PRUNE APHIDS: OVERWINTERING BIOLOGY AND BIOLOGICAL CONTROL

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INTRODUCTION

Reduced use of dormant season sprays in prune production in the Central Valley, due to human health risks of fog bound sprays and environmental risks of runoff into Central Valley river systems, has caused two different aphid species to be a management concern. The mealy plum aphid (MPA), *Hyalopterus pruni*, develops large populations on the undersides of prune leaves in the spring that sap tree vigor, slow the growth of young trees, aggravate the splitting of fruit in years with high June temperatures, and occasionally lower fruit sugar content. MPA populations generally migrate from older orchards to their summer host plant (cattails) around mid June, but in young orchards that have vigorous growth through the summer aphid populations will remain in the orchard all through the year. In contrast, the leaf curl plum aphid (LCPA), *Brachycaudus helichrysi*, rapidly builds populations on new foliage in spring causing affected spurs to develop tightly curled leaves that cause thinning of the fruit load on affected branches, and reduced growth of young trees. LCPA populations develop very early in the season, but their activity in the orchards is very brief with migration to their summer host plants (Asteraceae) occurring at the end of April or early May.

Dormant season oil alone has not proven to be very effective in controlling the aphids, and the use of within-season organophosphate insecticide treatments can often disrupt the natural control of the San Jose scale and mites. In addition, the need to severely restrict organophosphate use in compliance with the Food Quality Protection Act of 1996, may remove this option for prune growers in the near future. Ongoing research by Sacramento Valley farm advisors indicates that sufficient coverage with summer oils can reduce MPA populations temporarily within season in orchards, and that summer oil sprays timed at full bloom can provide a significant reduction in MPA in orchards where overwintering populations are high. Summer oil sprays have proved to be less successful against LCPA as this aphid is well protected within the tightly curled leaves.

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 it is important to know the timing of the return migrations of both MPA and LCPA, which can be monitored both through experimental evaluation of the critical photoperiods that trigger the switch to the sexual phase of the life cycle and by field trapping of aphids in prune orchards. For early spring

treatment of aphids it is important to know the timing of egg hatch for both aphid species, and this can 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 strains 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 strain 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 monitor the development of egg-laying oviparae of MPA and LCPA in orchards in the Fall, and complete estimation of the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.

2. To expand testing of the impact of potential insecticides, for use in fall or early spring treatments of aphids, on adult as well as mummies of *Aphidius transcaspicus*.

3. To continue to field release and monitor different strains of *Aphidius transcaspicus*, and to assay parasitoid populations for virulence against California MPA.

Of these three objectives, the focus of our research was on 1 and 3 this year, with details from 2 having been presented in the previous report for 2005.

PROCEDURES AND RESULTS

Objective 1. To monitor the development of egg-laying oviparae of MPA and LCPA in orchards in the Fall, and complete estimation of the timing of termination of diapause and subsequent thermal requirements for egg hatch in early spring for both MPA and LCPA.

Background

The life cycle of MPA and LCPA are typical for host alternating aphids. 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 egg-laying oviparous adults. 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 oviparae as their mature to become adult. Two management options are to replace dormant sprays either with fall sprays or with delayed dormant sprays after the rains. In both cases, the timing of these options is critical and dependent on a sound knowledge of the overwintering ecology of the aphids.

Methods

We monitored development of MPA from mid October through November in both 2006 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 30 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.

To determine the thermal requirement for egg hatch of MPA and LCPA, a large set of eggs were collected on cut twigs from an orchard in the Winters area on November 30, 2005. Small sections of spurs with eggs at bud bases were cut from the twigs collected and placed into Petri-dishes and held in an incubator at 2°C to allow chilling to bring about the termination of egg diapause. The eggs were removed from chilling on February 10, 2006 and for each aphid species either five (MPA) or 10 (LCPA) replicate sets of 10 eggs were placed at each of four constant temperatures (10.0, 12.2, 15.5 and 20.8°C) to await egg hatch. Eggs were monitored daily to note the number of eggs hatching on each day. The nymphs hatching from the eggs were also checked to ensure that they were MPA and LCPA respectively, rather than nymphs of any other aphid that may have laid eggs on the same twigs collected from the field. Linear regression was 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.

The date when diapause of MPA eggs was terminated in 2005 was also estimated, as this defines the date after which temperatures above the threshold for egg development begin to accumulate. Termination of diapause was estimated for MPA and LCPA by collecting spurs with overwintering eggs at regular two-week intervals from December 15, 2005 through to February 21, 2006. The spurs were trimmed and held in Petri-dishes as described above. For each sampling date, 8 sets of Petri-dishes containing 10 eggs each were held at 15°C to determine the median time from field collection to egg hatch for each Petri-dish, and the mean of the median times for each sample date. If eggs have not completed their diapause chilling requirement, they take far longer to hatch than would be expected from the thermal requirement for egg hatch. Thus the date of termination of diapause can be estimated from the collection date at which the actual degree day accumulation for egg hatch matches the estimated thermal requirement.

Results

In 2006, nymphal instars of oviparous MPA and winged gynoparae were already on the prune trees when sampling began on October 13 and were present in the orchard through to November 27 (Fig. 1). These results are similar to those reported from 2005, and serve to confirm that although the return migration of male aphids begins in mid October (from trap data collected in 2002-03), the majority of the developing oviparae do not mature until mid November. The first male was seen in the orchard on October 20, and their abundance peaked on November 9. It is also interesting to note that each gynopara produces only a small set of 5-6 nymphs following their migration back into the orchards, with sometimes more than one gynopara per leaf.

As in previous years, we were unable to find LCPA on the trees again this fall, despite visiting orchards that have a history of LCPA activity in spring. It seems likely that this is because LCPA is generally far less abundant than MPA and therefore much more difficult to detect through sampling. However, we were able to find some more eggs of LCPA this year and so it also seems likely that the return migration of LCPA takes place earlier in the fall than is the case for MPA.

By collecting aphid eggs from the field in early December in 2004 and 2005 and storing them at 2°C for 2 months, we were able to complete the diapause chilling requirement for the eggs and to accurately assess the thermal requirements for egg hatch of both MPA and LCPA. The results presented in Fig. 2 are based on data from both years combined. Fitting a linear regression to the data on rate of development in relation to temperature, we estimate the threshold temperature for development of MPA eggs to be 2.7°C and the thermal requirement or number of heat units that must be accumulated for egg hatch to be 238°D(C) (Fig. 2a). The threshold is shown by the temperature at which the fitted relationship crosses the x axis of the graph, and the thermal requirement comes from the reciprocal of the slope of the fitted relationship. The corresponding estimates for the LCPA are a greater threshold temperature of 4.4°C, but a much lower thermal requirement of 85°D(C) (Fig. 2b). These results indicate that once diapause has been terminated, heat units will accumulate much more rapidly for LCPA than for MPA to produce a rather earlier egg hatch date.

When collected from the field early in the winter of 2005-06 and brought into the lab at 15°C only a low percentage of eggs hatched for both MPA and LCPA, and they took much longer to hatch than normal. For example, for MPA eggs collected on December 15, 2005 it took 531°D(C) for egg hatch (Fig. 3a) compared to an estimated thermal requirement of 238°D(C). This is because, at that time, the eggs had not experienced sufficient winter chilling for diapause to be completed. For each successive set of eggs brought in from the field at regular intervals through the winter, the number of degree days (heat units) needed for egg hatch steadily declined. For the last collection date of February 21, 2006, it only required 70°D(C) for the MPA eggs to hatch (Fig. 3a) indicating that not only was diapause terminated by that time, but the eggs had already begun to accumulate heat units toward egg hatch in the field such that by the time they were brought back to the laboratory they required very few extra degree days to complete egg hatch. We can use this data set to estimate the date at which diapause was terminated by finding the date when eggs brought into the field required exactly the complete thermal requirement of

degree days to permit egg hatch. From Fig. 3a, it can visually assessed from the dashed line on the graphs that this occurred around the end of January or early February in both years.

A more accurate way to estimate to estimate the termination of diapause, however, is from those egg samples collected after the termination of diapause. Subtracting the degree days required for egg hatch in the laboratory from the estimated thermal requirement for egg hatch provides an estimate of the thermal units accumulated in the field before the eggs were collected. We can then work backwards in time from the date the eggs were collected to accumulate sufficient day degrees above the estimated threshold temperature for egg development to match the estimated thermal units accumulated in the field. This is accomplished using degree day accumulation based on records of maximum and minimum daily temperatures for the period recorded by the CIMIS weather station in Winters. Using this approach for MPA in 2005-06, the last two collection dates give estimates of the termination of diapause as February 5 and February 7 respectively. The corresponding estimate for the diapause termination date for LCPA in 2005-06 (Fig. 3b) is February 4. Thus it seems that both MPA and LCPA eggs may require the same level of chilling to break diapause.

It is interesting to note that estimated dates for termination of diapause for MPA eggs differed substantially for the two winter seasons monitored. The termination dates were estimated to be January 21, 2005 in contrast to February 4, 2006, a full two weeks difference between the two years. The reason for this difference is likely due to the greater number of chilling days that occurred in 2004-05. While the threshold temperature for accumulation of chilling in aphid eggs is totally unknown, we can make a simple comparison between years by looking at the number of days during which minimum temperatures fell below that threshold of 2.7°C for development of MPA eggs. Using this arbitrary metric, the number of chilling days between November 21 and January 31 was 24 in 2004-05 and 15 for 2005-06. Thus it is clear that the first of these two winters had greater potential for winter chilling that the second, and fits well with the earlier estimate for termination of MPA egg diapause in 2004-05.

While egg diapause is likely to have a variable termination date from winter to winter, the subsequent thermal requirement for egg hatch is expected to be constant. In this regard it is interesting to note that temperatures above the threshold for development of MPA eggs accumulated at a similar rate in both years such that egg hatch was estimated to have peaked 29 (February 19, 2005) and 28 (March 4, 2006) days after the termination of diapause respectively. The much lower thermal requirement for egg hatch of LCPA eggs generated an estimated peak egg hatch of 11 days post termination of diapause or February 15, 2006.

3. To continue to field release and monitor different strains of *Aphidius transcaspicus*, and to assay parasitoid populations for virulence against California MPA.

Methods

In May 2006, a survey was made of the occurrence of mealy aphids on *Prunus* trees in southern Spain and Morocco. These two regions were selected as the most likely origins of the invasive populations of MPA in California as determined by genetic fingerprinting. Both aphids and

mummies were collected from 13 locations in these regions and were sent or hand carried 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.

Field releases of parasitoids of the almond biotype of *Aphidius transcaspicus* collected from Israel in May 2005, and of new almond biotypes collected from southern Spain and Morocco in 2006 were carried out in prune orchards in the Sacramento Valley in July 2006 and in *Phragmites* reed beds in early October 2006. All parasitoids that were field released this year were reared on MPA on potted *Phragmites* rather than from black bean aphids on potted beans. Although it is more difficult to produce parasitoids continuously on MPA it avoids the problem encountered in 2004, when parasitoids reared on black bean aphids began to reject MPA as suitable hosts. 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 or reed beds to allow the parasitoids greater freedom of movement in selecting trees and resting sites.

The relative virulence of different populations of *A. transcaspicus* was assessed in the insectary. The parasitoid populations selected for this study were from Cyprus, Israel, Morocco and southern Spain spanning the full breadth of the Mediterranean region and representing the greatest levels of variation in genetic relatedness. Individual mated female parasitoids of similar size were introduced into a ventilated sandwich box lined with moist paper in which a single leaf blade of *Phragmites* supported 75 3rd instar MPA. The parasitoids were removed after 8h and the aphids were maintained on the *Phragmites* in the sandwich boxes, with the leaf blades being changed after 5 days. Two measures of virulence were recorded, the number of aphid mummies produced and the sex ratio of the parasitoid offspring produced. A set of 15 replicate females were used for each of the four parasitoid populations.

Results

As in 2005, I was issued a unique hand carry permit by USDA-APHIS for the importation of *A. transcaspicus* into California from the Mediterranean. This once again ensured that I was able to get live parasitoids back to our quarantine facility at UC Berkeley this year and we were able to set up two new colonies of *A. transcaspicus*, one from Morocco and the other from southern Spain.

As in 2005, we were not able to field release as many parasitoids this year, due to the lower production of parasitoids from MPA on *Phragmites*, but we did achieve a greater level of release than in the previous year. The generally lower level of abundance of MPA in orchards in 2006 also made it more difficult to find suitable release sites. A total of 2,520 parasitoids were field released during the 2006 field season (Table 1). The majority of the parasitoid releases this year were of two new almond biotypes collected from Morocco and southern Spain, but releases were also made of the almond biotype from Israel collected in 2005. Unfortunately, this latter biotype did not performed well in sleeve cages and so we concentrated on the new biotypes for both sleeve cage trials and open field releases. Of the new biotypes, the parasitoids from southern

Spain appeared a little more aggressive than those from Morocco, but both performed well in the sleeve cage trials producing good numbers of mummies.

One particularly important difference in field releases of parasitoids in 2006 was the decision to make an open release into *Phragmites* reed beds in the Sacramento delta in early October. These reed beds had heavy populations of MPA at that time and proved to be a fortuitous site for a parasitoid release. The release of 300 parasitoid individuals led to the production of more than 25 mummies 2 weeks later and subsequently to a second generation of parasitoid mummies by the end of October. This is the first time that we have seen substantial turnover of parasitoid generations in the field, but it remains unclear at this point whether it is the compatibility of the southern Spain population of parasitoids or the particularly favorable conditions of *Phragmites* reed beds that led to this success. This will be repeated and explored further in 2007.

Females from all four populations of *A. transcaspicus* produced an average of 31-39 mummies during an 8h period in the virulence assay (Fig. 4a) with an average proportion of males from 30-45% (Fig. 4b). The lack of significant variation between parasitoid populations in both of these metrics suggests that the genetic differentiation that we have found among parasitoid populations across the Mediterranean region does not translate into differentiation in virulence. This result contrasts with results obtained in 2005, but it is now clear that there is a strong relationship between virulence and body size in *A. transcaspicus*, and that our previous observations of variation in virulence among parasitoid populations was due to differences in size.

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. Alternatives to dormant season sprays include fall applications targeted against the egg-laying generation (oviparae) of aphids that return to the orchards in November, or delayed dormant treatments targeted against the first generation of aphids that emerge from overwintering eggs in March. During the last three years we have characterized the timing of the fall migration and overwintering biology of both MPA and the leaf curl plum aphid (LCPA), *Brachycaudus helichrysi* (Mills et al. 2005, 2006). Fall migrations of both aphid species begin with the gynoparae that return to the orchards in September (LCPA) or October (MPA) to produce a generation of nymphs that develop into the egg-laying oviparae. Male aphids then return to the orchards the mate with the oviparae in either October (LCPA) or November (MPA). The beginning of the male migrations provides an indication of the last date by which fall treatments must be applied. Aphid eggs overwinter in diapause which must be broken by chilling before heat units can be accumulated toward egg hatch. For MPA diapause is broken around late January or early February depending on the year, and then egg hatch occurs approximately four weeks later. For LCPA diapause is broken at the same time as for MPA, but egg hatch is more rapid taking approximately 10 days. Thus treatments for the first generation of aphids could begin in late February for LCPA or mid March for MPA.

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. From 2005, an analysis of the impact of four insecticides that could be used for aphid control in fall and spring applications on survivorship of *A. transcaspicus* 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.

Attempts to establish *A. transcaspicus* in prune orchards has not yet met with great success, but late summer releases of parasitoids (collected from southern Spain in 2006) in *Phragmites* reeds, one of the summer host plants of MPA, led to substantial field parasitism. It is not yet clear whether it was the origin of the parasitoids or the focus on *Phragmites* that led to this success. However, these preliminary results are very promising, and suggest the need for additional releasing and monitoring both in prune orchards and *Phragmites* beds

a. Releases in prune orchards

Parasitoid Strain	Open	Sleeve	Mummies
Almond, Israel 2005	500	100	0
Almond, Morocco 2006	800	160	175
Almond, S. Spain 2006	500	180	415
Total	1,800	420	590

b. Releases in *Phragmites* reed beds

Parasitoid Strain	Open	Sleeve	Mummies
Almond, S. Spain 2006	300	0	>25

Table 1. A summary of the releases and recoveries of parasitoids in prune orchards (a) and *Phragmites* reed beds (b) in 2006.

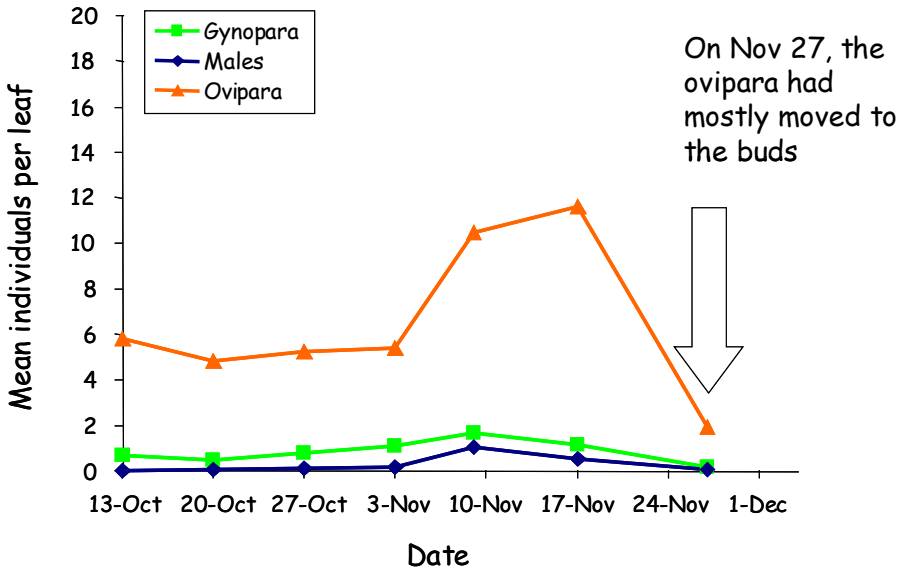
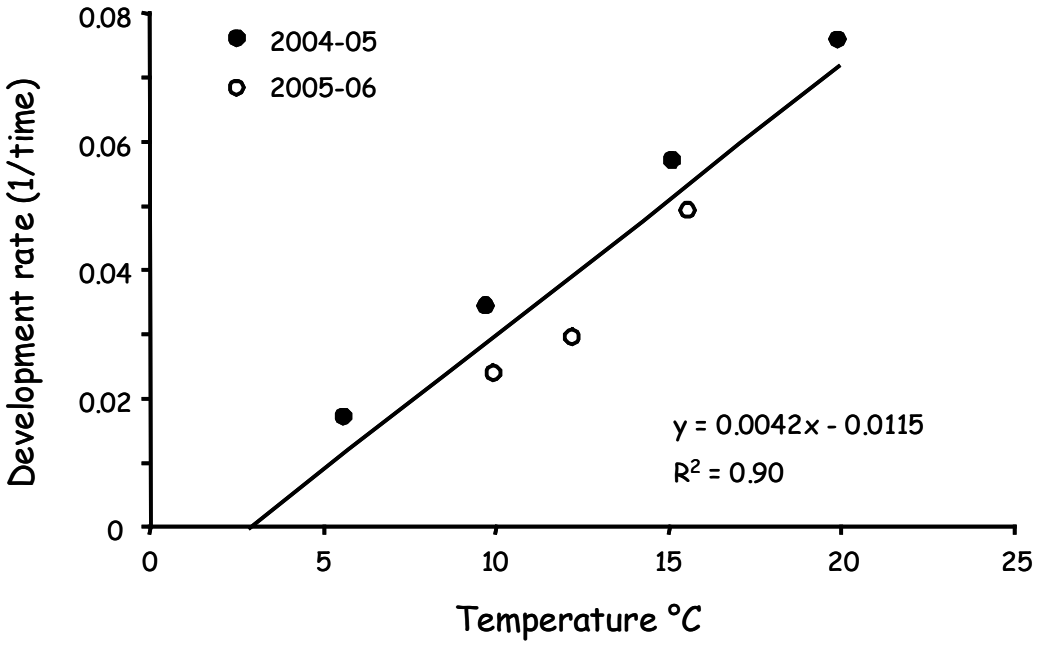


Fig. 1. The abundance of gynoparae, nymphs of egg-laying oviparae, and males of MPA in an orchard near Winters in October – November 2006.

a. Thermal requirements for MPA



b. Thermal requirements for LCPA

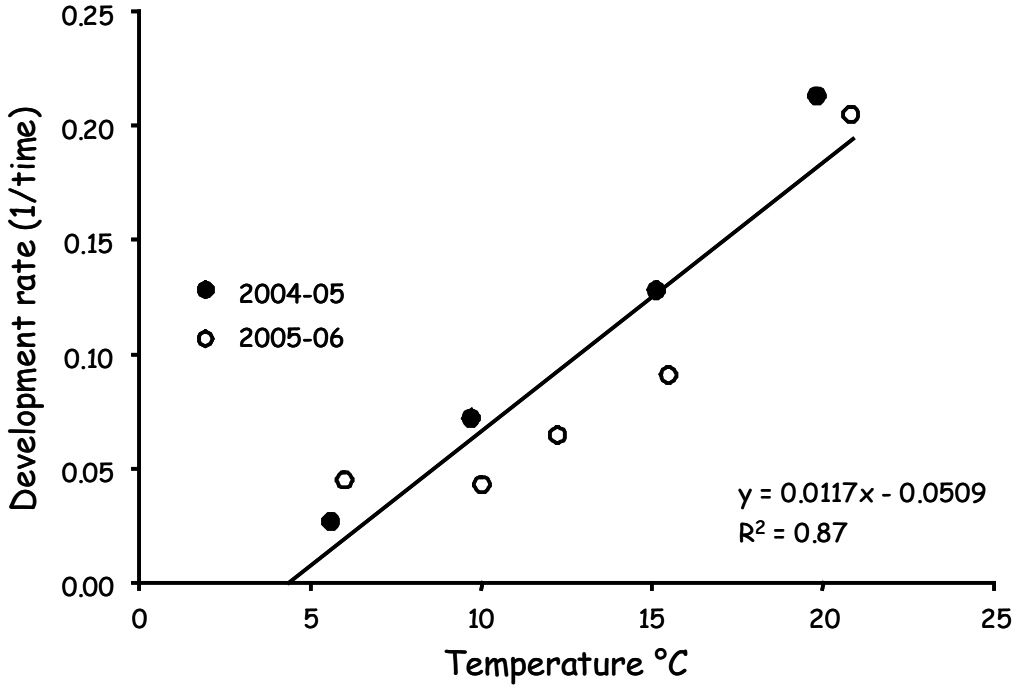
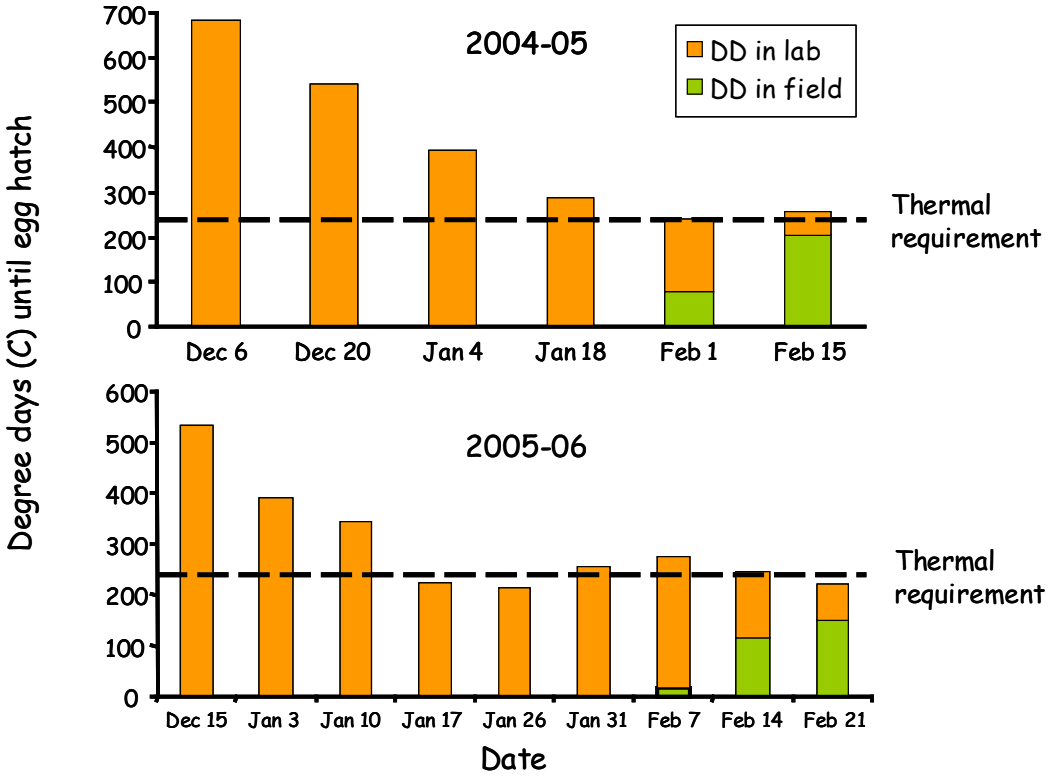


Fig. 2. Estimation of the threshold temperatures and the thermal requirements for development of MPA (a) and LCPA (b) eggs collected from Winters in December and chilled at 2°C until February.

a. Termination of diapause for MPA



b. Termination of diapause for LCPA

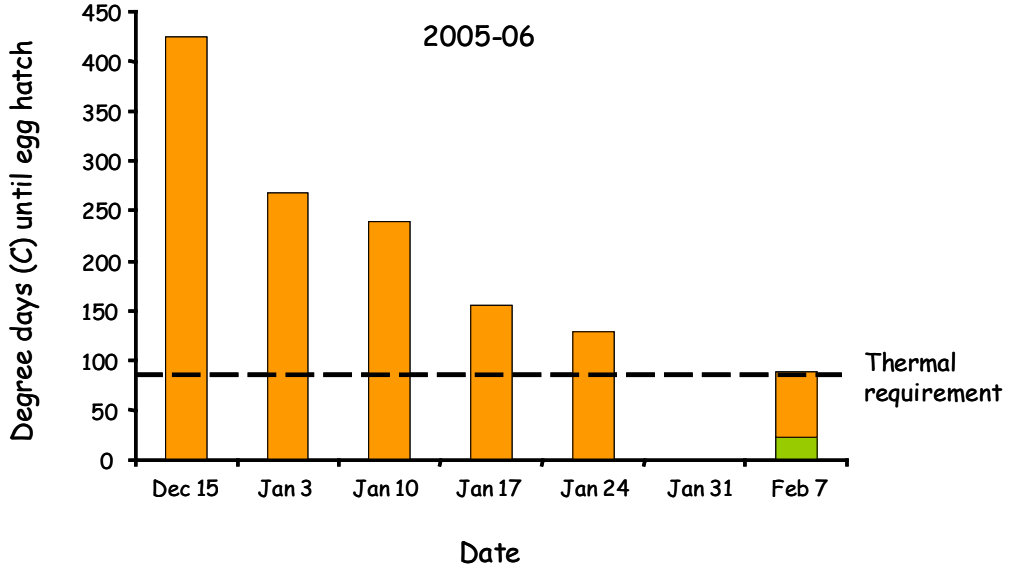
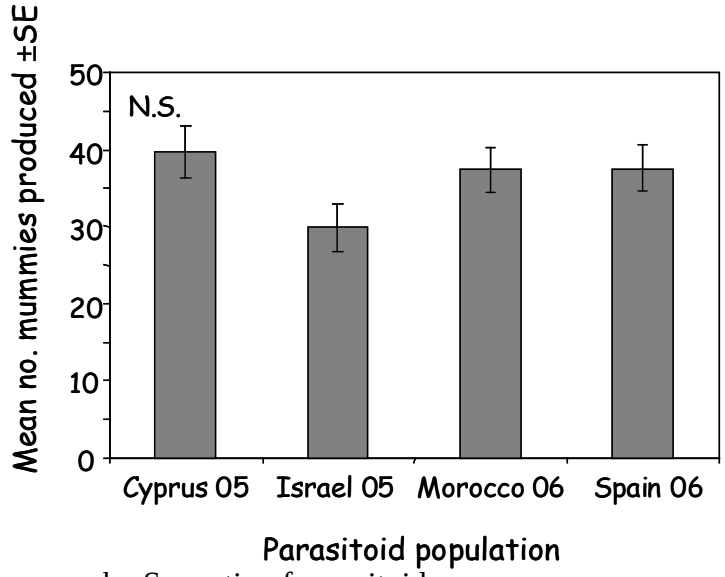


Fig. 3. Estimation of the date of termination of egg diapause for MPA (a) and LCPA (b) eggs collected from Winters at regular intervals through the winter of 2004-05 and 2005-06. The estimated thermal requirement for egg hatch (from Fig. 2) is presented as a horizontal dotted line.

a. Mummy production



b. Sex ratio of parasitoid progeny

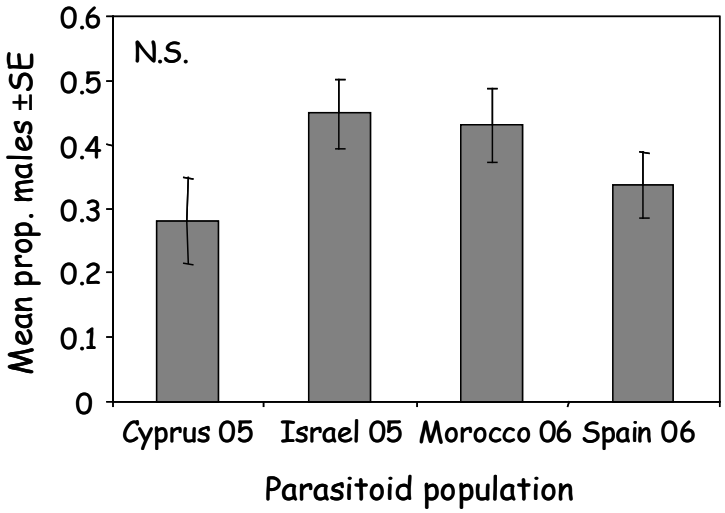


Fig. 4. The virulence of different populations of *Aphidius transcaspicus* based on two metrics, (a) the number of parasitoid mummies resulting from an 8h period of attack, and (b) the sex ratio of the progeny produced from these mummies.