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SURVEY AND EVALUATION OF POTENTIAL NATURAL ENEMIES OF *ANOPLOPHORA GLABRIPENNIS* AND *A. CHINENSIS* (COL.: CERAMBYCIDAE) IN ITALY

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SUMMARY:
Anoplophora glabripennis (ALB), and *A. chinensis* (CLB) (Col.: Cerambycidae), originating from the Far East, have been accidentally introduced in a few urban sites in North America and Europe where they are considered as serious threats to the urban and natural deciduous forests. In conjunction with eradication, biological control studies were initiated in order to find, to identify, and to evaluate the parasitoids that could successfully control these pests. In the CLB-infested area in Italy, the following parasitoids were inventoried: the egg parasitoid, *Aprostocetus anoplophorae* n. sp., very likely originating from eastern Asia, is specific to CLB; the larval ectoparasitoids, *Spathius erythrocephalus* (Braconidae), *Eurytoma melanoneura* (Eurytomidae), *Calosota vernalis* (Eupelmidae), *Cleonymus brevis* and *Trigonoderus* sp. (Pteromalidae), and *Sclerodermus* sp. (Bethylidae) attacked both *Anoplophora* hosts exposed in the study sites.

Key-words: *Anoplophora glabripennis*, *Anoplophora chinensis*, parasitoid, Italy

RÉSUMÉ:
PROSPECTION ET EVALUATION D'ENNEMIS NATURELS POTENTIELS D'*ANOPLOPHORA GLABRIPENNIS* ET *A. CHINENSIS* EN ITALIE
Anoplophora glabripennis (ALB), et *A. chinensis* (CLB) (Col. : Cerambycidae), originaires d'Extrême-Orient, ont été introduits accidentellement dans quelques sites urbains en Amérique du Nord et en France où ils représentent des menaces sérieuses pour les arbres d'ornement et les forêts. Conjointement aux efforts d'éradication, des études de lutte biologique ont été initiées pour inventorier, identifier, et évaluer les parasitoïdes qui pourraient faire échec à ces ravageurs. Dans la zone infestée par CLB en Italie, les parasitoïdes suivants ont été inventoriés : le parasitoïde d'œuf, *Aprostocetus anoplophorae* n. sp., très probablement originaire d'Asie orientale, est spécifique de CLB ; les ectoparasitoïdes larvaires, *Spathius erythrocephalus* (Braconidae), *Eurytoma melanoneura* (Eurytomidae), *Calosota vernalis* (Eupelmidae), *Cleonymus brevis* et *Trigonoderus* sp. (Pteromalidae), et *Sclerodermus* sp. (Bethylidae) ont attaqué les 2 hôtes *Anoplophora* exposés dans les sites d'études.

Mots-clés : *Anoplophora glabripennis*, *Anoplophora chinensis*, parasitoïde, Italie

INTRODUCTION

The two longhorned beetles *Anoplophora glabripennis* (Motschulsky), Asian Longhorned Beetle (ALB), and *Anoplophora chinensis* (Förster) (= *malasiaca*), Citrus Longhorned Beetle (CLB) (Coleoptera, Cerambycidae) have been accidentally introduced in North America (Haack et al., 1997) and Europe (Colombo & Limonta, 2001; Dauber & Mitter, 2001; Cocquempot & Hérard, 2003) where they are considered as serious threats to the urban and

natural forests, and are subject to eradication. Both pests are originating from Eastern Asia where they cause serious damages to many deciduous trees, mainly in the genera *Populus*, *Acer* and *Salix*. *A. chinensis* is also a major pest of *Citrus* in Japan (Adachi, 1994). In conjunction with the eradication programs, biological control studies were initiated in order to find, to identify, and to evaluate the parasitoids that could successfully control *Anoplophora glabripennis* and *A. chinensis* (Smith et al., 2003; Hérard et al., 2004). Recently a survey was attempted to find possible new associations between introduced *Anoplophora* spp. and natural enemies from the European fauna. We report here the first results of this survey and of the evaluation of some potential natural enemies based on both field and laboratory testings.

MATERIALS AND METHODS

Study sites and exposure cages. In 2000, the presence of *A. chinensis* was reported at Parabiago (MI), Italy, in the neighborhood of a nursery where bonsais imported from Eastern Asia were grown (Colombo & Limonta, 2001). Some evidence of much older CLB introductions was found later. A recent monitoring showed that the infested area extends in fact on more than 60 km^2 in the Northwest of Milan. In absence of *Citrus*, the pest attacks maples, preferably, and many other deciduous trees: 25 host-plant species were inventoried near Milan. At Parabiago, CLB adults start emerging in early June and are active till September. However, the main peak of egg laying activity lasts from mid-June through mid-July.

In 2003 and 2004, one of our study sites was selected at Parabiago, near the initial point of infestation. In 2005, two additional sites were selected, at Inveruno and at Gallarate, 11 and 16 km apart from the first study site, respectively. In each site, sentinel plants infested with eggs and early stage larvae of ALB or CLB were exposed for 2 weeks to the attacks of the responsive parasitoids living in these habitats. In each site, the plants were placed in a cage that was constructed of wire mesh and plastic netting to exclude birds and let the insects fly through it. The cages measured 1.8 m tall, 3.0 m wide, and 3.0 m deep. They were set up in woodlots selected for their secure fences and locked gates.

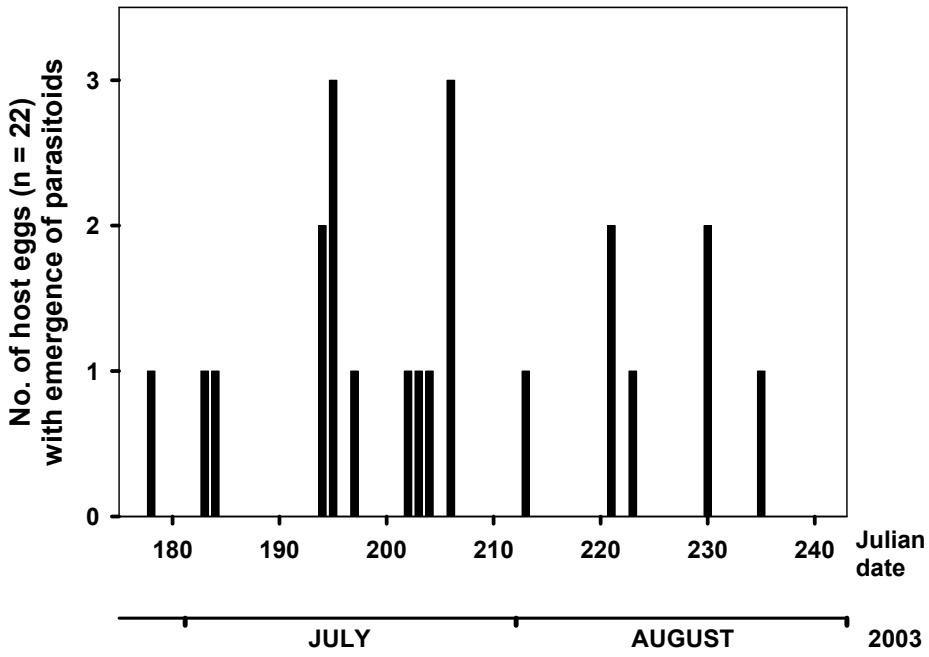
Sentinel plants. The sentinel plants were potted rooted cuttings of *Salix alba*. In the quarantine greenhouse of EBCL, at Montferrier-sur-Lez, France, they were prepared using pieces of branches 50 cm long and 4 - 9 cm diameter, driven in soil on 15 cm, in 10-liter plastic pots. After 3 weeks, when the cuttings began to root and to produce leaves, a pair of ALB or CLB adults was caged with a plant for egg deposition under bark during 5 days. Among the infested plants prepared for a particular period of exposure in the field, 2 sub-batches of plants had eggs with varied ages when they were installed in the field. Incubation of ALB and CLB eggs lasting 15 – 20 days at 22°C, the eggs in the first infested batch of plants hatched during the exposure in the field. In this way, early larvae were also exposed to the local parasitoids that were responsive to this particular stage of the hosts. At each date of a visit in the field, 96 infested plants were transported to Italy thanks to the official authorizations from the French and the Italian Plant Protection Services, which have been fully informed about our objectives and methodologies. A batch of 32 plants was exposed in each of the 3 sites. Among these plants, a sub-batch of 16 plants was infested with eggs of ALB and 16 other plants were infested with eggs of CLB. In each sub-batch, 8 plants contained freshly laid eggs, and 8 plants contained older eggs. Every 2 weeks, the 96 plants of the 3 sites were replaced by a new batch of freshly infested plants. The experiment lasted from early June through late September. The plants that were exposed in the field were carried back to the EBCL quarantine laboratory in France and dissected to separate the parasitized eggs and larvae. The pre-adult stages of parasitoids were maintained until adult emergence and identification, and used to create laboratory colonies for further rearing studies and evaluation.

RESULTS & DISCUSSION

Aprostocetus anoplophorae n. sp. (Hym.: Eulophidae)

For the first time in early 2002, at Parabiago, we found a gregarious egg parasitoid of CLB as hibernating larvae in unhatched host eggs. The number of larvae per host egg ranged from 5 through 36. The species was not known from Europe and Asia. This was a new species the description of which was made by Delvare et al. (2004) as *Aprostocetus anoplophorae* n. sp. Based on the available data, we could speculate that *Aprostocetus anoplophorae* was introduced in Italy together with its host, *A. chinensis*. In the laboratory, *A. anoplophorae* appeared to be highly specific to CLB and never accepted ALB as a host. In the field, it was always found in CLB eggs but not in the ALB eggs available in the next sentinel plants. *A. anoplophorae* overwinter as diapausing larvae in host eggs. From an overwintering cohort of host eggs collected in April 2003, adult parasitoids emerged late June through late August, 2003 (Fig. 1).

Figure 1: Emergence of the adults of *Aprostocetus anoplophorae* from their host eggs during 2003, following winter diapause
(Emergence des adultes d'*Aprostocetus anoplophorae* de leurs œufs-hôtes en 2003, à l'issue de la diapause hivernale)



The first signs of any parasitization activity could be noticed in early July. From data collected during 2003 through 2005, the rates of parasitism observed in the sentinel plants varied from 4.3% to 24.4%. It was shown that *A. anoplophorae* attacks most hosts in July and August. However, its parasitization activity could extend beyond. For instance, in early September 2003, we observed some *A. anoplophorae* adults emerging from the diapausing material that was collected during the previous winter as well as from the first summer generation. Obviously, both a diapausing winter generation and two summer generations can occur. Nevertheless, adults from the diapausing cohort, with late emergence in early September, seem to develop only one summer generation, which could attack the CLB eggs deposited in

late summer. It appears that *A. anoplophorae* has the features of a promising biological control agent against *A. chinensis*: 1. it shows a high level of host specificity; 2. it attacks hosts during a long period in summer; 3. both host and parasitoid phenologies are synchronized; 4. it displays 2 or 3 generations per year; 5. it is a gregarious species, which allows the production of many individuals from a few hosts.

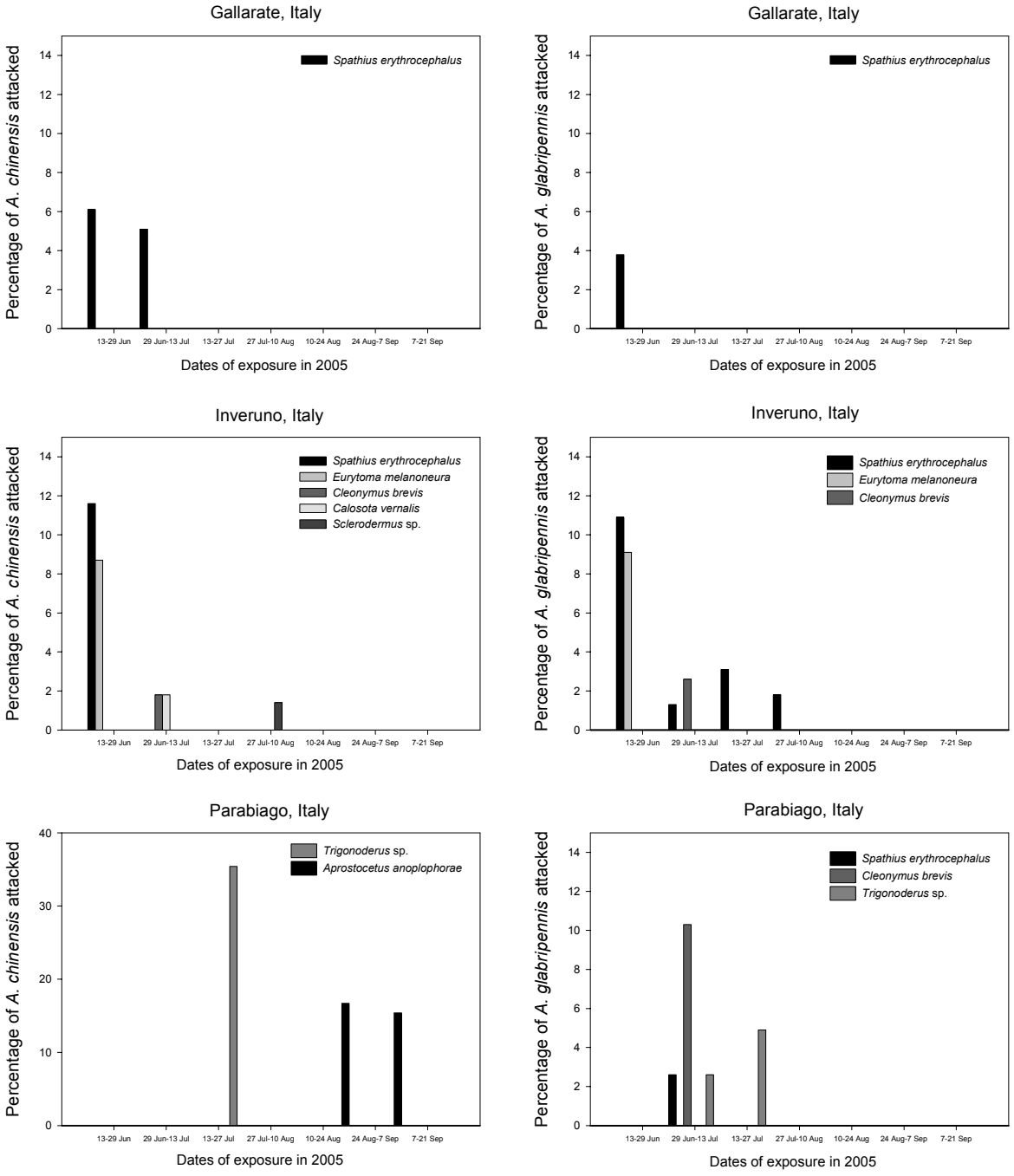
***Spathius erythrocephalus* Wesmael (Hym.: Braconidae)**
For the first time in early July 2004, at Parabiago, Italy, *Spathius erythrocephalus* larvae were found on newly hatched CLB larvae in a sentinel plant. During 2005, *S. erythrocephalus* was an omnipresent parasitoid in the sentinel plants in our study plots where it attacked L1 and L2 of both host species, CLB and ALB. This is the first mention of the new associations between *S. erythrocephalus* and both Asian hosts. The highest percentage of parasitism by *S. erythrocephalus* was 12% in both hosts, during the second half of June, 2005. In the 3 sites, the parasitoid attacked hosts from mid-June through mid-August, 2005 (Fig. 2). It is a gregarious ectoparasitoid which paralyzes its hosts before laying eggs on them. Longevity, fecundity, search rate of the braconid, and rearing methods are currently investigated at EBCL. From CLB hosts that were parasitized in the field in Italy in mid-July, 2004, *S. erythrocephalus* adults emerged in the quarantine laboratory on 14 August, 2004 (30 days after host parasitization). In the laboratory at 22°C, their progeny on the *Anoplophora* hosts developed, from egg through adult emergence, in about 30 days. Longevity of *S. erythrocephalus* females and males was 48.7 ± 4.8 days, and 42 days, respectively. Fertilized *S. erythrocephalus* females aged 5 to 42-day old were able to successfully attack both ALB and CLB hosts. Many hosts among the newly hatched larvae that were present under bark were attacked and paralyzed, but many of them did not receive an egg from the parasitoid. A high proportion of hosts were killed although they were not parasitized. Some of the smallest hosts, newly hatched larvae of *Anoplophora*, bore 2 larvae of the parasitoid, and ensured their complete development.

In Southern France, *S. erythrocephalus* is abundant on larvae of *Phymatodes testaceus* (L.) (Col.: Cerambycidae) under the bark of freshly cut branches of *Quercus ilex*. During fall, collections of this material showed that the number of eggs laid per host increases with the host size; 5–8 parasitoid cocoons are usually found on 10-12 mm length *P. testaceus* larvae. The ability of *S. erythrocephalus* females to detect and to attack very early stages of the targets is noteworthy. In southern France, this parasitoid does not seem going through a diapause as adults emerge quickly at room temperature from material collected during fall and winter. According to Dr. M. R. Shaw (pers. com.), if *S. erythrocephalus* and *Spathius curvicaudis* Ratzeburg are distinct species, *S. erythrocephalus* may be restricted to Cerambycidae only. So far, very little is known about the biology and the host range of this braconid. Further evaluation of the new associations between *S. erythrocephalus* and *Anoplophora* spp. is being considered.

***Eurytoma melanoneura* Walker (Hym.: Eurytomidae)**
For the first time, *Eurytoma melanoneura* was reared from L1-L2 of ALB and CLB exposed at Inveruno. Almost 9% of the larvae in each host species were attacked by the parasitoid during the second half of June 2005, in only one of the 3 sites (Fig. 2). This is the first mention of the new associations between *E. melanoneura* and both Asian pests. *E. melanoneura* is a solitary ectoparasitoid species known in the European fauna from xylophagous insects. Its biology and ecology are unknown, yet.

***Calosota vernalis* Curtis (Eupelmidae, Calosotinae)**
For the first time, *Calosota vernalis* was reared from an L2 of CLB exposed during the first half of July 2005 at Inveruno, Italy (Fig. 2). This is the first mention of a new association between *C. vernalis* and CLB. Only one specimen was recovered and we do not know yet if it would accept ALB as a host. Its biology and ecology are unknown. It is a solitary ectoparasitoid. *Calosota* species are known to attack xylophagous insects.

Figure 2:
Parasitization of early stages of *Anoplophora chinensis* and *A. glabripennis* exposed in sentinel plants in 3 sites of the area infested with *A. chinensis* near Milan, Italy, during 2005
(Parasitisme des premiers stades de développement d'*Anoplophora chinensis* et d'*A. glabripennis* exposés dans des plantes sentinelles dans 3 sites de la zone infestée par *A. chinensis* près de Milan, en 2005)



***Cleonymus brevis* Boucek (Pteromalidae, Cleonyminae)**
For the first time, *Cleonymus brevis* was reared from L2 of ALB and CLB exposed during the first half of June 2005 at Inveruno, and from L2 of ALB exposed at Parabiago at the same dates. At Inveruno, it attacked a very small proportion of the exposed hosts, when at Parabiago 10.3% of the hosts were parasitized (Fig. 2). This is the first mention of the new associations between *C. brevis* and both Asian pests. In our collections, it was observed as an ectoparasitoid, either solitary or gregarious. *C. brevis* is known from another xylophagous insect, the bark beetle *Hylesinus toranio* Danthoine (Col.: Scolytidae) on olive trees in southern Europe. Its biology and ecology are unknown, yet.

***Trigonoderus* sp. (Pteromalidae, Pteromalinae)**
For the first time, *Trigonoderus* sp. (very likely *T. princeps* Westwood) was reared from L2 of ALB and CLB exposed at Parabiago, Italy during July 2005. This is the first mention of the new associations between *Trigonoderus* sp. and both Asian pests. Rates of parasitism by *Trigonoderus* sp. in the sentinel plants ranged from 3 - 5% on ALB through 35.4% on CLB. This was the highest rate of parasitism observed in our sentinel plants (Fig 2). As most early stage larvae of CLB develop during July in Parabiago area, *Trigonoderus* sp. appears to be fairly well fitted to its control. It is a solitary ectoparasitoid, distributed all over Europe. *Trigonoderus princeps* Westwood is known in south-western Europe from the host *Parmena balteus* (L.) (Cerambycidae, Lamiinae), which lives on the common ivy, *Hedera helix*, on herbaceous plants as *Helleborus* spp. and *Euphorbia* spp., and occasionally on conifers. *T. princeps* is also known from the birch bark beetle, *Scolytus ratzeburgi* Janson, all over Europe.

***Sclerodermus* sp. (Hym.: Bethylidae)**
For the first time, a pre-ovipositing female of *Sclerodermus* sp. was found near its paralyzed prey, an L2 of CLB exposed at Inveruno during the first half of August 2005 (Fig. 2). The galleries under bark were in fact a dry habitat in a dying sentinel plant. This is the first mention of the new association between *Sclerodermus* sp. (a European species?) and CLB. In the Far East, *Sclerodermus guani* and *S. sechuanensis* are known to attack various cerambycids and were used in large scale release programs, successfully. Before we found in Italy a *Sclerodermus* sp. attacking spontaneously a small *Anoplophora* larva in the field, we have been bringing some attention to the associations between cerambycids and *Sclerodermus* spp., especially the association between *Sclerodermus abdominalis* Westwood (Hym.: Bethylidae) and *Phytoecia rufipes* (Olivier) (Col.: Cerambycidae) larvae in stems of sweet fennel *Foeniculum vulgare* Miller (Umbelliferae), in the south of France. We also studied an unidentified species of *Sclerodermus* recovered from *Mesosa nebulosa* ssp. *obscuricornis* Pic (Col.: Cerambycidae) in *Quercus* sp. at Azad Shahr, Iran, and an unidentified species of *Sclerodermus* recovered from *Purpuricenus schurmanni* Sláma in *Acer creticus* in Crete. These species were tested in the EBCL quarantine laboratory on ALB and CLB larvae. They performed much better in petri dishes or in dried plant material than in fresh healthy plants infested with the Asian hosts. Data from these tests will be presented separately. As a summary, our study showed that the wet habitat that are the galleries bored under the bark of living plants is not appropriate to the preservation of the hosts paralyzed or parasitized by *Sclerodermus* spp. and to survival of the egg laying females, which tend to get caught in the sap and finally die. *Sclerodermus* spp. may be much more effective parasitoids of cerambycids attacking dying or dead trees or branches.

Behavioral studies in the laboratory showed that a *Sclerodermus* spp. female paralyzes its host by venom injections and feeds on it to get a sexual maturation. The parasitoid female sticks its eggs on the host body in numbers varying with the host size. The newly hatched larvae stay externally and punch the host tegument to suck the hemolymph. The larvae develop gregariously. The parasitoid female stays near its developing offspring to groom it, and to defend it against predators. A very small proportion of parasitoid males are found among the offspring developed from one host. Generally, adult males are winged whereas

females are wingless, although wingless males and winged females can be observed. The males of *Sclerodermus* spp. show a protandry. They penetrate the cocoons of the females to copulate. Thus, most females are fertilized before leaving their cocoon. Longevity of males is short (2 – 3 weeks) whereas longevity of females is much longer (5 – 20 weeks, depending upon rearing conditions). Un-copulated females may lay eggs, but their progeny contains males, exclusively (arrhenotoky).

CONCLUSION

So far, 1 egg parasitoid and 6 early stage larval parasitoids were inventoried in the 3 sites selected in the urban habitats near Milan; their evaluation is in progress. Ten "new associations" between some parasitoid species from the European fauna and the Asian pests were identified. Among the larval parasitoids *Spathius erythrocephalus* and *Trigonoderus* sp. were the most abundant. Further evaluation will indicate if their particular biological, behavioral and ecological features are those of promising biological control agents.

The egg parasitoid *Aprostocetus anoplophorae* has a special place in the complex of natural enemies of CLB because the parasitoid and the pest very likely have the same origin, and the parasitoid seems to be very specific to CLB. Furthermore, the host and its natural enemy phenologies are closely synchronized. This parasitoid may be a key species in a biological control program against *Anoplophora chinensis*.

The positive responses of several local larval parasitoids to the presence of the *Anoplophora glabripennis* hosts that were exposed in our Italian study sites, let us assume that potential candidates for the biological control of this second Asian pest may be surveyed using the same technique in the European sites infested with this particular pest species.

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REFERENCES CITED

Adachi I., 1994 – Development and life cycle of *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) on *Citrus* trees under fluctuating and constant temperature regimes. *Applied Entomology and Zoology*, 29, 485-497.

Cocquempot C., Hérard F., 2003. – Les *Anoplophora* : Un danger pour les cultures ornementales et l'arboriculture françaises. *PHM, Revue horticole*, 449, 28-33.

Colombo M., Limonta L., 2001 – *Anoplophora malasiaca* Thomson (Coleoptera Cerambycidae, Lamiinae, Lamiini) in Europe. *Bollettino di Zoologia Agraria e di Bachicoltura*, 33, 65-68.

Dauber D., Mitter H., 2001 – Das erstmalige Aufreten von *Anoplophora glabripennis* Motschuslsky 1853 auf dem europäischen Festand (Coleoptera, Cerambycidae, Lamiinae). *Beiträge zur Naturkunde Oberösterreichs*, 10, 503-508.

Delvare G., Bon M.-C., Hérard F., Cocquempot C., Maspero M., Colombo M., 2004 - Description of *Aprostocetus anoplophorae* n. sp. (Hymenoptera, Eulophidae), a new egg parasitoid of the invasive pest *Anoplophora chinensis* (Förster) (Coleoptera, Cerambycidae). *Ann. Soc. entomol. Fr. (n.s.)* 40, 3-4, 227-233.

Haack R. A., Law K. R., Mastro V. C., Ossenbruggen H. S., Raimo B. J., 1997 – New York's Battle with the Asian long-horned beetle. *Journal of Forestry*, 95, 11-15.

Hérard F., Cocquempot C., Lopez J., Covi J., Maspero M., Colombo M., 2004 – Field study to evaluate the egg parasitoid *Aprostocetus anoplophorae* sp. n. (Hymenoptera: Eulophidae) on two *Anoplophora* hosts. *Proceedings of the United States Department of Agriculture interagency research forum on gypsy moth and other invasive species, Annapolis, MD, USA, 13-16 January 2004* (sous presse).

Smith M. T., Zhong-Qi Y., Hérard F., Fuester R., Bauer L., Solter L., Keena M., d'Amico V., 2003 – Biological control of *Anoplophora glabripennis* Motsch.: A synthesis of current research programs. *Proceedings of the United States Department of Agriculture interagency research forum on gypsy moth and other invasive species, 15-18 January 2002, Annapolis, MD, USA*, 87-91.