Family	Origin	Distribution
Alternanthera philoxeroides	Amaranthaceae	South America	S, SW, SE China, Henan, Hebei, etc.
Eichhornia crassipe	Pontederiaceae	Brazil	S, SW, SE China, Henan, Hebei, etc.
Spartina alterniflora	Gramineae	North America	Zhejiang, Jiangsu, Shanghai, etc.
Spartina anglica	Gramineae	North America	S. China sea coast provinces
Ambrosia artemisifolia	Compositae	North America	Tianjin, Hebei, Henan, etc.
Ambrosia trifolis	Compositae	North America	Henan, Hebei
Mekania micrantha	Compositae	North America	Shanghai, Hongkong, S. China
Eupatorium adenophorum	Compositae	Mexico	Sichuan, Yunnan, Chongqing, etc.
Eupatorium odoratum	Compositae	Africa	Guangxi, Hainan, Sichuan, etc.
Solidago canadensis	Compositae	North America	Zhejiang, Yunnan, Gansu, etc.
Xanthium spinosum	Compositae	South America	Southern China provinces
Lolium temulentum	Gramineae	Mediterranean	All provinces except Tibet
Sorghum halepense	Gramineae	Mediterranean	Chongqing, Sichuan, Fujian, etc.
Alternanthera retroflexus	Amaranthaceae	Africa	Chongqing & southern provinces
Conyza canadensis	Compositae	North America	Throughout China
Oxalis crymbosa	Oxalidaceae	America	Sichuan, Chongqing, etc.
Amaranthus spinosus	Amaranthaceae	Tropics	Henan, SW & SE China
Anredera cordifolia	Basellaceae	South America	Beijing, Jiangsu, Zhejiang, etc.
Daucus carota	Umbelliferae	Europe	Sichuan, Guizhou, Zhejiang, etc.
Bidens pilosa	Compositae	Tropical America	Chongqing, Hubei, Hunan, etc.
Galinsoga parviflora	Compositae	South America	Yunnan, Guizhou, Sichuan, etc.
Avena fatua	Gramineae	Mediterranean	Throughout China

* Note: The importance of these weeds is determined according to their distribution range, harmfulness to agriculture, forests, the environment, or ecosystems, etc.

DISTRIBUTION, DAMAGE AND IMPORTANT BIOLOGICAL CHARACTERISTICS OF AI WEEDS

A large proportion of the alien invasive weeds in China originated in America and Africa and some originated in Europe (Table 2). Many species arrived in China accidentally, through packing materials, hay, and animal fodder, or on the ballast of ships, while others were purposely introduced as ornamentals, forage, or for medicinal use (Liu, Dong & Shi *et al.*, 2006). Many of the purposely introduced species escaped from cultivation and due to rapid change of land use, spread widely. This continued change in land use, coupled with the spread of the introduced species, has made it possible for invasive species to increase their distribution range, sometimes to the extent that they crowd out native species and threaten natural habitats.

The alien invasive weeds in China caused immeasurable damage and economic loss to cultivated crops, forests, and biodiversity in ecosystems, depending upon the weed species. Some species decrease crop yields, cause losses in forest production, interrupt waterway transport, destroy flora of certain habitats, or adversely affect the health of human being and animals. Some alien invasive weeds can be harmful in more than one of these adverse effects. Thorough assessment of losses due to alien invasive weeds as a whole is presently lacking in China. The annual economic loss would be about 500 billion Chinese Yuan by conservative estimation according to investigations of some alien invasive weeds in a few provinces (Wan & Guo, 2005; Zhou, Zhang & Chen *et al.*, 2007).

The alien invasive weeds found in China are trees, shrubs, vines, and herbaceous plants, including wetland species. While most species are terrestrial, some are aquatic, and a few are both terrestrial and aquatical. These species flourish in a diverse number of habitatsthroughout China. Many of these species share the same characteristics, such as rapid growth in a variety of conditions, abundant seed production, highly successful seed dispersal, germination, and colonization, the ability to propagate vegetatively, a lack of natural enemies, and the ability to out-compete native species, as well as the high cost of removal and control (Sang, 2003; DiTommaso, Lawler & Darbishire, 2005; Sakai, Allenodorf el al., 2006; Xu, Li & Lu, 2006).

Although some species that are considered invasive may be beautiful, they can pose a threat to native vegetation and natural habitats. In most cases, a native plant with many of the same characteristics may be available to replace the invasive species. Many people choose non-natives for the beauty of the plant, plus many of these alien plants are hardy, disease free, and easily propagated with few if any insect pests. Unfortunately, these characteristics make them serious competitors when they are released into a new ecosystem or habitat.

STRATEGY AND MEASURES TO PREVENT AND CONTROL ALIEN INVASIVE WEEDS

Effective management of alien invasive weeds in natural and semi-natural systems is undoubtedly needed if we are to prevent their enormous impacts and subsequent economic losses. An integrated approach involving the combined use of a range of methods is usually necessary to control alien invasive weeds effectively (Cao, Guo & Zhang *et al.*, 2004; Liu & Wu, 2006). The various methods that are used in China are usually classified in five categories: 1) legal quarantine and prevention; 2) mechanical methods (*e.g.*, felling, burning, hand-weeding, hoeing, and other removal methods, etc.); 3) chemical methods (*e.g.*, using selective and environmentally safe herbicides and pesticides);

4) biological control (*e.g.*, using species-specific natural enemies (parasites, predators and pathogens) collected from the alien plant's country of origin, etc.); and 5) making use of a beneficial aspects of some alien invasive weeds (*e.g.*, weeds are collected for using as livestock food, green manure, or for extracting active biochemicals for medicine or pesticides).

Strategies for integrated weed management depends on the species under consideration (*e.g.*, features of individual species and the number and identity of species that occur together), features of the invaded systems, the availability of resources and other factors (Wan & Guo, 2005). To prevent transmission and spreadof potential alien invasive weeds into new areas, legal quarantine procedures have to be used. Mechanical and chemical control methods are short-term activities, but they are often effective methods for common emergency treatments. Rigorous and disciplined follow-up use of artificial weeding or chemical herbicides and rehabilitation are necessary in the medium term weed management. Biological control measures can provide effective control in the short and medium term in some cases, and they are usually the only really sustainable solution in the long term. The uses of alien invasive weeds as organic composts, or as raw materials to make animal fodder, medicine or phytopesticides are the by-products of weed management in China and this can help strengthen weed control in some cases.

BRIEF REPORT ON OUR STUDIES OF BIOLOGICAL CONTROL OF ALIEN INVASIVE WEEDS USING FUNGAL PATHOGENS

In recent years, we have been studying weed diseases in order to find fungal pathogens for controlling alien invasive weeds. The target plants are the four most important alien invasive weeds in China,, alligatorweed (*Alternanthera philoxeroides*), crofton weed (*Eupatoriium adenophorum*), water hyacinth (*Eichhornia crassipes*) and shamrock (*Oxalis crymbosa*). Progress has been achieved because potentially useful fungal biological control agents have been found for each of these weeds (Table 3). All of these fungi have been studied in detail and some results have been published (*e.g.*, Tan, Li & Qing, 2002; Zhou, Tan & Tian el al., 2007; Yin & Tan, 2008; Tan & Gu, 1995).

TABLE 3.

Potential Biological Control Fungi Selected for Four Target Alien Invasive Weeds

Target weed	Fungal pathogen	Weed disease	Potential for biological control
Alligator weed	*Colletotrichum gloeosporiodes* *Fusarium philoxeroides*	Anthracnose Fusarium wilt	Medium High
Water hyacinth	*Alternaria* sp. *Colletotrichum* sp.	Black spot Anthracnose	High Medium
Crofton weed	*Mycosphaerella* sp.	Leaf brown spot	Medium
Shamrock	*Cercospora* sp.	Leaf blight	High

The investigations and results are briefly outlined as follows:

1) All the diseases and pathogens of alien invasive weeds listed in Table 3 were described in detail. The two species of fungi on alligatorweed have been taxonomically named, but species names of the four fungi from the other three alien invasive weeds were not given yet, as their identification and taxonomy are still under investigation.

2) The in vitro growth conditions were tested and the best nutrient media, temperature, pH values, lighting schedule, etc. for fungal growth and sporulation were determined from these tests. Further experiments will be conducted to optimize conditions for mass production.

3) All of these fungi showed strong pathogenicity to their own host weed and they are highly specific as shown by pathogencity and host range tests. They did not infect all the plants tested (20-40 different crops and weeds) and could be safe if they are used as biological control agents in weed management.
4) All the fungi caused diseases through the mechanism of producing mycotoxins. Raw mycotoxins from Fusarium philoxeroides on alligatorweed, Alternaria sp. on water hyacinth and Mycosphaerella sp. on crofton weed were obtained through extraction experiments. These raw mycotoxins were also highly pathogenic (toxic) and host specific. Further investigations will be done to purify the toxins, to find out their molecular structures, to modify the molecules for improving their toxicity and biological control efficacy, and to study the genetic background of toxin production in the fungi.
5) Greenhouse and some field-plot experiments were carried out to demonstrate control efficacy of these pathogens and some mycotoxins. All the fungi and their toxins were able to induce serious diseases after inoculation, but the efficacy of weed control varied.
These results indicated the biological control potential for respective alien invasive weeds (Table 3). The potential of the three pathogens are high and thus are very promising for use in weed biological control. The other three pathogens have limited potential and are less promising. Further and extended experimental tests on biological control efficacy of these pathogens and mycotoxins are still needed.

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SESSION FOUR
PHYTOPATHOGENS
AND
BIOPESTICIDES

Endophytes Influence Growth, Competition and Protection of an Invasive Plant

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ABSTRACT

Invasiveness may be the result of 'novel weapons' that an invasive plant employs against evolutionary naive neighbors in its invaded range. According to this hypothesis, the same weapons are neither novel nor effective in the plant's native range because its neighbors there are adapted to its weapons. For example, much evidence indicates that invasive *Centaurea* spp. possess novel weapons because native plants from their invaded ranges (*e.g.* North America) grow more poorly in their presence than adapted plants from their native range (*i.e.* Eurasia). Although allelochemicals have been proposed as the putative weapons, he described results from pot experiments indicating that the only novel weapons possessed by *C. stoebe* are fungal endophytes. Endophytes inoculated into *C. stoebe* plants reduced the growth of the native neighbors (*i.e. Festuca idahoensis*, Idaho fescue) of its host, but increased the growth of adapted neighbors (*i.e. F. ovina*, European fescue). Competitive advantage of *C. stoebe* over *F. ovina* was significantly increased by five of the six most common endophytes, whereas endophyte-free *C. stroebe* had no greater competitive advantage pver *F. ovina.* Given the relatively high isolation frequencies of endophytes from seed, these novel weapons could easily have been co-introduced into their invaded range with their host.

Problems and Possible Solutions in Detection of Invasive *Phytophthora* Species that Threaten Forest Ecosystems

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ABSTRACT

Research on *Phytophthora* started as early as 1876 with the identification of the potato late blight pathogen (*P. infestans*). Over sixty species were recognized until 1996. However, as an outcome of surveys of natural ecosystems and nurseries the number of the newly recognized species increased considerably during the last decade. In addition, more practical tools became available such as Polymerase Chain Reaction (PCR), *Phytophthora* test kits and selective media which improved considerably the detection of new species (Erwin & Ribeiro, 1996).

Soil surveys in Europe and the United States demonstrated that a variety of unknown species exist (Balci *et al.*, 2007). The emergence of *P. ramorum* in forest ecosystems, parks and nurseries in Europe and California in the United States further provoked explorations of different environments for possible new *Phytophthora* species. Some of the newly described species appear to be adapted to specific site conditions, whereas others were widely distributed. However, their role in plant health is largely unknown and presents a challenge to manage the diseases caused by this group of pathogens.

Recent advances in *Phytophthora* research have demonstrated the ability of *Phytophthora* species to sporulate on foliage and spread aerially also in temperate forest settings. A number of *Phytophthora* species remained undetected because necrotic lesions on foliage associated with *Phytophthora* mimic lesions caused by a variety of other organisms. This also provides a major problem to detect *Phytophthora* based only on symptoms. The majority of plants with foliar infection remain alive or symptomless unless conditions are favorable for the pathogen to increase its inoculum. Hence, the foliar phase provides opportunities for the pathogen to spread to new areas undetected and build up inoculum to support an epidemic. In epidemic areas of California, certain evergreen plants were favoring sporulation of *Phytophthora* and remained unaffected by the pathogen further underscoring the critical role of the foliar phase in disease spread and incidence. Further intensive nursery surveys for *Phytophthora* demonstrated the presence of numerous species associated with soil and foliage of ornamentals including *P. ramorum*, indicating the pathway of introduction for exotic *Phytophthora* species.

Among the forest damaging *Phytophthora* species, perhaps the most notorious ones are *P. cinnamomi*, *P. ramorum*, *P. citricola*, *P. lateralis* and *P. alni*. Among these species, with exception of the limited impact of *P. citricola*, all other species cause extensive damage on certain forest trees in natural ecosystems. *P. cinnamomi*, *P. ramorum* and *P. citricola* are associated with numerous hosts around the

world, whereas *P. lateralis* and *P. alni* are restricted by host and geography. Their wide-spread distribution and occurrence provides some evidence about their ecology and impact. For instance, the Mediterranean climate type overlaps with the areas where *P. cinnamomi* and *P. ramorum* cause extensive damage in forest settings. In the field, lesions caused by *P. cinnamomi* have been shown to be limited with lower temperatures on infected oaks in France. Further, in the eastern United States, *P. cinnamomi* was restricted in soil to the northern 40° latitude, whereas for *P. citricola* no such limitation was evident (Balci *et al.*, 2007). *P. citricola* caused damage above this latitude where extreme winter temperatures are present. This further supports that pathogenesis of some species of *Phytophthora* is closely linked to environmental constraints. In addition, a different behavior was observed in areas where they spread. *P. ramorum* is mostly considered an pathogen of ornamentals in several countries of Europe, whereas, in coastal California in United States it is associated with wide-spread tree mortality. This behavioral difference further provides a challenge to the accuracy of disease prediction maps, which are mainly based on host type and optimum environmental conditions. Key epidemiological features that lead for an epidemic caused by a *Phytophthora* remain still unresolved.

Nurseries repeatedly have been shown to provide the main source of spread of various *Phytophthora* species. Thus, any management practice for *Phytophthora* disease should focus on reducing the movement of infected plant material. However, this is extremely difficult if we consider species of *Phytophthora* that can infect numerous plants. Unfortunately, the most damaging species of *Phytophthora* were those that cause damage on numerous hosts. In a *P. ramorum* epidemic area, evergreen plants were demonstrated to contribute to the inoculum buildup and expansion of the pathogen. Thus, plants that favor the sporulation of *Phytophthora* should perhaps be the main focus of management practices in nurseries. In this respect, *Rhododendron*, *Azalea* and *Viburnum* come into consideration while these plants are among the most widely marketed ornamentals.

Finally, in order to reduce damage caused by species of *Phytophthora* in forest and urban ecosystems, movement of plant and soil material should be reduced or regulated. Some management options are: 1) the use of seeds and germplasm for propagation purposes of exotic plants; 2) screening target plant species for *Phytophthora* to monitor the movement of species that exist; and 3) adoption of gardening practices such as natives instead of exotics. Monitoring certain plant species for *Phytophthora* under nursery and natural settings seems critical to manage future *P. cinnamomi-* or *P. ramorum*-like organisms before they become established.

Keywords: *Phytophthora ramorum*

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Phytophthora spp. in Oak-azalea Forests of Southwest China

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ABSTRACT

To investigate the diversity of *Phytophthora* in oak-azalea forests of southwest China, a preliminary survey was undertaken during the rainy season of 2004, 2005 and 2006 in Yunnan and Sichuan provinces. Six locations were established to detect *Phytophthora* spp. in forest streams and 350 symptomatic plant tissue samples were collected. Rhododendron leaves were placed in mesh bags and secured in watercourses for 7- to 10-day intervals from July to September to bait for *Phytophthora* species. Symptomatic plant tissues were plated on CARP media after collection, and then transported to and processed in the laboratory for sub-culturing and DNA extraction using a standard CTAB-based extraction protocol. All DNA samples were further purified and amplified using the ITS 6 and 7 rDNA primers developed specifically for use on oomycetes. While *P. ramorum* was not immediately identified from these samples, a total of 163 *Phytophthora* isolates were obtained, and further work on species identification and biological features is underway.

Keywords: *Phytophthora* spp., oak-azalea forests, southwest China

Pine Rust and International Quarantine

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ABSTRACT

Approaches to accurately predict potentially invasive forest pathogens could help avoid enormous environmental and economic impacts to forest ecosystems.

In the Northern Hemisphere, the invasive forest diseases are coniferous rust fungi pathogen consisting of a group of heteroecious host specific pathogens that have coevolved with their hosts. They require two different specific hosts to complete their life cycle. Rust phytogeography is diverse in North America and East Asia floras and tree rusts are among the most complicated taxa in the world (Chen 2003). Recently, the author has identified 3 major tree rust species and many subspecies. Twelve pine species and 24 *Ribes* species are involved in white pine blister rust life cycles in the US, Canada, China, Japan, South Korea, Pakistan, Ireland and Switzerland. Twenty-seven pine species and 28 oak species are susceptible to Pine-oak rust in North America and East Asia. Limb rust is distributed only in the U. S. A. and Canada. This rust infected 7 pines with a number of associated alternative hosts on Scrophulariaceae genera (Chen 2008).

In this paper, overviews of white pine blister rust, pine-oak rust and limb rust are presented, as well as their phylogeny, taxonomic position, host range; geographic distribution patterns are identified, respectively. The primary goal typically is to unveil ecological, behavioral, or evolutionary features of the pine rust themselves.

Sustainable Management of the Pine Sawyer (*Monochamus alternatus*) and Pine Wood Nematode (*Bursaphelenchus xylophilis*)

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The pine sawyer (*Monochamus alternatus* Hope), and pine wood nematode (*Bursaphelenchus xylophilus*) are very important pests in south China. Thousands of pine trees died from their injuries. The local ecological environment, natural scenery and economy have been threatened by these pests. Biologically, there is an intimate relationship between the two harmful pests. The pine sawyer not only damages pine trees directly, but also transports the pine wood nematode, which causes pine wilt disease. Generally, the pine sawyer transports the nematode in two ways, by adults at feeding and by female adults at ovipositing. However, transportation by humans could be responsible for spread of the nematode as well.

The pine sawyer often produces 1-3 generations but usually 2 generations every year in Guangdong province, China. Furthermore, the adults are always found in the forest all year (Song *et al.* 1992). In general, the pine tree will die within 40 days and the whole pine forest will be destroyed in 3-5 years after infestation with the nematode (Huang *et al.* 2006). In order to have sustainable management of the pests, it is necessary to understand the relationship between the nematode, the pine sawyer, and human activity. To achieve this goal, the authors studied systematically and constantly for ten years, and we present here the results.

Early diagnosis of pine wilt disease

Based on the knowledge of occurrence and epidemic regulations of the pine wood nematode, the study about early diagnosis of pine wilt disease was carried out.

There are 3 methods for early diagnosis:

1. Diagnosis by the appearance of the dead pine trees in the field except those that died of *Dendrolimus punctatus* Walker, *Hemiberlesia pitysophila* Takagi and *Hylobitejus xiaoi* Zhang. Sampling of the dead pine wood and separating the pine wood nematode.

Sampling of the dead pine trees directly and separating the pine wood nematode immediately.

Catching the adults of *M. alternatus* with the traps of A-3 sawyer beetle attractant, and isolating nematodes from the body of beetles.

2. Control and monitor the adults of *M. alternatus* by attractant

"A-3 attractant" and "YB-50 beetle trap" were invented based on a series studies on the special volatile materials of *M. alternatus*, healthy and dying pine trees (Huang 2004). The attractant is composed of terpenic compounds from plants, which do not harm humans and the environment, but can attract adult female beetles both before and after laying eggs. The attractant and traps were widely used in control and monitoring adult *M. alternatus* in 12 provinces of China.

The data showed that the A-3 attractant can monitor activity rhythm, the peak of eclosion and population density of the adult beetle. The population of *M. alternatus* was reduced 11.7 percent after using the attractant. The peak period of beetles caught by traps was April to June, which caught 85.6 percent, 71.5 percent, 93.2 percent of the totals for the year in 2002, 2003, 2004 respectively (Li, *et al.* 2006a; Li 2006b).

The National Standard of Forest Industry "Standard of Using *M. alternatus* Attractant" was founded based on our experiments. Some important guidelines were developed as follows:

Time of attractant application: Between the first to the last adult *M. alternatus* eclosion (March to October) in the central area of Guangzhou, where two generations of *M. alternatus* were observed, and during April to September in the area along the Yangtse River where *M. alternatus* produces only one generation.

Density of traps: For monitoring purposes, place one trap every 0.5hm^2; for control purposes, place one trap every 0.2hm^2- 0.3hm^2, and keep 80m–100m between traps.

3. Control pine sawyer beetles by releasing *Dastarcus helophoroides*

D. helophoroides spreads widely in the pine forest, its natural parasitic rate varied with the emergence time of sawyer bettles and their damage extent. Under natural conditions, in a single Chinese red pine tree, *D. helophoroides*' parasitic rate to larvae of pine sawyer can be up to 79.1 percent (Huang *et al.* 2003).

In Guangdong, *D. helophoroides* produces over two generations per year, their generations obviously overlapping, different stages are present in forest year round. Emergence of *D. helophoroides* occurs from July to September, oviposition period persists for 10–15 days, 5–6 days later, larvae turn into pupae and the latter persist for 15–33 days (Wang 2004). A single larva of pine sawyer can be parasitized by up to 30 *D. helophoroides.* If the parasites amount to only 1–3 *D. helophoroides* per sawyer larva or pupae, they can grow successfully.

D. helophoroides were released in the pine forest on Zhongling Mountain, Tianhe Borough, Guangzhou in July, 2005. The survey suggested a parasitism rate of 29.23 percent.

In octuber, 2005, a biological method was developed to control *M. alternatus* in Huangpu borough, Guangzhou. Wilted pine trees were covered with mesh. Several holes (0.5cm in diameter) were drilled in the net. The holes are big enough for *D.helophoroides* to pass in and out freely but small enough to prevent adults of *M. alternatus* from passing through. A survey 30 days later suggested a parasitism rate of 41.67 percent.

4. Cleanup epidemic trees and extinguish pests in woodlands

Biological characteristics and emergence of *M. alternatus* were studied systemically in Guangdong Province. Three times per year, from January to February, July, and from Octuber to November, wilted pine trees were removed from woodlands. Wilted trees, together with branches (those exceeding 1.0cm in diameter) of healthy trees were piled and enclosed in bags made of plastic film (0.8mm thick). Aluminium phosphide pellets used for fumigation were placed inside the bags at a dose of 20g/m^3 and kept in the bags for 48 hours.

5. Utilize epidemic trees harmlessly

In order to utilize infected trees harmlessly, a wood processing plant was built in Baiyun borough, Guangzhou. Wood is heated to 56°C. for 30 minutes in the heat-treatment factory. Logs from the infested forest and clear cut woods can be shipped out for use after treatment.

6. Modificate structure of pine forest

The relationship among pine forest, pest and the environment were studied. Based on a series of construction projects (construction of public forest, construction of amenity forest, etc.) in Guangdong, structure of pine forests were modified to improve their resistance to pine wilt disease. Thus, native broadleaf trees were replanted or interplanted in the pine forests, the reconstructed pine forests have a higher resistance to pine sawyer and pine wilt disease.

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Pine Wilt Disease: First Recognized Case of a Plant Disease Induced by a Mutualistic Nematode-bacterial Symbiosis

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ABSTRACT

Pine wilt disease has become a worldwide threat to pine forests. It was widely believed that *Bursaphelenchus xylophilus*, the pine wood nematode (PWN), was the only pathogen involved. Our recent work, however, indicates that the bacteria symbiotically associated with the nematode play a key role in the disease. We conducted a series of inoculation experiments. The results show that systemic toxins produced by the pathogenic bacteria associated with PWN cause pine wilt disease and tree death. This is the first experimentally confirmed plant disease induced by a mutualistic nematode-bacterial symbiosis. This new model of pine wilt disease will improve our knowledge of its molecular aetiology and increase our understanding of the symbiotic relationship between nematodes and associated bacteria.

Rapid Detection of Wood Biological Decay by Near Infrared Spectroscopy*

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ABSTRACT

The use of near infrared spectroscopy (NIR) coupled with multivariate data analysis (MVA) to detect wood biological decay was investigated in this paper. The results show that NIR spectroscopy coupled with pattern recognition of MVA could be used to rapidly detect biological decay in wood; the discriminant accuracy by the NIR model based on the training set for the non-decay, white-rot (*Trametes versicolor* (L: Fr.) Pilat (Madison R-105)) and brown-rot decay (*Gloeophyllum trabeum* (Pers.:Fr.) Murr. (Madison 617)) samples were 100 percent; and that of the samples (non-decay, white-rot and brown-rot decay samples) in the test set were 100 percent. The results suggested that near infrared spectroscopy (NIR) coupled with MVA can be used to detect and discriminate wood biological decay.

Keywords: near infrared spectroscopy (NIR), multivariate data analysis, wood biological decay, detection

Application of Toxic Mushrooms for Forest Disease Control

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ABSTRACT

The inhibiting effects of fungal extracts obtained by different extraction methods from culture fluids, filtered culture fluids and mycelial ultrasonic fluids of 8 toxic mushroom strains *Amanita virosa* Lamb. ex. Secr., *Lepiota clypeolaria (*Bull. Ex Fr.) Quil., *Lactarius vellereus* (Fr.) Fr., *Amanita pachycolea* Stuntz, *Amanita* sp., *Ramaria ephemeroderma* Sacc. *et* Syd., *Clitocybe dealbata* (Sow. ex Fr.) Gill., *Lepiota cristata* (Bolt:Fr.) Quel. on three of pathogens *Cytospora chrysosperma* (Pers.) Fr. (pathogen of poplar canker), *Alternaria alternata* (Fr.) Keissler (pathogen of poplar leaf blight), and *Sphaeropsis sapinea* Kieks (pathogen of pine shoot blight) were studied. The results show that all extracts have definite inhibiting effects on three of the pathogens' growth and germination.

*Amanita viros*a had the best inhibiting effect on *Cytospora chrysosperma* growth and spore germination. The growth-inhibiting rate of the extract extracted by alcohol at 50˚C. 2h from stationary mycelia was the highest one, at 52.05 percent. Next was the extract extracted from shaker-cultured filter fluid by heating 2h at 50ºC, the growth-inhibiting rate is 50.30 percent. The extracts showed evident inhibiting effects on *Cytospora chrysosperma* conidia germination , in which conidia had not germinated in 10 days, they are extracts extracted from stationary cultured fluid by dipping into n-butyl-alcohol or ethyl acetate 2 days, or by ethyl acetate and heated 2 hours at 50ºC; the mycelia extracts extracted by ethyl acetate or acetone by heating 2 hours at 50ºC, and mycelia aqueous extract by ultrasonic or heating. The inhibiting substances are types of amide, which growth-inhibiting rate and germination-inhibiting rate are 100 percent. The test of feeding extract to white mice shows that the safety dosage is <2mg/mL, which is 30 times more than the actual dosage for pathogen inhibition.

Lactarius vellereus had the best inhibiting effect on *Alternaria alternata* growth and spore germination. The inhibiting effect of the extract extracted by n-butyl alcohol was the best, the growth-inhibiting rate was 78.95 percent, and the germination-inhibiting rate was 91.47 percent. The inhibiting substance is Lactarin PP, which was first found from *Lactarius vellereus*. The molecular formula is C_8H_9NO, molecular weight is 135, chemical name is 1-(2-pyridine)-2-acetone. The growth-inhibiting rate and germination-inhibiting rate of Lactarin PP are 100 percent. Through feeding extract to white mice, LD50 = 8570.8mg/kg. it was showed that the extract had low toxicity. There were no remaining toxicity on the leaves of poplar and no touch toxicity to white mice.

Ramaria ephemeroderma has the best effectic inhibition to *Sphaeropsis sapinea* growth and spore germination. The extract extracted by ethyl acetate from culture liquid had the best inhibiting effect, the growth-inhibiting rate was 100 percent and germination-inhibiting rate was 91.63 percent. The growth-inhibiting rate of he extract extracted by ethyl acetate from culture liquid of *Lepiota cristata* was 100 percent, and the germination-inhibiting rate was 84.88 percent.

Keywords: toxic mushroom, forest diseases, control

Latest Progress, Problems, Suggestions and Expectations on Bio-pesticide Research

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ABSTRACT

The general improvement on bio-pesticides at home and abroad was introduced and new improvement on bio-pesticide in China was stated. Then the differences in concept, registration and research of bio-pesticides were compared, and on these grounds questions and suggestions were proposed for the research of bio-pesticides in China. In the end the development of bio-pesticides in the future in China was expected.

Keywords: bio-pesticide, research progress, registration, problems, development suggestion, expectation

1. General progress of foreign bio-pesticide

One goal was proposed at the World Environment and Development Convention held in Rio de Janeiro, Brazil in 1992 that by the end of the 20th century, the application area of bio-pesticide would account for 60 percent of the total to replace organic synthesized pesticides. However, it was not attained. Until 2002, according to the statistics of traditional definition, the application area of bio-pesticide accounted for 15 percent, while according to the statistics of generalized definition (containing transgenic plants), the percentage would be 41 percent.

Western developed countries like the United States, attached great importance to the development of bio-pesticides, at a very fast pace. According to the data at the website http://www.epa.gov/, from 1995 to 1996, 14-15 kinds of bio-pesticides were registered in the United States. By the end of 2005, there had already been 265 kinds, 1,160 products registered, including 159 kinds of biochemical pesticides, 70 kinds of microbial pesticides, and 23 kinds of transgenic pesticides. Sales of the products reached nearly $5.8 billion.

2. General progress of China's bio-pesticide

According to the data at http://www.chinapesticide.gov.cn/ and the information published inPesticide Registration Bulletin by the Institute of Pesticide Inspection, Ministry of Agriculture, since the early 1960s, when China gradually conducted industrialized production of *Bacillus thuringiensis* (Bt) insecticide and agro-antibiotics, more than 200 enterprises had produced bio-pesticides. Fifty kinds of active components of bio-pesticides were registered in 1999, and 146 kinds in 2005 (14 percent), which accounts for 15 percent of the total active components of pesticides; 530 kinds of products accounting for 9 percent of the registered pesticide products. Annual yield of preparations reached 120-130 thousand tons, accounted for 12 percent of the total production of pesticides. The annual production value reached about 2.3 billion yuan, accounted for 9 percent of the total production value. The application area reached 10 million acres, accounted for 9-10 percent of the total application area of pesticides (excluding transgenic pesticides). Forty-five kinds of plant pesticides, 120 products; 42 kinds of microbial pesticides (bacteria, viruses and fungi), 86 products; 29 kinds of antibiotics, 165 products; 39 kinds of growth regulator and pheromone, 160 products.

3. Progress of concrete classification of China's bio-pesticides

Research progress of China's bio-pesticides was introduced as follows, centering on microbial pesticides (bacteria, viruses, fungi), agro-antibiotics, plant bio-pesticides and biochemical pesticides (such as plant growth regulator)

3.1 Bacterial pesticides

3.1.1 *Bacillus thuringiensis* (Bt)(Cai Qiliang *et al.*, 2003; Yu Ziniu *et al.*,1995)

Through the research of Bt resources, more and more new useful Bt serum type and genes have been found. Bt was introduced to China in 1959 . To date, 41 H serum types and more than 50 subspecies have been discovered, accounting for 60 percent of the number (70 serum types) discovered by the whole world. Twelve subspecies were first discovered by China, as well as 50 new Bt genes, which accounted for 12.5 percent of total new genes discovered in the world.

The yield of insecticidal crystal protein has been greatly improved by the application of modern fermentation control technology. Through the research of fermentation control technology of *Bacillus thuringiensis* conducted by a technical group led by Hubei Bt center and Huazhong Agricultural University, the fermentation level of Bt with 40-ton fermentor has been improved from 3,000–4,000 IU/mL five years ago to 5,800IU/mL (*Helicoverpa armigera*).The fermentation level of toxin protein reached 5.5–6mg/mL and the production cost of fermentation liquid /ton (1,000IU/mL) has dropped from 350 yuan to 175 yuan, dropping by 51.4 percent.

A number of specific virulent strains and new engineered strains were screened and constructed applying various modern biotechnology means (*e.g.,* plasmid elimination, protoplast fusion, conjugation transfer, gene recombination), through which the insecticidal spectrum has been amplified and the period of validity has been extended. Five new international authorized subspecies were screened and identified, like the efficient strain Bt15A3 that can control *Helicoverpa armigera,* Laphygma exigua Hubner and *Hyphantria cunea* belonging to *Bt.subso colmeri* H21; Engineered strain YFM-4 and LCG-12 were constructed to kill Lepidoptera and Coleoptera. Engineered strain WG-001 that has a highly toxic effect on *Plagiodera versicolora* and *Plutella xylostella* was approved to enter a stage of small-batch production in 2003 and could already be registered to produce in 2005.The fermentation unit reached about 5,000IU/ul .

Through fierce market competition, China's Bt technology has reached the international advanced level. China has issued national inspection standard of Bt pesticide and all of the important technical indexes reached the international standard. Insecticidal standard of *Prodenia litura* was newly added. Nowadays, 13 kinds of Bt products (excluding remixed products) have been registered. BtI.var was worth stressing that it can control mosquitos and new formulations of WG, EO were newly registered.

3.1.2 Other bacterial pesticides

Thirteen kinds of other bacterial pesticides like *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas fluorescens*, *Bacillus licheniformis* have been registered. Engineered bacteria that carried bollworm virus genes were registered; the research and development of Bacillus subtilis attracted more attention. On the basis of “Caifengning” registered by Nanjing Agricultural University, plant endogenic bacteria BS2 and BS322 that can control rice blast have been developed.

3.2 Insect virus pesticides (Liu Gaoqiang *et al.*, 2004; Chen Jinxiu *et al.*,1997; Tan Zhoujin *et al.*, 2004; Wangling *et al.*, 2004)

3.2.1 More and more new useful varieties of viruses have been found through resource development

To date, 1,690 kinds of viruses have been found from 900 species of insects, containing 43 families and 11 orders, and more than 50 virus agents including *Helicoverpa armigera* NPV, tussock moth NPV, *Plutella xylostella* GV were developed to control agricultural pests occurred in cotton, soybean, tea, apple, vegetable and forest. According to incomplete statistics, more than 50 kinds of virus pesticides were registered in the world that 8 was in America,10 in Europe, 11 in Russia and 13 in China.

3.2.2 The research is among the advanced international level

The wild type baculovirus was modified by genetic engineering technology and its insecticidal properties have been improved. For example, Scorpion toxin Aalt gene was induced into *Trichoplusia ni* virus to construct recombinant virus TnAT35. Shortened Bt crylAb gene was induced into *Autographa californica* NPV to construct recombinant virus. *Helicoverpa armigera* HaSNPV and *Spodoptera litura* SpltMNPV genome sequences were gained, which laid a solid foundation for the further study of new insect virus genes

3.2.3 Scaled production has been formed

Provisional pesticide registration certificate was granted to “*Helicoverpa armigera* nuclear polyhedrosis virus SC” and scaled production was conducted by WENJIAN biological Co., Ltd in Jingmen, Hubei. (with annual output of 100 tons. Registration number: LS20020751); The factory that covered an area of 4,000m^2 with annual production capacity of 100 tons has been built up to produce *Prodenia litura* virus pesticides with new formulations.

3.3 Fungal pesticide

3.3.1 New breakthrough in the industrialized production process of fungal bio-control agent

Beauveria bassiana spore production process with low solid waste discharge rate (only 20-30 percent) was successfully developed; the fermentation level reached 600 trillion spores / m3 medium (of ordinary light-row cultivation, the rate amounted to 90-95 percent), and spore powder content reached 130-160 billions/g.

Low-temperature vacuum drying, crushing and tornado air separation processes were determined for the production of high pollen fungal pesticides. The micro-powder agents of the fungal pesticides were developed through this process containing more than 50 billion spores/ g for *Beauveria bassiana* and 20 billion/g for *Metarhizium anisopliae*. The water content was less than 8 percent, the rate of live spores was more than 90 percent.it can be widely used in ultra-low volume spray control for forest pests; most of the product quality of the developed nonwoven agent reached Japan's Nitto Denko Product Standards (sporulation 100 million spores / cm2) and the highest yield reached 400 million spores/ cm^2, much higher than that of the Japan's Nitto Denko Product. New technology of one-step liquid fermentation was applied to produce chlamydospore of *Trichoderma*.The culture temperature was 28°C and the yield of chlamydospore reached 4.2×10^8 CFU/ml after fermenting for 96 hours.

3.3.2 The stability of fungal pesticide products was improved because of new addictives and technological means so that in recent years there has been growing number of registered fungal pesticide varieties.

Since 1990s, resin, diesel and vegetable oil were researched and applied as dispersing agents or carriers of fungal spores emulsion to improve the dispersion and adhesion ability of the spore suspension, so as to improve the product stability. Anisopliae decoy agent was produced mixing insect feeding farm and sideline products with fungi, which improved the efficacy of the fungal pesticides. Also, through transgenic techniques, the stability and instant performance of anisopliae spores against pests were enhanced.

In recent years, the number of registered fungal pesticides has been increasing from 2 kinds before 2000 to the current 8, including five fungal insecticides owing to the improved stability of formulations. New formulations, such as *Beauveria bassiana* 72 percent WP, Anisopliae DP, flotation agent and WP, *Beauveria bassiana* OL, *Trichoderma* chlamydospore WP.

3.4 Agro-antibiotic (Zhu Changxiong *et al.*,2003)

Agro-antibiotic is a kind of bio-pesticides with highest industrialization and widest use. To date, China has achieved three main progresses.

3.4.1 New varieties of agro-antibiotics with independent intellectual property right were developed through screening a number of new compounds

Since 1990s, China has reported a number of new varieties of agro-antibiotics with independent intellectual property rights. Those that have declared patents include insecticidal antibiotics, like Jietacin and Meilingmycin, and bactericidal antibiotics like Ningnanmycin, Polarmycin, Yimeimycin, Streptomyces Men-myco-93-63, Wanlongmycin and Tianshanmycin, etc. Among them, Ningnanmycin, Zhongshengmycin and Agro-antibiotic 120 have been successfully developed and reached the primary stage of industrialization.

New pesticides were synthesized through transforming the structure of the Agro-antibiotics or taking it as the lead compound. The most typical example was transforming Avermectin into Ivermectin, Emamectin or Eprinomectin, which improved the efficacy of Avermectin and reduced its toxicity; Since a series of efficient bactericides and pesticides, like azoxystrobin, kresoxim-methyl, metominostrobin, picoxystrobin had been developed abroad taking the antibiotic Strobilurin as the lead compound, In China, Shenyang Institute of Chemical Engineering also developed microbicides SYP-L190 and kresoxim-methyl and put into production by two enterprises.

3.4.2 Old varieties were re-developed to expand their use and enhance the efficacy

The re-development of old varieties were mainly focused on raising the fermentation level of production strains and exploring or modifying new formulations to further lower the production costs; For example, the production levels of Abamectin, Ningnanmycin, Zhongshengmycin have been increased by 4 to 30 times through the national scientific and technical programme. The fermentor-based production level reached AVB1 4,500mg/L, 18,000 mg/L and 11,000 mg/L and the production cost reduced 3-10 times. The formulation of Abamectin has been gradually shifted from EC to ME or EW, which improved the performance of the product and reduced the environmental pollution; Efficient AS of Agro-antibiotic 120 was successfully developed, which made the application concentration that could take effect reduce 3-4 times.

Valiolamine is an important intermediate of anti-hyperglycemia drug - Voglibose. The conversion rate of validamycins-A to valienamine and valiolamine reached 65 percent and 50 percent respectively through strain screening and mutagenesis. The fermentation level of valienamine reached 2,500μg/mL.

3.5 Botanical bio-pesticides (Cao Haiqun *et al.*,2000)

3.5.1 More and more plant species suitable to be processed into pesticides

In the recent 30 years, active components of tens of insecticidal plants, like tobacco, derris, *Millettia pachycarpa* Benth, blood rattan, croton, toosendanin, *Melia azedarach*, celangulin were studied and more than 40 kinds of plant pesticides, like matrine, saponin, nicotine, rotenone, dual-alkali, fennel, veratryl base, cevadine and azadirachtin were developed, registered and batch produced.

3.5.2 The mechanisms of plant pesticides were studied and partly were clearly determined

α- pinene, camphor, L-menthol, glycosides, acids and quinones worked on the sensory nervous system of insects, and has repellent antifeedant and enticing role. Nicotine, pyrethrum and DL- cathinone enable the presynaptic packet of the central and peripheral nervous system of insects to release norepinephrine, causing the excessive release of norepinephrine, cardiovascular and appetite suppression .Furthermore, plant pesticide has the physiological functions of interfering insect endocrine system secreting molting and juvenile hormone, causing infertility and blocking breathing.

3.5.3 Plants with bactericidal role and functions of curing viral diseases

The extracts of tobacco, tea cakes, derris and tripterygium can inhibit spore germination and growth of certain pathogens or prevent the pathogens from invading plants. Litsea Cubeba Berry Oil has good control effect on main tea diseases, like tea red rust, *Verticillium* wilt, moire blight wilt as well as cotton blight wilt and *Verticillium* wilt.

3.5.4 Some new formulations were developed and the effect of the products was improved.

Aerosol of essential oils from *Sabina vulgaris* ant was developed. On this basis, after mixing the essential oil with crude pyrethrin that can enhance the efficacy, insecticide aerosol 1#, 2# with efficient insecticidal activity against house flies were developed. They reached A and B level of hygienic insecticide efficacy evaluation 0.15 percent podophyllotoxin ME and 1.15 percent podophyllotoxin cyhalothrin ME were developed. The latter has remarkable role in adding the control effect on the 3-year larva of *Plutella xylostella*, in field, the control effect of the mixtures against *Pieris rapae* was far better than the control agent 2.5 percent Lambda-Cyhalothrin.

3.6 Plant growth regulators

From the production of antibiotic fertilizer 5,406 with indigenous method to successful development and mass application of gibberellin, the plant growth regulator plays an important role in China's agricultural production. In particular, the role of gibberellin in promoting tillering, heading and early-maturing of the double cropping hybrid rice made its demand rise rapidly and manufacturers reached over 40, which headed itself in the varieties of plant growth regulators. Newly developed plant growth regulators produced by microbes also include oligosaccharide, cytokinin, abscisic acid and activated protein.

4. Comparison between China's and foreign bio-pesticides

4.1 Differences in concept

Taking Food and Agriculture Organization (FAO) of United Nations and the Environmental Protection Agency (EPA) of United States as an example, they regard that the biological pesticides include: biochemical pesticides (no direct toxic effect on control objects, but only special role like conditioning and interference; Must be natural compound structures), microbial pesticides (fungi, bacteria, viruses and protozoa used to control grass, rodent, pest and genetically modified microbial preparations), transgenic bio-pesticide (agricultural organisms with pesticidal function after genetically modified with exogenous genes) and natural enemy bio-pesticides (commercialized non-microbial live organisms).

According to the definition stated in the text of the "pesticide registration information requirements" approved by the Ministry of Agriculture in China, biological pesticides, refers to the direct use of physiologically active substances produced by organisms or living organisms themselves as biological pesticides, as well as the synthetic pesticide with the same structure as the natural compounds, also known as organism-derived pesticides, including biochemical, microbial, botanical pesticides, antibiotics, transgenic bio-pesticides and natural enemies biological pesticides.

The main difference between the concept of biological pesticides at home and abroad lies in that in foreign countries, agro-antibiotic was listed as chemical pesticides ,plant-derived pesticides as biochemical pesticides.

4.2 Differences in registration

At present, the comparison of China's registered varieties and numbers of biological pesticides with those of United States at table 1,2,3 showed the main differences.

Table 1. Varieties of Microbial Pesticides Registered in America (2005)

Number	Active Component	Number of Active Components	Number of Products	Note
1	Bacteria	35	220	13 different Bt species
2	Engineered bacteria	8	12	mainly Bt endotoxin
3	Yeast	1	2	
4	Fungi	17	40	
5	Protozoa	1	3	microsporidia
6	Virus	8	14	
7	Agent for plant protection	23	23	Plant with Bt *cry* gene
	Total	93	314	

Table 2. Biochemical and Plant Pesticides Registered in America (2005).

Number	Active Component	Number of Active Components	Number of Products	Note
1	Flower and plant volatile matter	18	92	Most are plant oil
2	Plant growth regulator and herbicide	27	295	

Number	Active Component	Number of Active Components	Number of Products	Note
3	Pheromone	44	235	
4	RE	17	106	
5	Other biochemical preparations	25	88	Harpin and polyoxin are under this item
6	Plant pesticide	10	30	
	Total	141	846	

Table 3. Bio-Pesticides Registered in China (2005)

Number	Active Component	Number of Active Components	Number of Products	Note
1	Bacteria	11	40	Two different Bt strains were clearly indicated
2	Transgenci strain	2	2	Mainly contain boll-worm toxin gene
3	Fungi	8	18	5 insecticides
4	Virus	13	26	6 kinds of active components were registered as mix-tures
5	Antibiotics	29	165	9 insecticides, 19 bacteria, 1 herbi-cide
6	Plant pesticide	45	120	
7	Biochemical pesti-cide	22	120	Gibberellin, oligo-saccharide, brassin-olide, Harpin
	Total	17	40	

4.2.1 The difference in the classification and quantity of the effective components

It was mainly shown in antibiotics, plant volatile substances, transgenic plant protection agents. In United States, only one antibiotic adriamycin was classified as biological pesticides, and a great many vegetable oils and transgenic plants were registered as pesticides; in China, antibiotics is the main biological pesticides, while transgenic plant has not yet been registered as pesticides.

4.2.2 Differences in use and functions

It was mainly manifested in the microbial pesticide registration, In United States, microbial strains of different types, targets and functions were classified and registered respectively, highlighting their uses and functions. For example, thirteen Bt strains were registered, while only two was registered in China. The fungi registration has the same characteristics.

4.2.3 The differences in product formulations

It was mainly manifested in the differences of the research level of the formulations, such as one active ingredient of biological pesticide registered in United States can be tapped into 5.5 products with different formulations and contents on average, while only 2.9 in China

4.3 Gap in the research level

The microbial pesticide *Bacillus thuringiensis* and agricultural antibiotics were taken to show the gap in the research level between home and abroad

4.3.1 *Bacillus thuringiensis*

Compared with the developed countries, Bt industry in China lagged behind mainly in the following areas.

In application areas, Bt products in China are mainly used for agricultural economic crops, while the cost of forest protection in Canada with Bt products amounted to 20 million US dollars, so Bt market in China should be further developed. Areas of forest protection, pests control in storage and health urgently need governmental policy support, as well as the co-development by forest protection workers, Bt technical researchers and manufacturers.

In industry, nowadays, there are still redundant manufacturers in China. As for small businesses, the fermentation production levels are too low, with single preparation, small-scaled production and high cost, the majority of small businesses rely largely on commercial profit of the Bt formulations. There are only 3-4 Bt enterprises with annual production value over 30 million yuan. While foreign Bt production enterprise, Certis U. S. A., branch of Japan's Mitsubishi Corporation, Bt sales of which amounted to 30 million US dollars in 2000.

On strain and production technology, since the production strains at abroad were rich, efficient engineered strains with broad spectrum, as well as liquid fermentation technology with stuff-fed technology have been widely applied in the fermentation process. The level of fermentation was relatively high; Low-temperature spray drying process was applied in the product preparation, with high recovery rate; There are various formulations, including DP, WP, SC, dense water, EO, EC, GR, DTTB, ES, ER, bio-coating agent. While in China, most Bt production strains belong to k type, with only four formulations; batch fermentation technology is mainly used in liquid fermentation process and centrifuge concentration technology is used in post extracting process, resulting in much loss of active ingredients, like enhancer. Furthermore, China's current spray drying equipment also restrict the improvement of recovery rate.

4.3.2 Agro-antibiotic

(1) There were very few new varieties of agro-antibiotics with utilitarian role in China

In Japan, 110 new antibiotics patents were declared annually from 1980-1994 on average, six for livestock antibiotics, ranking first in the world. After the 1990s, the United States equalled or surpassed Japan in the area of new antibiotics. From a global perspective, found that the most influential new agricultural antibiotic found in 1980s were insecticidal antibiotic abamectin (avermectin) and weeding antibiotics phthoxazollin. the most influential new agro-antibiotics found in 1990s were insecticidal antibiotic spinosad and bactericidal antibiotic antibiotics strobilurin. Avermectin was developed into one of the world's best practical insecticide by Merck and several other corporations of United States. Phthoxazollin was taken as precursor compound to successfully synthesize glyphosate series, the current best herbicides; Spinocad and strobilurin were likely to be developed into the world's best bio-pesticides and fungicides. The above varieties were all found by foreign companies and successfully developed.

Since 1990s, China has also reported a number of new varieties of antibiotics with their own intellectual property rights. Among them, the insecticidal antibiotics Jietacin and Meilingmycin and the bactericidal antibiotics including Ningnanmycin, Polaramycin, Yimeimycin, etc. Now only Ningnanmycin is practically used.

(2) The ability to synthesize new varieties of pesticides taking the structure of agro- antibiotics as the lead compound was poorer than that of foreign countries. Most technologies were imitated from abroad. The most typical example is to transform the Avermectin into Ivermectin and dimethylamine Emamectin (Emamectin benzoate), which not only improved the efficacy of Avermectin, but also reduced toxicity, widening the scope and field of the application of abamectin, but the technology was first developed and reported by American Merck corporation. Domestic researchers conducted related research imitating foreign findings. In addition, taking Strobilurin as lead compound, foreign company has developed series of products. While in China, only the fungicide SYP-L190, co-developed by Shenyang institute of Chemical Engineering and Dow AgroSciences, LLC of the United States belongs to the series of new pesticides fungicides.

(3) Re-development of the old varieties of agro-antibiotics was mainly focused on improving the fermentation level and perfecting the formulation, lacking of in-depth development and creation of new agents.

The most typical example is the Validamycin, or China's Jinggangmycin, an old variety. Japan's Takeda Pharmaceutical Co., Ltd. reported the new effective components G and H of Validamycin, which is a strong trehalase inhibitor.Since flying energy of many insects is supplied by glucose decomposed by trehalase, Validamycin could be developed into a new safe insecticide through further research; Furthermore, the byproduct of Validamycin - Valiolamine is an important intermediate of anti-hyperglycemia drug – Voglibose,which has great medical value. Flint, gained through Strobilurin transformation can be used to control many diseases occurred on grapes, pears, vegetables, peanuts, oats and turf. a seed treatment agent Helix that can control soil pests was developed through the change of the formulation of Flint.

5. 5 Problems lied in the research (Wu *et al.*, 2002; Zhu 2003)

5.5.1 Inadequate and scattered investment

Through the analysis of China's industrialized products, it took at least 12-15 years from the research establishment to the approval of the product and gained three certificates. The state allocated 1-1.5 million yuan as the supporting fund. According to the highest expense of investment of national "10th five-year-plan," the total expenditure for a decade on one variety of biological pesticides was only 4-5 million US dollars. While it also needs more than 10 years to develop a new pesticide of the same species, but the total funding would reach 80-100 million dollars, and annually over 8 million dollars. Japan is at the world leading level developing biological pesticide and the total funding for one bio-pesticide research would reach 20 million.

From the biological pesticides project listed in the existing agricultural national projects, we can see that the investment was small and dispersed. During the "7th five-year-plan" period, investment for a program on average was 10,000 yuan per year and 25,000 yuan during the "8th five-year-plan" period, the "9th five-year-plan" period, 45,000 yuan, the "10th five-year-plan" period, 80,000 yuan; adding the self-financing investment and wages, the annual investment was relatively doubled.

5.2 Underadvanced research level and low transformation rate of research results

The following areas of problems or phenomena were the main reasons for underadvanced research level, innovation ability of biological pesticides ,and the research results not being recognized by market.

5.2.1 Scientific research personnel lacking of spirit of collaboration prolonged the outcome

Many scientific research units and research staff still don't have perspective of market economy, who cling tenaciously to one project; from screening bacteria to plant mid-trial to field applications, completed by their own people, unwilling to cooperate with others, which lead to very slow step into market and lengthened research cycle.

5.2.2 Small investment in scientific research caused that the fundamental study was not deep enough and key technologies cannot be broke through

Because of less funding for research, the scientific research units were unable or unwilling to conduct the study of the mechanism of the product, environmental toxicology and behavioral tests that need high input, unable to break through key technologies of the industrial production, prolonging research cycle or making the research unforeseeable.

5.2.3 Institutional issues resulted in researches lagging behind the market demand and that the results did not have market competitiveness

R & D units only emphasize academic standards and pursue SCI paper; The study objectives, directions and requirements can not be fully consistent with the needs of enterprises and markets. It is found that there are a lot of semi-finished products that has application potential, but few varieties with the commercialization condition. Only a few have been developed into industrialized varieties or dominant species.

5.2.4 Unshared information and low-level duplication in research

Different Industries, departments and units mutually blockade information between each other. They snatch limited research funds through their respective way, resulting in scattered outlay and using a small amount of funding to repeat low –level work.

6. Suggestions on bio-pesticide research (Zhu *et al.* 2000; Zhu *et al.* 2004)

A large number of problems existed during the development course of biological pesticides of China, especially in the industrialization process, which have a direct impact on the transformation of biological pesticides and urgently need to be addressed. Herewith some suggestions are put forward for reference.

6.1 We are in dire need of developing biological pesticides that can control soil pests and soil-borne diseases to fill up the blank of domestic market

Soil pests and soil-borne diseases are important in agricultural production in China, severely affecting normal growth and foison of majority of crops. Since pests and pathogens survive in the soil, it is difficult to control the harm with general chemical pesticides. Therefore, high intense toxic pesticides are always used to prevent them, which aggravated soil pesticide residues. New and effective microbial pesticides or new formulations and edaphon restoration agent urgently need to be launched to meet the needs of the field.

6.2 Pay more attention to the research on the safety of biological pesticides while developing it.

In the currently limited number of pest-controlling biological pesticides, Abamectin has been banned in green vegetables and fruit production resulting from its high toxicity. Agro-streptomycin has been restricted for the sake of human health and safety due to its usage on humanity and agriculture at the same time. Therefore, to develop new efficient and secure varieties of bio-pesticides, we need to strengthen the research on the security and environmental compatibility so that we can stabilize and maintain the competitive edge and market share of biological pesticides in green agro-products production.

6.3 Researching and developing biological pesticides need collaboration

As China attaches more importance to food security and pollution-free agriculture, there is an increasing number of organizations focusing on the research and development of biological pesticides. This is conducive to biological pesticide development. But we must pay attention to collaboration, instead of repetitive low-level research. The research and development of biological pesticides include pre-production, on-production and post-production research. Research organizations should carry out the research in accordance with their specific advantages.

6.4 Research of integrated application technology of biological pesticides need to be strengthened to promote the development of bio-pesticides

By the end of 2002, including transgenic cotton, China's annual production output of biological pesticides only accounted for 11 percent of the total output of pesticides and only 12 percent of pesticide-applied fields took use of bio-pesticides. The promotion area reaches 50 million mu annually. Only four varieties have become regular pesticides, only accounting for 3.5 percent of commercialized biological pesticides. Why there is a large number of biological pesticides being commercialized, but only a few become regular pesticides. Despite many reasons, a main one that we did not attach importance to in the past was the research of application technology of bio-pesticide.

So in the future we need to strengthen the study of field integrated application technology on the basis of strictly controlling the quality of products. For different crops and ecological zones, we should bring forward different application methods, stabilize and enhance the efficacy of commercialized biological pesticides, reduce cost and promote their sound development.

6.5 Researches of large-scale fermentation technology and formulation processing technology of some varieties urgently need to be strengthened

According to statistics, there are currently about 200 enterprises in China producing biological pesticides and more than 140 kinds of commercialized effective components of biological pesticides , among which only four that the annual output value reached over 100 million yuan , eight that reached 10 million yuan, 13 that reached 10 million yuan. The main reasons that bio-pesticide cannot realize large-scale production lie in that the level of product formulations was low with poor stability and no breakthrough was gained in key technologies of the scaled production process, such as fermentation and preparation processing technology, resulting in high cost and lack of market competitiveness. Moreover, different types of biological pesticides have their specific differences: for example, because the virus can not be cultured in vitro , a large number of insects need to be fed during the production of the virus biological pesticides, which restricts large-scale production. Fungal biological pesticides, because no breakthrough has been gained to cultivate numerous resilient spores, the product shelf life and stability cannot meet pesticide registration requirements, resulting in some difficulty in large-scale production. For botanical pesticides, which need a large number of plants, large-scale production will be subject to the land, vegetation and ecological protection, and other restrictions; as for transgenic plants biological pesticides, the safety evaluation problem has also affected the investment interest of most entrepreneurs.

Moreover, most of the biological pesticides are live organisms or their metabolites. There are many variable factors in the production process, so in the production and amplification process, the test of large-scale industrial production and relatively long time must been gone through to find the key technologies to the large-scale production, and tackle problems and perfect through research.

Therefore, the key industrialized production technology of biological pesticides and research and breakthrough of the product stability and security technology not only became a bottleneck that determine whether large-scale industrialization of biological pesticides can be conducted in the future, but also a key point where scientific researchers and entrepreneurs should strengthen cooperation.

7. Expectations

7.1 The development of bio-pesticide will embrace great opportunities in the future

After China's entry into WTO, the international trade in food and agricultural products, in the face of harsh pesticide residue standards, will strictly limit the application of highly toxic pesticides, which provided great opportunities for the development of biological pesticides.

7.2 Biological pesticides have a great potential for development

At present, the bio-pesticides in the international market (excluding agricultural antibiotics and genetically modified plants) account for only around 2.5 percent that of total pesticide market share, among which Bt insecticide accounts for over 90 percent. In China, Bt insecticide only accounted for 2 percent of the market *Helicoverpa armigera* virus pesticides accounted for 0.2 percent, agricultural antibiotics accounted for 9 percent, botanical pesticides 0.5 percent. According to the common view of the experts at home and abroad, in the next 10 years, bio-pesticides will replace more than 20 percent of chemical pesticides. Therefore, the potential for the development of bio-pesticides is enormous.

7.3 The future of biological pesticides face a huge market

Forecast of near and medium-term market demand for biological pesticides in China: the planting area of wheat, rice, corn and cotton will reach about 1.4 trillion mu, and the cultivation area of vegetables and tobacco will reach 250 million mu. Annually, there would be about 300-400 million mu affected and destroyed by *Helicoverpa armigera, Plutella xylostella, Laphygma exigua* Hubner and other pests. There would be about 200-300 million mu affected by *Rhizoctonia solani* Kühn, *Magnaporthe grisea*, *Xanthomonas oryzae , Blumeria graminis* f.sp.*tritici*, plant nematode and virus diseases. So there will be a great demand for pesticides. According to the experts' prediction, by 2010, total sales of China's various types of biological pesticides is expected to be over 200,000 tons, which Bt insecticide 40-50 thousand tons, Agro-antibiotics 12-13 million tons, botanical pesticides 20-30 thousand tons, the virus insecticide 5-10 thousand tons, plant growth regulator 10-15 tons, others 10-20 thousand tons. The total sales are expected to reach over 3 billion yuan.

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Introduction to a New Agro-antibiotic Zhongshengmycin

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ABSTRACT

The research of Zhongshengmycin (96-C01-02-02B) is one of the most important projects during the period of the national "ninth five-year plan." To date, classification and identification of the Zhongshengmycin-producing strain, structure determination of effective components, mechanism and production technology, product safety assessment, registration and the research of field application technology have been accomplished. After years of field demonstration and promotion, Zhongshengmycin had proved itself to be the best bio-pesticide against crop bacterial diseases.

Keywords: Zhongshengmycin, *Streptomyces lavendulae* var. *hainanensis,* bacterial diseases

INTRODUCTION

Zhongshengmycin (commodity name Ke Jun Kang)- producing strain is a streptomycete, which was numbered B-7. It was separated from Hainan soil by the Institute of Medical and Pharmaceutical Biotechnology, Chinese Academy of Medical Sciences and developed into a microbial pesticide strain by the Institute of Biological Control, Chinese Academy of Agricultural Sciences. Beginning with screening the strain in 1986 to obtaining the temporary pesticide registration certificate in 1996, the research had been under the sponsorship of three Five-year Key Technologies R&D Programs, which is held by the Ministry of Science and Technology. "The achievement of Zhongshengmycin" passed through the technical identification in 1997. The identification committee regarded "the research results filled the domestic blank in the microbial pesticide preventing and controlling bacterial plant diseases. The research is among the leading level in China and reaches the international advanced level in application." The research results won the second prize in Sci-Tech Progress bestowed by the Ministry of Agriculture in 1999. The primary achievements were briefly introduced as follows:

1 Classification and identification of the Zhongshengmycin-producing strain

1.1 Morphological characteristics

The strain B-7 can grow on diversified classification media. On each of them, aerial mycelium and substrate mycelium can be well-developed. The mycelium has branches. On the top of aerial mycelium forms 1-4 round of curly spores. More than 10 conidiophore chains are formed. The spores are round or elliptic (0.6-0.8×1-1.2μm) and the surface is smooth .

1.2 Cultural characteristics

On the common classification media or International Streptomyces Plan stipulated media (ISP media), the strain B-7 forms powder aerial mycelium, the colour of which is ranging from gray white, gray with slight pink, gray with slight purple to dark purple. The colour of substrate mycelium is ranging from creaminess, light yellow to khaki. No soluble pigments are produced.

1.3 Physiological and biochemical characteristics

On the peptone-yeast, cream-iron agar medium (ISP6), the strain forms brown pigments, while on the lysine agar medium, it does not form a soluble pigment. It can liquefy the glutin, solidify and peptonize the milk, hydrolyze the starch and deoxidize nitric acid. But it cannot decompose cellulose. It first makes good use of glucose, Ganlujing, xylose, xanthorhamnin, arabiancinand then fructose, creatine, Jujing, Rujing, Banrujing. It cannot utilize tartaric acid, mannitol, Tongzi sugar, sodium acetate, sodium citrate. and possibly sucrose.

1.4 Chemical component of the cell wall

The cell wall contains DL-2,6- diaminopimelic, glucose and mannose through whole-cell hydrolyzation analysis.

1.5 Strain identification

The strain B-7 has the typical morphological characteristics and cell wall components of streptomyces. It is more close to the typical strain of *Streptomyces lavendulae* ISP5069 concerning the morphological characteristics, but when ISP5069 grows on the glycerol-asparagine agar medium, the aerial mycelium turns silver gray, while the substrate mycelium turns dark olive, with a light brown soluable pigment. On the inorganic starch agar, the aerial mycemium turns yellow gray, while the substrate mycemium turns dark olive. ISP5069 cannot utilize Arabian sugar, xanthorhamnin, xylose and inositol. It can utilize tartaric sodium, citric sodium as carbon sources. On these aspects, the strain B-7 is totally different.

The results above show: B-7 strain is similar to *S. lavendulae*, but has some differences, so it was named *Streptomyces lavendulae* var. *hainanensis*.

2 Identification of the effective components

There are six effective components in Zhongshengmycin. Among the compoments, there is a margin of one β-lycine. A paper chromatogram of the solvent system indicated that it belonged to typical alkaline water-soluble antibiotics. UV absorption spectrum of the mixture showed only the end was displayed. They have similar infrared spectrum and the typical absorption peak appear at 3400,1720,1660,1560,1340 and 1050 respectively. They are all levorotatory compounds.

3. Antimicrobial activity and toxicologic determination

3.1 Antimicrobial activity

Zhongshengmycin has a wide range of antimicrobial activities, and the activities of the six components are different.

Table 1. Antibiogram of the different components of Zhongshengmycin

Minimum antimicrobial concentration of the different components of Zhongshengmycin (μg/ml)

Test microbes	1	2	3	4	5	6	7
Erwinia aroideae	50	0.09-0.1	1-2	0.78	0.39	0.78	1.56
Erwinia tracheiphila	25	1.56	0.78	3-4	0.78	0.39	0.78
Pyricularia grisea Saccardo	12.5	3-4	0.39	0.39	0.1-0.2	0.1-0.2	3-4
Aspergillis niger	50	50	0.78	3-4	1-2	3-4	12.5
Candida albicans Berkhout	12.5	100	3-4	0.78	0.39	0.39	1-2
Saccharomyces sake Yabe	25	3-4	0.78	0.1-0.2	3-4	0.04	1-2
Proteus	50	6-7	3-4	1-2	1-2	3-4	6-7
Escherichia coli	100	50	12.5	6-7	6-7	12.5	3-4
Staphlococcus aueus	3-4	1-2	0.1-0.2	0.97	0.09-0.1	0.1-0.2	0.39
Sarcina	6-7	25	0.39	0.97	0.09-0.01	0.1-0.2	0.39
Bacillus subtilis	6-7	0.39	0.09	0.1-0.2	0.04	0.09-0.1	0.1-0.2
Mycobacterium tuberculosis	6-7	1-2	0.78	0.39	0.78	1-2	0.78
Mycobacterium phlei	3-4	0.1-0.2	0.39	0.9-1.0	1-2	0.39	12.5
Fusarium gramineanum Schwabe	12.5	0.097	0.1-0.2	0.39	0.39	0.39	
Urocystis brassicae	50	0.97	3-4	1-2	1-2	1-2	
Uredo panaacis	6-7	0.097	1-2	0.1-0.2	0.1-0.2	0.1-0.2	

3.2 The toxicity of Zhongshengmycin to animals and its residual dose determination in plants and soils.

Toxicity assessment of Zhongshengmycin to animals:

(l)Acute toxicity : White mouse oral toxicity test : medium lethal dose (LD_{50}) of the industrial sample amounts to 10,000 mg/kg, LD_{50} of the pure sample is 316mg/kg for males, and 237mg/kg for females. White mouse percutaneous toxicity test: LD_{50} of the industrial sample >l0,000mg/kg (2) No irritation to skin and eyes: skin test of the white rabbit showed that no erythema and edema formed. 1-24h after treatment of eyes, there were slight hyperaemia and edema in conjunctiva, no secretion. The cornea was normal. Restored after 48h.

(3)Cumulative toxicity: The cumulative coefficient is 5.8. It was calculated as follows: When half dead, the gross absorption volume of the sample divides LD_{50}

(4)Teratogenic test: Fed the mouse for 10 consecutive days from the 6th day to 15th day of its pregnancy with the dose of 0.766-15.32mg/kg. For each dose, filled 0.5mg /100g (weight) each time. There was no negative influence on the embryo. According to X2 examination, no significant differences were observed between treatments and the control due to the dead embryo, bone teratogenity and viscera.

(5)Micronucleus test: no damage to chromosomes, no teratogenicity to germ cells

(6)Mutagenicity test: No mutagenicity. With the method of Ames standard plate, results showed there was no significant difference between treatments and the control.

3.2.3 Determination of the residues in plant and soil

(1) Sample preparation

Chose the field with well-grown lotus cabbage and set the control and treatment areas. After spraying Zhongshengmycin, took the sample of the control soil, control cabbage, treated soil and treated cabbage for 10 consecutive days. The cabbage sample was crashed in the tissue crasher and soaked with water for 24h, then the cabbage residues were filtered out with gauze. After that, the filtrate was filtered with filter paper for two times and vacuum filtration was conducted with Brandt funnel. Finally, the filtrate was evaporated at the bath of 50°C to smaller volume and frozen dry.

The soil sample was dispersed with water, soaked for 24h, filtered with gauze and then filtered again with filter paper and Brandt funnel. Finally, the filtrate was concentrated and frozen-dry.

(2) Sample determination

Since Zhongshengmycin belongs to N-glycoside antibiotics and its UV spectrum was of end absorption, it could not be detected under the general UV spectrum scope. It could only be detected by the following method. Mix the sample with ophthalaldehyde and conduct derivatization reaction under a certain condition. Then we get the fluorescent product and separate it using reverse phase chromatography, detected by fluorescence detector. This method is super sensitive and the minimum detection dose is 6.9×10-2 pps with good repeatability. The relative standard deviation(CV) was 0.2372%. The recovery ratio was 97.09.

(3) Detection result

The detected content was 0.185μg/ml on the first day after spraying Zhongshengmycin. On the 7th day it was 0.001μg/ml and none on the 8th day. The results indicated that the residue dose of Zhongshengmycin was extremely low and that it could only last 7days, so it caused no pollution to the environment and no toxicity to human beings and livestock.

4 Antimicrobial Mechanism of Zhongshengmycin

4.1 Mechanism of Zhongshengmycin against crop pathogenic bacteria

The pathogenic bacteria of *Erwinia carotovora* was taken as the test bacteria. It was determined that Zhongshengmycin had little effect on the membrane of the pathogenic bacteria, but it could cause protoplasm to agglomerate at the concentration of 100μg/ml. The experiment of adding the isotope-marked precursor showed that Zhongshengmycin has little effect on DNA and RNA synthesis of the pathogenic bacteria at the concentration of 15.6μg/ml, but it could strongly inhibit the synthesis of the protein. We analyzed the effect of Zhongshengmycin on the content of intracellular macromolecules of *B. subtilis* through chemical method. Similar results were achieved compared with the former experiment, which indicated that the antimicrobial mechanism of Zhongshengmycin is to inhibit the synthesis of the bacteria protein. On this basis, further study was conducted by means of non-cell system, which showed that Zhongshengmycin could inhibit the polyU-directed synthesis of poly-phenylalanine. The inhibition ability had a close relationship with the concentration of Mg^{2+}, which elucidated the mechanism of Zhongshengmycin against pathogenic bacteria was to inhibit the formation of the peptides during the course of the synthesis of the bacterial protein.

4.2 Mechanism of Zhongshengmycin against crop pathogenic fungi: The pathogenic fungi of watermelon wilt were selected as the test fungi. The study showed that Zhongshengmycin could greatly cause the agglomeration of protoplasm in the mycelial cell and strongly inhibit the germination of pathogenic spores. At the concentration of 2.5μg/ml, it could completely inhibit the germination and kill the pathogenic fungi. It had a relatively strong ability of inhibiting the formation of spores. The inhibition rate was 94.7 percent at the concentration of 100μg/ml. It had a certain ability of inhibiting hyphal growth. The inhibition rate was 40 percent at the concentration of 100ug/ml.

4.3 Inductive resistance mechanism of Zhongshengmycin against rice bacterial leaf blight

Callus induction and suspension cell culture system of rice susceptible species Shennong1033 and anti-disease species CBB4 were established. Applying RNA spot blotting, we found that Zhongshengmycin had a relatively strong inductive effect on the transcription and expression of the key genes that encoded Proxidase (PO), phenylalanine ammonia-lyase (PAL) and Chalcone synthase(CHS), which were responsible for the plant defensive responses so that further disclosed the inductive resistance role Zhongshengmycin played at the molecular level.

5 Zhongshengmycin production

5.1 High-yield strain breeding

The high-yield strain has been gained through the protoplast and UV methods. When applied in a 20-ton fermentation tank, the fermentation unit of this high-yield strain could reach 5000±500μg/ml ,or even 6500μg/ml ,which was four times higher than that of the original one.

5.2 The study of the production technology has been accomplished.

The extraction rate could reach 85 percent for the liquid formulation and 70 percent for the powder formulation.

5.2.1 Production technology for the aqueous solution (AS) of Zhongshengmycin.

Spores on the agar slope→flask shaking (28°C 200rpm 24h) →blend the seeds together into the innoculation flask→seed tank fermentation (24h) →big tank fermentation (42~46h) →release→adding flocculants (30min)→plate filtration or centrifugation→adjust pH value for the filtrate→vaccum concentration→storage→potency determination→products.

5.2.2 Production technology of the powder formulation of Zhongshengmycin.

Spores on the agar slope→flask shaking (28ºC 200rpm 24h)→blend the seeds together into the innoculation flask→seed tank fermentation (24h) →big tank fermentation(42~46h)→release→plate or centrifuge filtration→adjust pH value for the filtrate→vaccum concentration→high-speed centrifuge spray→primitive powder crush→through 100-mesh sieve→package (primitive)→content determination→add assistant and carrier→blend even→package (products).

6 Application of Zhongshengmycin

The MIC and MBC of Zhongshengmycin against *Xanthomonus oryzae* are 0.19µg/ml and 0.4µg/ml respectively. The MIC and MBC of Zhongshengmycin against *Erwinia aroideae* are 1.56µg/ml and 3.12µg/ml respectively; for the pathogenic fungi of Apple perennial canker, the MIC and MBC are 1.56µg/ml and 3.12µg/ml respectively. The application concentration of Zhongshengmycin in the field is low. To control the above three diseases, the application concentration is generally 30-40µg/ml and the control effect can all reach 80-90 percent through their respective control route, which can keep even or be a little higher compared with the regular chemical pesticides.

6.1 Instruction to the usage of Zhongshengmycin

Table 2-1. Application instruction to Zhongshengmycin

Control object	Application period and method	Application concentration and method
Physalospora piricola Nose, *Gloeosporium* rot, moldy core, and spot stain, peach bacterial shot hole	Spray for the first time at the phage of flourishing florescence, then one time every 15-20d. Spray with Bordeaux liquid (mixture of $CuSO_4$, CaO and water) by turns at the late phage, 4-5 times for Zhongshenmycin in total.	30-40 µg/ml, spraying
Erwinia carotovora	Dressing and spray 2 times from the phage of seedling to lotus.	60µg/ml, dress 30-40µg/ml, spray
Solanaceae crops bacterial wilt	Irrigate roots at the beginning of the disease for 2-3 times in succession	30-40µg/ml, irrigating roots
Pseudomonas lachryninas Car., bean bacterial blight, Asparagus stem blight, pepper bacterial spot	Spray at the beginning of the disease	30-40µg/ml, spraying
Xanthomonas oryzae	Soak with warm water (55˚C, then let the temperature drop naturally for 36-48h), spray at the phage of seedling with 5 leaves and the 5th day before transfer respectively. Spray in the field when disease breaks out.	80µg/ml, soaking seeds, 30-40µg/ml spray
Xanthomonas citri Dowson	Spray once every 10 days since the bud phage, 6-8 times for all.	30-40µg/ml, spraying
Pseudomonas solanacearum	Soak seeds and irrigate the root at the beginning of the disease.	30-40µg/ml, irrigate roots

6.2 Current status of the application

As a new agro-antibiotic, Zhongshengmycin has gained its three certificates. It has been registered in Minstry of Agriculture to control more than ten diseases, such as rice bacterial blight, apple perennial canker, Chinese cabbage soft rot, cucumber bacterial angular leaf spot, bean bacterial blight, Asparagus stem blight, pepper bacterial spot, ginger blast, citrus canker, tomato bacterial wilt, etc. Its production and application was listed as one of the key programs of national agro-technical extension (98110203A). At the end of 2005, the application area had reached 50,000,000Mu throughout more than ten provinces in the nation. The production of Zhongshengmycin products is mainly in charge of Fuzhou Kaili Biological Products Co., Ltd. Two thousdand tons of 1% aqueous solution and 1,000 tons of 3 percent wettable powder are planned to produce each year. Blessed with the mature production and processing technology of Zhongshengmycin, as well as its stable application effect, it has become well-known in controlling the crop bacterial diseases in agricultural production.

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Keywords: program-efficient, low toxicity, new antibiotics used in plants, internal information

Evaluation of the Bactericidal Effect of Zhongshengmycin 3 Percent Wettable Powder on Six Bacterial Diseases

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ABSTRACT

The control effect of Zhongshengmycin 3 percent wettable powder (WP) on six pathogenic bacteria, including *Xanthomonas oryzae*, *Erwinia carotovora*, *Pseudomonas solanacearum*, *Pseudomonas lachryninas*, *Xanthomonas citri* and *Pseudomonas solanacearum*, was studied in the laboratory, in the field and in large scale demonstrations throughout China. The application effect was evaluated as well.

Keywords: Zhongshengmycin, bacterial diseases, application effect

INTRODUCTION

Zhongshengmycin is an antibiotic produced by *Streptomyces lavendulae* var. *hainanensis*, which was separated from Hainan soil by the Institute of Medical and Pharmaceutical Biotechnology, Chinese Academy of Medical Sciences. It was numbered B-7 and developed into a microbial pesticide strain by the Institute of Biological Control, Chinese Academy of Agricultural Sciences (Zhao and Wang, 1990). Through 15 years of study, two formulations, 1 percent aqueous solution (AS)that can control three bacterial diseases, and 3 percent WP that can control 10 bacterial diseases, were developed and registered in 1996 and 2001 respectively, and the annual output value has reached more than 10 million yuan. Effective resistant strains against *Erwinia carotovora* were screened out in 1986 and the fermentation efficacy improved from 400 mg/L of the wild strain to 8,500-11,000 mg/L using 30-ton fermentor in 2002 (Zhu *et al.*, 2002). Furthermore, the control range has expanded from *Physalospora piricola* Nose and *Xanthomonas oryzae* to ten species (*e.g.*, *Erwinia carotovora*, *Pseudomonas lachryninas* Car., *Xanthomonas citri* Dowson, *Pseudomonas solanacearum* Smith). This paper summarized and assessed the control effect of Zhongshengmycin on the above bacterial diseases, in order to further look into the prospect of the application and development of Zhongshengmycin.

MATERIALS AND METHODS

The antibiotic Zhongshengmycin was provided by Fuzhou Kaili Biological Products Co. Ltd.

Bioassay for Lab Control Effect of Zhongshengmycin on Bacterial Diseases

One gram of Zhongshengmycin 3 percent WP was added to 30 ml sterilized water, surged for 2 minutes and shortly centrifuged at 8,000 rpm. The supernatant was collected, filtrated with bacterial filter and the sterilized Zhongshengmycin liquid at the concentration of 1,000 μg/ml was gained. Ten test tubes were prepared for dilution. The first tube contained 9.5 mL medium, others 5 ml. 50 μg/ml Zhongshengmycin was gained by adding 0.5 ml 1000 μg/ml Zhongshengmycin into the first tube. 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 μg/ml were gained transferring 5 ml mixture from the former tube into the latter tube till the ninth one. 5ml mixture was removed from the ninth tube and the tenth tube was the control without Zhongshengmycin. So the concentrations of Zhongshengmycin from the first tube to the tenth one were 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 and 0 μg/ml. The cultured pathogenic bacterial suspensions (108 cfu/ml) 100 μl were added into each tube respectively. Media with the control agent Agro-streptomyces at the concentration of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19 μg/ml were prepared with the same method. They were cultivated statically for 24 hours at 30°C. The lowest concentration was identified as the minimum inhibition concentration (MIC) if there was no bacterial growth through eye observation. The lowest concentration was identified as the minimum bactericidal concentration (MBC) if there was still no bacterial growth after cultivating for another 48 hours. There were three replicates for each treatment and the whole experiment.

Bioassay for Field Control Effect of Zhongshengmycin on Bacterial Diseases

According to the field pesticide efficacy test methods regulated by the Institute of Drug Inspection, Ministry of Agriculture, P. R. China, a two-year-two-site efficacy test was conducted to evaluate the control effect of Zhongshengmycin on each object. Five treatments, including 37.5 μg/ml, 30 μg/ml, 25 μg/ml Zhongshengmycin, control agent (Agro-streptomycin, imported agent 53.8 percent Keshade dry suspension agent or Bismerthiazol) and control blank were set. Medicine three times. There were three replicates for each treatment. (Meng, 2002; Zhang *et al.*,1998; Xue, 2002).

RESULTS

Lab Control Effect of Zhongshengmycin on Bacterial Diseases

Table 1. The MIC and MBC of Zhongshengmycin against pathogenic bacteria.

Control objects	Zhongshengmycin (μg/ml)		Agro-streptomycin (μg/ml)	
	MIC	MBC	MIC	MBC
Xanthomonas oryzae	0.39	1.56	3.12	6.25
Erwinia carotovora	0.78	1.56	3.12	6.25
Xanthomonas citri	0.39	0.78	3.12	6.25
Pseudomonas lachryninas	0.78	1.56	3.12	6.25
Pseudomonas solanacearum	0.78	1.56	6.25	12.5
Xanthomonas axonopodis pv. *citri*	0.39	0.78	3.12	6.25

Table 1 shows that the MIC and MBC of Zhongshengmycin on the above pathogenic bacteria were 0.39-0.78 μg/ml and 0.78-1.56 μg/ml, four to eight times lower than those of Agro-streptomycin, which were 3.12-6.25 μg/ml and 6.25-12.5 μg/ml.

Field Control Effect of Zhongshengmycin on Bacterial Diseases

The control effect of Zhongshengmycin 3 percentWP on the above bacterial diseases in Fujian, Shandong, Jiangsu, Anhui, Liaoning and Beijing in the year of 2000 and 2001 are summarized in table 2.

TableE 2. The Integrative Control Effect of Zhongshengmycin on Bacterial Diseases

Treatment	**Control effect** (percent) *					
	Xanthomonas oryzae	*Erwinia carotovora*	*Pseudomonas lachryninas*	*Pseudomonas solanacearum*	*Xanthomonas citri*	*Xanthomonas axonopodis* pv. *citri*
Zhongshengmycin 37.5 μg/ml	73.8	83.6	74.4	76.8	71.8	94.4
Zhongshengmycin 30 μg/ml	67.5	74.7	66.7	60.7	67.5	89.7
Zhongshengmycin 25 μg/ml	55.8	66.8	57.8	50.3	61.8	80.1
Agro-streptomycin 250 μg/ml		82.4	55.3	70.4	62.5	
Kocide 800 times						83.5
Bismerthiazol 400 μg/ml	63.8					

*Note: The control effect of Zhongshengmycin on the above diseases was the average investigation result of four spots and twelve times at day 12 to day 15 after the third application of the pesticides.

Table 2 shows that Zhongshengmycin 3 percent WP had good control effect on the above bacterial diseases and the control effect reached 60-94 percent when diluted 800 and 1000 times to 37.5 μg/ml and 30 μg/ml respectively. Especially, the control effect of the former one reached over 70 percent, higher than that of regular control agents at the recommended concentration.

Large-scale Demonstration of Zhongshengmycin against Bacterial Diseases

Since Zhongshengmycin AS came into the market as a formal commodity in 1996, it has been promoted and demonstrated in many provinces throughout China. It had been promoted about

2.67×10^5 ha. by the end of 2000. In 2001, temporary pesticide registration certificate was granted to 3 percent WP and since its control range was widely expanded, the application area was correspondingly broadened and good control effect was gained (Ministry of Agriculture, 2005). See detail in table 3.

Table 3. The integrated statistics of the effect of Zhongshengmycin on bacterial diseases

Main application provinces	Control objects	Control effect (percent)	Application area (1,000 ha)	
			To 2000	To 2005
Shandong	*Xanthomonas oryzae*, *Pseudomonas solanacearum*, etc	60-85	33.3	400.0
Liaoning	*Erwinia carotovora*, *Xanthomonas oryzae*, *Xanthomonas citri*, etc	60-80	20.0	200.0
Beijing	*Xanthomonas oryzae*, *Erwinia carotovora, Xanthomonas citri*, etc	60-78	20.0	33.3
Henan	*Xanthomonas oryzae, Erwinia carotovora*, etc	65-80	53.3	333.3
Jiangsu	*Xanthomonas oryzae, Erwinia carotovora, Pseudomonas lachryninas, Xanthomonas citri*, etc	65-85	33.3	266.7
Anhui	*Pseudomonas solanacearum, Xanthomonas oryzae*, etc	60-85	6.7	133.3
Jiangxi	*Xanthomonas axonopodis pv. citri, Xanthomonas oryzae*, etc	65-80	6.7	133.3
Fujian	*Xanthomonas oryzae, Xanthomonas axonopodis pv. citri, Erwinia carotovora, Pseudomonas solanacearum, Pseudomonas lachryninas,* etc	60-90	6.7	233.3
Zhejiang	*Pseudomonas lachryninas, Xanthomonas citri, Xanthomonas oryzae,* etc	65-80	3.3	153.3
Hainan	*Pseudomonas lachryninas, Xanthomonas citri, Ralstonla solanacearum*, etc	65-85	3.3	266.7
Guangdong	*Pseudomonas lachryninas, Xanthomonas citri, Ralstonla solanacearum*, etc	65-85	1.3	133.3
Hubei	*Xanthomonas oryzae, Erwinia carotovora, Pseudomonas lachryninas*, etc	65-85	6.7	200.0

Table 3 shows that the new formulation of Zhongshengmycin had a good control effect on crop bacterial diseases and therefore has a good application and development prospect.

DISCUSSION

It was observed that Zhongshengmycin was safe to crops, with no side-effects. Since the residue of Zhongshengmycin would stay for seven days in soils and plants and it played a better preventive than curative role, 30-37.5 μg/ml of Zhongshengmycin 3 percentWP was recommended as the field application concentration. Spray every 7-10 days for three times consecutively at the early stage of crop disease.

After many years of promotion and application, Zhongshengmycin had proven itself to be one of the best pesticides to control bacterial diseases and had been well accepted by farmers, which proved the assessment made by Zhongshengmycin Scientific and Technological Achievements Identification Commission in 1997: "Zhongshengmycin research results have filled the blank in biological pesticides to control bacterial diseases, which was at the leading level in China and reached the international advanced level for application." (Ministry of Agriculture, 1997).

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Inhibitory Effects of Extracts of *Stellera chamaejasme* on Plant Pathogenic Fungi

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ABSTRACT

Six kinds of solvent were used to extract the active material from root of *Stellara chamaejasme* and fungi stasis effects of the extracts were tested with 8 fungi, *Fusarium graminearum*, *Botrytis cinerea* Pers., *Verticillium dahliae* Kleb, *Fusarium oxysporum* f. sp. *melongenae* Matuo & lshigami, *Monilinia fructicola*, *Physalospora piricola* Nose, *Alternaria mali* Roberts, *Valsa mali* Miyabe & Yamada. The results showed that different extracts had notable effect on different fungi tested. As a whole, extracts of *S. chamaejasme* had notable fungi stasis effect on 8 species, the highest inhibition rate was achieved in *V. mali*, Which is 85.27 percent, while the lowest was achieved in *V. dahliae* that is 60.34 percent. Six solvents extracts had different antifungal activity, three extracts have the highest inhibition rate on 8 fungi, including methanol extract, ethanol extract, ethyl acetate extract, which is 87.7 percent, 83.29 percent, 82.59 percent, the chloroform extract have the lowest inhibition rate, which is 47.03 percent. So we could conclude that *S. chamaejasme* can be used as antifungal plant to control plant pathogenic fungi and extract, ethanol extract, ethyl acetate extract can extract the higher active material.

Keywords: *Stellara chamaejasme*, extracts, fungi stasis

Two New Botanical Pesticide Products Kingbo and Vegard

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ABSTRACT

We all know botanical pesticides have some safety advantages, such as low toxicity, readily biodegradable, and non-residue, but botanical pesticides have obvious weaknesses, such as poor results, poor stability, higher prices, and complicated. To play to the advantages of botanical pesticides while overcoming its weaknesses, our company carried out serious and painstaking work.

I want to briefly talk about our company, Kingbo Biotechnology Company, Ltd. and the four components, 1) Inner Mongolia Ulan Buh Desert and the ecological base of our growing base of raw materials, 2) Inner Mongolia Kingbo-Biotechnology, Ltd. is the original drug from the factory, 3) Beijing Shunyi factory is our preparation processing base, 4) Beijing Kingbo-Biotechnology, Ltd. is the management, marketing and research and development centers.

No1 Kingbo, 0.6 percent oxymatrine and prosuler aqueous solution is certified Grade AA green food product materials (LSSZ0199010501AA), OFDC(P3103-915-177-2002) and IMO(Nr26666). It is extracted from *Sophora flavescens* Ait and 5 other species of traditional Chinese herbs. Kingbo has 8 years into the market. It can be used for vegetables, tea, fruit, horticulture and forests. Control Object is: Lepidoptera, *Plutclla xylostella*, cabbage armyworm, broad mite, apple geometrid, *Hyphantria cunea* etc. The application is for foliar spray, dilution 1ml to 500-1000L water.

No2 Vegard, 0.5 percent emodin aqueous solution, its the national research project results with independent intellectual property rights. For vegetables, fruit and gardening, powdery mildew control. It has the protection role, therapeutic role. the mechanism is to make plants resistant material, the resistance of plant capacity. The application is for foliar spray, dilution 1ml to 500-800L water.

SESSION FIVE
STRATEGY AND TECHNOLOGY FOR MONITORING AND CONTROLLING INVASIVE SPECIES FOR A GREEN OLYMPICS 2008

Occurrence, Monitoring and Control of Forest Invasive Species in the Capital Eco-region, China

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ABSTRACT

The 29th Olympic Games will be held in Beijing in 2008, and mottos of the Beijing 2008 Olympic Games are Green Olympics, People's Olympics and Hi-tech Olympics. Forestry will play an important role in the Green Olympics, but more and more forest invasive species have been threatening capital ecology safety, with the rapid development of foreign trade and carrying trade. So we will illustrate the situation of forest invasive species, clarify how to monitor them, with special emphasis on control methods.

Theory and Practice of Restoration and Construction of a Stable Forest Ecosystem with Insect Viruses

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ABSTRACT

The theory and practice of sustainable control of forest pest insects are reviewed in this paper. The status of forest pest insects and biological control techniques in China are introduced. The criteria of a stable ecological system and the methods of restoration and construction are also analyzed. Meanwhile, the experiment in which four species of insect viruses were used to successfully control forest pest insects indicate that the insect viruses play an important role in restoring and constructing a stable forest ecological system.

Supported by National Natural Sciences Fund, (project 30671688)

Keywords: forest ecological system, restoration and construction, forest pest insects, insect virus

1.The present situation of forest pest insects and diseases in China

China is one of the countries in which there are serious forest pest insects. Since 1949, big achievements have been attained in controlling the forest pest insects. However, the overall situation is still complex. There are more than 8,000 species of forest pest insects and diseases. Among them more than 100 species often outbreak and the infested area includes more than 9 million hectares and reduces forest growth to 17,000,000 cubic meters. The economic losses reach more than 880 hundred million yuan. Among them, the foliage feeding insect's damage is especially serious and the area of damage occurrence maintenance at a higher level for a long time which occupies 50 percent of the total area of forest pest insects and diseases.

Chemical pesticides were used extensively to control pest insects for a long time. The chemical pesticides not only seriously injured natural enemies and other beneficial organisms, but also did harm to the mass harmful insects and the harmless insect. In the end, the inherent food web and the ecological equilibrium in the forest were destroyed and the "3R" questions became more and more serious creating a vicious circle which is "the more want to control pest insects with the chemical pesticides the pest insects outbreak more year after year." In recent years, organic food became popular. So following factors must be considered when controlling pest insects and diseases: the foundation of harmony society, sustainable development, ecological balance, ecological security and biodiversity protection.

Therefore, it is extremely urgent to reduce the usage of the highly poisonous chemical pesticides and to promote biological control and the planting techniques of non-environmental-damage. The

World Environment and Development Congress requested that the output of the world biological pesticides accounted for 60 percent of the total output of the pesticides before 2000. State Forestry Administration of China requested: “Integrated pest management measures including protecting the natural enemies, planting the plants for bees and releasing essential natural enemy insects must be fully used. The non-environmental damage measures must be used as much as possible. Such as using the bionic pesticides, biological and the plant sources insecticides to control the pest insects. The proportion of the chemical pesticides must be gradually reduced. The chemical pesticides will be restricted totally in the area of state-level project. The 80 percent of foliage feeding insects must be controlled by biological agents. Spraying of chemical pesticides to control the foliage feeding insects in the big areas will be comprehensive stopped in the whole country before 2003.”

To date the goal has not been achieved. The ratio of biological agents in forest pest insect control only accounts for about 30 percent up to 2005, including application of bionic pesticides. The primary cause is the preponderance of the cost of chemical pesticides and the pest-disaster-relief goal of the forest manager, as well as the short-term benefit urging the forest manager to use chemical pesticides. Moreover, other factors are the important reasons why the forest manager selects chemical pesticides, such as: the biological pesticide products are few, selective, effective slowly and the price is higher, the methods of use is very strict.

2. The situation and the future of biological control

The development of bio-pesticides in China is unoptimistic now. The bionic pesticide that higher efficiency, less virulence, non-environmental damage is few. Mass research has been done in bio-pesticides, but the bio-pesticides application range and the amount of use are small in forestry, because of the higher cost and storage difficulties. As for microbial pesticide, the manufacturer of Bt (*Bacillus thuringiensis*) has reached more than 70 in China, the annual output reached 20,000 to 30,000 tons, but the application range and area of Bt pesticide is actually small in forest pest insect control. The primary reason is that forest plant diseases and pest insects occur in a complicated terrain, and the lower temperature during the traditional time of forest pest insects be controlled, these environment affect the efficiency of Bt control, as well as the existing preparation of Bt cannot be satisfied the request of forestry pest insects control and so on. The insect virus is a kind of important insect pathogenic microorganism, UN Food and Agriculture Organization and the World Health Organization both recommends the baculovirus for possible use in pest insect control for wide areas, and baculovirus is the first choice in bio-pesticides development in the 21st century. Until now the insect virus has surpassed more than 1,000 species, and more than 30 kinds of virus registered in 20 nations. Our country also made great progress in production and the application of insect virus, for example, the HaNPV (*Heliothis armigera* nuclear polyhedrosis virus), the *Laphygma exigua* virus, the *Prodenia litura* virus, the *Apocheima cinerarius* virus, the *Lymantria dispar* virus, the *Hyphantria cunea* virus, the *Ectropis obliqua* virus, the *Clostera anachoreta* virus, *Dendrolimus* spp. virus and the *Parocneria furva* virus pesticide. These insect viruses have already been produced and applied in a large scale, and then obtained a remarkable efficiency in economy, ecology and society. The application of fungus pesticide gets widespread attention all the time, using *Metarhizium flavovirtide* to control *Locusta mgratoria*, using *Beauveria bassiana* to control the pine caterpillars (*Dendrolimus* spp.) and the corn borer(*Dstrinia furnacalis*), has obtained a remarkable effect. At present, there are more than 20 big manufacturers of fungus in China, and the *Beauveria bassiana* pesticide has been widely applied in pest insects control, such as the control of *Dendrolimus punctatus*. But the quality of the product is unstable, and the registration is very sluggish. The protection and application of natural enemy insects have already made great progress, like *Chouioia cunea* are already produced and applied widely in many provinces, such as Hebei, Tianjin, Liaoning, Shandong, with good results. Controlling pine needle scale (*Hemiberlesia pirysophila*) with *Coccobius azumai*, breeding and application of Bethyloidea, Trichogrammatidae, *Harmonia axyridis*, *Rhizophagus grandis* Gyllenhal the natural enemy of red turpentine beetle (*Dendroctonus valens* Leconte), and *Dastarcus longulus* Sharp the natural enemy of longhorn beetles all get remarkable results in China.

3. The stability of forest ecosystem and its restoration and construction

The forest ecosystem is a relatively stable ecological system and sustainable forest protection is a strategy which we must follow in forest pest insect control in China in the new century. The forest plant disease and pest insects control technology based on the ecology principle, and harmony with the environment is very important. This technology is also an important constituent in forestry sustainable development in our country.

3.1 Stability of ecosystem

The ecosystem concept was first raised by English ecologist Tansley in 1935. It stated that the biological ingredient and the non-biological ingredient constitute an ecological functioning unit that have a mutual effect and dependency on each other for existence through the circulation of materials and energy flow. The ecosystem has the ability to maintain or restore its own structure and function to be relatively stable.

The stability of resistibility, refers to the ability that ecosystem resists external interference and maintains its own original condition of structure and function. In other words, the interior of ecosystem has certain ability for automatic control. Generally speaking, the purer ecosystem ingredient is and the more simple nutrition structure is, the smaller ability for automatic control is and the lower stability of resistibility is; and the principle is done vice versa. At the same time, ecosystem automatic control ability also has certain limitation, when the external disturbance has surpassed this limitation, the ecosystem relative steady state can be destructed.

The stability of resilience, refers to the ability that ecosystem can restore to its original condition after be encountered the external interference destruction. The factor to destruct the stability of ecosystem is various, which is mainly the natural vicissitude of environment, the humanity use the natural resource unreasonably, and which causes death of woodland, soil erosion, obsolescence of prairie, the land desertification, as well as the producing activity of humanity to pollute the natural environment. Along with the fast development of economic society in our country, the using intensity of water, land and biological resources enlarge day by day. The humanity's ultra-use the natural resources already became the important factor for the worse of ecological environment. The extensive increase of economy which is important reason to causes ecological environment to worsen, and the world economical trade be increasing the invasion risk of external harmful biology to ecological environment objectively in our country.

The structure relative stability that is the species and numbers of plants and animals keeping relatively stabilization in the ecosystem, but sometimes the community quantity of plants and animals are periodic variation generally.

The function relative stability refers to in the biological community the material input and the material output are relative equilibrium, and the energy input and the energy output are relative equilibrium.

The stability of ecosystem is the result of system interior self-adjustment, this self-adjustment depends upon the relationship of interspecies in the community and the struggles of the intraspecies in the population, that may reconstructed after break. In keeping to the rule of ecosystem, humanity can base its our own need to break the original balance of ecosystem and establish a higher efficiency state of equilibrium. (Zhu Zhongbao, 1991)(Jiang Youxu *et al.*, 1993)

3.2 Restores and constructs relative stable ecosystem and realizes persisting control of forest pest

Based on ecology theory and practice experience to control the pest insect for a long time, the people attempt many kinds of ways to restore and construct the stable forest ecosystem that has the insect but did not cause the disaster. For example, to close hillsides to facilitate afforestation, namely through the compulsory measure to prevent human and livestock to influence the forest community, then restores the stable forest ecosystem with natural regeneration ability of forest. Through this measure to control disaster of *Dendrolimus punctatus* already obtained the remarkable effect. This measure enhanced the forest shade density and the vegetation degree of coverage, and the microclimate in forest land also had the obvious change, temperature drop 3°C more. The insect community structure had a remarkable change, in which the species of foliage feeding insects increased about 2 times, the species of soil insect increased about 3 times, the species of pine tree pest insects also increased about 2 times. At the same time, species of natural enemies also obviously increased, in which species of natural enemies for *Dendrolimus punctatus* Walker achieves 31 families and 67 species, compared with the unclosed mountain area the species were only 26 families 39 species. Quantity of natural enemies also obviously increased, in which egg parasitic wasp population density compares unclosed mountain area to increase more than 7 times, bird population quantity also compares unclosed mountain area to increase above 8 times. This measure improved the ability of self-control for *Dendrolimus punctatus* population quantity obviously, reduced the frequency and the serious degree of the disaster (Chen 1990; Chen *et al.*, 1999).

This method also has certain limitations, such as slow execution, and can be carried out in mountainous areas with low populations of humans and livestock. The application afforestation measure may construct a relative stable forest ecosystem to control the pest insect, this measure includes planting a mixed forest with bait trees, to protect and replant the flowering plants and so on. Experiments indicated that *Dendrolimus punctatus* parasitism rate was enhanced to 12 percent in the areas with more flowering plants and the species of parasitic wasps increased to 2 families, so this method can control *Dendrolimus punctatus* occurrence effectively. For borer insect control, separate poplar trees with an isolation belt of Chinaberry-tree (*Melia azedaeach* L.) prevents *Anoplophora glabripennis* (Motsch), mixture plant with poplar tree and Chinaberry-Tree, poplar tree and tree-of-heaven (*Ailanthus altissima* Swingle), poplar tree and black locust tree (*Robinia pseudoacacia* L.), poplar tree and ash tree (*Fraxinus chinensis* Roxb.). Through the mixture method it may be possible to reduce the long-horned beetles, Cerambycidae, to harm the poplar tree effectively, in which poplar tree and Chinaberry-tree interplantings can reduce the damage rate of long-horned beetles to about 82 percent. The poplar tree interplantings with soft maple (*Acer saccharinum* Marsh), ashleaf maple or boxelder (*Acer negundo* Linn.), and sensitive poplar tree to the long-horned beetle to be possible to seduce the long-horned beetle gather effectively, and then to kill the pests. These afforestation measures all have a good effect to construct the relative stable natural secondary forest, the plantation, including avenue trees, and virescence tree of city (Chen *et al.,* 1999; Gao 2003). Except for reducing the insect population density effectively and reducing the disaster area, these measures are unable to influence to the quality of pest insect population seriously, once the feed be sufficiently the pest insects can also make a quite huge disaster.

Protection and release natural enemy insect and pathogenic microorganism may effectively control target pest insect, reduce the density of pest insects, at the same time increase the biodiversity of environment. For example, natural enemy resources for pine caterpillar are extremely rich in China, there are 6 phyla, 11 classes, 33 orders, 91 families, and 555 species (Chen 1990) in the pine ecosystem. The complex food chain with the pine caterpillars as core have certain functions in the control of the pine caterpillars, but because the natural enemy breeding cost is high and difficult, so to exert the function of the natural enemy, it had better to take the protective measures, such as introduction, migration, supplement release, to rich the species of natural enemy, to restore the quantity of natural enemy suitably, then make it to form a quite stable community.

Chemical control which is the essential method to restore the ecosystem, can reduce disaster losses fast and effectively and stop disaster spread especially after the disaster occurrence. Although since 1980s, pest insect chemistry control has already been obtained a very great progress in our country. Chemical pesticides that have long residual time and serious pollution are already forbidden to use, and have been already instead with the one that be used widespread and has highly effective, such as high effect Cypermethrin, Dimilim pesticides, as well as biological metabolism preparation such as Abamectin, Azadirachtin, Nicotine, Matrine and so on, but these preparations only be one emergency procedures, and only can achieve a short-term effect, its function to construct and restore the relatively stable ecosystem is not obvious, it also have the negative influence to the ecosystem stability in the long term (Wu *et al.*, 2004).

Since long ago, the importance of afforestation measure is emphasized all over the country in China, the next is biological control and chemical control, but so far succeeds with the afforestation measure primarily to control plant disease and pest insects has been seen fewer, the most important reason is that the nature is complex and diverse, difference in the geographical environment and in the floristic species is distinct, the ready-made pattern has not been possible to supply to choose for afforestation. Simultaneously, natural secondary forest transformation is difficulty, and the plantation are always taken for economy or avenue trees and virescence trees, as well as when afforestation in barren hill have be done in a difficult condition, available trees is also limited, adds the bad environmental condition and the congenital condition of ecosystem is unstable, so it's a long term to construct a stable ecosystem, and it need the union of manpower and the natural strength to realize gradually.

The forest ecosystem is a complex and the relative stable system, the species that can cause disaster are quite simplex in this ecosystem, so long as we can control this species well, we may protect relative stability of forest ecosystem without to transform the plant community.

During the process when we controlled plant diseases and pest insects, we took the plants as the core and the lowest loss of plants and economic as the goal, and the pests' mortality is our control target. We lacked to think the long-term persistent control of the pests. Therefore, the temporary victory was achieved through sacrifice of the environment. The long-term experience already indicated that the idea must be transformed to a new one which takes the pest insects as the core and takes the environment friendly bio-technology as measures. A relative complex stable forest ecosystem which can control the harmful biology will be restored and constructed rapidly, thus the plant resources will be protected and the ecosystem's relative equilibrium will be realized.

4. The insect virus pesticide is one important method to restore and construct stable forest ecosystem

The insect virus is one kind of microbiology in the nature which may affect the quantity and the quality of insect population. It has strong host specificity, high toxicity and stability. And it does not pollute the environment. Moreover, it is secure to other non-target biology in the ecosystem .So it is the important biotic factor to control the pest insects' population. The insect virus not only diffuse horizontally through the sick larva' excreta and the corpse, but also it can transmit vertically through lay eggs. In addition, the virus also can accumulate in the environment and the host population; it can control the pest insects for a long term by forming epidemic disease in the pest insect population through the external environment stimulation. The quite stable food chain relation of plant - pest insect - natural enemy is gradually established. Thus it can reduce the risk of the pest insect continuous outbreak and realized the pest insect's persistent control.

4.1 The function of DCPV in restoring the pine forestry ecosystem

Pine caterpillar is one of the serious foliage feeding insects in China. Research on it began in the 20th century 1930s. A systematic and thorough research has been carried out since 1981 when it is listed as China "65" science and technical plan. Since then a series achievement have been obtained. Among them, two measures were most successful, one measure was based on closing hillsides to facilitate afforestation and the other was based on microbiological control. The *Dendrolimus* cytoplasmic polyhedrosis viruses (DCPV)) was used to control pine caterpillars that the area achieved 200,000 hectares and the effect is very good. *Dendrolimus punctatus* Walker has 3-4 generations in Guangdong province in 1 year. 7.5×10^{11} to 1×10^{12} PIB/hectare DCPV were sprayed during May to June when the pest larval is at 4-5 instar of the first generation. Fifteen to twenty days later, the average mortality rate was 91.6 percent. In July 1×10^{12} to 1.5×10^{12} PIB/hectare DCPV were sprayed, the mortality rate reached 99-100 percent (Chen 1990; Chen *et al.* 1999).

The compound microorganism pesticide (3×10^{11} PIB DCPV +7.5×10^{9} IU Bt/hectare) were applied during April- May in Anhui, Hunan, Liaoning province, the average mortality rate was about 80 percent. During June-July, 1.5×10^{12} PIB DCPV + 3×10^{9} IU Bt/hectare were applied the mortality of the larva achieved above 70 percent.

For many years, the DCPV virus as well as the DCPV virus plus Bt compound microorganism pesticide have already become the most main biological agents to control pine caterpillars in China and have obtained very good control and ecology effect. The outbreak cycle of the insects was lengthened and the disaster area was reduced in the forest region where the virus was used. A relatively stable forest ecological structure were restored and constructed, which formed pine caterpillars as a core, diverse plant community, DCPV virus, birds, and insect natural enemies as inhibiting factors through the DCPV virus's application. The ELISA examination technology was applied to detect the surviving pine caterpillars in the forest region where the virus were used. The result showed that: the detection rate of DCPV is still very high in the forest after 6 years later. This result manifest that DCPV plays an important role in restoring the stable pine ecosystem and has good persistent effect. (refer to Table 1. and Table 2.) (Zhu Guangdan *et al.* 1999).

Table 1. ELISA detection of DpCPV in larvae collected from pine forest in Yunnan Province

Type	Sample	Spraying date (year/month/day)	Gathering date (year/month/day)	Insect number	Average examination value
Dendrolimus wenshanensis	□	1990.3.5	1994.2.18	24	0.20
	□	1991.3.24-26	1994.2.20	30	0.52
	III	1992.4.20-26	1994.3.2	30	0.94
Dendrolimus tehchangensis	□	1993.4 affected virus naturally	1994.2.26	30	0.82
	□	1993.4.16	1994.2.26	30	0.78
	control				0

Table 2. ELISA detection of DpCPV in larvae collected from pine forest in Guangdong Province

Collecting site	Sample	Spraying date (year/month/day)	Gathering date (year/month/day)	Insect number	Average examination value
Wangwu	□	1988.9	1994.3.23	13	0.53
	□	1988-1989	1994.3.24	9	0.79
	□	1987-1988	1994.3.2	10	0.65
	□	Outside of the spraying region	1994.3.24	16	0.38
Hebeiling	□	1989.5	1994.3.24	6	0.24
	□	1990.4	1994.3.24	8	0.67
	control				0

4.2 The function of AciNPV to ecosystem in frail forest environment area

Poplar loopworm (*Apocheima cinerarius* Erschoff) is an important forest foliage feeding insects in shelter forest belt of northern China and the Yellow River area. It is widely distributed in Xinjiang, Gansu, Inner Mongolia, Qinghai, Ningxia, Henan, Hebei provinces and Beijing. This pest insects mainly harm many trees including poplar, willow, mulberry, and birch. The area of distribution is more than 40 hectares every year.

This pest insect has one generation every year and it overwinters in a cocoon in the earth. At the end of February or beginning of March of next year when the soil temperature at 5-10cm deep is about 0°C the adults emerge from the earth. In mid-March they lay eggs. In mid-April the eggs hatch. In mid-May the mature larva pupate and the pupation period last for 9 months. The adults emerge generally at about 19 o'clock and can produce 300 eggs at most. The egg stage lasts about 13-30 day. Larval stage is 18-32 days and larva has a total of 5 instars. The larva feed on the tree's shoots and flower buds and the older larva eat the leaves. When the whole tree is defoliated, the larvae shift to neighboring trees by spinning and the wind. Because this pest insect emerges earlier than other pests and the natural parasite enemy is hibernating and after the natural enemy comes out this pest's larva is old enough to pupate. Its pupae are underground so its enemy is very few (Xiao 1983).

Apocheima cinerarius NPV(AciNPV) is one kind of baculovirus and it is strong pathogen to poplar loopworm. It can create epidemic diseases in the pest insect' population easily and can control the pest insect' population for a long time. The result of application for many years indicated that: 8.2×105PIB/ml were sprayed when the pest is at 3-instar larval stage .Ten days later, the average mortality rate was 84.4 percent. 1.6×10^6 PIB/ml were sprayed when the pest is at 3-instar larval stage .Nine days later, the average mortality rate was 92.3 percent. And it can control the pest insects for 3-5years normally and the longest control period is twenty years (Wang *et al.* 1983, 1988).

The elm trees are suitable for the earth of Inner Mongolia, therefore many pure elm plantation forests have been formed there. However it is very difficult to restore and form the stable ecosystem depending upon the forests' own internal regulative ability or vegetation transformation under this environmental condition and the plant community structure. So when the poplar loopworm were outbreak at there, it is difficult to control through the natural factors, therefore the insects were often occurs before the AciNPV was used. But when the AciNPV virus was used to control this pest insects through many spray ways, the stable forest ecosystem (not outbreak of poplar loopworm ecosystem) were constructed with the virus, birds and so on at this area (Li *et al.* 1995).

In Hetian Region, Xinjiang Province, Xinjiang poplar is the most suitable tree that can survive in the extremely frail ecological environmental condition. And almost all the local shelter forests and the roadside, home-side trees are composed of Xinjiang poplars. Because human and the livestock have already become the important factors in this ecosystem, it is possible that one more serious ecology consequence will be lead to if human's behavior is not proper. But it is very difficult to construct a stable ecosystem depending on measures such as planting trees, protection and release natural enemy. When the poplar loopworm invaded this ecosystem which the measures of people adopt at first was the massive apply of chemical pesticides in order to extinguish the pest. However, the measures caused the pest' natural enemy including birds perish massively and the pest disaster was more and more serious. At present, a new theory and measures has been adopted which take the pest insects as the core and protect birds and the natural factor with the AciNPV virus as a method to control the pest insects. Six years later, a relatively stable shelter, roadside, home-side forests ecosystem were being constructed and restored gradually. There has been constructing a ecosystem that the poplar loopworm exist but not cause disaster in the three counties of the Hetian Region (refer to Table 3) (Zhang *et al.* 2002; Shi Dengming *et al.* 1998).

The practice proved that the stability of the ecosystem is relative. A stable system may be constituted in the complex forest ecosystem, and a stable system may also be formed in the simple forest ecological structure in certain period. A relatively stable shelter forests, plantation forests ecological structure which also be formed pass through the pest insects as the core constructing and restoring a web of AciNPV, birds and others as inhibiting factors gradually in many regions of China.

Table 3. Results of using the AciNPV to control the *Apocheima cinerarius* in Xinjiang.

Year	Larvae density of 50 cm trees	Leaf survival rate (%)	Viral infection percentage (%)	Note
2003	29-82	70	90	The year sprayed the virus
2004	8	80	90	

Year	Larvae density of 50 cm trees	Leaf survival rate (%)	Viral infection percentage (%)	Note
2005	13	70	75	

4.3 The function of HcNPV to control the fall web worm

Since 1979 when the fall web worm (*Hyphantria cunea* Drury) was introduced to China, the pest insects outbroke successively in Liaoning Province, Shandong Province, City of Yantai , Shanxi Province, City of Tianjin, Hebei Province. Because its reproductive capacity is strong (500-2000 eggs) and has mixed feeding habits (It may do harm to 200 kinds of plants). It also has the storm feeding habits as well as its adult has strong ability to migrate. So it is a great threat to the agriculture, the forestry, the silkworm raise business and the city botanical garden of China.

HcNPV belongs to nucleopolyhedrovirus, baculoviridae. Every polyhedron body has several viral granules and its genetic material is double strand DNA. The HcNPV virus is one kind of effective biology pesticide to fall web worm. For example, The HcNPV virus (6.27×10^{11}-1.85×10^{12} PIB/ml) was applied during 1983 to 1985 in Liaoning Province when the larvae is at 2-3 instar and the total area is 3,000 hectares. The average mortality was 92.0 percent and the highest mortality is 98.9 percent (Ai *et al.* 1984; Ye *et al.* 1985; Yan 1987). The compound pesticide (HcNPV+Bt+ Chemical pesticides) were applied and the mortality is 87.7 percent ten days later (Liu *et al.* 1986). The compound microbiological pesticide made of HcNPV (2.5×10^{6} PIB/ml, 4.17×10^{6} PIB/ml,1.25×10^{7} PIB/ml) and Bt (8,000 IU/ml) was sprayed at Hebei and Shandong Province when the larvae is at 3-5 instars and very good control result has been obtained (refer to table 4.).

Table 4. Results of using the HcNPV to control the *Hyphantria cunea* in Shandong Province.

Treatment	The insect number	Mortality (%)		
		5 days	9 days	19 days
2.5×10^{6} PIB/ml	150	14	52	98.7
4.17×10^{6} PIB/ml	144	11.1	88.2	100
1.25×10^{7} PIB/ml	144	4.9	77.8	100
1.25×10^{6} PIB +8000IU Bt/ml	154	89.6	98.7	98.7
Control	146	1.3	4.1	6.2

Fall web worm is one kind of invasion biology and it has mixed feeding habits and strong adjustability to environment. So when it invades in a new ecological environment it is very easy to settle down and to erupt in a new environment where the effective control system has not been formed. Besides its own survival request, human's behavior is the main reason forces the pest to migrate to a new ecological environment. Regarding this pest insect's control, in the regions where measure can be adopted to eradicate it thoroughly the chemical pesticides was applied, in the other regions where the pest insect has already settled down which the corresponding proper control method has to be adopted from the long-term consideration. Because this pest insect's feeding habits is mixed and its reproduction ability is strong and live environment, the forestation measure is impractical means obviously, the barrier of prevention and control must be constructed by means of applying the HcNPV virus first, and then a new protection system be constructed through cultivating native natural enemy gradually.

4.4 The function of LdNPV in protecting and restoring the community of the natural enemy insects

Gypsy moth (*Lymantria dispar* (L.)) damage more than 500 kinds of plants in China including oak, poplar, pear, cherry, apple, willow, elm, birch, spruce and so on. And it mainly distribute in following areas: Heilongjiang, Jilin, Liaoning, Inner Mongolian, Shanxi, five provinces of northwest, Jiangsu, Sichuan, Guizhou, Taiwan, Hunan, Henan, Hebei, Shandong. This pest insects has one generation every year, the larva survives the winter in the egg which has completed the embryonic development, the larva hatches in the late April or the beginning of May of the next year. The first age larva can drift very far by the wind power. After second instar the larva ambushes in the fallen leaf and tree's withered leaf or the bark seam during daytime and comes out after the dusk. When the young larva is disturbed it will come down by the spinning and proliferate in the forest with the wind. The older larva has the strong ability of shift by crawl.

Gypsy moth is one kind of native forest pest insect in China and there are many natural limit factors including natural enemy insects, birds. According to the research result, it has 23 kinds of natural enemy insects in which there are 17 kinds of parasitical insects and 6 kinds of predatory insects. The accumulated parasitism rate achieved 30.1percent, include 5.2 percent to 10.9 percent is at egg stage, 9.55 percent at the larval stage and 19.08 percent at pupae stage (Feng *et al.* 1999). Gypsy moth virus has already become the important factor that influences gypsy moth's population in many regions. Therefore, a quite complex and stable food chain and ecosystem has already been formed in the nature, if there's no humanity's disturbance, it is very difficult for gypsy moth to erupt in this web system.

Lymantria dispar, NPV(LdNPV) belongs to nucleopolyhedrovirus, baculoviridae. Every polyhedron has several viral granules. Viral preparation (30-60mL, approximately equal to $3.75\text{-}7.5\times10^{11}$ PIB/hectare) was applied when 1-2 instars larvea account for 85 percent, pest insects started to die 6-8 days later, and the mortality reached the peak 12 days later, the contemporary mortality achieved above 80 percent. The LdNPV virus has already generally existed in Chinese forest ecosystem, but sometimes the gypsy moth outbreak in somewhere, maybe because the virus' reduction in natural environment and the improper measures were taken to control gypsy moth and other pest insects that induce the original ecological equilibrium was destructed and the ability of the virus to control the pest insects decline. If this circs happened the proper methods is supplement of the virus to control the pest. This method can reduce the gypsy moth populations effectively, moreover, its main goal is protection and restoration of the original stable natural ecosystem.

5. Conclusion and discussion

5.1 Restoration and construction of the stable ecosystem is the basic measure to actualize the harmful biology persistence control. Only when the ecosystem achieves the state of balance the harmful biology will become a beneficial link in the ecology chain.

5.2 The stable ecosystem is diverse and there are many methods to restore and construct it. A stable system may be constituted in the complex plants community's ecosystem through the forestation measure, uniformity a stable ecosystem may also be formed in the sole plant community's ecosystem with the harmful biology as the core.

5.3 The insect virus pesticide is one kind of effective method to restore and construct the relative stable ecosystem where there're pests but they are under control in the pest-existing regions. The insect virus is the factor of nature, the component of the ecosystem which can control the pest persistently, and it is safe to other biology and environment and in favor of other biology's survival and development. The insect virus pesticide is only one method to construct and restore the stable ecosystem, other methods can also be applied as the effective methods as long as they are useful to the goal pest insect control and do not create the destruction of the environment.

5.4 The method put forward in this paper is the preliminary result of the experiments we have been trying to control the foliage feeding insects for many years. And this method taking the virus as the main measure to restore and construct the stable ecosystem is mainly suitable for the forest ecosystem where the pest insects had occurred.

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