# PRUNE APHIDS: OVERWINTERING BIOLOGY AND BIOLOGICAL CONTROL

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### INTRODUCTION

Aphids have become an important problem in prunes in California with the development of the Bt (*Bacillus thuringiensis*) spray program to replace in-season use of organophosphate sprays for peach twig borer (*Anarsia lineatella*). Two different aphid species cause damage to prune trees in the Central Valley: the mealy plum aphid (MPA), *Hyalopterus pruni;* and the leaf curl plum aphid (LCPA), *Brachycaudus helichrysi*. The mealy plum aphid develops large populations on the undersides of prune leaves in the spring and migrates from older orchards to cattails by mid June, but remains in young orchards all through the year. These populations sap tree vigor, slow the growth of young trees, cause splitting of fruit, and appear to lower fruit sugar content. The leaf curl plum aphid rapidly builds populations on new foliage in spring causing affected spurs to develop tightly curled leaves. The aphids migrate from the orchards to their summer host plants (Asteraceae) in May, but are also implicated in reducing tree growth and fruit sugar content and causing the splitting of fruit.

Traditional dormant oil sprays in prune orchards applied in December to February contribute to rain induced runoff of insecticides into the surrounding watersheds, and are considered by the State Water Resources Control Board as a significant source of nonpoint source pollution with regard to water quality. In addition, use of within-season organophosphate insecticide treatments often disrupts the natural control of the San Jose scale and mites, adding further to management costs. The need to develop alternatives to dormant oil sprays for the control of aphids in prunes has generated interest in the late season and winter phases of the life cycles of both MPA and LCPA. One alternative is to be able to treat aphid populations either in the fall before the dormant period, or in early spring after the dormant period but before the aphids hatch from their overwintering eggs. For fall treatment of aphids we have determined that the start of the return migration of male aphids is mid October, but it remains unclear how late in the season egg laying occurs in the orchards, information that would facilitate decisions regarding the timing of treatments for aphid control. For early spring treatment of aphids it is important to know the timing of egg hatch for both aphid species, and this could be predicted through experimental evaluation of the thermal requirements of post-diapause overwintering eggs.

In addition, as both of the aphid species are exotic insects, originating in Eurasia, they have not been kept under control in California by specialized insect parasitoids. The recent establishment of *Aphidius colemani* in the Central Valley has led to substantial levels of parasitism of LCPA and a general reduction in damage caused by this aphid species. In recent years, we have also imported and field released different biotypes of *Aphidius transcaspicus*, a parasitoid of mealy aphids from the Mediterranean for control of MPA. The success of this approach is dependent upon finding a biotype that is compatible with MPA in California, adapted to climatic conditions in California, and effective in using cues presented by MPA for host location.

## **OBJECTIVES**

- 1. To complete our estimation of the initiation of the sexual phase of the life cycle for LCPA through rearing under controlled conditions at different photoperiods.
- 2. To estimate the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.
- 3. To test the impact of a range of potential insecticides, for use in fall or early spring treatments of aphids, on mummies and emerging adults of *Aphidius transcaspicus*.
- 4. To continue to field release and monitor biotypes of *Aphidius transcaspicus* for control of MPA, and to determine their climatic tolerance.

## PROCEDURES AND RESULTS

# **Objective 1.** To complete our estimation of the initiation of the sexual phase of the life cycle for LCPA through rearing under controlled conditions at different photoperiods.

### Background

One management option for the control of aphids in prunes is to break the life cycle of the aphids by preventing returning migrants from laying overwintering eggs on the twigs of the trees in the orchards. The life cycle of MPA and LCPA are typical of a holocyclic heteroecious aphid (Fig. 1). Overwintering eggs hatch in spring and a series of parthenogenetic generations take place on the prune trees before the aphids migrate to their summer host plants (early May for LCPA and mid June for MPA). The aphids then complete a series of parthenogenetic generations on the summer host plant before switching to the sexual phase of the life cycle in the fall. The sexual phase of the life cycle begins with the production of gynoparae, winged aphids that migrate back to prune orchards to give live birth to young nymphs that develop on the tree foliage into adult egg-laying oviparae. A couple of weeks after the gynoparae migrate back to the orchards, winged males are produced on the summer host plant and these males migrate back to prune orchards to mate with the adult egg-laying oviparae. Two management options for breaking the life cycle in the fall are (1) insecticide treatment, or (2) early defoliation of the prune trees using zinc sulfate to prevent the successful development of the egg-laying oviparae. In both cases, the timing for the management options is critical and dependent on a sound knowledge of the overwintering ecology of the aphids.

### Methods

We monitored aphid development through November, 2004 in a prune orchard in the Winters area to determine the age structure of the aphid populations and the likely timing of egg laying in

the orchards. At weekly intervals 10 leaves with colonies of developing oviparae were placed into ziplock bags and returned to the laboratory for assessment of the numbers of individuals present in the successive instars. The numbers of winged male aphids present on each leaf were counted, and the size of all nymphs was determined by measurement of the length of the hind tibia. Nymphs were then assigned to each of the five successive instars (four nymphal and one adult) assuming a linear increase in tibia length between instars. Thus the extreme range of observed hind tibia lengths was separated into five equal size classes to assign individuals to instars.

#### Results

One Nov 10, the majority of the nymphal oviparae were in the first two instars with smaller numbers in the later nymphal instars, but it is interesting to note that some adult oviparae were present already (Fig. 2). The leaves also supported an average of 2.4 gynoparae (the migrant adults that produce the generation of oviparae) and 0.8 males per leaf. Males appear to sit on the leaves with a colony of developing oviparae waiting for them to mature to the adult stage when mating takes place. By Nov 17, most of the oviparae had developed through to the 4<sup>th</sup> instar, although some younger nymphs were also present. There was no accumulation of adult oviparae on the leaves as they move off onto the twigs to lay eggs around the base of the buds. It is interesting to note, however, that the numbers of oviparae, gynoparae and males had declined substantially over the intervening week suggesting that predation, or other mortality factors, may still be important at this time of the year. By Nov 24 there were few oviparae left on the leaves indicating that the majority had matured to the adult stage and were egg laying on the twigs.

These results suggest that although the return migration of male aphids begins in mid October, the majority of the oviparae are not maturing in the orchard until mid November, suggesting that there is an extended interval in the fall when insecticide applications are likely to be successful in the disruption of egg laying in prune orchards.

# **2.** To estimate the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.

#### Methods

A preliminary set of MPA eggs were collected in a prune orchard in the Yuba City area on February 17, 2004. These field collected eggs were heavily predated and so many could not be used to assess the thermal requirements for egg hatch. However, the 26 viable eggs collected were split into three groups and incubated at 12, 17 and 23 °C respectively. The eggs were monitored daily for egg hatch and the duration of the interval between field collection and hatch noted.

To develop a more extensive analysis of the thermal requirements for egg hatch of both LCPA and MPA, we have collected spurs from orchards on December 7, 2004 that were identified as being likely to support overwintering aphid populations. A total of 300 viable LCPA were collected from prunes in the Orland area, and more than 1200 viable MPA eggs have been collected from orchards in the Yuba City area. These eggs will be stored at 4 °C, in tightly

fitting Petri dishes with sufficient moisture to prevent desiccation, to accumulate sufficient chilling to complete their overwintering diapause. Once diapause in completed by early February, the eggs will be removed from cold storage and sets of 5-10 Petri dishes will be incubated at a series of temperatures from 8 - 22 °C to determine the length of time needed for egg hatch. The Petri-dishes will be examined daily around the time that egg hatch occurs to determine the median time of egg hatch for each dish. The hatching aphid nymphs will also be examined carefully to ensure that each has the characteristics of either LCPA or MPA in case other aphid species such as green peach aphids or water lily aphids are also present on the field collected spurs. Linear regression will be used to examine the relationship between the rate of egg development and temperature, using the intercept on the x axis to estimate the lower threshold temperature for development and the reciprocal of the slope to estimate the thermal requirement for development.

It is also important to determine the date at which diapause of the aphid eggs is terminated, as this defines the date after which temperatures above the threshold for egg development begin to accumulate. This will be estimated for MPA during the 2004-05 winter period by collecting spurs with overwintering eggs at regular two-week intervals from the beginning of December through to mid February. The spurs will be treated and the eggs held in Petri-dishes as described above. A set of 5-10 Petri dishes from each sampling date will be held at 18°C to determine the median time of egg hatch. The date of termination of diapause will then be estimated from the point at which the relationship between development time and sample date switches from a steady linear decline to a more dramatic reduction.

#### Results

The preliminary results for the thermal requirements for egg hatch for MPA are presented in Fig. 3. Fitting a linear regression to the minimal data on rate of development in relation to the three temperatures tested, we estimate the threshold temperature for development to be 5.87 °C. The reciprocal of the slope of the regression indicates a thermal requirement for egg hatch of 66.23 °D(C), a value that is clearly to low to be correct. This suggests that by Feb 17, 2004, when the eggs were collected from the field, they had already completed their diapause and had begun to develop toward egg hatch in the field under the prevailing climatic conditions. By collecting most extensive sets of eggs from the field in early December, 2004 and storing them at a temperature below the threshold for development we will be able to avoid this proble during the coming winter, and accurately assess the thermal requirement for egg hatch of both MPA and LCPA without any intervening egg development.

In addition, our two-weekly collections of MPA eggs from the field that started in early December is an ongoing experiment that will allow us to pinpoint the end of diapause under field conditions to within a two-week period in late winter.

# **3.** To test the impact of a range of potential insecticides, for use in fall or early spring treatments of aphids, on mummies and emerging adults of *Aphidius transcaspicus*.

Methods

Although diapausing mummies of *A. transcaspicus* would be the most appropriate experimental subject for testing the impact of dormant or delayed dormant insecticide treatments, the long duration of diapause development adds to the difficulty of effectively monitoring the impacts of the insecticides. In view of this constraint, non-diapause mummies which have only a short interval before adult emergence were used. In view of the experiences of earlier studies with aphid parasitoids, the insecticides were applied through dipping rather than contact spraying to provide more consistent results. Four insecticides were tested, Asana XL with a recommended field application rate of 14.5 oz per care, Omni oil at a rate of 6 gallons per acre, Imidan 70W at a rate of 4.25 lb per acre, and Diazinon 4E at a rate of 2 pints per acre. In each case the full field recommended rate (100%) was tested together with a 50%, 10% and 0% (water control) rate.

Ten replicate sets of leaves with 10 parasitoid mummies each, standardized in age to 2-3 days old, were dipped for 3 secs in each of the treatment materials, allowing the material to run off and the leaves and mummies to air dry. Once dry, individual mummies were cut out from the leaves, leaving only a very small piece of leaf attached to minimize the surface of insecticide residue, and each set of 10 mummies was placed into a glass vial and kept at 20°C and a 16h photoperiod to allow emergence. Emergence and the sex of the emerging adults were monitored daily, and mummies removed from the vials as the adults emerged. Honey was provided every other day and the longevity of the emerged adults was recorded. This test provides evidence of both acute lethal effects on emergence and sex ratio from penetration of the mummy, and chronic sublethal effects on adult longevity.

#### Results

Omni oil had the greatest lethal effect on mummies of *A. transcaspicus*, resulting in no emergence even at the lowest 10% rate (Fig. 4a). Diazinon had a greater lethal impact than Asana, with zero emergence at the 100% rate in comparison to 28% emergence, and the 10% rate of Asana not reducing emergence in comparison to the control. Interestingly, Imidan had no lethal impact on *A. transcaspicus* at any of the rates tested. The sex ratio of adults that emerged from the treated mummies showed a very similar pattern to overall emergence (Fig. 4b). It was not possible to assess any effect for Omni oil, as there was so little emergence, but again the impact of Diazinon was greater than that of Asana, and there was no effect of Imidan.

In the case of sublethal effect on the longevity of emerging *A. transcaspicus*, the effect of Omni oil appeared to be limited from the very small sample available. The effect of the other products was again similar, with Diazinon reducing longevity to a greater extent than Asana, and no impact of Imidan on survivorship.

These results clearly indicate that if Imidan proves to be an effective product to use against aphids in the fall or early spring, it would have no detrimental impact on the survival of overwintering parasitoids that reside within aphid mummies during the winter.

# 4. To continue to field release and monitor biotypes of *Aphidius transcaspicus* for control of MPA, and to determine their climatic tolerance.

Previous Work

During early foreign exploration visits to the Mediterranean to collect parasitoids of MPA it had been noted that mealy aphids occur on almond, apricot, peach and plum trees in this region. The aphids appeared somewhat different, varying in overall size as well as in the relative shape of the siphunculi, suggesting the possibility of host race diversification. A more recent analysis of mealy aphid populations using molecular markers indicate that there are three species of aphid, one associated with plums, a second associated with almonds, and a third associated with apricots and peaches. In addition, it was noted that all populations of California mealy aphids belong to the plum species, and that in the Mediterranean, the plum species can occasionally be found on almond, apricot and peach, although the other two species appear to be entirely host-tree specific.

Collections of *Aphidius transcaspicus* from the Mediterranean also suggested significant variation in the number of flagellar segments of the antennae and thus the occurrence of cryptic species. In addition, almond biotypes collected from Spain and Israel, and plum biotypes from various parts of the Mediterranean, often failed to attack MPA when field released in prune orchards in California. However, a recent analysis of parasitoid populations again using molecular markers suggest that all belong to a single species, with no significant genetic variation associated with host plant, but some significant variation between geographical regions of the Mediterranean. Recent experiences with field releases also indicate that populations of parasitoids collected from almond in Greece in 2003 have proved to be the most virulent in attacking Californian aphids.

#### Methods

In May 2004, a survey was made of the occurrence of mealy aphids on *Prunus* trees in Tunisia and southern Italy and Cyprus. These regions were selected for their higher summer temperatures and low winter rainfall, more similar to those found in the Central Valley of California, in comparison to previous collections from eastern Spain (Fig. 5). Both aphids and mummies were collected from a total of 28 locations in the region and were sent to the quarantine facility at UC Berkeley for parasitoid rearing and analysis of host tree specific and geographic variation among mealy aphid populations in the Mediterranean.

To better understand the climatic tolerance of *A. transcaspicus* we determined the temperature and humidity tolerance of adult parasitoids under constant laboratory conditions by exposing batches of newly emerged adults for 72 h in glass vials with honey to a range of temperatures and relative humidities (using saturated salt solutions). Two different parasitoid biotypes were tested, one collected from almond in Spain in 2001, and the other collected from almond in Greece in 2003. Survivorship over the period of 72 h was used as the measure of parasitoid tolerance.

In addition, field releases of parasitoids of the almond biotype of *Aphidius transcaspicus* collected from Spain in May 2001, and of a new almond biotype collected from western Spain in 2003, were carried out in prune orchards in the Sacramento Valley from May 21 through August 26, 2003. Some of the parasitoids were released into sleeve cages on aphid infested branches where parasitism could be more closely monitored, while other were released openly into the orchards to allow the parasitoids greater freedom of movement in selecting trees and resting sites. Up to 4 successive releases were made in each of 4 orchards with a total of between 150 and 1000 individuals released each time.

#### Results

Although aphids and parasitoids collected into alcohol from Tunisia, southern Italy and Cyprus were successfully returned to UC Berkeley from the foreign exploration trip in May 2004, no live parasitoids were received into our quarantine this year. The reason for this is that Homeland Security no longer allows researchers to hand carry packages of living natural enemies into the country, requiring us to make use of a bonded carrier such as airline freight companies, or courier services. Although shipments of live parasitoids were sent from Tunisia, southern Italy and Cyprus, none of these packages arrived at our quarantine with the insects still alive. As a result we were not able to set up colonies of parasitoid biotypes from these regions, although the alcohol preserved material will help us to analyze the variation in aphid and parasitoid populations around the Mediterranean. We are currently working with USDA on this issue, and anticipate that either better methods of shipment or hand carrying will be in place by spring 2005.

The tolerance of the Spanish and Greek biotypes of *A. transcaspicus* differed in response to variation in both temperature and relative humidity (Fig. 6). Both parasitoid biotypes survived well at lower temperatures, but at constant temperatures between 20 and 30 °C survivorship over the 3 day period dropped dramatically (Fig. 6a). The Spanish biotype was slightly less tolerant of higher temperatures than the Greek biotype, and this corresponds to the lower July mean temperatures in eastern Spain in comparison to southern Greece (Fig. 5). Similarly, while both parasitoid biotypes survived well at constant relative humidities above 50%, the Greek biotype was more tolerant than the Spanish biotype to lower relative humidities (Fig. 6b). This again matches that difference in mean winter rainfall patterns in the two regions (Fig. 5). These results suggest that parasitoids collected from the eastern and southern Mediterranean may indeed provide a better match to the climate of the Central Valley.

A total of 10,200 parasitoids were field released during the 2004 field season. This level of release was greater than that achieved during the 2003 field season. The majority of the parasitoid releases this year were of the new almond biotype collected from southern Greece in May 2003, but releases were also made of biotypes from almond collected in Spain in 2001 and 2002. Unfortunately, none of the biotypes released performed well in the field this year with no mummies recovered from individuals that were released into sleeve cages. Closer examination of the parasitoid colonies in culture in the glasshouse at UC Berkeley indicated that while adult females probed nymphs of MPA as actively as in previous years, no progeny resulted. Thus it appears that although the aphids were probed no eggs were laid, indicating that the parasitoids were rejecting the MPA nymphs as suitable hosts. It appears that this change may have resulted from our need to maintain the parasitoid colonies on an alternative aphid host, the black bean aphid (Aphis fabae) during the previous winter when Phragmites plants used to support colonies of MPA died due to excessive aphid damage. While the parasitoid colonies continue to do well on black bean aphid, they no longer transfer back to MPA due to rejection of the latter as suitable hosts. Additional Phragmites plants are being collected from the field for future use, but new colonies of parasitoids from the Mediterranean will be needed to ensure compatibility with MPA before making any additional field releases.

#### CONCLUSIONS

The need to develop alternatives to dormant oil sprays for the control of aphids in prunes has generated interest in the late season and winter phases of the life cycles of both MPA and LCPA. While male aphids begin their return migration into prune orchards in mid October, observations this season have confirmed that the egg-laying oviparae produced by the retuning gynoparae continue to develop through to the adult stage throughout November. This indicates that fall treatments for the control of aphids could be as late as mid November and still have a substantial impact on overwintering aphid populations. Analysis of the termination of diapause under field conditions is underway for MPA, and field collected eggs of both MPA and LCPA have been collected for analysis of the thermal requirements for egg hatch in February 2005. These data will allow us to provide a predictive model for temperature dependent egg hatch that will clarify the window of activity for control treatments for aphids in early spring.

In addition to possibilities to develop acceptable treatments for aphids in the fall or early spring, the most cost-effective way to manage aphids in prunes, as invasive pests, is to establish effective biological control. An analysis of the impact of four insecticides that could be used for aphid control in fall and spring applications on survivorship of parasitoids within aphid mummies, and their subsequent adult longevity produced some interesting results. Surprisingly, Omni oil had the greatest detrimental effect, whereas there was no measurable impact from Imidan, with Asana and Diazinon falling in between. Thus Imidan would clearly be the product of choice with regard to compatibility with aphid parasitoids. Tests of the temperature and humidity tolerances of two biotypes of A. transcaspicus clearly indicated a better climatic match with the Central Valley for parasitoids from Greece versus Spain, indicating that future collections from the eastern and southern Mediterranean would be valuable in this regard. Then finally, a need to maintain colonies of A. transcaspicus on black bean aphids during the winter of 2003-04 resulted in our current parasitoid colonies rejecting MPA as a suitable host during the 2004 field season, and thus new parasitoid colonies will be needed for field releases in 2005. This was an unexpected consequence of using an alternative aphid host for colony maintenance, but additional stocks of Phragmites are currently being collected to ensure that we can maintain parasitoids on MPA throughout the year in future.

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Table 1. A summary of the releases and recoveries of parasitoids in prune orchards in 2004.



Fig. 1. A schematic diagram of the life cycles of the mealy plum aphid, that migrates between prunes and reeds or cattails, and of the leaf curl plum aphid, that migrates between prunes and composite plants such as Shasta daisy. The asexual stages of the life cycles are labeled A-D, while the sexual stages are labeled F-H.



Fig. 2. The development of oviparae through the four nymphal instars to the adult stage in an orchard near Winters in November, 2004. Hind tibia length classes represent the five instars, and the number of gynoparae (G) and males (M) per leaf are shown inset.



Fig. 3. A preliminary assessment of the thermal requirements for development of MPA eggs from eggs collected near Yuba City on Feb 17, 2004.



Fig. 4. The impact of 100%, 50% and 10% field rates of four insecticides in relation to controls on the emergence (a), percent females (b), and adult longevity (c) of *Aphidius transcaspicus* exposed to the products as 2-3 day old mummies.



Fig. 5. Climatic variation around the Mediterranean in relation to two locations in the Central Valley (Marysville and Modesto). Location variables are elevation (m), mean annual rainfall (cm), mean January temperature (°C) and mean July temperature (°C).



Fig. 6. The tolerance of adult female A. transcapicus from two different geographic biotypes to variation in temperature (a) at a constnt RH of 70%, and to variation in relative humidity (b) at a constant temperature of 21  $^{\circ}$ C.