The case (to date) for biological control of emerald ash borer, *Agrilus planipennis, by a* native parasitoid, *Atanycolus hicoriae*

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Dead and dying ash, SE Michigan.

Emerald ash borer (EAB) and biological control: a context for this study.

Researchers have been keenly interested in the possibility that EAB populations might be reduced or managed by natural enemies. Natural enemies might include predators (e.g., ladybird beetles feeding on aphids) or pathogens (an insect disease, e.g., the fungus that infects gypsy moth). Many of the most important natural enemies are *parasitoids*, insects that feed on or within a single host insect, killing it. Typically these are the larvae of small to tiny wasp or fly species. Biological control projects have contributed to reductions in foliage feeding forest pests including gypsy moth (via an introduced fungal pathogen) and browntail moth (via a fly parasitoid). Wood-boring pests like EAB are probably more difficult to target with natural enemies, because the susceptible larvae are beneath an "armor" of tree bark. Although there are many natural enemies that attack borers, we are currently unaware of a clear example of biological control for a wood boring beetle pest. So why do we think that biological control of EAB is a useful area for research? First, if biological control of wood-borers *did* commonly occur, we might not know about it—species that are prevented from reaching pest status are unlikely have been studied well enough to demonstrate biological control. A second reason to invest in biological control research is that the potential benefit is so large. If EAB could be reduced to non-pest levels by natural enemies, we would avoid billions of dollars of future costs.

One approach to the use of natural enemies is *classical biological control*, based on the idea that in their native range, potential pest insects are suppressed by the natural enemies with which they evolved. So in the case of EAB, we might expect to find effective natural enemies in China, where EAB is native. In fact, we do know that EAB is far less damaging in China than it has been to date in the US. Could natural enemies be at least part of the explanation for this difference? Currently, USDA APHIS is evaluating several promising biological control candidates (wasp parasitoids) found in China (see other research reports posted at http://emeraldashborer.info).

Another approach is to evaluate native natural enemies. Given that EAB has many close relatives that are native (two-lined chestnut borer and bronze birch borer are common species in the *Agrilus* genus), we might expect the natural enemies of these species to also attack EAB. Recently, we have been studying one of these, *Atanycolus hicoriae,* a slender half-inch wasp that might contribute to significant mortality of EAB. Prior to

2007, *A. hicoriae* had been noted as a parasitoid of EAB in a single published study that reported barely detectable parasitism (< 0.1%). Then, in the fall of 2007, *A. hicoriae* was again observed at Seven Lakes State Park. Preliminary studies found parasitism to be widespread and locally intense; the site-wide average parasitism was near 20%. In 2008, we initiated a study to characterize the biology of *Atanycolus* sp. and to gain insight into its interaction with EAB at the population level.

Life cycle of *A. hicoriae*

A. hicoriae is a small wasp in the family Braconidae. It is a *solitary ectoparasitoid*; its larvae develop singly while feeding externally on the EAB host. Adults attack EAB larvae by inserting a long ovipositor through the bark of a tree and laying an egg on a larva. The wasp larva then emerges and feeds externally on the EAB larva, eventually forming a cocoon from which an adult will later emerge by chewing through the bark (see Fig 1 below).

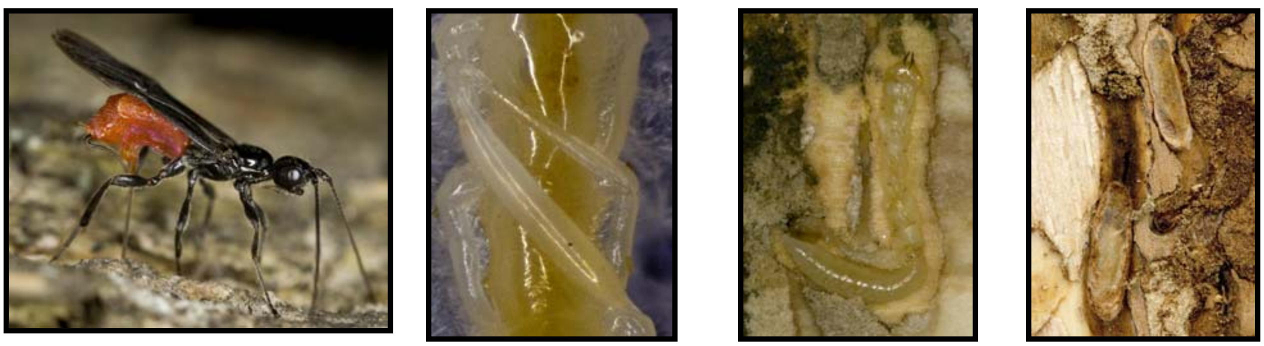

Fig. 1. A. hicoriae life cycle L-R: adult ovipositing through bark; wasp egg on EAB larva; wasp larva feeding on EAB; cocoons in EAB galleries

To evaluate the life cycle of *Atanycolus* sp. in relation to EAB, we selected 8 ash stands at Seven Lakes State Park in Fenton, MI, and 3 stands at Commons Park in Grand Blanc, MI (Fig 2). Beginning in May 2008, trees at each stand were dissected at biweekly intervals and EAB and *A. hicoriae* life stages counted.

The questions we were asking in this study were: How well does the life cycle of *A. hicoriae* match with that of EAB? Do susceptible EAB larvae occur when *A. hicoriae* adults are seeking hosts? We were also interested in whether *A. hicoriae* has more than one generation per year; multiple generations would mean much more rapid reproduction.

Fig. 2. Study sites in Genesee Co.

EAB life stages. EAB have the usual insect life stages: egg, larva, pupa, and adult. We distinguish between small and large larvae for this study because only the larger individuals are susceptible to parastisim. We also note a *prepupal* larval stage. These are individuals that have excavated a pupation cell in the wood; they are then no longer susceptible to parastism. The relative abundance of EAB life stages observed at our study sites is outlined schematically in Fig. 3. Beginning in spring, there are two generations of EAB present.

Insects that overwintered as prepupae (Fig. 3, #1) pupate in May (2), and then emerge as adults in June (3).

A much smaller proportion of the EAB present in the spring are slow-growing larvae destined to complete development in spring of 2009. Most of these are small (4); over the spring and summer they grow ( ) and eventually become prepupae in the fall (6).

Meanwhile, the EAB adults begin to lay eggs (7) which progress to small larvae (8) . Some of these develop to large larvae and then prepupa (9 10) and will emerge as adult in 2009; others remain as small or large larvae that will not emerge as adults until spring 2010.

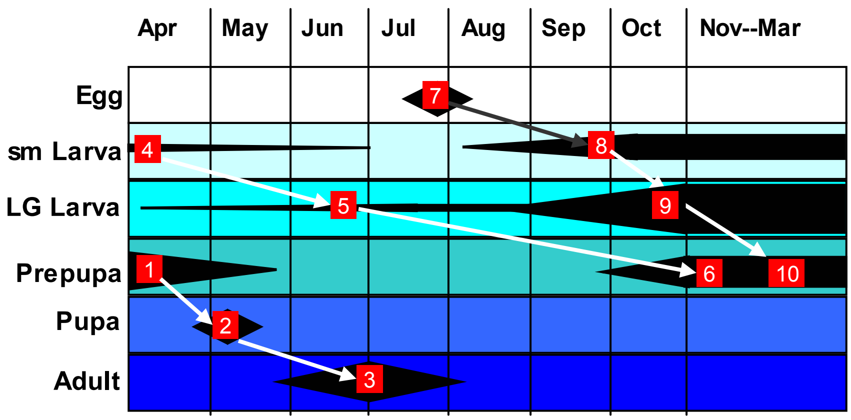

Fig. 3. Relative abundance of EAB life stages at study sites. Larval stages were documented; adult and egg stages are inferred. See text for explanation.

***A. hicoriae* life stages.** The occurrence and relative abundance of *A. hicoriae* observed at our study sites is outlined schematically in Fig. 4. In the spring, all *A. hicoriae* are in cocoons (#1) from which adults emerge in May (2). These adults mate and lay eggs (3) which progress to the larval stage within days (4). Larvae then form cocoons, some of which will generate a second generation of adult wasps (6); others apparently remain through the winter to emerge the following spring (7).

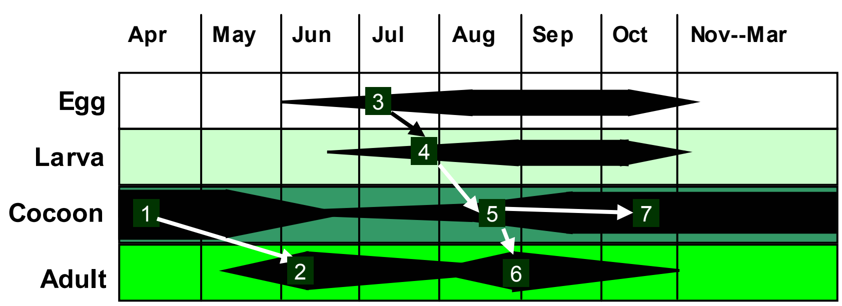

Fig. 4. Relative abundance of *A. hicoriae* life stages at study sites. Larvae and cocoons were observed; adult and egg stages are inferred. See text for explanation.

Synchrony of host and parasitoid. Our study indicated that for the EAB and *A. hicoriae* at our sites, the life cycles of host and parasitoid were not well matched. The overwintered *A. hicoriae* adults had fully emerged by mid June, about 2 months before the the large larvae that *A. hicoriae* requires as hosts were present. However there were some EAB larvae available as hosts in May and June: the small fraction of the population that was beginning a second year of development. The occurrence of a second 2008 generation of *A. hicoriae* meant that there were some adult wasps present in the field late in the season to target the new EAB cohort. However our data indicate that this was only partial; many of the cocoons formed in from first generation larvae will not emerge until 2009. Overall, for EAB emerging in 2008 we calculated parasitism rates of 24% (Site CP) and 56% (Site SL). For larvae still developing at the end of the 2008 season, parasitism rates were 15 and 16.% for sites CP and SL respectively.

Other observations

In several further experiments, we determined that:

- *A. hicoriae* adults are long-lived. Provided with honey and water, females lived for an average of 31 days, and a maximum of 100 days. Mean survival for males was 15 days. The male: female ratio was approximately 1:6.
- *A. hicoriae* readily parasitizes other *Agrilus* species. We encountered parasitized *Agrilus liragus* (bronze poplar borer) in aspen at the SL site. We also obtained parasitism for *A. bilineatus* (two-lined chestnut borer) in infested oak logs introduced at SL.
- *A. hicoriae* has its own natural enemy. We observed hyperparasitism (parasitism of a parasitoid) of *A. hicoriae* by another wasp species, *Eurytoma sp.* We confirmed this relationship by rearing *Eurytoma* from an *A. hicoriae* cocoon.

Conclusions. We have documented parasitism of EAB by *A. hicoriae* at two locations over two field seasons. We found that the life cycle of *A. hicoriae* is poorly synchronized with EAB that are on a one year development cycle (progressing from egg to prepupa in the late summer and fall). However for the small fraction of EAB that are on a two year cycle, vulnerability to parasitism by *A. hicoriae* is much higher. We know from other studies that there are some EAB populations that are primarily in a two year cycle, which would enhance their vulnerability to *A. hicoriae.*

The relatively high rate of parasitism—10-100-fold greater than observed in previous studies—suggests one of two scenarios. One, there may be an environmental constraint on native *A. hicoriae* populations that is absent or reduced at our sites. Two, there may have been an adaptation event producing a new *A. hicoriae* biotype with a greater affinity for EAB. For either scenario, we can't know whether the parasitism we have observed is near the maximum likely to occur, or is an early example of parasitism that might be far more significant with optimal conditions and/or further adaptation of the parasitoid.

We will continue to monitor *A. hicoriae* parasitism at our study sites, and survey elsewhere within the range of EAB, in the hope of detecting additional examples of locally significant parasitism. In cooperation with the US Forest Service in East Lansing, MI, we will also initiate lab studies to address questions about the basic biology of the wasp. Details of the work reported here will be forthcoming in a manuscript in progress.