Evaluation of *Psyllobora vigintimaculata*
(Say) (Coleoptera:Coccinellidae) for Biological Control of
Powdery Mildew Fungi (Erysiphales)

By

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I must extend my most gracious thanks to my immediate family,

without whose love and support I would not have had the

strength or courage to move cross-country and pursue a new career

in the academic world.

To my dearest Kassandra, my beloved Pandora, and the funny, furry Sammy J:

I love you with power and patience.

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Arthropod Biology

The Biology and Natural Occurrence of *Psyllobora vigintimaculata taedata* (Coleoptera: Coccinellidae), a Mycophagous Ladybird in the Urban Landscape of California

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ABSTRACT Biological control of powdery mildew (PM) plant pathogens may offer a solution to traditional agro-chemical problems such as pesticide resistance, worker safety, pesticide residues and non-target effects. However, mildew consumption by arthropod mycopredators is seldom studied and is therefore poorly understood. The coccinellid tribe Psylloborini is composed entirely of obligate consumers of various PM fungi and is represented in the western hemisphere by members of the genus *Psyllobora* Chevrolat. In western North America *Psyllobora vigintimaculata* Say is an obligate feeder of PM in natural and managed systems. Here we describe the insect's biology and degree-day phenology determined using individuals from a laboratory colony observed at constant temperature. In addition, the natural occurrence of this beetle has been documented in native and exotic landscape plants prone to PM over the course of one year through weekly presence-absence and density sampling. The insect was observed feeding on PM on more than 25 plant species in 13 different plant families. Positive correlations were found between the presence of PM in the landscape and the presence and number of *P.vigintimaculata* life stages on the afflicted plant. Additionally, positive correlations were observed between PM density and the presence and relative density of insects, suggesting an aggregative numerical response. Presence-sampling data indicates activity from late February through mid-December in Davis, California. The potential of *P. vigintimaculata* for biological control and management of PM is discussed.

KEY WORDS powdery mildew *Psyllobora vigintimaculata* biological control

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. bisoctonotata* 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^{o}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^{o} 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 chicoracearum*) 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): $DD=\sum(T_{mean}-T_0)_{day}$.

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 varietals, 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 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 ($0.03m^3$) 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 α 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 tubercules (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^{o}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^{o}C. Using this as T_0 in the equation $DD=\sum(T_{mean}-T_0)_{day}$ 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^{o}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.

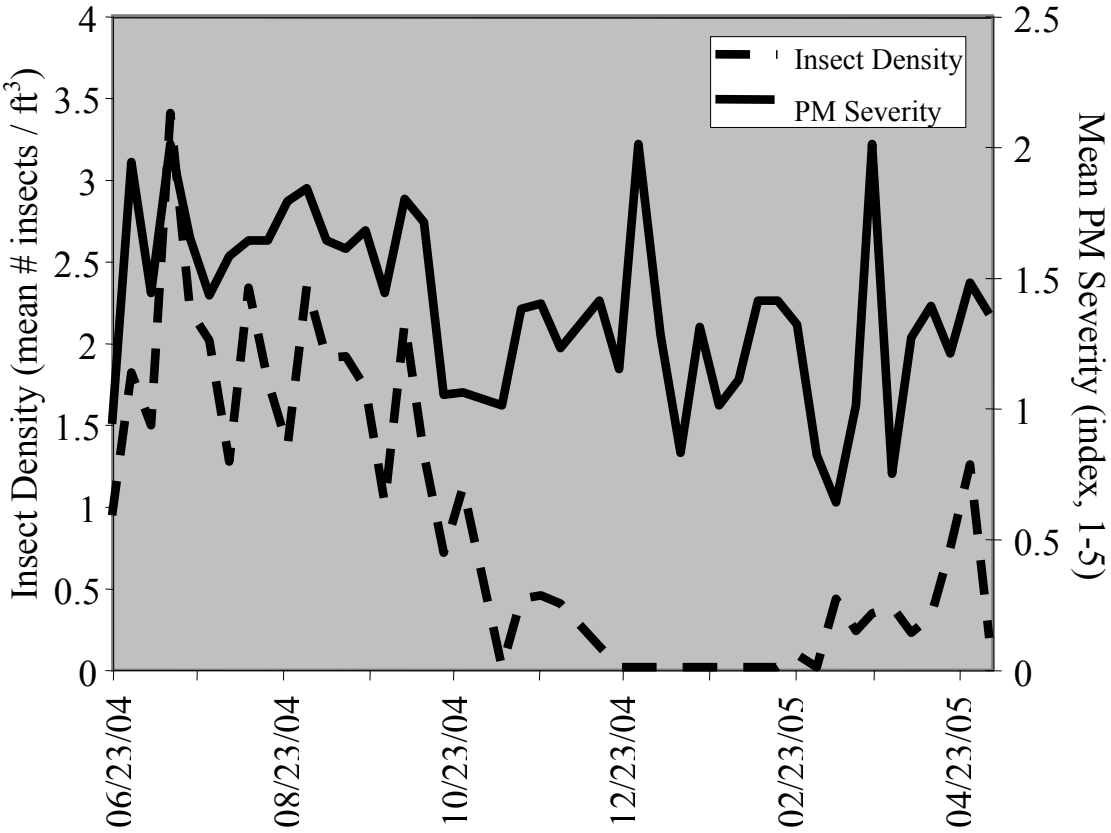

Figure 6. Seasonal density of powdery mildew (sensu latu) and its consumer, *Psyllobora vigintimaculata taedata*, in urban gardens of Davis, California.

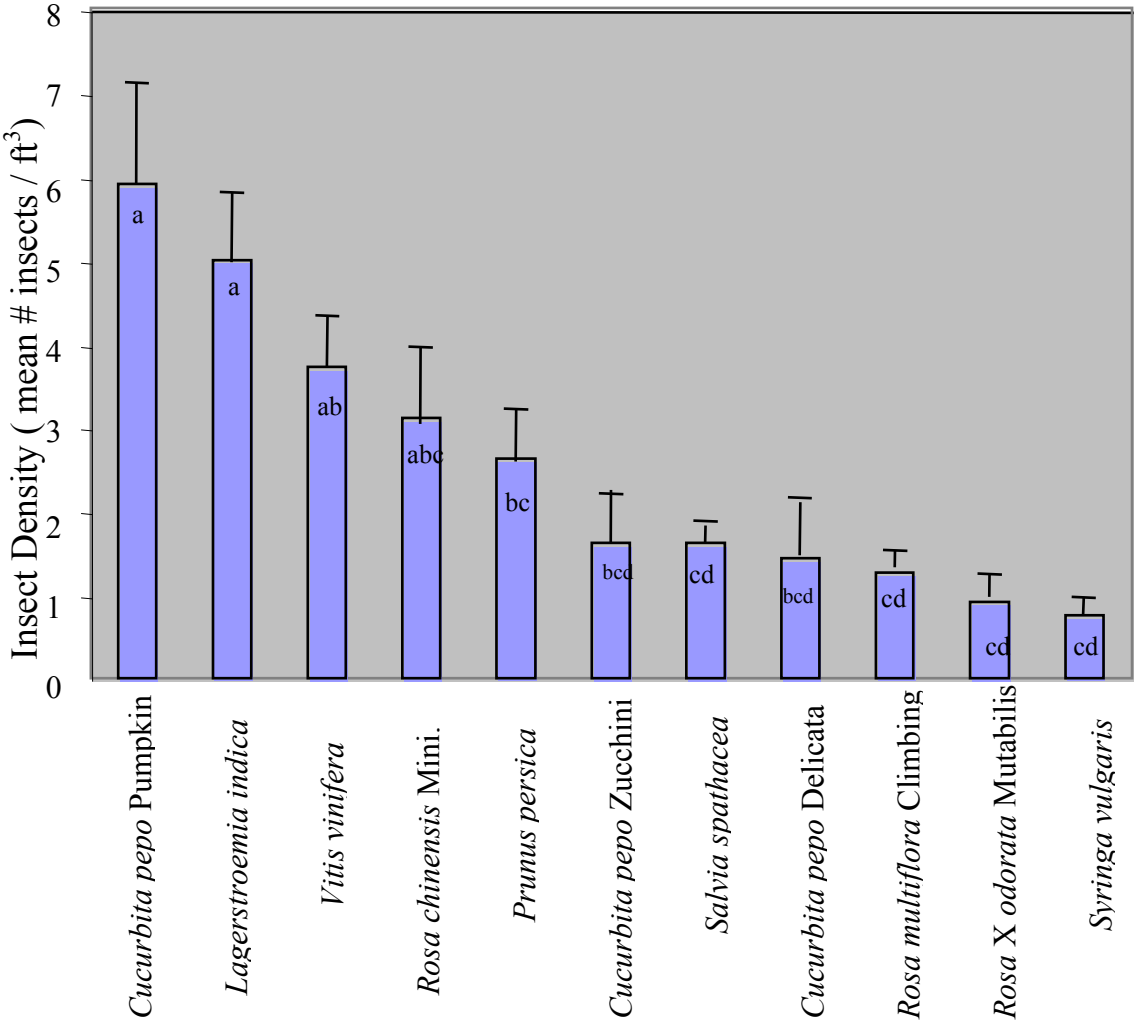

Figure 7. Mean density of *Psyllobora vigintimaculata taedata* individuals on various plant species infected with powdery mildew (sensu latu) during insect's active season. Student's t LSD mean separation, t = 2.91, $\alpha = 0.0038$ or 0.05 / 13 comparisons.

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: $DD=\sum(T_{mean}-T_0)_{day}$

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
4th Instar	24.3	3.24	1.94
Pupa	59.4	7.92	4.75
Total (egg-adult)	234.9	31.32	18.79

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

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

Effects of Selected Fungicides on a Mycophagous Coccinellid

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Abstract. The adults and larvae of the small ashy gray ladybird, *Psyllobora vigintimaculata taedata* Say (Coleoptera: Coccinellidae), are obligate consumers of the hyphae and conidia of various powdery mildew fungi. This insect is present in natural and managed systems, and has been observed to reduce mildew densities through consumption. Powdery mildew is a serious plant disease warranting chemical control in many horticultural systems. Five different commercial fungicides, including sulfur, a strobilurin, two sterol biosynthesis inhibitors and the microbial agent *Bacillus subtilis* were applied to *P. vigintimaculata* adults and second instar larvae in the laboratory to gauge contact toxicity. Sulfur was extremely toxic to adults, resulting in the death of all individuals after 24 hours. The application of the strobilurin (trifloxystrobin) and a demethylation inhibitor (myclobutanil) also resulted in significant adult mortality. Sulfur and myclobutanil caused severe contact toxicity to larvae. Piperalin, an amine fungicide, exhibited no measurable effect on adults or larvae when compared to untreated controls. This bioassay provides information on the ability to integrate chemical fungal management tactics with arthropod biological control agents.

Keywords. Powdery mildew; *Psyllobora vigintimaculata*; *Bacillus subtilis*; sulfur; myclobutanil; trifloxystrobin; piperalin; biological control; fungicide compatibility

1. Introduction

Powdery mildew (PM) (Erysiphales) is an economically and environmentally important plant disease worldwide. The disease affects all aboveground portions of plants, and can lead to yield losses (Miller et al, 2003), decreased aesthetic value and plant death. These fungi utilize plant nutrients, resulting in a disruption of respiration and a decrease in photosynthesis (Yorinori et al, 2004). Disease management typically employs fungicides. This approach can be costly and sometimes ineffective due to the development of resistance in the fungi. Powdery mildew pathogens have shown resistance to benzimidazoles, sterol inhibitors, demethylation inhibitors (DMI) and strobilurins in both laboratory and field observations (Gubler et al, 1996; del Pino et al, 1999; Heaney et al, 2000; McGrath, 2001). In order to manage this resistance phenomenon in a chemical-based control program, chemical class rotation in addition to the use of other control strategies is necessary. Biological control of PM is a largely unexplored management tactic, and may offer an alternative control strategy. Microbial agents such as the spore-forming bacterium *Bacillus subtilis* have achieved control of PM equivalent to that obtained through fungicide applications in recent greenhouse trials (Chase, 2004). Other microbial agents, such as the related *Bacillus pumilis* and the fungal hyperparasite *Ampelomyces quisqualis* Ces. also show promise as commercially available and effective PM control options (Falk et al., 1995). Additionally, arthropod agents have been considered as actual consumers of PM in novel approaches to biological control of plant pathogens. Tydeid mites (Acari: Tydeidae) associated with grapevines were observed to reduce severity of PM on wine grapes (English-Loeb et al, 1999). The Psylloborini tribe of coccinellid beetles is comprised of obligate feeders of PM hyphae

and conidia (Davidson, 1921). The cosmopolitan genus *Psyllobora* (Coleoptera: Coccinellidae) is representative of this tribe, and has been observed feeding on at least seven PM genera on many different plants as both larvae and adults. Soylu et al (2002) observed a 92% reduction in PM conidia on leaves occupied by *Psyllobora bisoctonotata* Mulsant larvae when compared to control leaves in the laboratory. The small ashy gray ladybird, *Psyllobora vigintimaculata* Say (Figure 1), is native to western North America, and common in California ecosystems. It has been recorded feeding actively on PM in greenhouses and in the urban landscape on many different plant/fungus host complexes (personal observation). Evaluation of this insect's potential for use as a biological control agent for PM, especially in controlled systems such as greenhouses, is currently underway. An understanding of the compatibility of this coccinellid with commonly used PM fungicides is essential in order to fully evaluate the role this insect may play in PM control in managed systems.

Sulfur, an elemental fungicide, insecticide and acaricide, is recognized by the USDA National Organic Program (USDA-NOP) as permissible material for plant disease management using its standards. For organic growers of wine grapes in California, where PM is a chronic problem (Delp, 1954), sulfur is one of the only registered materials available. Sulfur has been shown to have direct and indirect negative effects on local beneficial arthropods. High toxicity of sulfur residues to predatory mites (Acari: Phytoseiidae) has been documented in vineyards (Kreiter et al, 1998) and in strawberry fields (Coop and Croft, 1995), often resulting in a secondary pest outbreak of spider mites (Acari: Tetranychidae). Contact toxicity of sulfur to egg parasitoids (Hymenoptera: Mymaridae) was observed in wine grapes by Martinson et al (2001). Since *P.*

vigintimaculata is present feeding on the PM of grapevines, *Uncinula necator* (personal observation), it is interesting to consider the effect sulfur applications may have on its survival and its ability to control PM.

In systems lacking the need for organic certification there are many more options for PM control materials. Strobilurins, strong natural antibiotics that inhibit fungal respiration, are widely available as synthetic derivatives in commercial fungicide formulations. Demethylation inhibitors and amines, subgroups of sterol biosynthesis inhibitors (SBI), disrupt sterol synthesis at single biochemical sites and are commonly used as effective commercial powdery mildew fungicides. There are concerns regarding the effects these chemicals may have on natural enemies present in the crop. Investigations have shown a decrease in adults and larvae of aphidophagous coccinellids after applications of strobilurins (Michaud, 2001) for PM control. Demethylation inhibitor fungicides have elicited a toxic response in phytoseiid mites on apple trees (Raudonis et al, 2004).

The objective of this laboratory bioassay was to determine the effects of direct contact of commonly used PM fungicides on the survival of *P.vigintimaculata* adults and the successful development of larvae to the adult stage.

2. Materials and Methods

2.1 Insects

A rotating colony of *Psyllobora vigintimaculata taedata* was constantly maintained using *Erysiphe chicoracearum*, the causal agent of powdery mildew affecting Compositaceae. This fungus was used as a food source growing on either *Gerbera*

jamesonii Adlam or *Zinnia elegans* Jacquin. This colony was held at an average of 25°C using four insect cages, composed of wood and insect screening under high-pressure sodium lighting (1000 W), with each containing discrete life stages of uniformly aged individuals. Colony yield included newly emerged adults, daily deposited eggs or actively foraging larvae of uniform age every five days. A vacuum aspirator was used to remove adults. Second instar larvae were removed using a fine paintbrush. All insects were stored in a refrigerator (13°C) with excised leaves containing PM lesions for one day prior to treatment.

2.2 Experimental Units

Individual insects were placed in a petri dish (55mm X 15mm) arena containing filter paper moistened with deionized water. Insects were sprayed directly while in these arenas. After treatment each insect was moved to a clean arena and given food in the form of an excised leaf disc (10mm D) of *Gerbera jamesonii* or miniature rose leaflet containing PM. Fresh food and fresh deionized water (0.3ml) were added daily at 24-hour increments. Units were held in an incubator (Percival Scientific) at constant 25°C and average 50% relative humidity under fluorescent lights with a 14-hour photoperiod. The number of surviving insects was recorded every 24 hours until 96 hours post treatment for adults and until pupation for larvae. An initial observation of mortality was made one hour after treatment, and this was also used in analysis. Pupae were retained in the incubator to assess emergence success.

2.3 Treatments

Three commercial fungicides labeled for greenhouse use were selected: trifloxystrobin (QoI-strobilurin:oximino-acetate; Compass 0 50WDG, Olympic

Horticultural Products), piperalin (amine:piperidine; Pipron, SePro Corporation) and myclobutanil (DMI:triazole; Systhane WSP, Dow AgroSciences). Elemental sulfur in the form of wettable powder (Safer Garden Sulfur) and the microbial fungicide *Bacillus subtilis* (Rhapsody AS, Agraquest) were the treatments representing reduced risk or organic certified materials. An untreated control and a water-treated control were included as treatments. There were 22 adults and 15 larvae assigned to each treatment. Each individual represented a replication. Materials were applied directly to the insect body. An airbrush spray tower was used to administer 0.5ml of solution to each individual. Treatments were applied in their numerical treatment number order (Table 1). Between treatments 15ml of deionized water was used to triple flush the spray apparatus, and prior to the first application of each treatment 0.5ml of the pending treatment was used to flush the water.

2.4 Statistical analysis

Survival success was analyzed using the log-rank and Wilcoxon $\chi 2$ survival analysis (JMP© Start Statistics, SAS Institute, 2005) and final treatment means for percent mortality and successful pupation were compared by Student's t tests utilizing the Bonferroni Procedure for alpha manipulation, therefore reducing the probability of a Type I error from 0.05 to 0.0083 (Holm, 1979; Morikawa et al, 1996). Insects observed dead at end of observation were assigned a censor factor for survival analysis.

3. Results

3.1 Adults

Sulfur was toxic to *P.vigintimaculata* adults, resulting in 91% mortality at one-hour post treatment. All individuals treated with sulfur died within 24 hours of treatment. Trifloxystrobin and myclobutanil caused intermediate levels of mortality significantly greater than the control. Since there was no difference in response between the untreated control and the water treated control groups these treatments were pooled to form a control treatment group containing 44 individuals. Survival analysis revealed a highly significant overall mortality effect of tested materials on adult beetles (Figure 2: Log-Rank $\chi^2 = 111.05$, $p > \chi^2: < 0.0001$; Wilcoxon $\chi^2 = 108.69$, $p > \chi^2: < 0.0001$; model $\alpha = 0.05$, df = 5, n = 154). There were no significant differences between the control and *B. subtilis* or piperalin (Table 2).

3.2 Larvae

The mortality effect of sulfur was extreme, with all treated larvae dead one hour after treatment. Survival analysis showed a highly significant mortality effect of treatment materials as compared to the untreated control (Figure 3: Log-Rank $\chi^2 = 63.93$, $p > \chi^2: < 0.0001$; Wilcoxon $\chi^2 = 63.35$, $p > \chi^2: < 0.0001$). All treatments except piperalin and the untreated control exhibited some immediate mortality at the first observation event. Although no significant differences were found between the untreated control and the water control ($p=0.0563$ with $\alpha'=0.0083$), 20% mortality was observed in the water control one hour after treatment. This was attributed to a drowning effect. Therefore, the untreated control and the water control were not combined in the analysis of larval mortality, and tested materials were compared to

the water treatment when inferring toxicity. Myclobutanil was the only treatment, other than sulfur, which significantly impacted larval survival and development as compared to the water control (Table 2). Total larval mortality for individuals treated with myclobutanil, a DMI, was 80%. Some beetles initiated pupation at 96 hours post treatment, and by hour 120 all surviving individuals had formed pupae. There was no detectable effect of tested materials on emergence, as all retained insects successfully emerged as adults after 5-8 days.

4. Discussion

Myclobutanil was responsible for a measurable and significant toxic effect while piperalin, another SBI, was no different from untreated units. Both materials act on sterol biosynthesis in the target fungi, but at different sites. Sulfur is clearly identified as a toxic agent to this insect when directly applied to adults or larvae. In reduced-risk pest and disease management there is an emphasis on the retention and conservation of the native and established beneficial arthropods. We have found *Psyllobora vigintimaculata taedata* to be a common PM predator in many outdoor and greenhouse situations. Our data suggest that sulfur is not compatible with *P. vigintimaculata taedata*, while piperalin has no effect. The sulfur used in these experiments was formulated for retail homeowners for ready-to-use application and consisted of wettable powder pre-emulsified in a concentrated solution. Many commercial greenhouse operations utilize elemental sulfur as a powder that is volatilized through burning, therefore exhibiting a different leaf concentration and settling pattern than seen with sprayed materials.

Whether this method of application would increase or decrease sulfur's impact on the beetle is unknown.

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Figure 1. An adult *Psyllobora vigintimaculata* grazes on a patch of euonymus powdery mildew, *Oidium euonymi-japonici*.

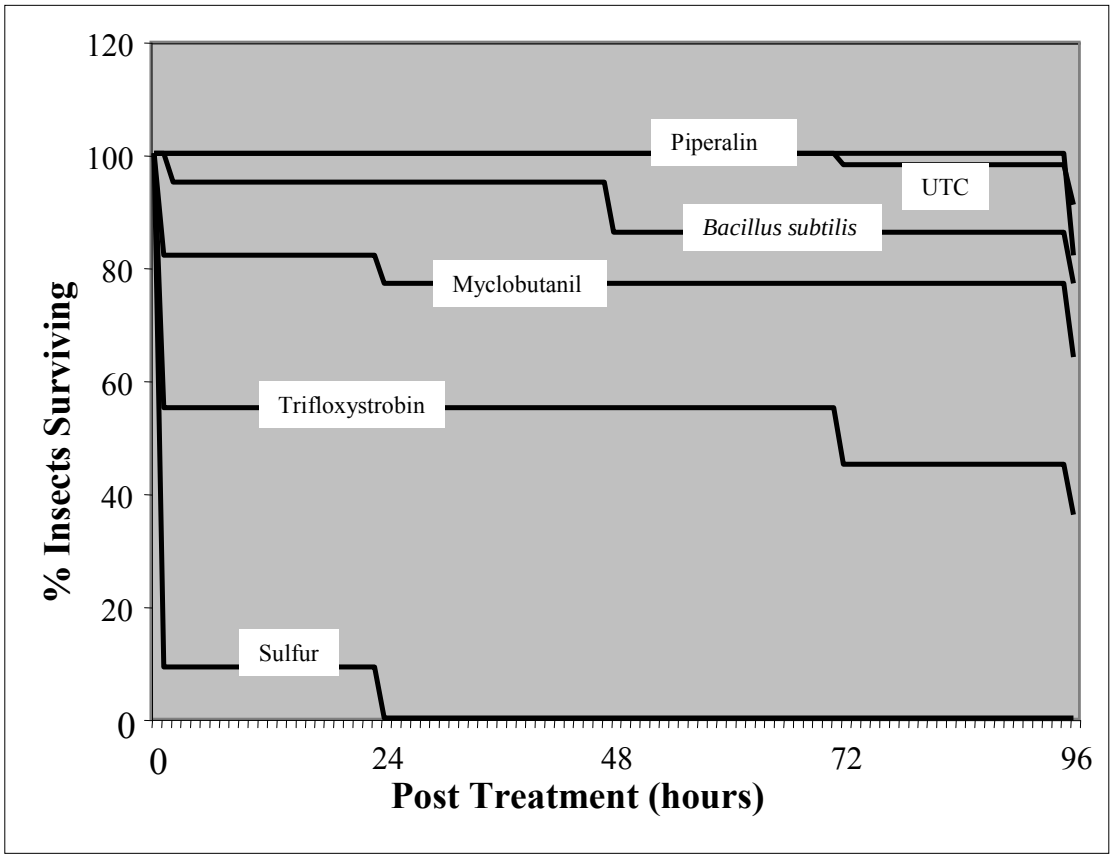

Figure 2. Survival analysis of *Psyllobora vigintimaculata* adults directly treated with selected fungicidal materials. Insects were observed for 96 hours. Log-Rank χ^2 = 111.05, p > χ^2: < 0.0001; Wilcoxon χ^2 = 108.69, p > χ^2: < 0.0001; model α = 0.05, df = 5, n = 154.

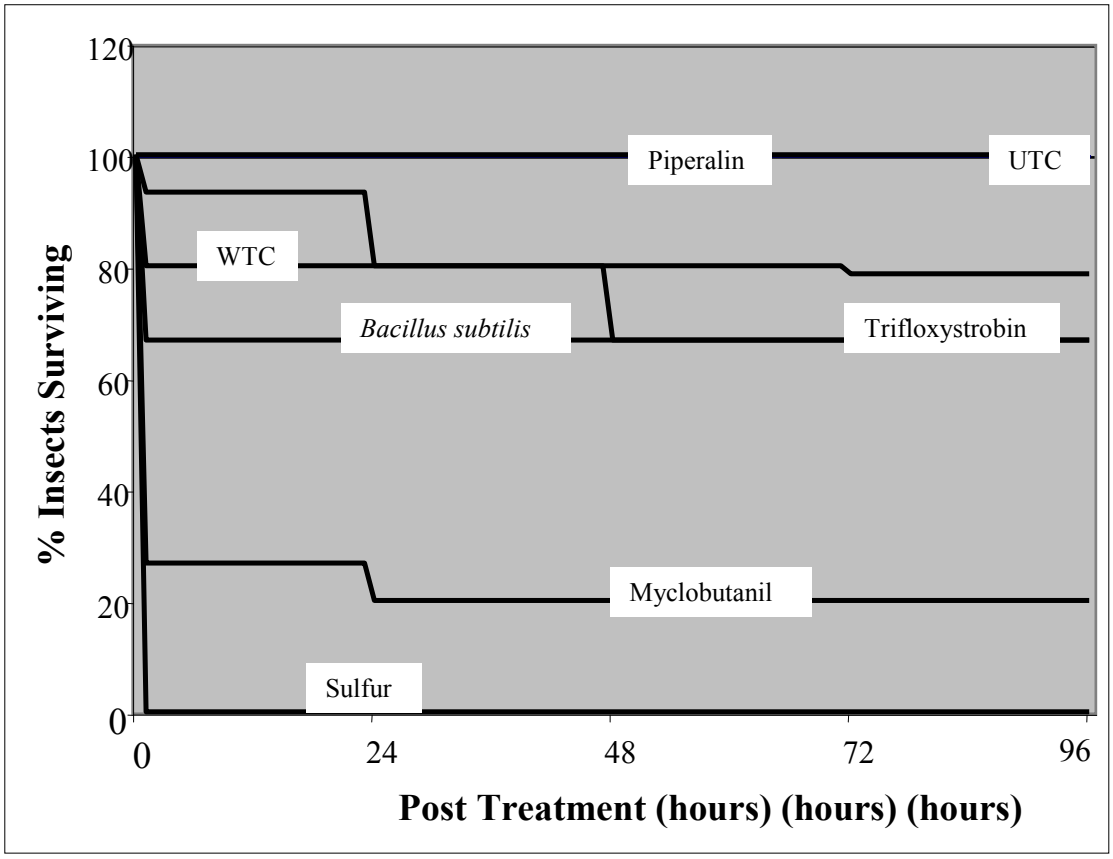

Figure 3. Survival analysis of *Psyllobora vigintimaculata* second instar larvae directly treated with selected fungicidal materials. Insects were observed for 120 hours. Pupation began at 96 hours post treatment. All pupating insects emerged successfully. Log-Rank $\chi^2 = 63.93$, $p > \chi^2$: < 0.0001; Wilcoxon $\chi^2 = 63.35$, $p > \chi^2$: < 0.0001; model $\alpha = 0.05$, df = 6, n = 105.

Table 1. Active ingredient, trade name and concentrations of topical treatments to adults and larvae of *Psyllobora vigintimaculata taedata* to gauge contact toxicity.

#	Product	Active ingredient	Concentration (mg A.I. per liter)
1	Untreated (UTC)	N/A	N/A
2	Water (WTC)	N/A	N/A
3	Rhapsody AS	*Bacillus subtilis* spores	135
4	Safer® garden Sulfur	Elemental sulfur	3800
5	Compass 050 WDG	Trifloxystrobin	70
6	Systhane WSP	Myclobutanil	116
7	Pipron	Piperalin	532

Table 2. Survival of adults and second instar larvae of *Psyllobora vigintimaculata taedata* after direct contact with selected fungicides. Means in each column followed by the same letter are not significantly different according to comparisons for each pair of means using Student's t and Bonferroni alpha adjustment procedure so that $\alpha' = \alpha / n-1$ where n is the number of comparisons and the desired α is 0.05.

treatment	mean hours ± SE survival[a] of adults after treatment	mean hours ± SE survival[b] of larvae after treatment
untreated control	95.5 ± 0.5a	120 ± 0.0a
water-treated control	-	93.0 ± 13ab
Bacillus subtilis	88.4 ± 4.3ab	80.3 ± 15b
Elemental sulfur	3.09 ± 1.4d	1.00 ± 0.0c
Myclobutanil	75.5 ± 8.3b	26.3 ± 13c
Trifloxystrobin	50.6 ± 10c	89.7 ± 12ab
Piperalin	96.0 ± 0.0a	120 ± 0.0a
t	2.61	2.69
α'	0.01	0.0083

[a] Adults were given fresh water and food daily, and observed for 96 hours total.

[b] Larvae were given fresh water and food daily, and observed for 120 hours total.