

Prospects for Inundative Release of Natural Enemies for Biological Control of *Anoplophora glabripennis*

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Introduction

Anoplophora glabripennis is native to China and Korea, where it is a serious pest of deciduous broadleaf tree species [1], particularly poplar and willow, as well as elm and maple. It is widespread in China, found in parts of at least 25 provinces. ALB is thought to have been accidentally introduced into the U.S. in solid wood packing materials originating from China, with breeding populations discovered in New York City and Long Island (1996), Chicago (1998) [2, 3], and Jersey City, NJ (2002). By September 1, 2002, 5,888 and 1,545 trees had been found infested, cut and removed in New York and Chicago, respectively [4, 5]. For the lumber, maple syrup, tourist and other forest-related industries, costs in survey, detection and management costs, and lost revenues could mount into the billions of dollars [3]. Furthermore, a recent report projects that if ALB spreads to urban trees across North America, there could be a loss of 35 percent of total canopy cover (1.2 billion trees) and a compensatory value loss of \$669 billion [6].

Objectives

Eradication of ALB currently relies primarily upon: (1) detection of infested trees that are then cut and chipped, and (2) injection of trees with systemic insecticides that target adult beetles should they feed on trees and/or larva feeding within infested trees. However, detection of infested trees relies solely on visual surveys, which are labor-intensive and costly, and only ca. 30-60 percent effective. Furthermore, use of systemic insecticides is labor-intensive and costly, and thought to be primarily effective against adult beetles. Therefore, the objectives of this research are to develop and utilize biological control agents: (1) that possess good dispersal and host searching capability; (2) that are highly effective parasitoids; (3) that can be efficiently reared and released in large numbers; and (4) for which an operationally feasible distribution system and protocol can be developed for effective delivery within the APHIS eradication program. The biological control approach of choice for eradication, which is the focus herein, is inundative release of parasitoids. Inundative biological control is the release of large numbers of mass-produced biological control agents to reduce pest populations without necessarily achieving establishment.

Sources of Natural Enemies

The potential sources of natural enemies of ALB under consideration are as follows:

	Natural Enemies of ALB	Congeners of Non-Indigenous Natural Enemy Species	Natural Enemies of Related Cerambycids
Exotic Natural Enemy Species	Asia Europe		Asia Europe
Native Natural Enemy Species	North America	North America	North America

Prioritized selection of natural enemies attacking other cerambycid species is based upon: (1) the phylogenetic, ecological and behavioral relatedness of their cerambycid hosts to ALB, including North American congener species [7]; and (2) the ALB life stage attacked, with emphasis on parasitoids that attack eggs and early larvae instars. Project components are composed of at least five primary objectives, each with a series of systematic lines of research: (1) parasitoid efficacy evaluation (i.e. mobility/dispersal; host searching; parasitism rate); (2) nontarget evaluation (i.e. identify; bioassay; permit acquisition); (3) development of mass rearing technology (i.e. substitute hosts; nutritional ecology; artificial diets); (4) development of release technology and protocols (i.e. prerelease conditioning; delivery systems & protocols); and (5) implementation (i.e. mass rearing; inundative release; monitoring efficacy, spread & establishment).

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: 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 germari*. Also among the first group are larval parasitoids, including: the tachinid *Bullaea* sp., the bethylid *Scleroderma guani*, a parasitoid of *Saperda populnea*, *Semanotus bifasciatus* and *Semanotus sinoauster*; the braconids, *Ontsira palliates*, a parasitoid of *Semanotus bifasciatus* and *Semanotus sinoauster*, and *Zombrus bicolor* 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. germari* [8, 9]. However, no egg parasitoids have as yet been reported from ALB or *A. nobilis* [10, 11]. Also among this second group are several larval parasitoids, including the braconids *Ontsira* sp. parasitizing *A. chinensis* larvae, and *Ontsira anoplophorae* Kusigemati and Hashimoto., parasitizing *Anoplophora malasiaca* on citrus in Japan; as well as the Colydiidae beetle *Dastarcus longulus*, a larval-pupal parasitoid of ALB, *A. nobilis*, *B. horsfieldi*, *A. germari*, *Monochamus alternatus*, and *Trirachys orientalis* [12].

To date, investigations by Yang, Smith et. al. have found no egg parasitoids of ALB. Therefore, 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.

***Scleroderma guani*:** Results from studies of *S. guani*, to date, have shown that this species is an idiobiont ectoparasitoid, and females first paralyze their host by stinging, 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 percent 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 likely be used to specifically target ALB 1st to 3rd instar larvae.

***Sclerodermus* spp. (Hymenoptera: Bethyilidae):** Two species, *Sclerodermus guani* and *S. sichuanensis*, have been used in large scale release programs for biological control of woodboring Coleoptera in the Far East. Releases of *S. guani* were made against the cerambycids *Saperda populnea* (64.5 percent control) [13], *Semanotus bifasciatus* in fir forests (62-64 percent parasitism, with a reduction of infested trees from 39 percent down to ca. 2 percent) [14], and *Monochamus alternatus* in pine forests for indirect control of pine wood nematode (*Bursaphelenchus xylophilus*) [15]. In the latter case, parasitism ranged 26-47 percent, averaging 35 percent, and control of withering caused by the nematode was reduced 68-87 percent. In addition, *S. guani* was released against the anobiid beetle *Ptilinus fuscus*, in poplar, but the degree of efficacy was not clearly stated in the abstract [16]. Releases of *S. sechuanensis* were made against *M. alternatus* and *S. bifasciatus* with rates of parasitism ranging 36-100 percent [17]. Zhou et al. [18] conducted studies on the biology of *S. sechuanensis* and stated that it could reproduce on a number of hosts such as *Semanotus sinoauster*, Hymenoptera and lepidopteran larvae. It was not clear from the abstract whether attacks on Hymenoptera and Lepidoptera occurred in nature, or were limited to laboratory trials. Attacks on surrogate hosts in the laboratory do not necessarily indicate that such attacks would occur in nature [19], but if such a broad host range were exhibited under field conditions, the introduction of *S. sechuanensis* could not be entertained. Mass rearing of *S. guani* has been reported [20 and references cited therein], and studies are currently continuing.

***Dastarcus longulus*:** Results from studies of *D. longulus*, to date, have shown that it is an ectoparasitoid, with females laying eggs in frass and sawdust in the 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* reach between 50-70 percent, and in locations where *D. longulus* is established in relatively high numbers, ALB is apparently under natural control.

Additional investigations, which are in progress, have focused on development of mass rearing technologies for both of these species. Given their respective optimal preferences for different sized larvae, 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, nontarget studies are planned and will be conducted at BIIR. Preliminary results of the initial studies have been published [21, 22, 23]. In addition, two manuscripts on *D. longulus* have been written and will soon be submitted for journal publication, and additional manuscripts are in preparation. It should also be mentioned that investigations of a braconid species, not previously described, have been initiated. Studies conducted under natural field conditions (Smith and Bancroft, unpublished), have thus far suggested that it may be an egg or early larval parasitoid of ALB.

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, 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 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 *Dolichomitrus*

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 in *S. populnea* galleries by dissection of branches: *Odinia xanthocera* (Odiinidae), *Lasiambia baliola* (Chloropidae), and *Thaumatomyia elongatula* (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 were planned for 2002 included:

- (1) Continue exploration and develop of an inventory of early stage parasitoids of *S. populnea* and *S. carcharias* across Europe. During summer 2002, 80 sites with low to high levels of infestation by *S. populnea* and *S. carcharias* were visited across France, Germany, Denmark, Sweden, and Finland. In northern Europe, *S. populnea* has a 2-year cycle. The populations of adult hosts, including egg-laying females, and of the solitary early-stage parasitoid *Euderus albitarsis* (Hym.: Eulophidae) had been abundant during summer 2001 in most of the visited sites. In contrast, during summer 2002, the populations of host and parasitoid adults were extremely low in most sites simultaneously, suggesting that this natural enemy is available in the field during odd years mainly, in this part of its native area. However, in Finland we identified a very few sites where the development cycles of the host and its parasitoid appeared to be asynchronous compared with what was observed in most other sites (host oviposition and attack by *E. albitarsis* would occur during even years). During winter 2001-2002, it was determined that *E. albitarsis* hibernates as pupae in the gallery of its host, as first or a second instar larva of *S. populnea*. Concerning *S. carcharias*, in Finland, the last week of June and the first week of July, 2002 was a period of intense activity of the adults (feeding on leaves, and ovipositing). This was also

the time of emergence of the gregarious egg parasitoid *Euderus caudatus* (Hym.: Eulophidae). *E. caudatus* was obtained from 17 out of the 34 sites where its host was collected. During winter 2001-2002, it had been determined that *E. caudatus* hibernates as full-grown larvae in the host eggs.

(2) Finalize *S. populnea* and *S. carcharias* rearing techniques using rooted cuttings. Rooted cuttings of *Populus deltoides*, *P. tremula*, *P. alba*, and *Salix* sp. were successfully grown in a greenhouse (initiated fall 2001) using a soil mixture and watering regime. However, infestation of the rooted cuttings by *S. populnea* did not equally affect the various host plants. For example, it was revealed that *P. alba* is the best host plant in terms of survival following pest attack. In addition, no problem of unexpected secondary pest was observed on this host plant. Rooted logs of *Salix* sp., 4 to 10 cm in diameter and 50 cm in length, were successfully cultured for the purpose of rearing *Lamia textor* (European Cerambycid, Lamiinae, Lamiini) (initiated March 2002), and *S. carcharias* (initiated July 2002). Survival rate of the rooted logs was affected by the number of oviposition scars and developing larvae. For example, if long survival of the logs is desired, using logs of the above mentioned size, a maximum number of two oviposition scars per log should be allowed. If the logs are maintained to allow the cerambycid larvae to only reach a medium size, deposition of no more than five cerambycid eggs per log should be allowed. In some instances, survival rate of logs was also affected by the occurrence of a secondary pest, a Homoptera encrusted in crevices of bark, which developed from eggs that were not visible when the logs were collected in the field. Careful washing of the infested logs was insufficient to solve this problem. Thus, it is suggested that logs be cut and rooted at least 6 months prior to exposure to cerambycids. Following collection in the field, the logs should be sprayed with an insecticide (with low residual activity) and then isolated in sleeve cages to totally eliminate any risk of inadvertent infestation by secondary pests.

(3) Implement ALB rearing techniques in 5-10 cm diameter rooted cuttings. The same type of logs of *Salix* sp. were successfully used to rear ALB and CLB (*Anoplophora chinensis*).

(4) Test *Saperda* spp. parasitoids on ALB, in quarantine at Montpellier, France. *Euderus albitarsis* (Hym.: Eulophidae), and *Dolichomitus populneus* (Hym.: Ichneumonidae), two larval parasitoids of *S. populnea*, were tested on ALB. *D. populneus* was also tested on CLB and on *L. textor*, a European cerambycid we are now rearing. We think that *L. textor* may eventually be used as a surrogate for the production of parasitoids in the

laboratory. The egg parasitoid *Euderus caudatus* was tested using rooted cuttings of *Salix* sp. infested with eggs of its original host, *S. carcharias*, rooted cuttings infested with eggs of *L. textor*, and rooted cuttings infested with eggs of ALB. All infested rooted cuttings that had been exposed to parasitoids were placed in a sleeve cage made of organdy until dissection. Infestation of rooted cuttings and rate of parasitization of the hosts will be studied during winter 2002 by collecting data during dissection of the plant material. Dissections will not be completed before the end of 2002. Thus, results of the tests are not yet available.

(5) Survey ALB and *Anoplophora chinensis* populations in sites where these two species were accidentally introduced in Europe, for possible occurrence of parasitism by native species. The ALB population that occurred in Braunau (Austria) until January 2002 was submitted to a program of eradication by the Austrian local authorities. Before the operation of eradication was made, branches of infested maple trees were given to us by our Austrian colleagues for dissection. Unhatched eggs of ALB and one L3 larva were placed on diet. No parasitoids emerged from these pest stages.

The CLB population that established at Parabiago (near Milan, Italy) was visited in December 2001 and February 2002. Infested trees were cut and the stumps dissected. Larvae of various stages were sampled and placed on artificial diet until adult eclosion. None of these larvae were found to be parasitized. Three unhatched eggs of CLB were separated during dissection of the stumps. One was old, empty, and showed an exit hole of a parasitoid. The two other eggs were fresh and unhatched. One of these two eggs was dissected and revealed the presence of larvae of a gregarious parasitoid. The second egg was maintained intact. The parasitoid larvae found in the first egg were subjected to DNA analysis by Marie-Claude Bon, molecular biologist at EBCL, Montpellier, France. This analysis showed that the parasitoid has a high probability of being a Eulophid belonging to the genus *Aprostocetus*. A comparison of the data available from existing gene banks allowed us to determine that the probability of this *Aprostocetus* to belong to the European fauna was much greater than the probability that it belonged to the Asian fauna. The second unhatched CLB egg was kept until mid July 2002 when eight adult chalcids emerged. The specimens were submitted to Gérard Delvare, chalcid taxonomist at CIRAD, Montpellier, France, who identified them as a new species of *Aprostocetus* (sub-genus *Aprostocetus*). Additional DNA analyses and comparisons with fresh material of other European *Aprostocetus* were planned. DNA analyses of Asian material and comparisons with our European material is also foreseen. Additional

collections of CLB infested material are planned during winter 2002-2003 in Italy to find more parasitized eggs, and to try to determine the original host of this *Aprostocetus* 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 nontarget effects on North American ecosystems.

North America

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, Bethyidae, Encyrtidae, Eulophidae, Gasteruptionidae, Pteromalidae, Eupelmidae, and Eurytomidae; and Tachinidae and Sarcophagidae flies [24]. Natural enemy species that appear to be consistently associated with Cerambycidae are *Avetianella longoi* (egg parasitoid), and tachinids in the genus *Billaea*, and the braconid *Iphiaulax imposter*.

This third line of research is being pursued because host specificity is an important consideration in obtaining regulatory permission to make releases of nonindigenous natural enemies. Host specificity can be either phylogenetically or ecologically driven. Phylogenetic host specificity is associated with host-parasite groups that clearly have had a long history of co-adaptation. Examples include weevils with ichneumonids in the genus *Bathyplectes*, and aphids with parasitoids in the subfamily Aphidiinae. Ecological host specificity, on the other hand, is prevalent among natural enemies that attack hosts that live in concealed places, such as gallmakers, leafminers, and woodborers. With these kinds of herbivores, host specificity might be determined by such secondary considerations as the host plant, or size or shape of the gall; thus, ecologically similar but taxonomically unrelated host species may have similar or nearly identical parasite complexes. Thus, it might prove difficult to find imported natural enemies that will not attack a wide range of nontarget organisms.

A. Natural Enemies of ALB: Smith and Fuester recently initiated investigations of potential natural enemies of ALB in North America. To date, they have found a dipteran parasitoid associated with ALB-infested Norway maple trees from New York (1998), which has not yet been identified. Progress in this area has been slow due in part to the lack of access to, and/or short supply of ALB-

infested logs originating from the U.S. infestations. In addition to searching for natural enemies associated with ALB-infested trees in New York and Chicago, efforts to identify and evaluate parasitoids of selected North American cerambycids are in the planning stages.

B. Congeners of NonIndigenous Natural Enemies:

North American congeners of nonindigenous natural enemies found attacking *A. glabripennis* or other *Anoplophora* species in Asia or Europe is another approach that offers several advantages. Among potential congeners of *Sclerodermus* under consideration are *Sclerodermus carolinensis*, whose hosts include *Dicerca lepida* (Buprestidae: Buprestini) and *Urographis fasciatus* (Cerambycidae: Lamiinae); and *Scleroderma macrogaster*, whose hosts include *Megacyllene antennatus* (Cerambycidae: Clytini), *Scolytus rugulosus* (Scolytidae: Scolytini), and *Xyletinus peltatus* (Anobiidae: Xyletininae). While no North American *Dastarcus* species are reported, 85 species of Colydiidae are reported, with some parasitizing cerambycids [25]. Other common hosts include scolytids and platypodids. Colydiids are reported as predators, feeding on small wood-borers, parasitoids, and feeders of decaying plant matter. Other possible congeners of nonindigenous natural enemies of *Anoplophora* are under consideration, including *Ontsira anoplophorae*, a braconid parasitoid of *A. malasiaca*.

C. Potential Native Cerambycid Sources: Because host plant recognition is frequently an important component in the sequence of events (host location → host acceptability → host suitability) that determine host utilization, natural enemies of cerambycids that attack trees preferred by *A. glabripennis* might prove to be promising candidates for inundative releases against this pest. Prime candidates would include the sugar maple borer, *Glycobius speciosus* (Say); poplar borer, *Saperda calcarata* Say; poplar-butt borer, *Xylotrechus obliteratus* LeC.; and cottonwood borer, *Plectrodera scalator*. Other common cerambycids that could yield natural enemies include banded ash borer, *Neoclytus caprea*; hickory borer, *Goes pulcher*; and locust borer, *Megacyllene robiniae*.

Summary: Inundative Release of Natural Enemies and Eradication

Although most of the applied research on parasites and predators in biological control has been focused on the suppression of pest populations, Knipling [26] has proposed using the parasite augmentation technique to eradicate pests from prescribed areas, especially in concert with autocidal techniques. At present, mass rearing of the ALB is not possible and autocidal techniques therefore seem problematic, so inundative releases might have to be a stand-alone strategy or

combined with other techniques. The parasite complexes of forest insects usually contain parasitoid species that are well adapted to low host densities and others that predominate when outbreaks of the host occur [27]. Therefore, it might be necessary to use two or more species in an eradication program, including high density specialists in the beginning to knock down populations, and other species with the highest host-finding ability at the end of the program. Compared to most insects, ALB is a relatively low fecundity species, so it might be possible to find natural enemies with sufficient biotic potential and searching capacity to use inundative releases of natural enemies to eliminate localized ALB infestations.

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