Biological Control of *Anoplohora glabripennis* Motsch.: A Synthesis of Current Research Programs

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Introduction

Anoplophora glabripennis Motschulsky (Asian longhorned beetle) (ALB) (Coleoptera: Cerambycidae: Lamiinae: Lamiini), is among a group of high-risk exotic woodborers native to Asia, specifically China and Korea (Nowak et al. 2001). In China, *A. glabripennis* is considered one of the most important forest pests, having been reported from 25 provinces and extending from 21°- 43° N Latitude and 100°-127° E Longitude (Yan 1985). This region extends across climatic zones that correspond to the climatic zones in North America from southern Mexico to the Great Lakes, and includes virtually all of eastern U.S. Feeding by larvae in the cambium and xylem causes widespread mortality among many deciduous broadleaf tree species in China (Yang et al., 1995), particularly *Populus* spp., *Salix* spp. and *Ulmus* spp. (Xiao 1992).

Within the U.S., *A. glabripennis* has been intercepted in 14 states, but established infestations are currently only known to exist in New York City and on Long Island (first discovered in 1996), and in Chicago, Illinois (first discovered in 1998). Utilizing the most effective method currently proven to limit its spread, approximately 5,286 and 1,509 infested trees have been located, cut and removed in the New York and Chicago infestations, respectively, as of May 2001 (U.S. Forest Service 2001). Furthermore, *A. glabripennis* has thus far been reported to attack 18 deciduous tree species in 12 genera within these two U.S. infestations (Cavey et al. 1998; USFS 2001). Most notably among these are maples (*Acer* spp.). In addition to the ability to attack and kill apparently healthy trees, *A. glabripennis* also structurally weakens trees, which poses a danger to pedestrians and vehicles from falling limbs or trees.

Although quarantines and eradication programs exist in New York and Chicago, *A. glabripennis* possesses the potential for introduction into the urban, suburban and forest landscapes, particularly in eastern U.S. Based upon field data from nine U.S. cities, national tree cover data and proposed host preference of *A. glabripennis*, the estimated potential tree resources at risk to *A. glabripennis* attack ranges from 12 to 61% of the city tree population, with an estimated value of $72 million-$23 billion per city. The corresponding loss in canopy cover that would occur if all preferred hosts were killed ranges from 13-68%, with an estimated maximum potential impact of 34.9% of total canopy cover, 30.3% tree mortality (1.2 billion trees), and value loss of $669 billion (Nowak et al. 2001).

Therefore, efforts to develop control strategies that represent alternatives to the felling and chipping of infested trees were initiated within the past three years. Included among these are biological control strategies, which are the focus of this paper. These strategies have two broad objectives. The first objective is focused on the development of mass rearing and mass production technologies, coupled with inundative release and application technologies, respectively, for various biological control agents found to be effective against *A. glabripennis*. The resulting technologies are intended to complement existing or other currently developing technologies (i.e. insecticidal controls) for use in the eradication program. The second objective is to develop technologies that could be utilized in managing *A. glabripennis* populations should eradication fail to succeed. As such, this paper will provide an update on the biological control research, with the exception of fungal pathogens, which is the subject of a companion paper in these proceedings.

Research Summaries

Nematodes **(Solter and Keena).** Entomopathogenic nematodes may offer an alternative and/or complementary method for the control of ALB, specifically targeting the larval and/or pupal stages. Therefore, four entomopathogenic rhabditoid nematode species, *Steinernema carpocapsae*, *Heterorhabditis bacteriophora*, *H. indica*, and *H. marelatus*, were tested for their ability to kill and reproduce in larvae of the ALB. The larvae were permissive to all four species but mortality was higher and production of infective juveniles (IJ) was greater for *S. carpocapsae* and *H. marelatus*. The lethal dosage of *H. marelatus* was determined to be 19 IJ's for second and third instar larvae, and 347 IJ's for fourth and fifth instar larvae. *H. marelatus* infective juveniles, applied via sponges to oviposition sites on cut logs, located and killed host larvae within 30 cm galleries, and reproduced successfully in several of the larvae. *H. marelatus* killed fifth instar host larvae in 2-6d [@ 500 (n=3) and 2000 IJ/larva (n=5)]. *H. marelatus* reproduction within fifth instar host larval cadavers ranged from 158-321 x 10^3 nematodes per cadaver. While the *S. carpocapsae* isolate is currently being evaluated in ID-50 and LD-50 studies and data have not as yet been quantitatively analyzed, it appears that *S. carpocapsae* kills its hosts somewhat faster than for *H. marelatus*. However, it also appears that *S. carpocapsae* reproduction within the dead hosts may be lower than for *H. marelatus*. Results of the initial studies have been published (Solter et al. 2001).

Bacillus thuringiensis **(D'Amico).** *Bacillus thuringiensis* (Bt) was evaluated as a microbial insecticide against larval and adult ALB. Studies in which existing commercial Bt products ("whole" Bt requiring activation) were incorporated into diet and fed to larvae and adult ALB, showed that they were not effective against either ALB life stage. Voltage clamp assays resulted in the identification of several Cry toxins that were effective against ALB larval midgut in vitro, especially Cry 1B. Assay of available Cry 1B showed that it is not effective against ALB larvae in vivo. It was noted that midgut environment in vivo may not be suitable for Bt MOA, which may cause a discrepancy between in vitro work and bioassays. Brush Border Cell Assays (conducted by D. Dean, OSU) provided positive results that led to preparation of Cry toxin for use against adult ALB. This preparation, as well as new commercial products will be evaluated against adult ALB in 2002.

Microsporidia **(Bauer).** Working with Deborah Miller and Houping Liu, a new microsporidium isolate from ALB larvae was collected in Henan Province, China (December 2001), but it is as yet unidentified. Infection prevalence of larval samples was ca 2% (n=97).

Predators and Parasitoids **(Smith et.al.; Herard et.al.; Bauer et.al.).** A number of natural enemies of the Cerambycidae have been reported worldwide, including predators belonging to the Cucujidae, Ostomidae, Cleridae, Colydiidae, and Elateridae beetles; Asilidae, Xylophagidae, and Rhagionidae flies; Phymatidae and Reduviidae bugs; and predaceous thrips and carpenter ants, as well as parasitoids belonging to the Braconidae, Ichneumonidae, Bethylidae, Encyrtidae, Eulophidae, Gasteruptiidae, Pteromalidae, Eupelmidae, and Eurytomidae, wasps; and Tachinidae and Sarcophagidae flies (Linsley 1961). As such, identification of effective parasitoids for use in eradication and/or management of ALB has been underway, both within the country of origin (China in particular), as well as outside the country of origin (including Europe and North America). Two approaches are being utilized in these efforts: (1) evaluation of natural enemies known to attack ALB in China; and (2) evaluation of possible new associations between ALB and natural enemies of other cerambycids in China, Europe and North America. However, in order to focus efforts on those natural enemy species with a greater probability of providing biological control of ALB, selection and prioritization of these natural enemies are based largely upon the relatedness of their respective longhorned beetle hosts to ALB at three levels: (1) phylogenetic relatedness; (2) ecological relatedness (e.g. climate; habitat; host tree species and condition); and (3) behavioral relatedness (e.g. larval feeding behavior) (Smith 1999). Certain aspects of each of these likely play a major role in determining the potential efficacy of a given natural enemy to control ALB, as well as other *Anoplophora* species.

China. In China, parasitoids have been identified that are known to attack longhorned beetles that share a common host tree with ALB, as well as those known to attack ALB and/or other *Anoplophora* species. Among the first group are several egg parasitoids, including: the encyrtids *Oophagus batocerae* and *Zaommoencyrtus brachytarsus*, parasitoids of *Batocera horsfieldi*, and *Austroencyrtus ceresii*, a parasitoid of *Ceresium sinicum*; and the eulophid *Aprostocetus prolixus*, a parasitoid of *Apriona germarii*. Also among the first group are larval parasitoids, including: the tachinid *Bullaea* sp., the bethylid *Scleroderma guani*, a parasitoid of *Saperda populnea*, *Semenotus bifasciatus* and *Semenotus sinoauster*; the braconids, *Ontsira palliates*, a parasitoid of *Semenotus bifasciatus* and *Semenotus sinoauster*, and *Zombrus bic*olor and *Zombrus sjoestedti*, larval parasitoids of cerambycid spp.; and the ichneumonids *Xylophrurus coreensis*, *Schreineria* sp and *Megarhyssa* sp., larval parasitoids of cerambycid spp. Among the second group is the egg parasitoid *Aprostocetus fukutai* (Eulophidae), which parasitizes both *Anoplophora chinensis* and *A. germarii* (Liao et al, 1987; Wang and Zhao, 1988). However, no egg parasitoids have as yet been reported from ALB or *A. nobilis* (Yan and Qin, 1992; Zhou, 1992). Also among this second group are several larval parasitoids, including the braconids *Ontsira* sp. parasitizing *A. chinensis* larvae, and *Ontsira anoplophorae* sp. nov., parasitizing Anoplophora malasiaca on citrus; as well as the Colydiidae beetle Dastarcus longulus, a larval-pupal parasitoid of ALB, *A. nobilis*, *B. horsfieldi, A. germarii, Monochamus alternatus*, and *Trirachys orientalis* (Qin and Gao, 1988).

To date, investigations by Smith et al. have found no egg parasitoids of ALB. Therefore, their efforts have focused in large part on two of the species mentioned above, *S. guani* and *D. longulus*. Primary objectives have been to evaluate their relative efficacy to parasitize ALB, and to develop mass rearing technology. Results from studies of *S. guani*, to date, have shown that *S. guani* is an idiobiont ectoparasitoid, and females first paralyze their host by stinging, which immobilizes the host, and then lay eggs on the host body. Larvae are gregarious while developing on their host. After hosts are consumed, mature wasp larvae spin cocoons and pupate. An average of 45 adult *S. guani* emerged from a single mature host larva of *Saperda populnea*. In nature, *S. guani* was found parasitizing 41.9 - 92.3% of *S. populnea* larvae in poplar stands in many areas. Parental wasps remain with their young until they have completed their development and emerged as adult wasps. Should their eggs or larvae become separated from the host, parental wasps have been observed to return them to the host. Most female wasps are apterous, and *S. guani* usually parasitizes longhorned beetle species whose larvae are small, ca. 15 mm in length. Therefore, *S. guani* would be used to specifically target ALB 1st to 3rd instar larvae. Results from studies of *D. longulus*, to date, have shown that it is an ectoparasitoid, with females laying eggs in frass and sawdust in host gallery or on the host gallery wall. First instar larvae possess thoracic legs and crawl in search of a host. Upon finding an acceptable host, the larvae lose their thoracic legs and attach to the body of its host for feeding. It feeds singly or gregariously on its host, and as many as 30 individuals of this parasitoid are capable of successfully completing their development on a single ALB larva or pupa, which usually kills the ALB within 10 days. In many areas, parasitization rates of ALB by *D. longulus* has found to reach between 50-70%, and in locations where *D. longulus* is established in relatively high numbers, ALB is said to be under natural control. Investigations focused on development of mass rearing technologies for both of these species are in progress and are promising. Given their respective optimal preferences for different sized larvae, as well as the wingless nature of *S. guani*, inundative releases of these two species, in tandem, appears to offer a possible complementary approach to the existing strategies in the ALB eradication program. However, prior to releases, non-target studies are planned and will be conducted at BIIR. Results of the initial studies have been published (Smith et.al. 1999; Yang and Smith 2000, 2001)

Europe. Herard et. al. recently initiated investigations of potential natural enemies of ALB in Europe, with an initial emphasis on studies of *Saperda populnea* (L.) and *Saperda carcharias* (L.). These two species were selected because they share common traits with ALB: (1) both are Lamiinae; (2) both attack trembling aspens, poplars, and willows, trees that are among the preferred hosts of ALB in China; and (3) both attack healthy trees. While no egg parasitoids have been found in France to date, the eulophid *Euderus caudatus* has been reported as an egg parasitoid of *S. populnea* and *S. carcharias*. Two early larval parasitoids have been found thus far: a tachinid (not yet been identified) from France (southern and eastern) and Finland, and the eulophid *Euderus albitarsis* from southern Finland, where it was found parasitizing 1st instar *S. populnea* larvae. Two parasitoids whose adults emerged from full-grown larvae of *S. populnea* were found in 2001: the tachinid *Billaea irrorata* and the ichneumonid *Dolichomitus populneus*, previously mentioned from *S. populnea* and *S. carcharias*. Although *B. irrorata* emerges fairly late during its host development, its ability to attack very early larval instars will be elucidated. Rate of parasitism by each species in the various sites has not as yet been determined.

In addition, the following predatory Diptera larvae were found by dissection of branches in *S. populnea* galleries: *Odinia xanthocera* (Diptera, Odiniidae), *Lasiambia baliola* (Diptera, Chloropidae), and *Thaumatomyia elongatula* (Diptera, Chloropidae). While no braconids have been found to date, four species are known parasitoids of *S. populnea* and one species is known to parasitize *S. carcharias*. Among tachinids, two other species are known (one from *S. populnea* and one from *S. carcharias*). Among Ichneumonids, 22 other species are known from *S. populnea*, and 11 other species are known from *S. carcharias*. Consequently, it appears that the biocomplex of enemies of these two cerambycids in Europe constitutes a great reservoir of species that can be tested against *Anoplophora* spp.

In concert with identifying and selecting candidate natural enemies for evaluation against ALB, development of laboratory rearing techniques for the cerambycid species, specifically on live plant material, was initiated. Studies that are planned for 2002 include: (1) continue exploration and develop an inventory of early stage parasitoids of *S. populnea* and *S. carcharias* across Europe; (2) finalize *S. populnea* and *S. carcharias* rearing techniques using rooted cuttings; (3) implement ALB rearing techniques in 5-10 cm diameter rooted cuttings; (4) test *Saperda* spp. parasitoids on ALB, in quarantine at Montpellier; and (5) survey ALB and *Anoplophora chinensis* populations in sites where these 2 species were accidentally introduced in Europe, for possible occurrence of parasitism by local species. The anticipated product(s) from these studies are parasitoids of the Western Palearctic region cerambycids that show promise as efficacious biological control agents against early stages of ALB, and which can be used in the Nearctic region without significant non-target effects on North American ecosystems.

North America: Smith and Fuester, and Bauer et al. recently initiated investigations of potential natural enemies of ALB in North America. Both groups, to date, have found a dipteran parasitoid associated with ALB-infested trees (Bauer et al. from Chicago, 2001) (Smith et al. in Norway maple trees from New York, 1998), which has not yet been identified. In addition, however, to searching for natural enemies associated with ALB-infested trees in New York and Chicago, efforts to evaluated parasitoids of selected North American cerambycids are underway. To reiterate, in an effort to identify North American natural enemies that are most likely to adapt to ALB, Smith (1999) proposed that priority should be given to those natural enemy species whose cerambycid hosts are most similar/related to ALB (phylogenetically, ecologically and behaviorally).

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