

Natural Presence, Behavior and Feeding Efficiency of the  
Mycophagous Coccinellid *Psyllobora vigintimaculata* in an  
Urban Garden Setting within California's Central Valley

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## Introduction

The fungi belonging to the Erysiphales, Ascomycota, commonly known as powdery mildews (PM), are all obligate biotrophs. As an order, they have been shown to infect almost 10,000 species of angiosperm plants in 169 families (Amano, 1986). Since many of these host plants are valued as crops, PM is collectively considered one of the most important plant pathogens worldwide. Significant yield losses due to PM infection have been recorded in the following agronomic crops; soybeans, *Glycine max* L., wheat, *Triticum aestivum* L., barley, *Hordeum vulgare* L. (Phillips, 1984; Conner et al, 2003; Nordeng et al, 1988), horticultural crops; strawberry, *Fragaria X ananassa* Duch., wine grapes, *Vitis vinifera* L., cucumber, *Cucumis sativus* L. (Miller et al, 2003; Ypema and Gubler, 1997; Abood et al, 1991), and ornamental plants; roses, *Rosa* spp., crepe myrtle, *Lagerstroemia indica* L., poinsettia, *Euphorbia pulcherrima* Willd ex. Klotzsch (Alvarez et al, 2000; Liberato and Barreto, 2004; Celio and Hausbeck, 1998). Disease management typically involves regular applications of fungicides. This approach, coupled with the high rate of asexual sporulation in PM, has led to documented resistance to benzimidazoles, sterol inhibitors, demethylation inhibitors (DMI) and strobilurins in both laboratory and field experimentation (Gubler et al, 1996; del Pino et al, 1999; Heaney et al, 2000; McGrath, 2001).

Biological control of PM may offer solutions to this resistance phenomenon and other pesticide-related issues such as crop residues, effects on nontarget organisms, and worker health and safety. There are several commercially available microbial biological control agents including the spore-forming bacterium *Bacillus subtilis* and the fungal hyperparasite *Ampelomyces quisqualis* Ces. Little is known, however, of the potential of arthropod agents to control or reduce disease through consumption of PM. Work by English-Loeb et al (1999) evaluated the ability of a tydeid mite (Acari:Tydeidae) to reduce the incidence of PM in riparian grapevines, *Vitis riparia* Michx. Powdery mildew has been considered an important alternative nutrient source to some predatory mites (Acari:Phytoseiidae) and may help to maintain populations in absence of prey (Zemek and Prenerova, 1997). All members of the Psylloborini Casey (Coleoptera:Coccinellidae) are obligate consumers of various PM conidia and hyphae at all mobile life stages (Gordon, 1985). No-choice feeding assays by Davidson (1921) highlighted the prey refusal and subsequent death of *Psyllobora vigintimaculata taedata* LeConte individuals offered spider mites (Acari:Tetranychidae), aphids (Homoptera:Aphididae) or armored scale insects (Homoptera:Diaspididae). However, records of aphidophagy or phytophagy within Psylloborini persist in contemporary literature, usually as part of natural surveys (Omkar and Pervez, 1999; Yurtsever, 2001). In a review of coccinellid taxonomy, Gordon (1985) suggested records such as these to be a result of inaccurate observation. Clearly, a dearth in the scientific literature of research addressing the mycophagy exhibited by these insects has led to some biological inaccuracies.

The cosmopolitan genus *Psyllobora* Chevrolat is represented in temperate and subtropical regions worldwide, in natural and managed systems, and may play a role as a native biological control agent of PM (Prasad and Rai, 1988; Cruz, 1989; Almeida and Milleo, 1998; Hoffman et al, 1997; Tezcan and Uygun, 2003). Soylu et al (2002) recorded a reduction in PM conidia of 92% when comparing leaf areas grazed upon by *P. bisoetonotata* Mulsant with non-fed-upon areas, suggesting a real and measurable PM

removal through consumption. Further work with this species (Ahmad, 2003) revealed a large host range. The insect was recorded feeding on the mildew of 52 different plant species belonging to 24 families. This tendency for wide prey acceptance within Erysiphales coupled with the obligation to feed on mildew at all life stages may prove to be an important attribute of *Psyllobora* in relation to biological control. Colonization of our greenhouses in Davis, California by the subspecies *Psyllobora vigintimaculata taedata* LeConte raised questions regarding the insect's seasonality and natural presence in the local urban landscape.

The objective of this work was to observe and document the biology and natural occurrence of *P. vigintimaculata taedata* in various managed horticultural systems in Davis, California with respect to host plants, PM severity and the environment throughout the course of the year. It is hypothesized that since previous observations of the genus (Soylu, 2002; Ahmad, 2003) show a wide acceptance of mildew species as food on a variety of plants, then plants in the landscape infected with PM should harbor *P. vigintimaculata taedata* during its active seasons. In addition, we hypothesized that as PM severity increases at a specific location, then incidence and density of this PM predator will also locally increase.

## Materials and Methods

A rotating colony of *Psyllobora vigintimaculata taedata* was maintained in the laboratory in a series of insect rearing cages held at an average 25°C. Plant material with PM was grown separately under high-pressure sodium lighting (600W) with a 12-hour photoperiod in a humidified (50-80%RH) growth room utilizing ebb-and-flood hydroponic tables. Periodic inoculations with the crop-specific PM conidia were made either by an applied spore solution or brushing spores from infected plants. Infected plants, either *Gerbera jamesonii* Adlam, *Zinnia elegans* Jacquin or *Rosa* spp., were exposed to caged adults at regular intervals for egg deposition. After oviposition the adults were shaken off and the egg-laden plants were moved to another cage where larval development would occur. Towards the end of the fourth instar the larvae began to wander in search of a pupation site. At this time the plants were moved to a pupation cage and cut at the soil line. An inverted black plastic tray was situated in the pupation cage to offer shelter from the light and to act as a pupation platform for the wandering larvae. Pupae were harvested by removing those formed on the platform, or adults captured as they emerged and flew towards the top of the cage and the light. In this rotated manner the colony provided harvestable eggs, pupae or adults of uniform age and culture at five-day intervals.

**Biological Observation.** Egg masses deposited on the same day were removed from the colony and transferred to an incubator (Percival Scientific I-30 BL) kept at constant 25° C under fluorescent lights. Upon eclosion, the first-instar larvae were individually transferred with a fine paintbrush to observation petri discs (55mm X 15mm) containing an excised *Gerbera jamesonii* leaf portion infected with PM (*Erysiphe chiocearum*) as food and filter paper moistened with deionized water. Observation discs were returned to the incubator and monitored every 24 hours, noting visible exuviae or active molting in order to establish stadia durations. Fresh water and a new leaf portion containing PM were added each day until successful pupation. Observations

were terminated upon emergence and adult beetles were collected and redistributed to the colony or released in greenhouses. A general developmental threshold for larvae was estimated in the laboratory using mixed-age larvae and a floral refrigerator (True GDM-23F,C). Larvae were caged with PM-infected plant material and placed in the refrigerator at 20° C. Temperature was manually ramped down (1° C/30 minutes) using the refrigerator dial, and larvae were examined at each interval to assess mobility and feeding. This test was repeated with three different groups of mixed-age larvae on subsequent days. The mean temperature at which larvae discontinued movement and feeding was used as the developmental threshold,  $T_0$ , in all degree-day calculation. Accumulated degree-days were determined as the sum of the daily differences in mean temperature and the developmental threshold using the following adapted rectangular degree-day formula (Arnold, 1959):  $DD = \sum (T_{\text{mean}} - T_0)_{\text{day}}$ .

**Natural Occurrence.** Various landscape plants known to be susceptible to PM were identified in and around the UC Davis campus and plotted on a municipal map in order to establish a sampling circuit that encompassed some geographic variability and the largest variety of plant species. All sampling areas were managed urban gardens or landscapes, planted with exotic ornamentals, native plants or horticultural food crops. Sampling was initiated to describe the presence of *P. vigintimaculata* in an established urban landscape setting, where many different plant/mildew complexes are likely to be encountered and where desirable management may call for chemical applications or physical disturbances to the plants. This study was done in cooperation with the UC Davis Arboretum, a 40-hectare public botanical garden showcasing more than 4000 plant species alongside Putah Creek. Garden types included a native California garden, a showcase of Central Valley landscape perennials, a native southwest USA garden, a wedding gazebo garden with only white flowering plants, and a Mediterranean terrace container garden. The UC Davis Student Farm, an organic production operation and public educational resource providing fresh seasonal fruits and vegetables to public subscribers, also cooperated in the study. Sites utilized within the farm included an organic vineyard of mixed wine and table grape varieties, the organic subscription garden, and the children's ecological teaching garden. Foundation plantings of various shrubs on campus grounds were also included. The final compendium of sampling sites comprised a circuit of approximately 10 kilometers, navigable by bicycle or foot. A sampling protocol was developed, in which presence/absence and density measurements were recorded for both PM and *P. vigintimaculata* on a weekly basis for one full year for each plant at every sampling site. Sampling began July 1, 2004.

Mildew severity in the field was estimated visually, and a PM severity index from 1 (a very slight infection) to 5 (a very heavy infection) was assigned to each sample. Leaf samples were regularly collected and examined for real PM density in the laboratory in order to correct and account for possible subjectivity and error surrounding the severity index in the field. To achieve this, PM density and severity have been measured in several ways. In some cases leaves were analyzed visually or digitally to assess a percentage of leaf area occupied by PM mycelia (Miller et al, 2003). In cases where density of conidia is the desired measurement, a method of counting conidia on a haemocytometer was utilized and described by Chellemi and Marois (1991) and further adapted by Ypema and Gubler (1997) to achieve a density measurement expressed in conidia per  $\text{cm}^2$  of known leaf area. Since we were working with different mildew

species on many hosts it was decided to combine these approaches by multiplying the measured density within mycelial patches by the percentage leaf area affected to obtain a final density, expressed to the nearest integer in an index fashion. This real density term was then fit as a dependent variable to the sample's PM severity index from the field through linear regression in order to establish the index's validity and warrant its use as a measurement.

Insect presence or absence was determined through manual examination of the sample plant or through yellow sticky card trap catches, which were especially useful for small-leaved plants and during the cooler season when insect densities were low. Insect presence data also included a separation of observed life stages, so that presence of eggs, larvae, pupae, adults and mating adults were recorded at each sample site. Since the sampling circuit involved about 30 different plants, there was no uniform density unit such as a shoot or leaf. Instead, an estimation of the number of insects per cubic foot ( $0.03\text{m}^3$ ) was used to compare densities between plants or sites. When only eggs were encountered, a subsample of one egg mass was taken back to the laboratory for eclosion and positive identification.

Weather measurements included the daily high and low temperature, average daily relative humidity, and measurable precipitation. These figures were available electronically through the California Irrigation Management Information System (CIMIS) weather station located in west Davis. Bivariate scatter diagrams, multivariate correlation matrices and linear regression with insect presence or insect density as the dependent variable (JMP© Start Statistics, SAS Institute, 2005) were used to establish relationships between weather and organism occurrence as well as between PM density and insect occurrence or density. All plants harboring *P. vigintimaculata* life stages actively consuming PM were identified to species and recorded with respect to season and condition of the relationship. In order to determine whether host plant significantly affected the occurrence of the beetle, mean comparisons by host plant were performed by Student's t tests, using Bonferroni  $\alpha$  adjustment for Type I error avoidance (Holm, 1979).

## Results and Discussion

**Biological Observation.** Masses of one to seven elongate, oval whitish eggs (0.7mm X 0.25mm) were deposited, with the long axis always perpendicular to the substrate (Figure 1). Eggs were placed directly on the leaf, petiole or stem of the infected plant, or sometimes on a hard surface in the laboratory, such as the sides of a petri dish. Laboratory and field observations of hundreds of eggs over the course of the year indicated that virtually all deposited eggs were fertile, as there were very few hatch failures over a range of conditions. There were four larval instars followed by a final ecdysis and subsequent pupation. The first instar hatchling had an oval translucent whitish gray body (0.8mm X 0.25mm), somewhat dorso-ventrally flattened, with many white hairs borne from the thoracic and abdominal tubercles (Figure 2). Following the first molt the larva's color was a much darker gray with a median cream colored or yellowish stripe, but as the larva neared the end of the instar the color gradually paled to that of almost white (Figure 3). This phenomenon was observed after each molt until pupation, and was consistent with Davidson's original observations (1921). The size of larvae gradually increased after each molt so that the measure of a new second instar was

1.7mm X 0.55mm, the new third 2.3mm X 0.6mm, and the new fourth 2.8mm X 0.8mm. Just before the final molt the average fourth instar larva measured 3.3mm X 1.3mm. The body shape and markings did not change from the second instar to the fourth instar, but markings did become more pronounced. During the latter parts of the fourth instar the larva stopped feeding and attached itself to a pupation substrate, usually the abaxial surface of a large leaf or petiole. This process required about a third of the duration of the fourth instar. The pupa that follows the fourth molt was also oval in shape, though much shorter and somewhat convex (2mm X 1.3mm X 1mm). Pupae were similar in color to the larvae, with the addition of gray wing pads, and a transverse row of black spots on abdominal segment three and sometimes smaller, lighter spots on segments two, four and five (Figure 4). The emerging adult was similar in shape and size to the pupa (female 2.8mm X 1.5mm X 1mm; male 2.2mm X 1.3mm X 0.8mm) though more typically convex. The elytra were a base color of cream or yellowish, each marked with three dark brown spots and two light brown blotches. The pronotum was of similar cream color with five brown spots arranged in an arc. The legs and antennae were golden yellow and all ventral surfaces dark brown to black (Figure 5). Under laboratory conditions (25°C, 12-hour photoperiod) adults usually spent a full day virtually immobile and in close proximity to the recently exited pupa. Upon emergence beetles were very pale or white. After several hours the elytra darkened and the pattern of maculation became visible. The observed developmental temperature threshold for the larvae was 12.5°C. Using this as  $T_0$  in the equation  $DD = \sum (T_{\text{mean}} - T_0)_{\text{day}}$  we were able to calculate the required accumulated degree-days for each stage. Development from egg deposition to emergence of an adult required 235 degree days (Table 1). At 20°C this equals about seven days for the egg, four and a half days each for the first and second instars, three and a half days each for the third and fourth instars, and seven days duration from pupation to emergence for a total of about 32 days from egg to emerged adult. At 25°C this process is accelerated to 20 days. In California's hot central valley during the summer outdoor mean daily air temperature may reach 28°C, and *P. vigintimaculata* would complete development from egg to adult in just over two weeks (15.2 days). Davidson (1921) reported that adult females usually commence oviposition at least ten days after emergence. We found that this was not always the case, especially in the insect colony. Some females began to mate sometimes on the second day after emergence, and some began to deposit eggs as soon as five days after emerging.

**Natural Occurrence.** Life stages of *P. vigintimaculata taedata* were observed feeding on the PM of 26 plant species in 13 different families, and may be associated with several other plants where adult beetles were caught on sticky cards in the proximity of mildew infection (Table 2). The insect was never seen on sampled plants not harboring PM. It is interesting to note that insects were also not detected on several chronically infected plants including *Euonymus japonica* L. and the California natives *Heteromeles arbutifolia* Lindl. and *Eschscholzia californica* Charm. Perhaps there are mildews within the Erysiphales or certain host plants that are not palatable to *P. vigintimaculata* or offer insufficient nutrition. Insect activity was regularly detected until the middle of December, when California's Mediterranean climate includes daily fog, rainfall and sustained low temperatures (daily mean 7°C). Adult beetles were once again encountered in late February (Figure 6), as rains became less frequent and temperatures began to rise (daily mean 11.1°C). Previous observation by Davidson (1921) on this

subspecies notes that the insects overwinter as adults in small aggregates. This is consistent with our records, in which the adult is the last noticeable life stage late in the season and the first noticeable life stage in early spring.

The PM severity index developed for field assessment was positively correlated to real measured PM density ( $R^2=0.70$ ,  $y= 0.086x + 1.09$ ,  $n=53$ ) and a one-way ANOVA showed a significant positive influence of the field rating on real density ( $F=55.96$ ,  $df=1$ ,  $52$ ,  $P < 0.0001$ ). Therefore, the field rating was considered a reliable measure of PM density and was further utilized as a comparative tool.

Regression analysis to detect an aggregative numerical response of *P. vigintimaculata* to increasing PM severity showed a significant positive correlation between PM density and insect density ( $R^2=0.29$ ,  $y= 1.30x - 0.264$ ,  $n=410$ ). Additionally, there were more different *P. vigintimaculata* life stages present as mildew density increased ( $R^2=0.28$ ,  $y= 0.597x - 0.038$ ,  $n=393$ ). There were no eggs observed on plants harboring PM at severity level 2 or lower. This suggests that adult insects are unlikely to deposit eggs in a location where there is an inadequate food supply for the larvae to complete development. There were also significant differences in the mean insect density between plant species (Figure 7). Although not tested, this appeared to be positively correlated to PM severity, and was seasonally different. In early spring the largest populations were seen on roses, followed by grapevines and crepe myrtle trees in the summer, and field cucurbits in autumn.

The mycophagous coccinellid *Psyllobora vigintimaculata taedata* is present in many managed agricultural and horticultural systems in Davis, California as a consumer of PM between the end of February and the middle of December through a wide range of temperatures. Insect density correlated positively to mildew density. This suggests a positive aggregative numerical response of a consumer to its food source. This measurement is an important criterion for effective biological control agents (Solomon, 1949). Its presence on single or small groups of PM-infected plants in a large and variable landscape suggests the ability of the adult insect to locate PM infections aerially through specific olfaction or by following an aerial spore gradient. This ability to locate a food source is a key element to successful biological control. Additionally, in a protected system such as a greenhouse it is possible that this insect could be utilized for early detection of PM outbreaks by conventional insect monitoring methods such as yellow sticky cards. Since some PM complexes in the field lacked the presence of *P. vigintimaculata* throughout the year despite extensive mildew severity it is likely that there are restrictions or preferences in the PM diet of this insect.

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Figure 1. A typical cluster of *Psyllobora vigintimaculata taedata* eggs deposited on the abaxial surface of a leaf infected with powdery mildew.



Figure 2. Newly hatched first instar larva of *Psyllobora vigintimaculata taedata*.



Figure 3. Late second instar larva of *Psyllobora vigintimaculata taedata* on a grape leaf.



Figure 4. Typical pupa of *Psyllobora vigintimaculata taedata* fixed to the abaxial surface of a grape leaf.



Figure 5. An adult *Psyllobora vigintimaculata taedata* feeding on a patch of *Oidium evonymi-japonici*, the powdery mildew of euonymus.

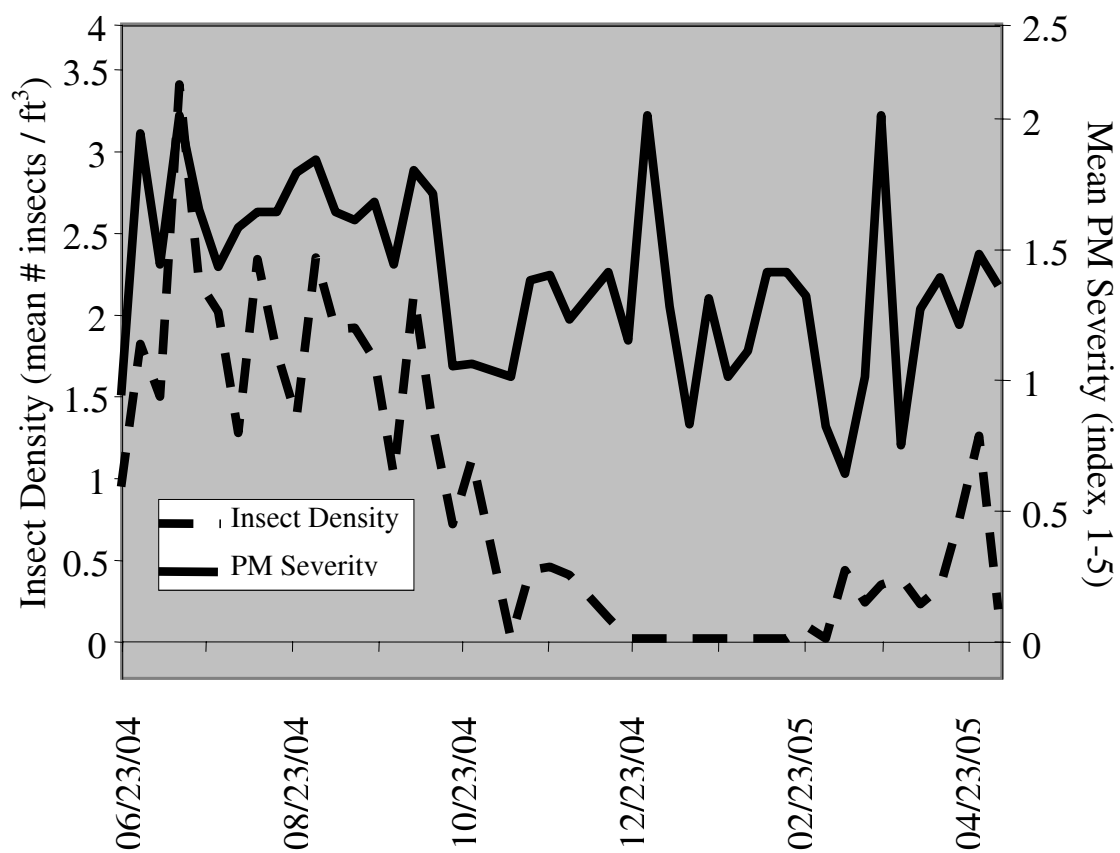


Figure 6. Seasonal density of powdery mildew (*sensu lato*) and its consumer, *Psylllobora vigintimaculata taedata*, in urban gardens of Davis, California.

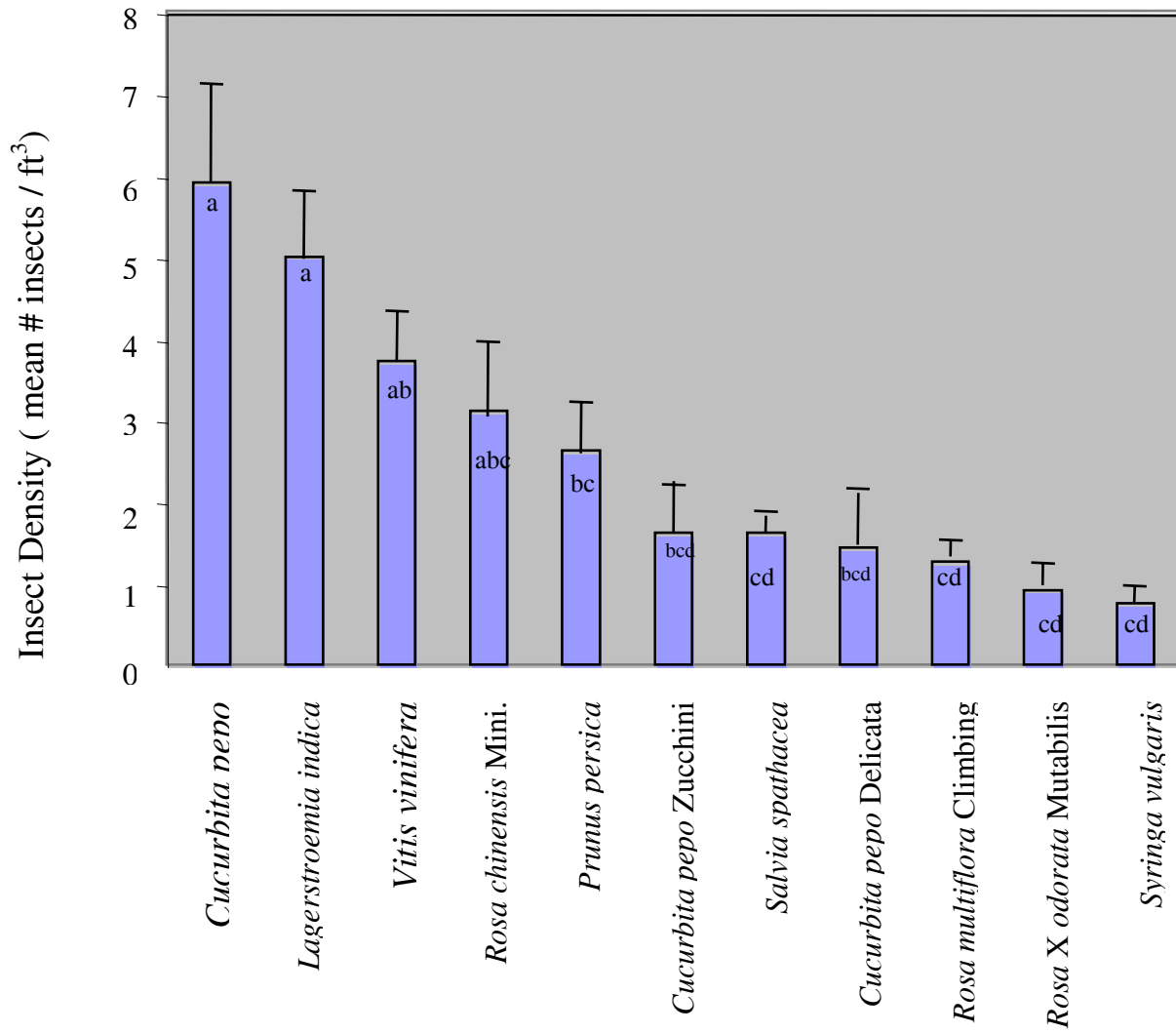


Figure 7. Mean density of *Psyllobora vigintimaculata taedata* individuals on various plant species infected with powdery mildew (sensu lato) during insect's active season. Student's t LSD mean separation,  $t = 2.91$ ,  $\alpha = 0.0038$  or  $0.05 / 13$  comparisons.



**Table 1. Generalized life cycle of *Psyllobora vigintimaculata taedata* with stadia degree-day requirements . Based on developmental threshold ( $T_0$ ) of 12.5°C and formula:  $DD = \sum(T_{\text{mean}} - T_0)_{\text{day}}$**

Life Stage	Degree-Days	Duration (days) @20°C	@25°C
Egg	59.4	7.92	4.75
1st Instar	32.4	4.32	2.59
2nd Instar	32.4	4.32	2.59
3rd Instar	27.0	3.60	2.16
4 <sup>th</sup> Instar	24.3	3.24	1.94
Pupa	59.4	7.92	4.75
<b>Total (egg-adult)</b>	<b>234.9</b>	<b>31.32</b>	<b>18.79</b>

**Table 2. Plant species on which *Psyllobora vigintimaculata taedata* was recorded feeding on powdery mildew in the landscape of Davis, California, 2004-2005**

<b>family</b>	<b>species</b>	<b>month(s) observed</b>
Asteraceae	<i>Dahlia coccinea</i>	November-December
	<i>Gerbera jamesonii</i>	April-December
	<i>Zinnia elegans</i>	June-August
Celastraceae	<i>Euonymus japonici</i>	November-December
Cucurbitaceae	<i>Cucumis sativa</i>	July-October
	<i>Cucurbita</i> spp.	July-December
Dipsacaceae	<i>Scabiosa columbaria</i>	December, April-June
Fagaceae	<i>Quercus agrifolia</i>	December
Lamiaceae	<i>Mentha spicata</i>	March
	<i>Monarda punctata</i>	November-December
	<i>Salvia spathacea</i>	June-December
Lythraceae	<i>Lagerstroemia indica</i>	April-November
Oleaceae	<i>Syringa vulgaris</i>	August-November
Plantaginaceae	<i>Plantago lanceolata</i>	July-September
Platanaceae	<i>Platanus X acerifolia</i>	August-November
Rosaceae	<i>Prunus persica</i>	June-November
	<i>Rosa</i> spp.	February-December
	<i>Spiraea douglasii</i>	September
Vitaceae	<i>Vitis californica</i>	August-October
	<i>Vitis vinifera</i>	July-October