BIOLOGICAL CONTROL OF MEALY PLUM APHID

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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 the mealy plum aphid (MPA), *Hyalopterus pruni* to be a significant problem. This aphid 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 and *Phragmites*) 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.

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*. 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.

While substantial levels of parasitism have led to a general reduction in the spread of LCPA aphid colonies both within and between trees in prune orchards in recent years, there remains a need to establish an effective population of *Aphidius transcaspicus* to provide successful biological control of MPA. We have made extensive surveys of mealy aphids and *A. transcapicus* throughout the Mediterranean in recent years and have been able to document the occurrence of three different mealy aphid species with distinct host tree preferences. Each of these aphid species is attacked by what appears to be a single parasitoid species, *A. transcaspicus*, although there are genetically distinct subpopulations or strains of the parasitoid occurring in different geographic regions. We have now established that the invasive MPA in California probably originated from southern Spain, and have focused collections of parasitoids this year on this region assuming that local populations of parasitoids would be better adapted

climatically and to the MPA genotypes from this region. Attempts to establish parasitoid populations in prune orchards in early summer has not yet met with great success, but summer releases of parasitoids in *Phragmites* reed beds, one of the two summer host plants of MPA, led to substantial field parasitism in late 2006. 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 were very promising, and suggested the need for additional releasing and monitoring both in prune orchards and *Phragmites* beds.

In addition, recent toxicological assays of the impact of registered insecticides on juvenile *A. transcaspicus* within mummified aphids have shown strong differential compatibility. With introduced parasitoids likely to form a central component of aphid management in prunes, it is important to be able to identify selective insecticides that are least likely to compromise the action of the parasitoids. Aphid parasitoids are most likely to compromised during either the pupal (aphid mummy) or adult stages, and thus an additional component of this project is to use simple laboratory assays to determine the effect of different insecticides on both emergence from mummies and adult longevity.

OBJECTIVES

1. To continue to field release and monitor different strains of *Aphidius transcaspicus* in prune orchards and *Phragmites* beds.

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*.

PROCEDURES AND RESULTS

1. To continue to field release and monitor different strains of *Aphidius transcaspicus* in prune orchards and *Phragmites* beds.

Methods

In May 2007, parasitized mealy aphids were collected from *Prunus* trees from a number of locations in southern Spain. This region was selected as the most likely origin of the invasive populations of MPA in California as determined by genetic fingerprinting. Mummies were collected from 7 locations and were sent or hand carried to the quarantine facility at UC Berkeley for parasitoid rearing.

A dominant hypothesis from the biological control literature is that parasitoids from the same location as the source of an invasive pest will be better adapted to that particular genotype of the pest and to the same climatic conditions that supported the successful invasion of the pest.

Field releases of parasitoids from subpopulations collected from Israel in May 2005, Cyprus in July 2005, Morocco in 2006 and southern Spain in 2007 were carried out in prune orchards in the

Sacramento Valley from early May to mid June 2007 and in *Phragmites* reed beds from July to early November 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.

We also carried out a series of greenhouse and field assays of the relative virulence of the different subpopulations of *A. transcaspicus*. The parasitoid populations selected for these assays were the same four subpopulations 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. For the greenhouse assays, 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 subpopulations. For the field assays, between 20 and 30 parasitoids were released into sleeve cages either on limbs of prune trees in orchards or clumps of *Phragmites* reeds in reed beds. The sleeves were removed after two weeks to count the number of mummies produced in relation to the number of parasitoids initially released. The number of replicates for each subpopulation was determined by the availability of parasitoids from the respective colonies and as a result it was not possible to produce a balanced experimental design with an equal number of replicates for each subpopulation.

Results

As in previous years, 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* from southern Spain.

In contrast to the two previous years, we were able to field release many more parasitoids this field season, due to the better production of parasitoids from aphids on *Phragmites*. A total of 3,575 parasitoids were field released during the 2007 field season (Table 1). The majority of the parasitoid releases this year were from the two eastern Mediterranean subpopulations from Cyprus and Israel, but releases were also made of western Mediterranean subpopulations from Morocco and Spain.

One particularly important difference in field releases of parasitoids in 2007 was the decision to make both sleeve cage and open releases into *Phragmites* reed beds in the Sacramento delta. These reed beds had heavy populations of aphids and proved to be an effective alternative strategy for parasitoid establishment in California. A single open release that was made in

Phragmites in October 2006 resulted in the production of a number of mummies, indicating the potential of establishing parasitoids on the summer host plants. Early juvenile stages of the parasitoid are carried in the bodies of migrant gynoparous and male aphids as they return from their summer host plants to prune orchards in the Fall. Thus the establishment of parasitoids on summer hosts would not only reduce populations that are able to make the return migration, but would also provide an effective means of naturally introducing parasitoids in prune orchards for overwintering. Parasitoids are likely to emerge from aphid mummies in prune orchards early in spring when they can have the greatest potential impact on aphid populations.

From the greenhouse assays of the relative virulence of the four subpopulations of *A. transcaspicus*, individual females produced an average of 31-39 mummies during an 8h period in the virulence assay (Fig. 1a) with an average proportion of males from 30-45% (Fig. 1b). Despite the 15 replicates there was no significant variation between parasitoid subpopulations in both of these metrics suggesting that the variation between individual wasps was too high. It appears that this variation for some subpopulations was due to differences in body size of the individual wasps, with larger females producing a greater number of mummies. The southern Spain subpopulation, that we expected to be best adapted to the use of California aphids, did not perform any less effectively in terms of mummy production and even slightly better than some subpopulations in terms of production of female progeny. However, these greenhouse assays provide little evidence that the southern Spain subpopulation is the best to use fro introductions into California.

In contrast, the field based assays of relative virulence did provide greater evidence of distinctions between parasitoid subpopulations in the production of mummies in field cages (Fig. 2). For assays in prune orchards the performance of the southern Spain subpopulation was significantly lower than that of both the Cyprus and Israel subpopulations. Similarly, for assays in *Phragmites*, the Cyprus subpopulation showed the greatest performance along with the Morocco subpopulation, although in this case the differences were not significant due to the large variation and small number of replicates for the latter. Nonetheless, both sets of assays suggest that in contrast to our expectation that the subpopulation from southern Spain should perform the best, the Cyprus subpopulation, which is not associated with the aphid genotypes that are most closely related to Californian aphids, appears to be the most virulent of the subpopulations tested.

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*.

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 non-diapause mummies which have only a short interval before adult emergence were used. Sets of leaves with attacked mummies, 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 to minimize the surface of insecticide residue. 10 replicate sets of 10 mummies were placed in glass vials and kept at 20°C and a 16h photoperiod to allow emergence. Emergence was monitored daily, and mummies removed from the vial as the adults emerged.

Testing of adult parasitoids was based on mated females of *A. transcaspicus*. 10 replicate sets of 5 female parasitoids were sprayed in Petri-dishes with a Potter tower for topical exposure, transferred to insecticide-dipped glass vials for residue exposure, and fed on honey surface sprayed with the same products for oral exposure. Acute mortality was estimated 48h after exposure, and survivorship will be checked again after 4 and 7 days in comparison to control parasitoids sprayed with water.

Materials that were tested were those that showed potential for dormant or delayed dormant application in prunes, as well as those that are most frequently used in season. Each of these materials was tested at a full field rate (100%) and at dilutions of 50% and 10% and compared to a water alone control treatment.

Results

This component of the project is currently underway and only limited results are available at this time. Four insecticides have been completed for the mummy emergence assays and are shown in Fig. 3. Surprisingly, Omni oil had the greatest detrimental effect on the emergence of adult wasps from the aphid mummies. In contrast, there was no measurable impact from Imidan, with Asana and Diazinon falling in between. As such products do not necessarily have the same impact on foraging adult parasitoids as on aphid mummies, it will be interesting to see whether the same patterns emerges from the adult assays that are currently in progress. In general, the impact of more traditional insecticides on adult parasitoids has been greater than on emergence of adults from aphid mummies, and we expect the same to be true for this study.

CONCLUSIONS

The need to develop alternatives to dormant oil sprays for the control of aphids in prunes has generated interest in establishing parasitoids that will provide long term control of both leaf curl aphid and mealy plum aphid in prune orchards. *Aphidius colemani* has been successfully established against leaf curl plum aphid and populations of this aphid have diminished in recent years as a result. Early spring populations can still occur in orchards in the north and the west side of the Sacramento Valley, but these populations are generally confined to individual branches, they remain small, and seldom develop to spread between branches and trees within orchards. The establishment of a strain of *Aphidius transcaspicus* that could achieve the same impact on mealy plum aphids is an important goal that would be of tremendous value to the prune industry as the most cost-effective way to manage these aphids in prunes.

Attempts to establish *A. transcaspicus* in prune orchards have not yet been successful. The parasitoids do produce large numbers of mummies when confined in sleeve cages, but when open released they appear not to stay in the orchards. The reason for this movement out of the

orchards is likely to be due to the relatively large numbers of aphids present, which may confuse the parasitoids that have experienced much lower aphid abundance in the region of origin. An important breakthrough, however, occurred late in 2006 when we found open releases of parasitoids in *Phragmites* reeds, one of the summer host plants of mealy plum aphid, led to substantial field parasitism. The same observations have been made during open releases in *Phragmites* this year and this provide an excellent opportunity for establishment of *A. transcaspicus* in California. Not only could the parasitoids reduce the numbers of aphids migrating back to prune orchards in the fall, but returning migrants would also carry early juvenile stages of the parasitoids with them to overwinter as mummies in the orchards and to emerge early in spring when aphid abundance is at its lowest and most vulnerable to parasitism.

Virulence assays carried out both in the greenhouse and in field cages suggest that the parasitoid subpopulation from southern Spain, the location in which mealy plum aphids are most closely related genetically to mealy plum aphids in California, is not necessarily to best source of parasitoids to use for introductions, with a subpopulation from Cyprus providing the best performance this year.

Fall sprays targeted against egg-laying aphids in prune orchards in November offers a very promising alternative to dormant sprays until parasitoids can be established in California and provide a more long term impact on aphid populations. In this context it is important to know which insecticides are most compatible with *A. transcaspicus*. Surprisingly, Omni oil had the greatest detrimental effect and Imidan the least, with Asana and Diazinon falling in between. These preliminary results suggest that products do differ in their compatibility and that pyrethroids might be preferable to organophsphates, but further work is needed to clarify these initial observations.

	Prunes		Phragmites		
Parasitoid subpop.	**Open**	**Sleeve**	**Open**	**Sleeve**	**Total**
Cyprus	0	90	1,225	240	1,555
Israel	300	90	700	75	1,165
Morocco	0	0	400	50	450
Spain	100	230	0	75	405

Table 1. A summary of the releases of *Aphidius transcaspicus* in prune orchards and *Phragmites* reed beds in 2007, indicating the geographic origin of the subpopulations of parasitoids used and whether releases were into sleeve cages or open.

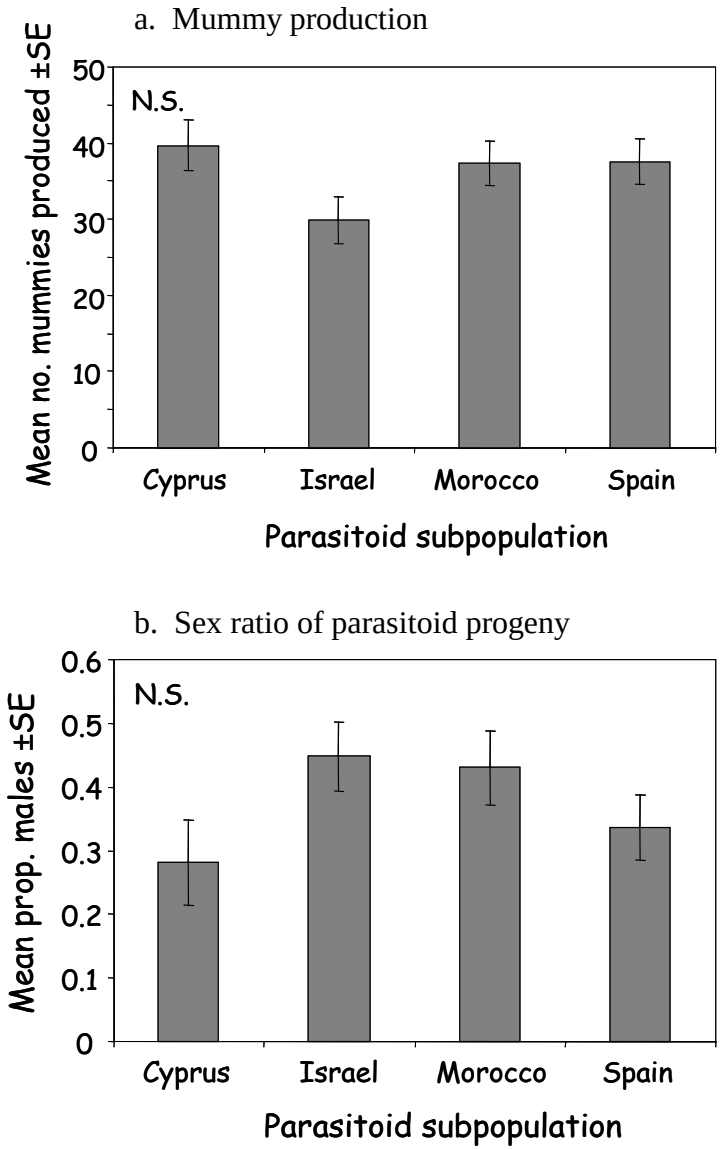


Fig. 1. A greenhouse virulence assay for different subpopulations of *Aphidius transcaspicus* based on two metrics, (a) the number of parasitoid mummies resulting from an 8h period of attack, and (b) the sex ratio of the progeny produced from these mummies.

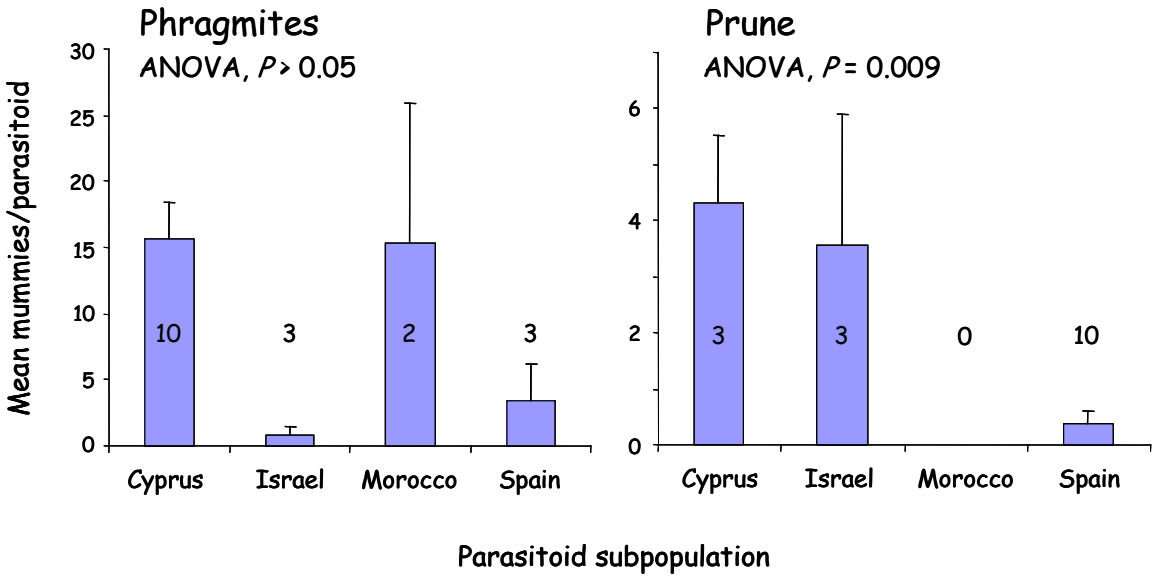


Fig. 2. A field cage virulence assay for different subpopulations of *Aphidius transcaspicus* in both *Phragmites* reed beds and prune orchards, based on number of mummies produced per parasitoid released into the sleeve cage. Inset numbers are number of replicates.

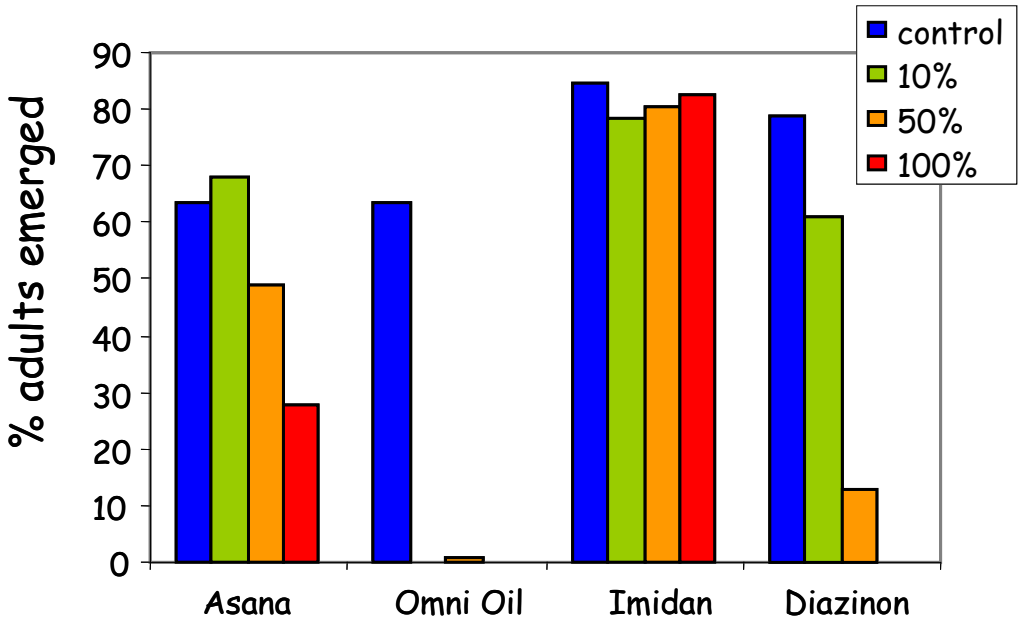
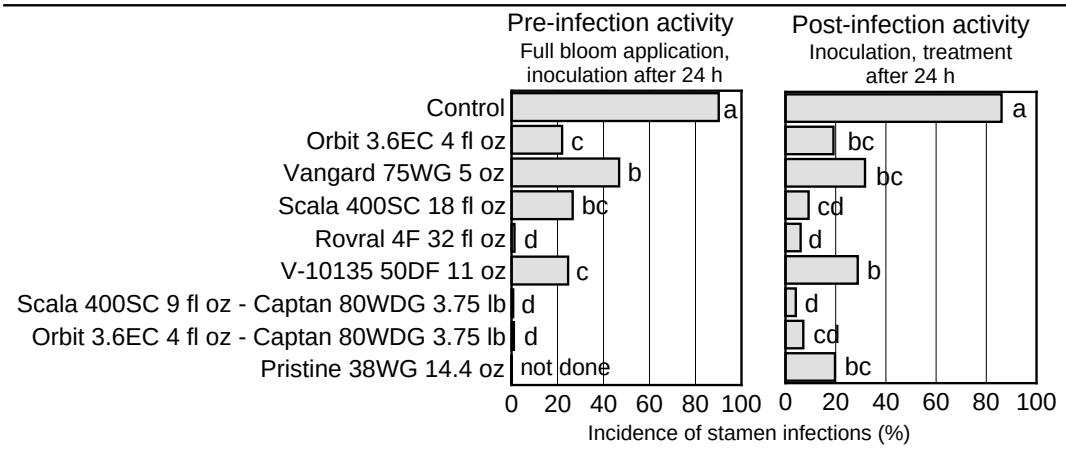


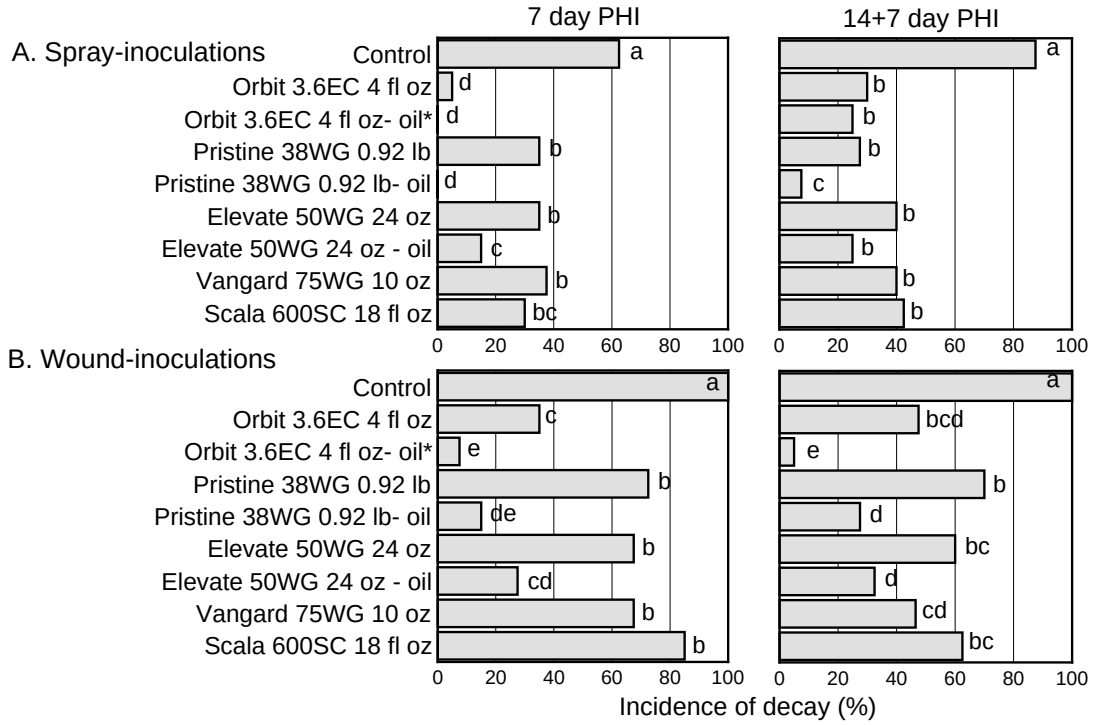
Fig. 3. Laboratory assays of the compatibility of different insecticides with *Aphidius transcaspicus* indicating the percentage of adult wasps that emerge successfully from treated mummified aphids. Insecticides were used a the full field rate (100%) and two dilutions (50% and 10%) in relation to controls.

Fig. 1. Efficacy of pre- and post-infection treatments with selected fungicides for management of blossom blight of French prune



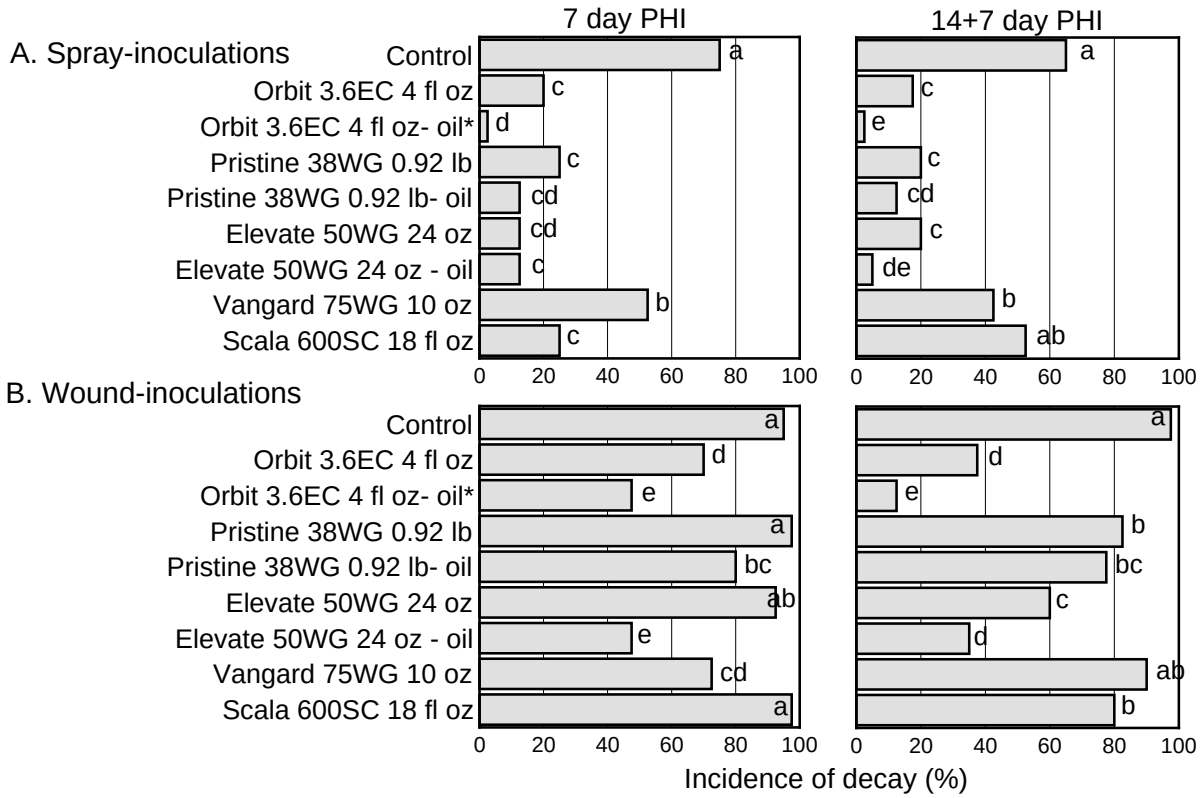
For evaluation of the pre-infection activity, blossoms were collected in the field and treated in the laboratory using a hand sprayer. After 24 h blossoms were inoculated with a spore suspension of *M. laxa* (10K/ml). For evaluation of the post-infection activity, blossoms were collected and inoculated and were treated with a hand-sprayer after 24 h. Blossoms were evaluated for stamen infections after 3-4 days of incubation at 20 C.

Fig. 2. Efficacy of 7 and 14+7 day preharvest fungicide treatments for management of postharvest brown rot decay of French prune - Field trial at UC Davis



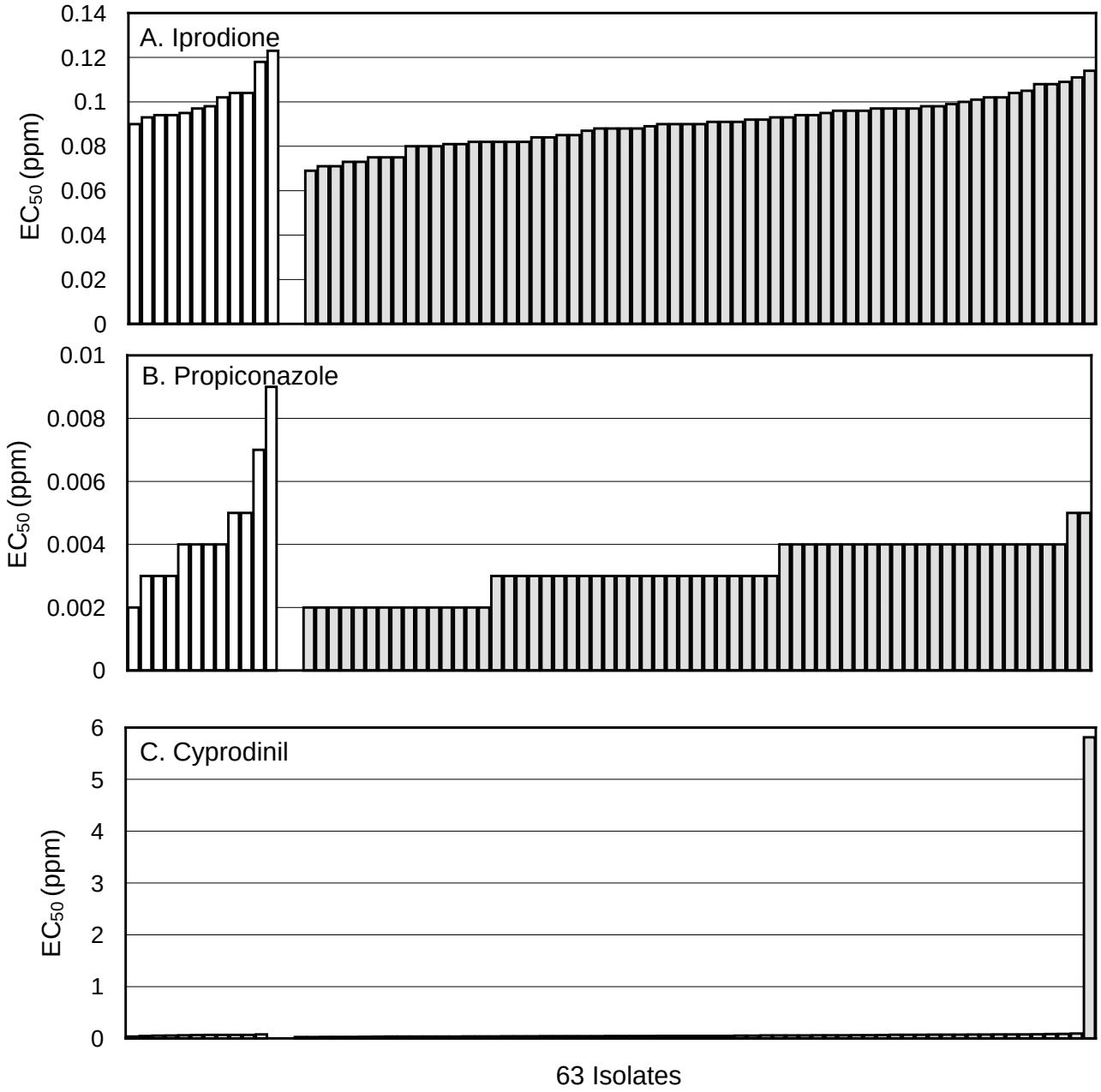
Preharvest treatments were applied on 8-14 and 8-20-07 using an air-blast sprayer at a rate of 100 gal/A. After harvest, fruit were wound- or spray-inoculated using a spore suspension of *M. fructicola* at a concentration of 30K/ml. Fruit weree then incubated at 20C for 7 days. *Superior 415 Spray oil was used.

Fig. 3. Efficacy of 7 and 14+7 day preharvest fungicide treatments for management of postharvest brown rot decay of French prune - Field trial in Colusa Co.



Preharvest treatments were applied on 8-14 and 8-21-07 using an air-blast sprayer at a rate of 100 gal/A. After harvest, fruit were wound- or spray-inoculated using a spore suspension of *M. fructicola* at a concentration of 30K/ml. Fruit weree then incubated at 20C for 7 days. *Superior 415 Spray oil was used.

Fig. 4. In vitro sensitivities of isolates of *M. fructicola* against seleccted fungicides Comparisons of isolates collected in 2007 to isolates collected in the past -



EC_{50} values for 63 isolates of *M. fructicola* were determined using the spiral gradient dilution method. The 12 isolates on the left side of the histogram (vertical bars not shaded) were collected from different stone fruits in 1999 or before and serve as references. The remaining isolates were collected from prune in 2007.