INTEGRATING PARASITISM INTO CODLING MOTH CONTROL IN WALNUTS

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ABSTRACT

As an exotic pest, the codling moth has very few natural enemies in California. The aim of the project is to increase parasitism of the codling moth in walnuts by importing new parasitoid species from Eurasia. A total of 7 different parasitoid species have been acquired from several locations in Central Asia, China and Europe. Only 3 of the 7 species have been successfully maintained in culture at Berkeley and despite extensive development of rearing methods for some of the larval parasitoids, they have not proved amenable to insectary propagation. The larval ectoparasitoid Hyssopus pallidus functions as a potential antagonist against other larval parasitoids and so has not been field released. The cocoon parasitoid, Liotryphon caudatus, has been field released in 34 locations since 1992 and recoveries have been made from several walnut orchards in the Sacramento Valley. A new parasitoid, Mastrus ridibundus, obtained from Kazakhstan in 1994 will be field released in 1995, and more extensive collections of parasitoids from Kazakhstan in 1995, through the assistance of a newly established collaborator in Almaty, will allow direct field release of additional parasitoid species in 1996.

OBJECTIVES

Codling moth, Cydia pomonella (L.), is a key pest in walnut production in California. The recent development of multiple resistance to insecticides in codling moth populations has caused growers and researchers to re-examine walnut pest management practices. It is unlikely that codling moth management will be able to rely solely on chemical insecticides, whatever products may arise in the future, due to the perennial problem of resistance and the development of secondary pests. A more robust and sustainable management program for codling moth must integrate a range of complementary controls.

Although the codling moth has been known in California for more than 100 years, it is an exotic pest in North America, and does not have a typical natural enemy complex working to maintain a natural balance in the abundance of this pest. As a result its abundance is constrained only by the availability of susceptible fruit and nuts and it continues to exert considerable pressure on walnut production in California. In Central Asia, the original home of the codling moth, walnuts are seldom attacked due to the low level of codling moth pressure in this region. The importation and establishment of specialized parasitoids of the codling moth from its native Eurasian region would help to restore a natural balance to codling moth populations in California and contribute significantly to the control of codling moth in walnut orchards adopting an integrated management program with minimal reliance on insecticides.

The overall aim of this project is to restore the natural balance in codling moth populations in walnut orchards in California through the importation and release of specialized parasitoids from the region of origin of the codling moth in Eurasia. The objectives for the project are:

1. To acquire and import specialized parasitoids of the codling moth from the Eurasian region into quarantine in Berkeley.
2. To establish a laboratory rearing of the parasitoids for potential field release.
3. To field release the selected parasitoid species and monitor their establishment and impact on codling moth in walnut orchards in California.
PROCEDURES AND RESULTS

Objective 1. To acquire and import specialized parasitoids of the codling moth from the Eurasian region.

The origin of the codling moth is Central Asia, perhaps Kazakhstan, where apple forests commonly grow in the foothills of the Tien Shan mountains and the name of the capital city Almaty translates as Mother Apple. Due to the rapidly changing political and economic climate in Central Asia foreign exploration for parasitoids of the codling moth in Kazakhstan has proven difficult and so material has also been collected from the Sinkiang Province in N.W. China and parts of Central Europe. Although codling moth appears to be exotic in Sinkiang Province, which lies to the south of the Tien Shan mountain range, its proximity to Kazakhstan makes it likely that parasitoids may also have moved into this region. Two climatically distinct enclaves in Central Europe are the Rhone Valley in Switzerland and the Burgenland of Austria, where the fauna and flora are more characteristic of Central Asia (east of the Ural mountains) than of Europe. Previous studies indicate that the parasitoid complex of codling moth in these two areas is more diverse than elsewhere in Europe. This season we have been able to gain the confidence of a local scientists, A. Slivin, in Almaty who is eager to collaborate on the collection of parasitoid material in the region. This link provides a unique opportunity to be able to make larger scale collections of parasitoids from this important region (Fig. 1).

Parasitized codling moth larvae can be trapped as they descend from fruit to find pupation sites under the bark of the trunk or scaffold branches of a tree by using corrugated cardboard banding. The banding is placed out on the trees in July and recovered in October. The lack of availability of corrugated cardboard in Kazakhstan and China has reduced the collection potential in these regions. In Kazakhstan various improvisations have been used, such as cloth and clothing bands, but only limited material can be collected this way. An alternative in both China and Kazakhstan has been to search the tree bark for natural overwintering sites of the codling moth. The diapausing codling moth larvae are then transferred from the field to small rolls of 3/4 inch banding in which they spin another cocoon for overwintering. The small rolls are stored in 16 ounce food cups and overwintered outdoors or in cold storage (approximately 2°C). The parasitoid species emerging from these collections are detailed in Table 1.

Objective 2: To establish a laboratory rearing of the parasitoids for potential field release.

As is typical in classical biological control, the number of parasitoid individuals collectable from foreign field sites in any one field season is limited both by the lower abundance of the pest and the practical difficulties of collecting. This necessitates the development of rearing procedures for each of the parasitoid species obtained to enable the production of a sufficient numbers of parasitoid individuals for field release.

A continuous culture of codling moth larvae is maintained in the insectary at the Laboratory of Biological Control in Berkeley. In previous years the codling moth larvae have been reared on artificial diet in 1 oz. creamer cups but this past season it has proven to be more efficient to rear the larvae on organically grown thinning apples. Granny Smiths and Pippins are collected early in the season and stored in a cold room at 2°C. The Granny Smiths will store well for a couple of months but the Pippins can be stored throughout the year. Larvae are reared on apples in enclosed cardboard trays (24 x 24 x 3 inches), the non-diapausing culture maintained at 25°C and a 16 hr daylength, the diapausing culture at 20°C and a 12 hr photoperiod. Non-
diapausing codling moth are used for the rearing of larval parasitoids, whereas diapausing prepupae can be stored for the rearing of cocoon parasitoids.

The parasitoid species that have been held in culture in Berkeley are noted in Table 2. Additional notes on each of these parasitoids are as follows:

*Liotryphon caudatus*: This solitary ectoparasitoid of cocooned prepupal codling moth has been maintained in culture in Berkeley since July 1991. It attacks codling moth prepupae under the bark by paralyzing them and laying an egg externally on the paralyzed host. Parasitoid development is completed in 3-4 weeks depending on temperature. An effective rearing procedure has been developed for the production of *L. caudatus* on diapausing codling moth cocoons. Mature codling moth larvae are collected into strips of corrugated cardboard 3/4 x 12 inches where they spin cocoons. The strips of cocooned hosts are then placed in a parasitoid oviposition cage for 24 hr before being incubated in 5 gallon emergence drums to await parasitoid adult emergence.

*Hyssopus pallidus*: This gregarious ectoparasitoid of late instar codling moth larva has been maintained in culture in Berkeley since September 1991. The small parasitoid adults enter infested fruit to find their hosts and will attack all later larval instars of the codling moth. The host larva is paralyzed and then a series of eggs are laid externally on the host. Parasitoid development is completed in 2-3 weeks depending on temperature. This parasitoid species can readily be reared on larger codling moth larvae (diapausing or non-diapausing stock) in glass vials and does not require the presence of the host plant to secure host attack in captivity.

*Trichromma enecator*: This solitary larval-pupal endoparasitoid of the codling moth has been reared with limited success in Berkeley since June 1992. The parasitoid appears able to attack all larval instars of the codling moth but will only attack larvae inside of fruit. Development of the parasitoid is completed inside the host pupa under the bark. In order to develop practical rearing procedures we have investigated a range of rearing methods. Host larvae of all stages exposed to parasitoids in artificial arena are occasionally attacked but it is clear that the female parasitoids are attracted to the exudations that accumulate on the surface of attacked fruit and that in the absence of these exudations the attack behavior of the parasitoids is disrupted. However, success in rearing *T. enecator* on thinning apples infested with codling moth larvae has so far been limited. When a tray of attacked thinning apples are presented to the parasitoid in an oviposition cage they show very little interest in attacking the available hosts. Presentation of a single attacked apple to a single parasitoid female in a smaller arena results in active probing behavior by the parasitoid, but the success of parasitism remains limited. It seems unlikely that this species can be reared in sufficient numbers to secure field establishment.

*Microdus rufipes*: This solitary endoparasitoid attacks young larvae of codling moth (1st and 2nd instar) and kills prepupae inside their cocoons. It is one of the most important parasitoids in Kazakhstan, where it regularly achieves levels of parasitism between 40-60%. We have tried to maintain this species in culture in Berkeley since May 1993. In 1992, we were unable to achieve successful mating in quarantine, but this problem has been overcome in transferring the culture to our insectary. Detailed observations during the season indicate that this species will only attack host larvae in apples and that, like *T. enecator*, the exudates produced by codling moth larvae on the surface of the apple are essential for parasitoid attack. However, like *T. enecator*, we have been unsuccessful in being able to multiply this species in the insectary either using larger oviposition cages or single females exposed to individual infested apples. It seems that the only way to establish this important parasitoid in California walnut orchards is to make large scale field collections in Kazakhstan for direct field release from
quarantine in Berkeley. Our newly established link with a collaborating scientist in Almaty should ensure that large scale collections will be possible in 1995.

*Microdus conspicus*: This endoparasitoid is very similar to the last species in both morphology and biology. It has been obtained only in very small numbers from our codling moth collections from Eurasia and is probably not a specialized codling moth parasitoid. It was previously released in the U.S. against the oriental fruit moth but apparently never established. In insectary rearing it does not do as well on codling moth as *M. rufipes* and we have not been able to maintain a continuous culture.

*Pristomerus vulnerator*: This solitary larval-prepupal endoparasitoid was maintained in culture in quarantine in Berkeley for only two generations. The adult parasitoids mated successfully in captivity but could not be induced to attack codling moth. Additional attempts during 1993 and 1994 using infested thinning apples also proved unsuccessful. Similar difficulties in culturing this parasitoid have been experienced in Canada during the 1960s. It seems probable that this species is not well adapted to the attack of codling moth and uses this host only incidentally. We will not be considering this species for field release in California.

*Mastrus ridibundus*. This gregarious ectoparasitoid of codling moth cocoons was obtained from Kazakhstan for the first time in 1994. Its biology is similar to that of *Liotryphon caudatus* in that it attacks the prepupal stage of the codling moth. This is a poorly known parasitoid, but local sources of literature from Kazakhstan indicate that it is often responsible for relatively high levels of parasitism. In contrast to *L. caudatus*, however, *M. ridibundus* is gregarious producing 4-7 individuals on a single codling moth host. This species also differs from *L. caudatus* in its behavior; it crawls inside the cardboard strips used for codling moth cocooning in the insectary rather than ovipositing through the strips, and it is frequently active on the floor of the rearing cages. This suggests that *M. ridibundus* is more likely to attack cocoons that spin up in the crotch of branches or at the base of the trunk, whereas *L. caudatus* is adapted to the attack of cocoons concealed beneath the bark of the main trunk. Preliminary host range tests in quarantine indicate that this parasitoid is relatively host specific and it is readily reared in captivity in diapausing codling moth cocoons. It is anticipated that we will be able to secure a field release permit for this parasitoid in time for releases to be made during the field season of 1995.

**Objective 3:** To field release the selected parasitoid species and monitor their establishment and impact on codling moth in walnut orchards in California.

Before field releases of exotic parasitoid species can take place the necessary State and Federal permits for the release of exotic natural enemies must be obtained. Permits are only granted after an environmental assessment has been made for each parasitoid species. We currently hold permits for the release of *Liotryphon caudatus, Microdus conspicus, Microdus rufipes, Trichomma enecator* and *Hyssopus pallidus*. In addition, we anticipate that we will have enough information on *Mastrus ridibundus* to be able to secure a permit for its field release in 1995.

During the 1993 and 1994 field seasons we concentrated on field releases of *Liotryphon caudatus*, since this parasitoid species could be produced in sufficient numbers to warrant a concerted effort in field monitoring. The parasitoids emerging from insectary rearing were kept in large (3 x 3 x 3 ft) well-ventilated cages for mating and were fed with honey during their 7 day pre-oviposition period. The parasitoids were then exposed to codling moth
cocoons for one or more days before being field released to ensure that each had experienced host attack.

From 50 to 400 individuals were released at one time at a field site, depending on the size of the orchard. Releases took place from mid June to the end of October and were confined to orchards in which insecticides were not being applied. A total of 1200 parasitoid individuals were field released in 1992, just over 7,000 individuals were released in 1993 and a further 6,000 individuals in 1994. The releases have taken place at 34 sites in the northern and central regions of the State. The releases took place in walnut, pear and apple orchards to maximize the probability for establishment of the parasitoid in the region. The walnut release sites are listed in Table 3. In general, the success of biological control introductions is increased by releasing individuals in as wide a geographic range and over as diverse a set of sites as possible.

The establishment and impact of the parasitoids is being monitored by banding the trees in the orchards to recover codling moth larvae descending from the fruit to pupate. Approximately 100 trees in each release site were banded in 20 of the 27 release sites in 1993. The majority of the banding made use of 1" bands, thinner than the more usual 3" bands, but better for the recovery of L. caudatus that appears to avoid ovipositing through the larger bands but can reach all host cocoons from the edge of a thinner band. The parasitoid was recovered from only three walnut sites in the Sacramento Valley, but it seems likely that there is greater activity of these parasitoids on naturally occurring cocoons than in the bands. Evidence from Washington State (Tom Unruh, USDA-ARS, Yakima) suggests that monitoring parasitism of L. caudatus is not easy. Following parasitoid releases in an apple orchard in Yakima, parasitism of codling moth cocoons under the bark of apple trees reached 15% while parasitism in bands remained undetectable. In 1994 we stapled individual sentinel cocoons (in diapause), inside a small section (3-4 flutes) of corrugated cardboard strip, onto trees in the release orchards in place of continuous bands. From 200-250 sentinel cocoons were used at a release site and were left in place for 3-4 weeks following the parasitoid release. Recoveries of parasitised cocoons were substantially improved using this monitoring technique. Recoveries were made in the majority of walnut orchards monitored and parasitism reached 25% in one orchard in Gridley.

Within-season recoveries of L. caudatus has been noted from several release sites, spanning from the coast through the Sacramento River to the foothills of the Sierras. This is very encouraging preliminary evidence that the parasitoid is attacking codling moth cocoons over a broad climatic range but we have yet to determine how extensive these attacks are throughout the release region.

CONCLUSIONS

Parasitoid species that are specialized natural enemies of the codling moth in Eurasia have been imported into quarantine at Berkeley from several different regions. Rearing techniques for the parasitoids have proved very effective for the production of the cocoon parasitoids Liotypeon caudatus and the newly acquired Mastrus ridibundus, but we have been unable to develop sufficiently effective rearing techniques for many of the larval parasitoids. Extensive field releases of L. caudatus from late 1992 through 1994 has led to within season recoveries from codling moth bands and sentinel cocoons in several different climatic zones. These preliminary recoveries are very encouraging but it is still too early to assess whether the parasitoid is established and if so, to what extent it is impacting the codling moth populations.

Field releases of an additional parasitoid species, M. ridibundus, are anticipated during the 1995 field season, and the newly established collaboration in Almaty in Kazakhstan is expected
to lead to the importation of sufficient material for direct field release, without need for insectary multiplication, in 1996. The opportunity to import and establish the dominant parasitoids from Kazakhstan (*Mastrus ridibundus* and *Microdus rufipes*) is an important step in the management of codling moth throughout the region. Information gained from this region over the last few years indicate that the parasitoid complex in the vicinity of Almaty is quite different from that observed through the rest of the Eurasian region. The parasitoids from Almaty have the greatest likelihood of being able to suppress the regional pressure from codling moth in California and indicate the need to concentrate parasitoid collections from this region.
<table>
<thead>
<tr>
<th>Location</th>
<th>Parasitoid species (Family)</th>
<th>Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soviet Crimea</td>
<td><em>Liotryphon caudatus</em> (Ichneumonidae)</td>
<td>1991</td>
</tr>
<tr>
<td>France</td>
<td><em>Hyssopus pallidus</em> (Eulophidae)</td>
<td>1991</td>
</tr>
<tr>
<td></td>
<td><em>Ascogaster quadridentatus</em> (Braconidae)</td>
<td>1993</td>
</tr>
<tr>
<td></td>
<td><em>Pristomerus vulnerator</em> (Ichneumonidae)</td>
<td>1993, 1994</td>
</tr>
<tr>
<td></td>
<td><em>Trichomma enecator</em> (Ichneumonidae)</td>
<td>1993, 1994</td>
</tr>
<tr>
<td>Western Switzerland</td>
<td><em>Ascogaster quadridentatus</em> (Braconidae)</td>
<td>1992, 1993, 1994</td>
</tr>
<tr>
<td></td>
<td><em>Liotryphon caudatus</em> (Ichneumonidae)</td>
<td>1992</td>
</tr>
<tr>
<td></td>
<td><em>Microdus conspicuus</em> (Braconidae)</td>
<td>1992, 1993</td>
</tr>
<tr>
<td></td>
<td><em>Liotryphon caudatus</em> (Ichneumonidae)</td>
<td>1992</td>
</tr>
<tr>
<td></td>
<td><em>Microdus rufipes</em> (Braconidae)</td>
<td>1993</td>
</tr>
<tr>
<td></td>
<td><em>Trichomma enecator</em> (Ichneumonidae)</td>
<td>1992, 1993</td>
</tr>
<tr>
<td>Sinkiang (China)</td>
<td><em>Microdus rufipes</em> (Braconidae)</td>
<td>1993</td>
</tr>
<tr>
<td>Kazakhstan</td>
<td><em>Mastrus ridibundus</em> (Ichneumonidae)</td>
<td>1994</td>
</tr>
<tr>
<td></td>
<td><em>Microdus rufipes</em> (Braconidae)</td>
<td>1993</td>
</tr>
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</table>
Table 2. Codling moth parasitoid species in culture at Berkeley

<table>
<thead>
<tr>
<th>Parasitoid species</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Liotryphon caudatus</em> (Ichneumonidae)</td>
<td>Since 1991</td>
</tr>
<tr>
<td><em>Trichomma enecator</em> (Ichneumonidae)</td>
<td>Temporary since 1992</td>
</tr>
<tr>
<td><em>Pristomerus vulnerator</em> (Ichneumonidae)</td>
<td>Not raised in captivity</td>
</tr>
<tr>
<td><em>Hyssopus pallidus</em> (Eulophidae)</td>
<td>Since 1991</td>
</tr>
<tr>
<td><em>Microdus rufipes</em> (Braconidae)</td>
<td>Temporarily since 1993</td>
</tr>
<tr>
<td><em>Microdus conspicuus</em> (Braconidae)</td>
<td>Temporarily since 1993</td>
</tr>
<tr>
<td><em>Mastrus ridibundus</em> (Ichneumonidae)</td>
<td>Since 1994</td>
</tr>
</tbody>
</table>

Table 3. Release sites for *Liotryphon caudatus* in walnut orchards 1992-1994

<table>
<thead>
<tr>
<th>Yolo Co.</th>
<th>Solano Co.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rio Oso</td>
<td>Winters (3 sites)</td>
</tr>
<tr>
<td>Sutter Co.</td>
<td>Glen Co.</td>
</tr>
<tr>
<td>Bear River</td>
<td>Sacramento River</td>
</tr>
<tr>
<td>San Joaquin Co.</td>
<td>Butte Co.</td>
</tr>
<tr>
<td>Linden</td>
<td>Oroville</td>
</tr>
<tr>
<td>- Ripon</td>
<td>Gridley</td>
</tr>
<tr>
<td>Stanislaus Co.</td>
<td>Colusa Co.</td>
</tr>
<tr>
<td>Crows Landing</td>
<td>Colusa (2 sites)</td>
</tr>
<tr>
<td>- Turlock</td>
<td></td>
</tr>
<tr>
<td>Tehama Co.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Red Bluff</td>
</tr>
</tbody>
</table>
Fig. 1. Region of origin of the codling moth and the source of parasitoids for importation to California