The Feasibility of Using *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae), a Mycophagous Ladybird Beetle, for Management of Powdery Mildew Fungi (Erysiphales)

By

ANDREW MASON SUTHERLAND
B.S., University of Florida, 2001
M.S., University of California, Davis, 2005

DISSERTATION
Submitted in partial satisfaction of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in
Entomology
in the
OFFICE OF GRADUATE STUDIES
of the
UNIVERSITY OF CALIFORNIA, DAVIS

Approved:

_____
Walter D. Gubler

_____
Frank G. Zalom

_____
Michael P. Parrella

Committee in Charge
2009

Andrew Mason Sutherland

June 2009

Entomology

The Feasibility of Using *Psyllobora vigintimaculata* (Coleopotera: Coccinellidae), a Mycophagous Ladybird Beetle, for Management of Powdery Mildew Fungi (Erysiphales)

Abstract

Obligate mycophagy on the hyphae and conidia of powdery mildew (PM) fungi (Erysiphales), as exhibited by the beetle tribe Halyziini (Coleoptera: Coccinellidae), may be utilized through augmentative biological control and/or decision support for disease management. Mycophagous coccinellids, a previously understudied group, are cosmopolitan and ubiquitous worldwide in regions where PM fungi are prevalent. Based on biological records, the host range of Halyziini is estimated to be wide, perhaps encompassing all PM genera. A tabular list of these host complexes is provided.

Quantification of the PM consumption rate and feeding capacity of *Psyllobora vigintimaculata*, a western North American species within Halyziini, was attempted through laboratory measurement, linear modeling, and extrapolation via population simulation modeling. The feeding capacity of larvae was measured by means of digital photography and image analysis. These larval consumption data were then used to construct a vector with age-specific feeding capacities. Population development of a hypothetical cohort was predicted using a Leslie matrix and previously established biological parameters. These models were combined to create a release-rate calculator

for a system with known leaf area and PM severity. Model output was evaluated through a comparison with observed data resulting from a caged feeding efficacy trial.

Adult *P. vigintimaculata* have been recorded to respond in an aggregative manner to increasing levels of PM severity. This phenomenon, coupled with the observation that adults also are locally attracted to yellow sticky cards, led to the hypothesis that local insect density could serve as an indicator of PM and as a decision support device for PM management. This positive correlative relationship between insect density and disease severity was confirmed through three successive years of monitoring in a commercial vineyard setting. In some cases, beetle catches were more sensitive to annual anomalies in disease phenology than was an established predictive model based on weather data.

Mechanical transmission of PM conidia by movement of spore-laden insects such as mycophagous coccinellids may represent an important means of new infection centers, possibly compromising the service of PM removal by these potential biological control agents. A series of growth chamber experiments were instituted in order to test the hypothesis that *P. vigintimaculata* contributes to disease development and severity via mechanical transmission. There was no difference in PM transmission rate or severity over time when uninfected plants were exposed to infected plants either with or without the presence of a small population of *P. vigintimaculata*.

ACKNOWLEDGEMENTS AND DEDICATION

Many mentors, colleagues, funding agencies, family members and friends have come together to help me through this process of extended education. Graduate school is said to be a journey where one's travels are more important than the destination. The most valuable information gleaned in such an endeavor is, in this case, the experience of the logistics and methodology of science. For such a journey, many guides are necessary.

I wholeheartedly thank my major professor, Michael P. Parrella, for all his straightforward advice and suggestion over the years. I have always respected the frank and candid way in which he conducts his academic program and his extension of scientific ideas. He has been patient and forgiving while expecting timely, quality work.

I have also been blessed with many wise and compassionate mentors during my time at UC Davis. My dissertation committee members Frank G. Zalom and W. Doug Gubler have provided me with valuable inquisition and constructive criticism during key moments in my graduate career such as thesis proposal and dissertation construction. Professors Les Ehler, Peter Cranston, Penny Gullan, Rick Karban and Jay Rosenheim have all introduced me to new concepts and new ways of seeing the entomological world. I also want to thank Harry Kaya for his advice on the construction of a review article. Thanks to Alison Berry for my newfound love of teaching. I have also received much help in the areas of experimental design and the scientific method from Roy Kaspi.

My fellow graduate students have served me well as confidantes and colleagues over the years. Many discussions over lunch or a beer have led to major improvements in

my objectives and hypotheses. In this respect, special thanks to Dominic Reisig, Hillary Thomas, Ian Pearse, Michael Branstetter, and Sara Diamond. Group study sessions helped in my preparation for the Departmental Exam and the Linnaean games.

Over the years, I have received funding from various donors, and I would like to acknowledge the collective facilitation of my degree through these sources. Thanks to the UC Davis Entomology Department, the UC Davis Environmental Horticulture Department, Elvenia K. Slosson Endowment Fund, Jastro-Shields Graduate Research Award, Jack Pickett Agricultural Scholarship, Association of Natural Biocontrol Producers, the UC Statewide IPM program, and the Entomological Society of America.

In my humble opinion, emotional support and camaraderie are absolutely necessary in order to retain sanity during completion of a graduate degree. Money and time were short, but thanks to my family and friends, I survived. Kassandra helped me decide that graduate school was a good decision in the first place, and she embarked on an adventure with me to settle into a new place far away. Without her ambition and zeal for life I would not be writing this now. She also taught me that life and love can never be taken for granted. This dissertation is dedicated to Pandora, who has given me almost daily laughter and taught me patience. My immediate family in Florida has succeeded in being a driving force in my success and the reason for some great visits back and forth between the two coasts. To all my smart, crazy and oft debaucherous friends in and around Davis; Rob, John, Noel, Brad, Megan, Jessie, Butch, Brent, Paul, please don't stop the music. To Julia, you've given me youth, vitality and the promise of new love.

TABLE OF CONTENTS

TITLE PAGE……i

ABSTRACT……ii

COPYRIGHT……iv

ACKNOWLEDGEMENTS AND DEDICATION……v

TABLE OF CONTENTS……..vii

CHAPTER 1: **Mycophagy in Coccinellidae: Implications for Biological Control**

Title page……1

Abstract……2

Introduction……..3

Origin of mycophagy in coccinellids……...3

Facultative and obligate mycophagy in coccinellids……...4

Brief taxonomic history of the Halyziini (= Psylloborini)……...7

Biology and ecology of Halyziini……9

Biological control and the possibility for integrated disease management (IDM)……12

Literature Cited……..15

Figures……29

Tables……32

CHAPTER 2: **Biological Control through Powdery Mildew Consumption**

Title page……...38

Abstract…..…….39

Introduction and Historical Review……...40

Materials and Methods: laboratory-based linear consumption model……...43

Materials and Methods: population-based simulation model……46

Results: laboratory-based linear consumption model……49

Results: population-based simulation model…….51

Discussion……..51

Literature Cited……..54

Figures……57

Tables…….61

CHAPTER 3: **Indication for and Decision Support for PM Management in Vineyards**

Title page……...64

Abstract……..65

Introduction……66

Materials and methods……...69

Results……71

Discussion……..73

Literature cited……...76

Figures……79

CHAPTER 4: **Evaluation of the possibility for mechanical transmission of powdery mildew conidia by the obligate mycophage *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae)**

Title page……...84

Abstract……..85

Introduction……86

Materials and methods……...89

Results and discussion……...91

Literature cited……...93

Figures……98

CHAPTER 1

Mycophagy in Coccinellidae: Implications for Biological Control

(partially published and extracted from: Sutherland, A.M., Parrella, M.P., 2009. Mycophagy in Coccinellidae: A Review and Synthesis. Biological Control, in press.)

Abstract. Mycophagy, though often overlooked, represents an interesting and unique ecological niche within the Coccinellidae. Facultative mycophagy has been reported from the aphidophagous Coccinellini and the polyphagous Tytthaspidini. Members of Halyziini, a cosmopolitan tribe of the Coccinellinae, are obligate mycophages specializing on the powdery mildew fungi of Erysiphales, a ubiquitous order infecting almost 10,000 angiosperm plants worldwide. Various researchers have recorded this mycophagous habit during the past 150 years, resulting in a large list of host-powdery mildew complexes around the world harboring these insects. Members of the Halyziini possess several attributes conducive to biological control, including host specificity (obligation), widespread native distribution, and strong aggregative response to host density.

Keywords. *Psyllobora*, *Halyzia*, *Illeis*, *Macroilleis*, *Vibidia*, mycophagy, powdery mildew, Erysiphales, plant pathogen, biological control, integrated disease management

Introduction

Although the overwhelming majority are predators of other arthropods, the Coccinellidae are not all purely entomophagous insects (Giorgi et al., 2009; Lundgren, 2009b). Phytophagy within the Epilachninae and mycophagy (both facultative and obligative) within the Coccinellinae have evolved from a common coccidophagous ancestor (Giorgi et al., 2009) that, in turn, may have been derived from an ancient mycophagous group, the Cerylonid series, from which all coccinellids are descended (Sasaji, 1968; Leschen, 2000; Giorgi et al., 2009). Phytophagous ladybirds (Giorgi et al., 2009; Weber and Lundgren, 2009) are generally regarded as pests, but the beneficial or detrimental economic position of the mycophagous Coccinellinae is less clear. This chapter will review some of the important historical literature associated with mycophagous coccinellids, concentrating primarily on obligate mycophages. In addition, we will discuss the taxonomy, biology, ecology, and possible utility of this clade of ladybird beetles as biological control agents.

Origin of mycophagy in coccinellids

Coccinellids belong to the cerylonid series of Cucujoidea, and based on current phylogenetic data may be a sister taxon to Alexiidae or Endomychidae (Slipinski and Pakaluk, 1991; Giorgi et al., 2009). Most members of this series are mycophagous. However, the vast majority of the Coccinellidae are predators on sternorrhynchan insects, and Giorgi et al. (2009) conclude that basal Coccinellidae were coccidophagous. Leschen (2000) and several others (Lawrence and Hlavac, 1979; Crowson, 1981; Thomas, 1993) suggest that honeydew production by the commonly sternorrhyncan prey

of this family may have been the ecological opportunity for evolution of predatory habits. Honeydew, a digestive by-product composed of carbohydrates and proteins, often accumulates on plant substrates where hemipteran insects feed and supports the growth of a specific group of Ascomycete fungi commonly known as sooty molds. Leschen (2000) proposed a simple model whereby ancestral mycophagous beetles first accepted sooty molds as food items, then specialized as sooty mold consumers, and finally accepted the insects indirectly producing the mold as food items. This idea is strengthened by the fact that many predators of Hemiptera, including many coccinellids, also feed on honeydew and sooty mold to this day (Majerus, 1994; Lundgren, 2009a; Lundgren, 2009b).

Facultative and obligate mycophagy in coccinellids

Within the Coccinellidae, mycophagy can be viewed as a derived condition, and it has only been reported from the Coccinellinae (Giorgi et al., 2009). A molecular phylogenetic analysis by Giorgi et al. (2009) suggests that the Halyziini arose within the generally aphidophagous tribe Coccinellini. They conclude that, in spite of distinctive mandible shape, presumably related to mycophagy, both Halyziini and the poorly-known Tytthaspidini (see below) have distinctive features which provide further evidence for a derived condition.

Facultative mycophagy may be commonplace in the largely aphidophagous tribe Coccinellini Weise (Majerus, 1994). These predators are often polyphagous, feeding on pollen, nectar, honeydew, fungi, fruit and foliage, but specific animal foods (e.g. aphids) are necessary to complete development (Hodek, 1973; Lundgren, 2009b). This distinction between “essential” and “alternative” foods (Hodek, 1973) is important when

discussing the polyphagy of the tribe. Additionally, at least some members of the tribe do not have a mandatory minimum level of predation: *Coleomegilla* Timberlake and its allies can complete development on pollen alone (Lundgren and Wiedenmann, 2004; Michaud and Grant, 2005). Facultative mycophagy, or mixed feeding on pollen, mildews (Erysiphales) and aphids has been reported in *Rhyzobius litura* (F.) (Ricci, 1986) and *Propylea quatrodecimpunctata* (L.) (Turian, 1971; Hukusima and Itoh, 1976). Upon finding fungal spores in the gut of Coccinellini, many researchers have suggested incidental or accidental consumption of sooty mold fungi during honeydew grazing (Zoebelein, 1956; Putman, 1964; Carter and Dixon, 1984). However, Triltsch (1999) found *Alternaria* Nees conidia and *Puccinia* Persoon uredospores more frequently than aphids in the gut of *Coccinella septempunctata*. These fungi are plant pathogens, and since the spores were found in both the presence and absence of aphids, it is likely that they represent an important seasonal food for the aphid predator.

Also within the Coccinellinae there is a poorly-known group of polyphagous coccinellids that regularly include fungi in their diets along with pollen, arthropods and possibly some plants (Hodek and Honek, 1996; Samways et al., 1997; Lundgren, 2009a). These interesting mycophilous polyphages, closely allied to the Coccinellini, have recently been deemed a separate tribe, the Tytthaspidini (Fursch, 1996; Kovar, 1996), containing three genera; *Tytthaspis* Crotch, *Bulaea* Mulsant and *Isora* Mulsant. Some authors, while recognizing their polyphagous habit, place these genera within Coccinellini (Hodek and Honek, 1996; Kuznetsov, 1997). Many times, however, they have escaped consideration during systematic treatment of the Coccinellidae, perhaps due to geographic obscurity or a dearth of specimens, and so are absent from phylogenies

(Sasaji, 1968; Vandenberg, 2002). Ricci (1982) uncovered fungal spores of *Alternaria* and *Cladosporium* Link ex Fries in the gut contents of *Tytthaspis sedecimpunctata* (L.) along with pollen, Acari and Thysanoptera remains. Mixed feeding in the same species on pollen, mildew and aphids was documented by Ricci et al. (1983). Turian (1969) also observed *Tytthaspis* feeding on Erysiphales and termed the behavior "micromycetophagy".

The cosmopolitan tribe Halyziini Mulsant (=Psylloborini, see Pakaluk et al., 1994) is comprised entirely of mycophages (Gordon, 1985), although some workers have reported aphidophagy (Schilder and Schilder, 1928; Borner and Heinze, 1957; Fulmek, 1957; Omkar and Pervez, 1999) or phytophagy (herbivory on higher plants) (Yurtsever, 2001). Davidson (1921) performed a series of simple no-choice feeding experiments with a variety of food items to establish *Psyllobora vigintimaculata* (Say) (Figure 1) as an obligate mycophage. Members of Halyziini feed on powdery mildew (PM) fungi (Ascomycotina: Erysiphales), a ubiquitous and diverse group of obligate plant parasites known to infect 9838 species of mostly dicotyledonous angiosperm plants worldwide in both natural and managed systems (Amano, 1986). Despite the wide host range of the order, individual species or biotypes within Erysiphales tend to be quite host-specific, often infecting only one species or genus of plant (Amano, 1986). Thus, the evolution of PM has closely followed the evolution of their hosts (Takamatsu, 2004). Similar environmental conditions are required for all PM to infect and develop, and unlike many other plant pathogenic fungi, spores can germinate and infect hosts under very low atmospheric humidity (Takamatsu, 2004). Positive osmotic potential is detrimental to the thin-membraned spores. Free water as overhead irrigation has been proposed as a control

measure (Sivapalan, 1993; Liu, 2001; Korner and Challa, 2003). Different PM fungi often infect many unrelated plants in an ecosystem simultaneously when conditions are favorable for PM germination and development. The ability of the Halyziini to feed on other fungi has not been reported in the literature. Other lower fungi including yeast (Saccharomycetales) and rust fungi (Uredinales) were refused in simple laboratory no-choice trials with *Psyllobora vigintimaculata* (Sutherland and Parrella, unpublished). We suspect that PM fungi are common and abundant enough worldwide for this group of beetles to maintain a relatively specialized diet in many different climates and ecosystems.

The specialized feeding exhibited by the Halyziini and Tytthaspidini is apparently facilitated by unique mandibular morphology. The typical bifed mandibular apex of all Coccinellinae is modified in the Halyziini such that the ventral tooth is further divided into a row of additional teeth (Samways et al., 1997). Furthermore, the inner mandibular cutting edge of Coccinellini is smooth, while in the fungal-feeding tribes it is covered in minute teeth, forming a comb. These structures are presumed to help the insects to rake fungal spores from conidial towers and spore-laden hyphae growing on leaf surfaces (Ricci, 1982; Lawrence, 1989; Samways et al., 1997). In the polyphagous Tytthaspidini these comb or rake-like structures may also serve as tools for removing individual pollen grains, and fungal spores may be an alternative or incidental food source. The specialized mycophages within Halyziini will be emphasized in the ecological and biological discussions of mycophagy in Coccinellidae below.

Brief taxonomic history of the Halyziini (= Psylloborini)

The obligate mycophages of the Coccinellidae are so similar morphologically to the other members of the Coccinellinae that they have often been overlooked as a distinct group. There are questions over whether the Halyziini is a distinct clade deserving of tribal status, since it is nested within the predatory Coccinellini (Giorgi et al., 2009). Mulsant (1850) studied the paraphyletic tribe Trimere, raised by Dejean (1837) and containing 22 coccinellid genera, and proposed the branch Halyziares which included the genera *Psyllobora* Dejean, *Halyzia* Mulsant, *Vibidia* Mulsant, *Thea* Mulsant (= *Psyllobora* Chevrolat in Dejean, 1837), *Illeis* Mulsant and *Propylaea* Mulsant. This represented the first attempt to taxonomically segregate mycophagy in the family. Chapuis (1876), however, considered *Psyllobora* to be a subgenus of H*alyzia* within the group Coccinellites, in turn nested within the Coccinellides Aphidophages. A major revision of the taxonomy of North American coccinellids, published in 1899 (Casey), organized the family into 16 tribes, one of which was Psylloborini. Korschefsky (1932), Sasaji (1968), Kovar (1996) and Kuznetsov (1997) have all retained the tribal name and nested it within the subfamily Coccinellinae. Twelve genera were identified by Kuznetsov (1997) within the tribe, including *Cleobora* (Mulsant), *Eothea* Iablokoff-Khnzorian, *Halyzia*, *Illeis*, *Macroilleis* Miyatake, *Metamyrrha* Capra, *Microneda* Crotch, *Neohalyzia* Crotch, *Oxytella* Weise, *Protothea* Weise, *Psyllobora* and *Vibidia*. The taxon Halyziini (from Halyziares Mulsant) was resurrected by Pakaluk et al. (1994) and recently adopted in Vandenberg's (2002) phylogeny of the family along with Coccinellini under Coccinellinae. The division between Coccinellini and Halyziini is sometimes vague, as evident by Pope's (1988) consideration that *Illeis* resides within

Coccinellini and by the recent movement of *Protothea* into Coccinellini (Poorani and Slipinski, 2005).

Biology and ecology of Halyziini

Halyziini is a truly cosmopolitan taxon. It seems that any locale in which there are plant-parasitic PM fungi also contains mycophagous coccinellids to consume them. The most widespread genus, *Psyllobora* (= *Thea*), is found in Europe, the Americas, Asia and Africa. A second geographically extensive genus, *Illeis* (= *Leptothea*), is found in Asia, Australia and Japan. Three other genera; *Halyzia*, *Vibidia* and *Macroilleis*, are Palearctic and Indomalayan in distribution. Given this wide tribal distribution, together with the obligation to feed on highly visible and important plant parasites, it is difficult to understand how these insects could remain understudied. The biology and ecology of this tribe were established by a series of historical observations, which are summarized in Table 1.

In 1874 the German taxonomist Kaltenbach noted that *Psyllobora vigintiduopunctata* (L.) was found on *Astragalus* L. leaves covered with the PM fungus *Erysiphe holosericea* (Wallroth) Link, yet he believed the beetles to be feeding on mites amongst the mildew, and so reported no mycophagy. Albert Koebele recorded mycophagy by *Illeis galbula* (Mulsant) while in Australia as early as 1893 (Timberlake, 1943). Prior to this, members of Halyziini had been regarded as aphidophagous (Chapuis, 1876). In Europe, Weise (1900), Martelli (1910; 1914) and Lichteinstein (1917) observed *Psyllobora vigintiduopunctata*, *Vibidia duodecimguttata* (Poda), and *Halyzia sedecimguttata* (L.), respectively, all to have mycophagous habits involving PM

fungi. In the United States *Psyllobora vigintimaculata* was commonly associated with rose and apple PM, *Sphaerotheca pannosa* (Wallroth) Léveillé and *Podosphaera oxyacanthae* (de Candolle) de Bary, respectively, and was reared in the lab for biological observation and "essential" host determination (Davidson, 1921). Davidson (1921) predicted up to five generations a year in California's Central Valley based on phenological observations and described a typical coccinellid life cycle; with elongate, oval eggs deposited on PM-infected plant parts, four stadia, a pupa, and a preovipositional period leading up to reproductive adulthood. Later life cycle studies with members of the Halyziini yielded results consistent with Davidson's (Liu, 1951; Almeida and Milleo, 1998; Sutherland, 2005; Cividanes et al., 2007), but Dharpur et al. (1990) indicated that *Illeis cincta* (F.) had five stadia in India. Perhaps most interesting were Davidson's laboratory feeding experiments: in a series of experiments, groups of newly hatched or PM-fed larvae were offered various arthropod prey, including aphids (*Chromaphis juglandicola* Kaltenbach, *Macrosiphum rosae* L., *Aphis gossypii* Glover, *Myzus persicae* Sulzer), spider mites (*Tetranychus* Dufour), coccids (*Saissetia oleae* (Olivier)), and diaspidids with "armor" removed (*Aspidiotus* Bouche). These offerings always resulted in dead, starved larvae while cohort larvae fed on rose PM developed and pupated. Adult beetles offered these prey items also refused them, and lived much longer than larvae, but also eventually succumbed to starvation. In Brazil, where the genus *Psyllobora* is represented by 17 species (Almeida, 1985), both *Psyllobora hybrida* Mulsant and *Psyllobora confluens* (F.) were recorded feeding on *Microsphaera caricae* (Maublanc) Hansford, a PM infecting castor bean, *Ricinus* L. (Lima, 1931). In China the food of *Halyzia hauseri* (Mader), *Halyzia sanscrita* (Mulsant) and *Illeis cincta* was

determined to be apple PM, *Podosphaera leucotricha* (Ellis & Everhart) E.S. Salmon, and PM consumption was quantified (Liu, 1951). Over the past 20 years, publications from around the world (Table 1) have provided data on halyziine biology as well as information regarding their biological control potential; Brazil (Almeida, 1985; Almeida and Milleo, 1998; Cividanes et al., 2007), China (Wu and Guo, 1987), India (Prasad and Rai, 1988; Dharpur et al., 1990; Krishnakumar and Maheswari, 2004), Cuba (Cruz et al., 1989), Italy (Ratti, 1996), Argentina (Bado and Rodriguez, 1998), Japan (Takeuchi et al., 2000), Turkey (Soylu and Yigit, 2002), Syria (Ahmad et al., 2003) and the United States (Sutherland, 2005; Sutherland and Parrella, 2006; Sutherland and Parrella, 2009).

Anderson (1982) tracked the seasonal habitat utilization of *Illeis galbula* near Sydney, Australia and found that the insect used one PM complex extensively (*Oidium* Saccardo on *Lonicera fragrantissima* Lindley & Paxton) during breeding, another (PM on *Senna pendula* [Willdenow] =*Cassia coluteodes*) sporadically, and an evergreen tree, *Ficus rubiginosa* Desfontaines ex Ventenat, as a protective overwintering site. Anderson (1982) found quantities of red *Ficus rubiginosa* trichomes in the insect's gut along with large air bubbles during winter. The author suggested that the trichomes could have been ingested accidentally along with latex, honeydew or water consumed at the overwintering site. The seasonal occurrence of the Japanese species *Illeis koebelei* Timberlake is thought to be synchronized with the abundance of essential fungi (Takeuchi et al., 2000), and the authors recorded the beetle's feeding on 11 PM species, documenting seasonal changes in host use and breeding complexes. A similar situation was observed in *Psyllobora vigintimaculata* in California (Sutherland, 2005), with natural populations shifting to different PM-complexes throughout the year based on PM availability. When

reviewing the literature on the relationships between halyziine species and PM, it appears that they are quite general in their acceptance of most PM fungi species as food (Table 2). Ahmad et al. (2003) in Syria and Turkey and Sutherland (2005) in California recorded 57 and 26 plant species, respectively, that served as hosts for PM fungi consumed by *Psyllobora*. However, there may be preferences or restrictions in host range for some species. For example, the PM genera *Uncinula* Léveillé and *Uncinuliella* Zheng & Chen (both now known as *Erysiphe* sect. *Uncinula*; Braun et al., 2002), and *Erysiphe* R. Hedwig ex DeCandolle were never associated with *Illeis koebelei* in field observations made by Takeuchi et al. (2000). However, larvae were later successfully reared on a diet of *Erysiphe kusanoi* (Sydow & P. Sydow) Braun & Takam (=*Uncinula kusanoi* Sydow & P. Sydow) in the laboratory. Sutherland (2005) found no *Psyllobora vigintimaculata* on severely PM-infected *Euonymus japonica* Thunberg and *Eschscholtzia californica* Chamisso throughout the year, and attributed this to differences in plant species rather than PM genera (*Oidium* and *Erysiphe*, respectively). Ratti (1996) reported *Psyllobora vigintiduopunctata* to feed and reproduce on *Oidium*-infected *Euonymus japonica* in Italy, but the same PM complex was conspicuously devoid of *Psyllobora* in California. Clearly the host ranges of these beetles is not completely known, and may specifically depend on the taxon, the geographic location, the host plant species, the PM species, and the other PM complexes available in local space and time.

Biological control and the possibility for integrated disease management (IDM)

The PM pathogens (Erysiphales) are collectively considered one of the most important plant pathogens worldwide since many of their hosts are valued as agricultural

and ornamental plants. Conventional management of PM employs regular applications of chemical fungicides. This approach can be costly and sometimes ineffective due to the development of resistance in the fungi (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 fungicide-related issues such as residues in food crops, effects on nontarget organisms, impacts on farm worker health and safety, etc. Control of PM using commercially-available microbial controls, equivalent to that obtained through chemical fungicide applications, has been found with the spore-forming bacterium *Bacillus subtilis* (Ehrenberg) Cohn (Bacillales: Bacillaceae) and the pycnidial fungal hyperparasite *Ampelomyces quisqualis* Cesati (not currently assigned to order or family) (Chase, 2004; Falk et al., 1995). Interest in the development of *Pseudozyma flocculosa* (Traquair, Shaw & Jarvis) Boekhout & Traquair (Ustilaginales: Ustilaginaceae) as a biofungicide has been prompted by results against the PM *Sphaerotheca fuliginea* (Schlechtendal) Pollacci (Traquair et al., 1988; Paulitz and Belanger, 2001).

Little is known of the potential for arthropods to control or reduce PM through consumption. The biology of PM fungi is unique: fungal growth is exposed on the leaf surface as a hyphal mat and only the haustorium, a structure used for nutrient acquisition, is found to penetrate the host's cuticle in most species (Takamatsu, 2004). This may allow arthropod biological control to be a viable option. However, it is unknown whether PM colonies can recover from complete removal of the hyphal mat through growth from haustoria. Work by English-Loeb et al. (1999) in upstate New York demonstrated that the tydeid mite *Orthotydeus lambi* (Baker) (Acari: Tydeidae) reduced the incidence of PM in riparian grapevines, *Vitis riparia* Michx. Abundance of these mites is thought to

be mediated by the host plant through acarodomatia (tufts of hair or invaginations on the abaxial leaf surfaces) which offer protection and a favorable microclimate for the mites (Norton et al., 2001). Larger arthropods able to consume greater amounts of PM, such as the coccinellid members of Halyziini, may offer superior PM removal and suppression. The Halyziini possess several characteristics deemed necessary by Solomon (1949) for successful biological control. The widespread distribution of the tribe ensures that most locations with PM problems already have a mycophagous species present, so that conserving and/or augmenting populations already in place may be all that is needed. Regular field observations (Sutherland and Parrella, 2009) have revealed that adult *Psyllobora vigintimaculata* are able to locate isolated, low-density PM infections in a large and heterogeneous landscape. This suggests that beetles can detect and respond to cues resulting from PM infection. Also, *Psyllobora* species have an observed tendency to aggregate on plant parts most heavily infected with PM (Dharpur et al., 1990; Yurtsever, 2001; Sutherland and Parrella, 2009) and consume as a group (Figure 3).

Biological control of a plant pathogen through consumption by an arthropod may be difficult. Many bacteria and fungi, including PM, have periods of intense asexual sporulation in which the infective population grows geometrically. Insects' generational time requirements may be several orders of magnitude longer than these microbes. Nevertheless, a handful of workers have gone beyond observation to speculate on the possible utility of these beetles for biological control of PM (Liu, 1951; Wu and Guo, 1987; Cruz et al., 1989; Dharpur et al., 1990; Soylu and Yigit, 2002; Krishnakumar and Maheswari, 2004; Sutherland and Parrella, 2006).

Literature Cited

Ahmad, M., Younis, G., Ali, N., 2003. Biology of the coccinellid *Psyllobora (Thea) bisoctonotata* Muls. (Coleoptera: Coccinellidae) a predator of powdery mildew fungi. Proceedings of the Eighth Arab Congress of Plant Protection, Elbeida City, Libya.

Almeida, L.M., 1985. Study of 17 species of the genus *Psyllobora* Chevrolat, 1837 (Coleoptera: Coccinellidae). Acta Biologica Paranaense, Curitiba. 14, 47-102.

Almeida, L.M., Milleo, J., 1998. The immature stages of *Psyllobora gratiosa* Mader, 1958 (Coleoptera: Coccinellidae) with some biological aspects. Journal of the New York Entomological Society 106, 170-176.

Amano, K., 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo.

Anderson, J.M.E., 1982. Seasonal habitat utilization and food of the ladybirds *Scymnodes lividigaster* (Mulsant) and *Leptothea galbula* (Mulsant) (Coleoptera: Coccinellidae). Australian Journal of Zoology 30, 59-70.

Bado, S.G., Rodriguez, S.M., 1998. Aspectos morfologicos y biologicos de una vaquita micetofaga *Psyllobora bicongregata* B. (Coleoptera: Coccinellidae). Revista de la Facultad de Agronomia Universidad de Buenos Aires 3, 181-184.

Borner, C., Heinze, K., 1957. Aphidina. In: Sorauer, P. (Ed.), Handbuch der Pflanzenkrankheiten. Berlin-Hamburg.

Braun, U., Cook, R.T.A., Inman, A.J., Shin, H.D., 2002. The taxonomy of powdery mildew fungi. In: Belanger, R.R., Bushnell, W.R., Dik, A.J., Carver, T.L.W. (Eds.), The powdery mildews: A comprehensive treatise. St. Paul, APS Press, pp. 13-55.

Carter, M.C., Dixon, A.F.G., 1984. Honeydew: An arrestant stimulus for cocinellids. Ecological Entomology 9, 383-387.

Casey, T.L., 1899. A revision of the American Coccinellidae. Journal of the New York Entomological Society 7, 71-169.

Chapuis, F., 1876. Genera des Coleopteres ou expose methodique et critique de tous les genres proposes jusqu'ici dans cet ordre d'insectes. (Coccinellides), Paris 12, 149-259.

Chase, A.R., 2004. Making the grade: New fungicides for ornamentals. Society of American Florists Proceedings for 20th Annual Conference on Pest Management on Ornamentals, 1-5.

Cividanes, T.M., Cividanes, F.J., Matos, B.A., 2007. Biologia de *Psyllobora confluens* alimentada com o fungo *Erysiphe cichoracearum*. Pesquisa Agropecuaria Brasileira, Brasilia 42, 1675-1679.

Crowson, R.A., 1981. The biology of Coleoptera. (Academic Press: New York.).

Cruz, B., Gonzalez-Avila, M., Soto, L., 1989. *Psyllobora nana* (Coleoptera: Coccinellidae), biological control agent of dusty mildew. Ciencias de la Agricultura 36, 151.

Davidson, W.M., 1921. Observations on *Psyllobora taedata* LeConte, a coccinellid attacking mildews. Entomology News 32, 83-89.

Dejean, P.F.M.A., 1837. Catalogue des coleopteres de la collection M. le Conte Dejean. 3e. ed. Paris, 1-458.

del Pino, D., Olalla, L., Canovas, I., Cazorla, F.M., deVicente, A., Tores, J.A., 1999. Resistance to fungicides of *Sphaerotheca fuliginea* strains isolated from southern Spain. International Powdery Mildew Conference, 1st, Avignon, France, 42.

Dharpur, S.R., Rao, M.K., Sagar, R.B.S., 1990. New record of a mycophagous beetle, *Thea cincta* Fabr. on powdery mildew of niger. Journal of Oilseeds Research 7, 124-125.

English-Loeb, G., Norton, A., Gadoury, D.M., Seem, R.C., Wilcox, W.F., 1999. Control of powdery mildew in wild and cultivated grapes by a tydeid mite. Biological Control 14, 97-103.

Falk, S.P., Gadoury, D.M., Cortesi, P., Pearson, R.C., Seem, R.C., 1995. Partial control of grape powdery mildew by the mycoparasite *Ampelomyces quisqualis*. Plant Disease 79, 483-490.

Fulmek, L., 1957. Insekten als Blattlausfeinde. Annalen des Naturhistorischen Museums, Wien 61, 110-227.

Fursch, H., 1996. Taxonomy of Coccinellidae. Coccinella 6, 28-30.

Giorgi, J. A., Vandenberg, N. J., McHugh, J. V., Forrester, J. A., Slipinski, S. A., Miller, K. B., Shapiro, l. R., Whiting, 2009. The evolution of food preferences in Coccinellidae. Biological Control, in press.

Gordon, R. D., 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93, 1-912.

Gubler, W.D., Ypema, H.L., Ouimette, D.G., Bettiga, L.J., 1996. Occurrence and development of resistance in *Uncinula necator* to triadimefon, myclobutanil and fenarimol in California grapevines. Plant Disease 80, 902-909.

Heaney, S. P., Hall, A.A., Davies, S.A., Olaya, G., 2000. Resistance to fungicides in the QoI-STAR cross-resistance group: Current perspectives. Conference Proceedings: British Crop Protection Council Conference on Pests and Diseases, pp. 755-762.

Hodek, I., 1973. Biology of Coccinellidae. Academia Publishing and W. Junk, Prague, 260 pp.

Hodek, I., Honek, A., 1996. Ecology of Coccinellidae. Kluwer Academic Publishers, Netherlands, 464 pp.

Hukusima, S., Itoh, K., 1976. Pollen and fungus as food for some coccinellid beetles. Research Bulletin of the Faculty of Agriculture, Gifu University 39, 31-38.

Kaltenbach, J.H., 1874. Die Pflanzenfeinde aus der Klasse der Insecten. Julius Hoffmann, Stuttgart.

Korner, O., Challa, H., 2003. Process-based humidity control regime for greenhouse crops. Computers and Electronics in Agriculture 39, 173-192.

Korschefsky, R., 1932. Coleopterorum Catalogus. XVI {120}, Coccinellidae II. W. Junk. Berlin, pp. 565-570.

Kovar, I., 1996. Phylogeny. pp. 19-31. In: Hodek, I., Honek, A. (Eds.), Ecology of Coccinellidae. Kluwer Academic Publishers, Netherlands, 464 pp.

Krishnakumar, R., Maheswari, P., 2004. Management of powdery mildew in mulberry using coccinellid beetles, *Illeis cincta* (Fabricius) and *Illeis bistigmosa* (Mulsant). Journal of Entomological Research 28, 241-246.

Kuznetsov, V.N., 1997. Lady beetles of the Russian Far East. Memoir No.1, Center for Systematic Entomology, Sandhill Crane Press, Gainesville, 248 pp.

Lawrence, J.F., 1989. Mycophagy in the Coleoptera. In: Wilding, N., Collins, N.M., Hammond, P.M., Webber, J.F. (Eds.), Insect-Fungus Interactions. Symposium of the Royal Entomological Society, London 14, 2-23.

Lawrence, J.F., Hlavac, T., 1979. Review of the Derodontidae (Coleoptera: Polyphaga) with new species from North America and Chile. Coleopterists Bulletin 33, 369-414.

Leschen, R.A.B., 2000. Beetles feeding on bugs (Coleoptera, Hemiptera): repeated shifts from mycophagous ancestors. Invertebrate Taxonomy 14, 917-929.

Lichteinstein, J.L., 1917. Observations sur les Coccinellides mycophages (Col.). Bulletin of the Society of Entomology of France, Paris, 298-302.

Lima, A.C., 1931. Coccinellideos mycophagos. (Coccinellidae: Psylloborini). Revista Entomologia, Rio de Janeiro. 1, 428-430.

Liu, C.L., 1951. Studies on the feeding capacity and life history of a mycophagous coccinellid, *Halyzia hauseri* Mader, in Kunming. Annales Entomologici Sinici, 1, 1-15.

Liu, H.L., 2001. Management of rose powdery mildew with automatic water sprays. Plant Protection Bulletin Taichung 43, 7-16.

Lundgren, J. G., 2009a. Relationships of Natural Enemies and Non-prey Foods. Springer, Dordrecht, The Netherlands.

Lundgren, J. G., 2009b. Nutritional aspects of non-prey foods in the life histories of predaceous Coccinellidae. Biological Control, in press.

Lundgren, J. G., Wiedenmann, R.N., 2004. Nutritional suitability of field corn pollen as food for the predator, *Coleomegilla maculata* (Coleoptera: Coccinellidae). Journal of Insect Physiology 50(6): 567–575.

Majerus, M.E.N., 1994. Ladybirds. Harper Collins Publishers, London, 367 pp.

Martelli, G., 1910. Sulla micofagia del Coccinellide *Thea vigintiduopunctata* L. Bollettino del Laboratorio di Entomologia, Portici 4, 292-294.

Martelli, G., 1914. Notizie su due Coccinellido micofagi. Bollettino del Laboratorio di Zoologia, Portici 5, 151-160.

McGrath, M.T., 2001. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Disease 85, 236-246.

Michaud, J.P., Grant, A.K., 2005. Suitability of pollen sources for the development and reproduction of *Coleomegilla maculata* (Coleoptera: Coccinellidae) under simulated drought conditions. Biological Control 32, 363–370.

Mulsant, M.E., 1850. Species de Coleopteres trimeres securipalpes. Annales des Sciences physiques et naturelles de Lyon 2, 1104 pp.

Norton, A.P., English-Loeb, G., Belden, E., 2001. Host plant manipulation of natural enemies: leaf domatia protect beneficial mites from insect predators. Oecologia 126, 535-542.

Omkar, Pervez, A., 1999. New record of coccinellids from Uttar Pradesh. Journal of Advanced Zoology 20, 106-112.

Pakaluk, J., Slipinski, S.A., Lawrence, J.F., 1994. Current classification and family-group names in Cucujoidea (Coleoptera). Genus 5, 223-268.

Paulitz, T.C., Belanger, R.R., 2001. Biological control in greenhouse systems. Annual Review of Phytopathology 39, 103-133.

Poorani, J.K., Slipinski, A., 2005. Notes on the genus *Protothea* Weise with redescription of *P. quadripunctata* (Mulsant) (Coleoptera: Coccinellidae: Coccinellini). Annales Zoologici 55, 329-333.

Pope, R.D., 1988. A revision of the Australian Coccinellidae (Coleoptera) Part 1. Subfamily Coccinellinae. Invertebrate Taxonomy 2, 633-735.

Prasad, R., Rai, M., 1988. New record of a mycophagous beetle, *Thea cincta* Fabr., on powdery mildew of linseed. Farm Science Journal 3, 89-90.

Putman, W.L., 1964. Occurrence and food of some coccinellids (Coleoptera) in Ontario peach orchards. The Canadian Entomologist 96, 1149-1155.

Ratti, E., 1996. Coleoptera associated with *Oidium evonymi-japonici* (Arc.) Sacc. (fungi imperfecti) in the urban gardens of Venice. Bollettino del Museo Civico di Storia Naturale di Venezia 45, 47-51.

Ricci, C., 1982. Sulla costituzione e funyione delle mandibole delle larve di *Tytthaspis sedecimpunctata* (L.) e *Tytthaspis trilineata* (Weise). Frustula Entomologica 3, 205-212.

Ricci, C., 1986. Seasonal food preferences and behavior of *Rhyzobius litura*. In: Hodek, I. (Ed.), Ecology of Aphidophaga. Academia, Prague & Dr. W. Junk, Dordrecht, pp. 119-123.

Ricci, C., Fiori, G., Colazza, S., 1983. Regime alimentare dell'adulto di *Tytthaspis sedecimpunctata* (L.) (Coleoptera: Coccinellidae) in ambiente a influenza antropica primaria: prato polifita. Atti XIII Congresso Nazional Italiano Entomologia Sestriere-Torino, 691-698.

Samways, M.J., Osborn, R., Saunders, T.L., 1997. Mandible form relative to the main food type in ladybirds (Coleoptera: Coccinellidae). Biocontrol Science and Technology 7, 275-286.

Sasaji, H., 1968. Phylogeny of the family Coccinellidae (Coleoptera). Etizenia, Occasional Publication of the Biological Laboratory Fukui University 35, 1-37.

Schilder, F.A., Schilder, M., 1928. Die nahrung der Coccinelliden und ihre Beziehung zur Verwandtschaft der Arten. Arbeiten aus der Biologischen Reichsanstalt fur Land- und Forstwirtschaft 16, 213-282.

Sivapalan, A., 1993. Effects of impacting rain drops on the growth and development of powdery mildew fungi. Plant Pathology 42, 256-263.

Slipinski, S.A., Pakaluk, J., 1991. Problems in the classification of the cerylonid series of Cucujoidea (Coleoptera). In: Zunino, M., Belles, X., Blas, M. (Eds.), Advances in Coleopterology. Silvestrelli and Cappelletto, Torino, pp. 79-88.

Solomon, M.E., 1949. The natural control of animal populations. Journal of Animal Ecology 18, 1-35.

Soylu, S., Yigit, A., 2002. Feeding of mycophagous ladybird, *Psyllobora bisoctonotata* (Muls.) on powdery mildew infested plants. Biological Control of Fungal and Bacterial Plant Pathogens, IOBC-WPRS Bulletin 25, 183-186.

Sutherland, A.M., 2005. Evaluation of *Psyllobora vigintimaculata* (Say) (Coleoptera: Coccinellidae) for Biological Control of Powdery Mildew Fungi (Erysiphales). M.S. thesis, University of California, Davis.

Sutherland, A.M., Parrella, M.P., 2006. Quantification of powdery mildew consumption by a native coccinellid: Implications for biological control? In: Hoddle, M.S., Johnson, M.W. (Eds.), California Conference on Biological Control 5 (Riverside). Proceedings, 188-192.

Sutherland, A.M., Parrella, M.P., 2009. Biology and co-occurrence of *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae) and powdery mildews in an urban landscape of California. Annals of the Entomological Society of America, in press.

Takamatsu, S., 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. Mycoscience 45, 147-157.

Takeuchi, M., Sasaki, Y., Sato, C., Iwakuma, S., Isozaki, A., Tamura, M., 2000. Seasonal host utilization of mycophagous ladybird *Illeis koebelei* (Coleoptera: Coccinellidae). Japanese Journal of Applied Entomology and Zoology 44, 89-94.

Thomas, M.C., 1993. A new species of *Cryptolestes* Ganglbauer from the Middle East and a new synonym (Coleoptera: Laemophloeidae [Cucujidae sensu lato]). Israel Journal of Entomology 27, 113-118.

Timberlake, P.H., 1943. The Coccinellidae or ladybeetles of the Koebele Collection. Part 1. Hawaiian Planters' Record, Honolulu. 47, 1-67.

Traquair, J.A., Shaw, L.A., Jarvis, W.R., 1988. New species of *Stephanoascus* with *Sporothrix* anamorphs. Canadian Journal of Botany 66, 929-933.

Triltsch, H., 1999. Food remains in the guts of *Coccinella septempunctata* (Coleoptera: Coccinellidae) adults and larvae. European Journal of Entomology 96, 355-364.

Turian, G., 1969. Coccinelles micromycetophages (Col.). Mitteilungen der Schweizerischen Entomologischen Gesellschaft 42, 52-57.

Turian, G., 1971. *Thea 22-punctata* et autres Coccinelles micromycetophages. Nature du pigment elytral jaune. Mitteilungen der Schweizerischen Entomologischen Gesellschaft 44, 277-280.

Vandenberg, N.J., 2002. Family 93. Coccinellidae Latreille 1807. In: Arnett, R.H. Jr., Thomas, M.C., Skelley, P.E., Frank, J.H. (Eds.), American Beetles. Volume 2. Polyphaga: Scarabaeoidea through Curculionoidea. Boca Raton, FL: CRC Press LLC, pp. 371-389.

Weber, D. C., Lundgren, J. G., 2009. Assessment of the ecological roles of the Coccinellidae. Biological Control, in press.

Weise, J. 1900. Berichtigung von Beobachtungen über die Nahrung unserer Coccinelliden. Deutsche entomologische Zeitschrift 2, 297-301.

Wu, X.B., Guo, X.L., 1987. Primary study on control of powdery mildew by ladybugs. Journal of Northeast Forestry University China 15, 13-17.

Yurtsever, S., 2001. A preliminary study on the ladybirds (Coleoptera: Coccinellidae) of Edirne in north-western Turkey. Turkish Journal of Zoology 25, 71-75.

Zoebelein, G., 1956. Der Honigtau als Nahrung der Insekten. Zeitschrift für angewandte Entomologie 38, 369-416.

Figure 1. Adult *Psyllobora vigintimaculata*, a North American mycophagous coccinellid, grazing on a patch of powdery mildew fungi (photo by Jack Kelly Clark).

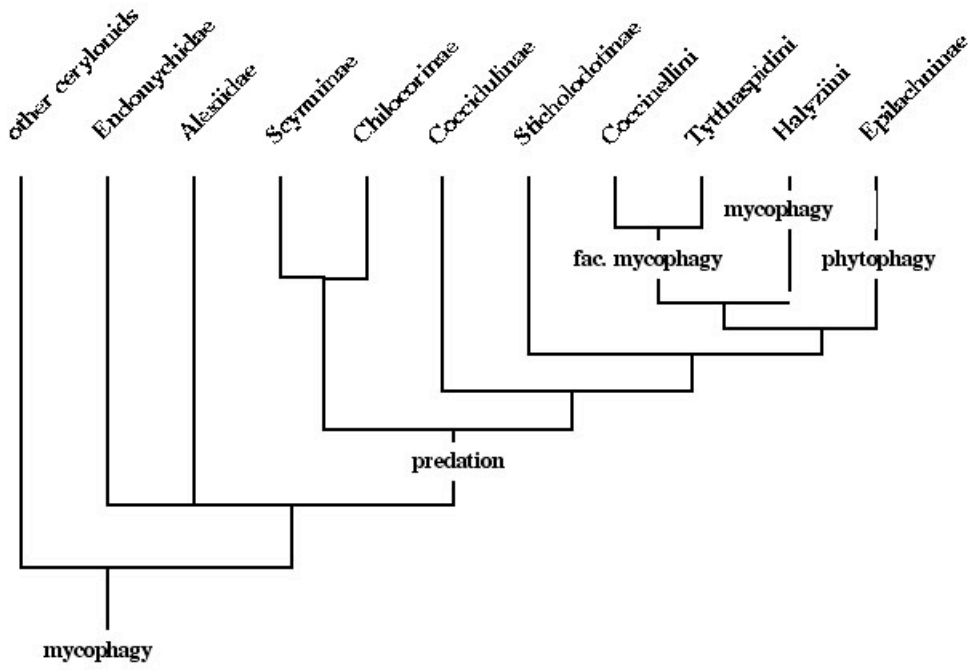

Figure 2. Consensus diagram for the cerylonid series and Coccinellidae (adapted from Sasaji (1968), Slipinski and Pakaluk (1994), Kovar (1996) and Leschen (2000)). Secondary mycophagy has evolved in the Coccinellinae, with facultative mycophagy found in Coccinellini and Tytthaspidini, and (possibly obligate) mycophagy in Halyziini.

Figure 3. Aggregation of *Psyllobora vigintimaculata* larvae feeding together on a patch of the PM *Erysiphe chicoracearum* infecting *Zinnia elegans*.

Table 1. A summarized chronological listing of biological and ecological observations and experimentation involving the mycophagous coccinellids of the tribe Halyziini and their food source, powdery mildew (PM) fungi.

Publication	**Species of Halyziini**	**Specific Topic**
Koebele, ~1893[†]	*Illeis galbula*	observation of mycophagy
Weise, 1900	*Psyllobora vigintiduopunctata*	observation of mycophagy
Martelli, 1910, 1914	*P. vigintidupunctata*, *Vibidia duodecimguttata*	observation of mycophagy, PM species determination
Lichteinstein, 1917	*V. duodecimguttata*, *Halyzia sedecimguttata*	observation of mycophagy, PM species determination
Davidson, 1921	*P. vigintimaculata*	biology, phenology and host range testing
Strouhal, 1926	*H. sedecimguttata*, *P. vigintiduopunctata*, *V. duodecimguttata*	biological observation, morphological description, and taxonomic key
Lima, 1931	*P. hybrida*, *P. confluens*	observation of mycophagy, PM species determination
Liu, 1951	*H. hauseri*, *H. sanscrita*, *I. cincta*	PM species determination, consumption quantification
Savoiskaya, 1961	*P. vigintiduopunctata*, *V. duodecimguttata*, *H. tschitscherini*	observation of mycophagy
Anderson, 1982	*I. galbula*	natural host range and utilization studies
Almeida, 1985	*Psyllobora* spp. (17)	biological descriptions
Wu and Guo, 1987	unknown	PM control efficacy
Prasad and Rai, 1988	*P. cincta*	biological observation
Cruz et al, 1989	*P. nana*	biological observation, suggestion of biocontrol
Dharpur et al, 1990	*P. cincta*	biological description
Ratti, 1996	*P. vigintiduopunctata*	biological observation

Bado and Rodriguez, 1998	*P. bicongregata*	biological and morphological descriptions
Almeida and Milleo, 1998	*P. gratiosa*	biological and morphological descriptions
Takeuchi, 2000	*I. koebeli*	Field phenology, natural host range and utilization
Soylu and Yigit, 2002	*P. bisoctonotata*, *P. vigintiduopunctata*	Biological observation, consumption quantification, host range observation
Ahmad et al, 2003	*P. bisoctonotata*	Natural phenology and host range observations
Krishnakumar and Maheswari, 2004	*I. cincta*, *I. bistigmosa*	PM control efficacy, release rate determination
Sutherland, 2005	*P. vigintimaculata*	Natural biology, phenology, host range determination, fungicide compatibility
Sutherland and Parrella, 2006	*P. vigintimaculata*	Consumption quantification, release rate determination
Cividanes et al, 2007	*P. confluens*	Biological observation and description
Sutherland and Parrella, in press	*P. vigintimaculata*	Natural biology, phenology, host range determination

† from Timberlake (1943)

Table 2. Powdery mildews, and their plant hosts, on which Halyziini (Coccinellidae) beetles were observed to feed.

Plant family	Plant species	Powdery mildew genus	Location	Halyziine species (reference)
Aceraceae	*Acer macrophyllum*	*Sawadaea*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Adoxaceae	*Sambucus racemosa*	*Erysiphe (=Microsphaera)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
Apiaceae	*Ainsworthia trachycarpa*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Ammi majus	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Torilis arvensis	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Torilis nodosa	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Asteraceae	*Calendula arvensis*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Centaurea calcitrapa	*Leveillula*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Chrysanthemum coronarium	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Cichorum intybus	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Cirsium arvense	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Conyza albida	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Cosmos bipinnatus	*Podosphaera (=Sphaerotheca)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Dahlia coccinea	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Erigeron naudinii	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Gerbera jamesonii	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Guzotia abyssinica	*Podosphaera (=Sphaerotheca)*	India	*Psyllobora cincta* (Dharpur et al., 1990)
	Helianthus annuus	*Erysiphe*	Cuba	*Psyllobora nana* (Cruz et al., 1989)
		Podosphaera (=Sphaerotheca)	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Matricaria chamomilla	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Picris echioides	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Sonchus oleraceus	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Urospermum picroides	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Xanthium strumarium	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Zinnia elegans	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Balsaminaceae	*Impatiens balsamina*	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Bignoniaceae	*X Chitalpa tashkientsis*	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Brassicaceae	*Rapistrum rugosum*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)

	Sinapis arvensis	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Sisymbrium officinale	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Cannabaceae	*Celtis sinensis*	*Erysiphe (=Uncinula)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
Caprifoliaceae	*Lonicera fragrantissima*	*Oidium*	Australia	*Illeis galbula* (Anderson, 1982)
Celastraceae	*Euonymus japonica*	*Oidium*	Italy	*Psyllobora vigintiduopunctata* (Ratti, 1996)
Chenopodiaceae	*Chenopodium opulifolium*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Convolvulaceae	*Calystegia sepium*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Convolvulus arvensis	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Cornaceae	*Benthamidia florida*	*Erysiphe (=Microsphaera)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
Cucurbitaceae	*Cucumis sativa*	*Erysiphe*	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Cucurbita spp.	*Erysiphe*	Brazil	*Psyllobora lenta* (Almeida, 1985)
		Erysiphe	Argentina	*Psyllobora bicongregata* (Bado and Rodriguez, 1998)
		Erysiphe	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Podosphaera (=Sphaerotheca)	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Trichosanthes kilirowii	*Podosphaera (=Sphaerotheca)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
Dipsacaceae	*Scabiosa columbaria*	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Euphorbiaceae	*Euphorbia heterophylla*	*Leveillula*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Ricinus spp.	*Ersiphe (=Microsphaera)*	Brazil	*Psyllobora hybrida* (Lima, 1931)
Fabaceae	*Ceratonia siliqua*	*Oidium*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Melilotus indica	*Erysiphe (=Microsphaera)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Onobrychis caput-galli	*Erysiphe (=Microsphaera)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Onobrychis christa-galli	*Erysiphe (=Microsphaera)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Senna pendula	Unidentified	Australia	*Illeis galbula* (Anderson, 1982)
	Trigonella hamosa	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Fagaceae	*Quercus agrifolia*	*Ersiphe (=Microsphaera)*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Quercus lobata	*Ersiphe (=Microsphaera)*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Fumariaceae	*Fumaria judaica*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Fumaria officinalis	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Geraniaceae	*Erodium malacoides*	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Erodium moschatus	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Hydrangeaceae	*Hydrangea hortensis*	*Oidium*	Brazil	*Psyllobora gratiosa* (Almeida and Milleo, 1998)
Lamiaceae	*Clerodendrum trichotomum*	*Podosphaera (=Sphaerotheca)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)

	Mentha spicata	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Monarda punctata	*Neoerysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Salvia spathacea	*Oidium*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Linaceae	*Linum usitatissimum*	*Oidium*	India	*Psyllobora cincta* (Prasad and Rai, 1988)
Lythraceae	*Lagerstroemia indica*	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Malvaceae	*Abelmoschus esculentus*	*Erysiphe*	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Erysiphe	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Podosphaera (=Sphaerotheca)	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	Brazil	*Psyllobora confluens* (Cividanes et al., 2007)
	Alcea rosea	*Leveillula*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Malva neglecta	*Leveillula*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Moraceae	*Morus* spp.	*Phyllactinia*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
		Phyllactinia	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Leveillula	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Phyllactinia	India	*Illeis bistigmosa* (Krishnakumar and Maheswari, 2004)
		Phyllactinia	India	*Illeis cincta* (Krishnakumar and Maheswari, 2004)
Oleaceae	*Syringa vulgaris*	*Ersiphe (=Microsphaera)*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Papaveraceae	*Papaver rhoeas*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Plantaginaceae	*Plantago lanceolata*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Veronica persica	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Platanaceae	*Platanus X acerifolia*	*Sawadaea*	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Sawadaea	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
Poaceae	*Avena sterilis*	*Blumeria*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Phalaris paradoxa	*Blumeria*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Polygonaceae	*Polygonum aviculare*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Rumex conglomeratus	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Ranunculaceae	*Ranunculus scandicinus*	*Erysiphe*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Rosaceae	*Malus* spp.	*Podosphaera*	USA	*Psyllobora vigintimaculata* (Davidson, 1921)
		Podosphaera	China	*Halyzia hauseri* (Liu, 1951)
		Podosphaera	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Prunus spp.	*Podosphaera*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
		Podosphaera	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Podosphaera (=Sphaerotheca)	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Podosphaera	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)

	Pyracantha coccinea	*Oidium*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
	Rosa spp.	*Podosphaera (=Sphaerotheca)*	USA	*Psyllobora vigintimaculata* (Davidson, 1921)
		Podosphaera (=Sphaerotheca)	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
		Podosphaera (=Sphaerotheca)	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Podosphaera (=Sphaerotheca)	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Spiraea douglasii	*Ersiphe (=Microsphaera)*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Stephanandra incisa	*Podosphaera (=Sphaerotheca)*	Japan	*Illeis koebelei* (Takeuchi et al., 2000)
Solanaceae	*Capsicum annuum*	*Leveillula*	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
		Leveillula	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
	Solanum lycopersicum	*Erysiphe*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
		Leveillula	Turkey	*Psyllobora bisoctonotata* (Soylu and Yigit, 2002)
	Solanum melongena	*Leveillula*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Verbenaceae	*Verbena officinalis*	*Podosphaera (=Sphaerotheca)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
Vitaceae	*Vitis californica*	*Erysiphe (=Uncinula)*	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)
	Vitis vinifera	*Erysiphe (=Uncinula)*	Syria	*Psyllobora bisoctonotata* (Ahmad et al., 2003)
		Erysiphe (=Uncinula)	USA	*Psyllobora vigintimaculata* (Sutherland & Parrella, in press)

CHAPTER 2

Biological Control through Powdery Mildew Consumption

(partially published as: Sutherland, A.M., Parrella, M.P., 2006. Quantification of Powdery Mildew Consumption by a Native Coccinellid: Implications for Biological Control? Proccedings of the Fifth Annual California Conference on Biological Control (CCBC V). 25-27 July 2006, Riverside, California.)

Abstract. The coccinellid tribe Halyziini (Psylloborini) is entirely comprised of obligate consumers of powdery mildew (PM) fungi (Ascomycota: Erysiphales). A western North American species, *Psyllobora vigintimaculata*, was evaluated for its ability to consume spores and hyphae of PM in greenhouses, in an attempt at augmentative biological control. Individual larvae were reared on PM-infected leaf discs in a laboratory incubator to quantify the removal of PM due to this insect's feeding. The leaf area cleaned and the background growth of PM were measured with image analysis software using periodic digital images. Leaf discs exposed to neonate larvae for 192 hours showed a significant decrease in PM growth as compared to an untreated control, and leaf discs exposed to 3rd instar larvae for 96 hours showed a significant decrease in infected leaf area. A simple model based on these data predicts that an individual larva cleans 6.3±3.3cm^2 leaf area of all visible PM hyphae and conidia from the time of egg eclosion until pupation. Secondly, a predictive exponential consumption model was created utilizing known biological attributes of the insect, a modified Leslie matrix, the previously-described individual larval consumption model and data from a feeding efficacy trial conducted in the greenhouse. The model has then been employed to simulate the population growth and subsequent removal of visible mildew colonies by the insects in a controlled horticultural system. When compared against real data from the efficacy trial, the model underestimates consumption, especially in the early time-periods. The model's limitations and assumptions are discussed, as well as the possibility of using such a model as a calculator to determine the insect release-rate necessary to bring about control in a system with known parameters.

Key words: powdery mildew, biological control, release rate, matrix modeling

Introduction and Historical Review

The fungi belonging to the Erysiphales (Ascomycota), commonly known as powdery mildews (PM), are all obligate biotrophs. As an order, they have been recorded to infect close to 10,000 species of angiosperm plants in 169 families (Amano, 1986). Since many of these host plants are valued as agricultural commodities, PM are collectively considered one of the most important plant pathogens worldwide. Chemically based disease management programs have historically led to resistance by the fungi to several key fungicides (Gubler et al, 1996; McGrath, 2001). Biological control of PM may offer solutions to this growing resistance problem in addition to issues such as residues in food crops, worker health and safety, and negative effects to nontarget organisms. There are several commercially available microbial biological control agents including the spore-forming bacterium *Bacillus subtilis* and the fungal hyperparasite *Ampelomyces quisqualis* Ces. Very little is known of the potential for arthropod agents to control or reduce the disease through consumption.

All members of the beetle tribe Psylloborini Casey (Coleoptera: Coccinellidae) are obligate consumers of various PM conidia and hyphae in all mobile life stages (Gordon, 1985). The cosmopolitan genus *Psyllobora* Chevrolat is represented in natural and managed systems in temperate and subtropical regions worldwide, and may be utilized as a native biological control agent of PM (Cruz et al, 1989; Almeida and Milleo, 1998). The small ashy gray ladybird, *P. vigintimaculata* Say, is native to northern and western North America. It has recently been recorded as locally common in Davis, California, feeding on various PM associated with horticultural and agronomic crops in the landscape as well as in protected greenhouse culture (Sutherland, 2005a). The overall objectives of our work are the evaluation and development of

P. vigintimaculata for biological control of PM, and the assessment of its compatibility with chemically managed systems such as greenhouse horticulture (Sutherland, 2005b).

In this system, the task of quantifying PM consumption is facilitated by the fact that PM-infected leaf areas, once fed upon by members of the Halyziini, are visibly discernable and easy to separate from those areas not fed upon (Figure 1). The first published attempt to quantify mycophagy was made by Liu (1951), working with *Halyzia hauseri* feeding on the PM *Podosphaera leucotricha* infecting apple in China. Insect developmental periods, total feeding periods, and estimated daily feeding capacity (cm^2) were determined through daily observations. From this information, Liu (1951) estimated that the feeding capacity for *Halyzia hauseri* from egg eclosion until death was 99.72 cm^2. He also presented the comparative ratio 1:2:5:5:5 representing the relative total amounts of fungi consumed during each stage respectively, the 1^{st} through 4^{th} instars and adults, showing that the last two instars consumed a similar amount to the adults.

Soylu and Yigit (2002) stained okra leaves infected with PM *Erysiphe cichoracearum* with lactophenoltryphan blue and examined them using light microscopy, revealing that larvae and adults of *Psyllobora bisoctonotata* (Mulsant) fed upon mycelia as well as conidia and conidiophores on the leaf surface. Spore solutions were made from infected leaf areas exposed to larvae and compared with those unexposed to larvae via the counting of conidia with a haemocytometer. The authors reported a 92% reduction in conidial density in leaf sections fed upon by the beetles. Leaf area cleaned by *P. bisoctonotata* was quantified using excised leaf sections and a leaf surface scaler. Third and fourth instars were the most efficient consumers in terms of leaf area cleaned per unit time.

In India, Krishnakumar and Maheswari (2004) measured PM control provided by *Illeis cincta* and *Illeis bistigmosa* (Mulsant). They used potted mulberry plants, uniformly infected with the PM *Phyllactinia corylea* (Pers.) Karst., exposed to adult beetles, and sampled over time to determine percent infection, percent disease control and the percent disease index (PDI) (Food and Agricultural Organisation, 1967). In addition, they compared the control offered by the beetles to the control provided by both the fungicide dinocap (0.2%) and neem oil emulsion (2%). A dramatic reduction in PDI (from 92.8 to 32.4) was recorded 10 d after five pairs of *Illeis cincta* were released per plant. No such reduction was observed when only two pairs of beetles were released. In the comparison with fungicides, the authors reported that the PDC was statistically similar 20 d after treatment in plants receiving beetles or an application of fungicide. Also, the PDI slowly increased over time in plants treated with fungicides, while PDI slowly decreased in plants receiving beetles.

The success of any biocontrol agent depends directly on its ability to locate the pest, reproduce in response and in relation to the pest, and to function or consume based on the relative abundance or density of the pest (Solomon, 1949). We are interested in evaluating these responses for *P. vigintimaculata*. The prey item of this insect, spores and hyphae of PM fungi, sometimes exist in dense colonies of millions of spores and sometimes as minuscule patches in isolated areas on specific plants. Previous work has shown that adults of this species can be found associated with low density, isolated PM patches (Sutherland, 2005a), suggesting their ability to locate food by responding to long-range stimuli during flight. It is established that large coccinellids such as *Coccinella septempunctata* L. respond to olfactory cues from their aphid prey, and can even distinguish between aphid species based on volatiles (Sengonca and Liu, 1994). Additionally, landscape surveys have demonstrated strong positive correlations

between presence and density of PM in the landscape and the presence and density of *P.vigintimaculata* life stages (Sutherland, 2005a), suggesting an increase in reproduction and/or aggregation by the insect in response to increases in its food item. Work presented here addresses aspects of the functional response of *P. vigintimaculata*. Specifically, we quantify the average amount of leaf area visibly cleaned of hyphae and conidia by a *P. vigintimaculata* individual during its entire larval development. Our experimental objective was to create a simple linear model based on the total visible PM removed by *Psyllobora vigintimaculata* during its larval development. Next, we aimed to couple this with a population model in order to predict the visible PM removed by a fluctuating population of mixed-age *P. vigintimaculata*, taking pathogen growth, crop leaf area, and time into consideration. The population growth and population structure at some point in time of any organism with known life stages, durations of life stages, developmental rates, mortality coefficients and fecundity can be determined through the use of matrix mathematics, specifically of the type defined by Leslie (1945). By combining the linear individual consumption model with a Leslie vector-matrix population model we hope to construct a population-level consumption model that can be used to quantifiably predict the ecological service of PM removal offered by this insect species in any horticultural system with known parameters.

Materials and Methods: laboratory-based linear consumption model

Organism Rearing

Plant material (*Zinnia elegans*) with PM was grown under high-pressure sodium lighting (600W) in a humidified (50-80% relative humidity) growth room utilizing ebb-and-flood hydroponics. Periodic inoculations with conidia from the PM fungus *Erysiphe chicoracearum*

were made either by spore solution or by brushing from infected plants. A colony of *P. vigintimaculata* was maintained in the laboratory in a series of insect rearing cages. Sufficiently infected *Zinnia* plants were exposed to caged adults at regular intervals for egg deposition. Afterwards, the egg-laden plants were retained in the cage to facilitate larval development. Towards the end of the fourth larval instar the plants were moved to a final pupation cage and cut at media level. Adults were captured as they emerged and flew towards the light at the top of the cage, as in a reverse Berlese funnel. In this manner the colony provided harvestable eggs, larvae or adults of uniform age at five day intervals.

Experimental Units

Egg masses deposited on the same day were removed from the insect colony and transferred to an incubator (Percival Scientific I-30 BL) kept at 20±5^{o} C and 50-90% relative humidity under fluorescent lights. Excised PM-infected *Zinnia* leaves from the hydroponic PM colony were cut with a scalpel to conform to a circular template (55mm diameter). These leaf discs were then placed on filter paper on top of ~10mm agar (USP, 150 mesh, Bio-serv®) in a vented petri dish enclosure (55mm diameter X 30mm height). Upon egg eclosion, the 1st instar larvae were individually transferred with a fine paintbrush to one half of the dish enclosures. The other half of the dishes received no insects and represented untreated controls, subject to normal fungal growth and development. All dishes were then assigned a reference number and returned to the incubator in a randomized fashion (random.org). This methodology maintained turgidity of leaf discs and allowed for development of insects and fungi for up to ten days. Larvae were removed and returned to the colony after they had completed two larval instars (approximately eight days). A similar set of experiments was established for newly molted third instar larvae. Fresh PM-infected leaf discs were created as above and introduced into the dish

enclosures and incubator. These larvae were allowed to develop until pupation, at which time the pupae were returned to the colony and observation was terminated. Together, the two cohorts of *P. vigintimaculata* larvae used in this feeding quantification trial represented one complete larval development period, from egg eclosion until sucessful pupation.

Image Analysis

Software based on existing image analysis algorithms (Assess Image Analysis Software for Plant Disease Quantification, The American Phytopathological Society, 2002), coupled with digital photography, was used to quantify the PM present on *Zinnia* leaf discs throughout the study. A digital camera (Nikon D70, Japan) equipped with a macrophotograpy lens (Sigma 150mm 1:2.8 APO MACRO DG HSM D, Japan) mounted on an adjustable tripod (Bogen, Manfrotto®, Italy) was used to capture digital images of each leaf disc just prior to insect release and every 48 hours thereafter. The described software was used to separate PM from the background leaf disc based on pixel saturation (Lamari, 2005), and then to express disease as the percentage of leaf area covered by visibly discernable PM colonies (%PM). This derived percentage was used as the response variable at all times.

Statistical Analysis

Experimental units were arranged as in a completely randomized design (CRD) within the incubator, and were re-randomized after each image acquisition event. Differences in %PM between units containing larvae and those left untreated were determined through use of the one-way analysis of variance (ANOVA) (JMP© Start Statistics, SAS Institute, 2005). Data were collected to reflect the increase in leaf area affected (Δ%PM) and the percentage increase in relation to initial severity ($\Delta\%PM/\%PM_{initial}$). These variables were also analyzed using

ANOVA to detect treatment differences. All percentage-based variables could be expressed as leaf area (cm^2) by multipling by the area of an entire leaf disc ($23.7cm^2$).

Model Construction

In order to estimate the total amount of leaf area cleaned (LAC) of PM by one *P. vigintimaculata* individual during larval development we constructed a basic model that included a measure of the normal PM growth (G) that should occur in the absence of feeding. Additionally, we treated the data resulting from the young larva (egg-2nd instar) and old larva (3rd instar-pupa) trials as continuous, with the sum representing the estimated response for the entire larval duration. Therefore, the constructed model is as follows:

$$LAC_{total} = LAC_{young} + LAC_{old} + G_{young} + G_{old},$$

$$\text{where } LAC = (\%PM_{initial} - \%PM_{final}) * 23.7cm^2 \text{ for larva units,}$$

and $G = \text{mean}(\%PM_{final} - \%PM_{initial}) * 23.7cm^2 \pm$ standard error for all untreated units.

Materials and Methods: population-based simulation model

The constructed model consisted of several key components; temperature-dependant growth rates, fecundity, estimates of mortality probabilities, consumption constants for each life stage (as determined by the linear larval consumption model), population composition over time (as determined through use of the Leslie vector-matrix model); and several driving variables including the total crop leaf area, the initial PM severity (expressed as % leaf area visibly infected) and the number of adult female *P. vigintimaculata* released into the system.

In order to simulate population growth of *P. vigintimaculata* over time we used the concept of thermal unit accumulation. The thermal units, or degree-days required for each step in the insect's development were previously determined (Table 1) through laboratory observation

of the life cycle at different static temperatures and by using a lower developmental threshold, T_0, of 10°C within the rectangular accumulation formula $DD=\sum(T_{mean}-T_0)_{day}$. (Arnold, 1959). Fecundity data (~7 eggs/female/day) were available from similar previous observation (Sutherland, 2005a). Mortality at each life stage was arbitrarily assumed to be 5%.

In order to estimate the total amount of leaf area cleaned (LAC) of PM by one *P. vigintimaculata* individual during larval development we utilized the previously mentioned linear consumption model that included a measure of the normal PM growth (G) that should occur in the absence of feeding.

The Leslie growth matrix model describes development, age-specific mortality, and reproduction. The major variables/parameters are $N_{x,t}$, which represents the number of organisms in age x at time t; S_x, the survival probability of organisms in the age interval from x to x+1; and M_x, which is the average number of offspring produced in age interval x to x+1 minus mortality. From these variables we have two base equations:

$$N_{x+1,t+1} = N_{x,t} * S_x \qquad \text{(development and mortality)}$$

$$N_{0,t+1} = \sum N_{0,t}*M_0 + \ldots N_{x,t}*M_x \qquad \text{(\# individuals in the first age class).}$$

These two equations can be combined into one matrix equation:

$$N_{t+1} = A*N_t \quad ,$$

where N_x is a vector of age distribution at time t and A is the transition matrix. This equation can be iterated to simulate as many time steps as necessary. Since the development rate of insects is not constant, and since each age class requires differing amounts of thermal units in order to move up to the next, we modify the model in the form of a distributed delay. The transition matrix will have non-zero diagonal elements so that some proportion remains in the same age class when time increases. This rate can be adjusted by changing the relative values of

the diagonal and sub-diagonal elements. The sub-diagonal elements of this model indicate the proportion that moves up to the next age class during each time step. From this conceptual design an Excel spreadsheet was adopted and modified that required only the input of an initial population (eggs), a probability coefficient for each stage to remain in that stage (diagonal), a probability coefficient for each stage to move up a stage (sub-diagonal), and a measure of fecundity of adult females (Table 2). This spreadsheet program could then simulate generations (or thermal unit steps) and generate an age distribution vector, which we will call [P] in subsequent formulae and equations.

The final model structure, which intends to deal with a dynamic population and different feeding rates for different age classes, is as follows:

$$\%PM * LA = [LAC] * [P] * A,$$

where %PM refers to the initial PM severity, LA refers to the crop leaf area, [LAC] is a vector of larval feeding rates at all four instars, [P] is the population vector from the Leslie matrix that tells us the population composition, and A is the number of adult females released. Control of PM in a given system is reached when the equation is satisfied. A release-rate calculator equation was generated by solving for A such that

$$A = (\%PM * LA) / ([LAC] * [P]).$$

There are several key assumptions made with this model:

1. PM growth is built into the individual consumption model and is based on laboratory observations in an incubator. Greenhouse conditions may be dramatically different and may change over time.
2. The leaf area within a system does not change over time.
3. All larvae of the same age consume the same amount.

4. PM spore density is uniform.
5. Adult beetle sex ratio is exactly 50/50.
6. Mortality, development and fecundity are uniform over each age class for all individuals.
7. Adult beetles do not consume PM.

This last assumption is especially problematic since we know the adults of both sexes do indeed consume PM, but since we have no data on how much is consumed it is not included in the model. Consumption data incorporated into the linear feeding model were based entirely on the larval experience. In nature, or in a large variable system such as a greenhouse, we expect the adults to be concerned with dispersal and reproduction in addition to feeding.

In order to compare the behavior of this model to real data, we have carried out a short and simple experiment, doubling as a feeding efficacy trial, to generate the needed data. Uniformly-infected Transvaal daisy, *Gerbera jamesonii*, were placed in community cages (five plants per cage) within a greenhouse at the UC Davis campus. One of three treatments; 0, 25 or 50 beetles released; was then instituted. Weekly leaf samples were taken from each plant for a total of five weeks in order to assess the %PM of the three treatments over time. A destructive harvest was initiated at the end of the trial and leaves from each plant were measured with a leaf area meter (LI-3100, LiCor Inc.) to determine the final (and assumed initial) leaf area of each plant.

Results: laboratory-based linear consumption model

1st & 2nd Instar Trial

There was no significant initial treatment difference in %PM (df=1,18; F=0.18; p=0.68). Both treatments exhibited an increase in %PM until 96 hours after larval introduction, when

%PM began to stabilize and decline (Figure 2). After 192 hours of observation %PM was significantly lower on leaf discs containing larvae as compared to untreated leaf discs (df=1,18; F=6.48; p=0.02). When larvae were removed after 192 hours the mean increase in infected leaf area in untreated units (4.38±0.95cm^2) was significantly higher (df=1,18; F=6.74; p=0.02) than that in larva units (1.17±0.8cm^2). Additionally, PM-affected areas increased an average of 54.3% relative to initial size (9.54cm^2, or 40.3% of 23.7cm^2) in untreated units but only 19.5% relative to initial size (10.1cm^2, or 42.6% of 23.7cm^2) in larva units.

3rd & 4th Instar Trial

There was no significant treatment difference in %PM at the onset of observation (df=1,18; F=0.33; p=0.57) or 48 hours following release, but the mean %PM on leaf discs containing larvae began to decrease immediately (Figure 3). After 96 hours there was a significant difference in %PM between treatments (df=1,18; F=7.79; p=0.01), with a %PM decrease in larva units as compared to a %PM increase in untreated units thereafter. Area infected by PM increased on average 14.9% relative to initial size (11.1cm^2, or 46.9% of 23.7cm^2) in untreated units but actually decreased 12.3% relative to initial size (11.8cm^2, or 49.7% of 23.7cm^2) with larvae present; a significant difference (df=1,18; F=12.38; p=0.002).

Consumption Model

Each larva cleaned an average of 3.22±1.8cm^2 of *Zinnia* leaf area of all visible traces of the PM from the time of egg eclosion until molt initiation at the end of the 2nd instar during a period of 192 hours. PM in larva units actually increased during this time by 4.9% to give a calculated LAC_{young} of –1.17cm^2/unit, but this was offset by a PM background growth, G_{young}, of 4.38 cm^2/unit. In the second trial each larva cleaned an average of 3.10±1.5cm^2 leaf area of PM from the start of the 3rd instar until pupation during a period of 96 hours. The LAC_{old} was

calculated as 1.69cm^2/unit and the G_{old} as 1.41cm^2/unit. The background PM growth rate was similar in both trials, at 0.023 and 0.015cm^2/hour, respectively. The model predicts that an average *P. vigintimaculata* larva will clean 6.32±3.3cm^2 of *Zinnia* leaf area of PM spores and hyphae during development.

Results: population-based simulation model

Simulated %PM data tended to underestimate the actual PM removal as recorded through the digital photography method for both release rates, but especially for the 25 beetle release treatment (Figure 2). The initial %PM was higher in the units where 25 beetles were released (40.7%) when compared to the initial value in the 50 beetle release units (30.0%) and the untreated units (12.4%). The simulated data for the 50 beetle release rate units was very close to the observed data at the end of the trial (8.54% vs. 8.38%), but was much higher than observed for the 25 beetle release units (30.0% vs.13.7%). Additionally, observed data displayed more fluctuation in the early phase of the experiment with a pronounced PM severity peak one week after beetle release and another small peak or plateau at three weeks after beetle release.

When using the constructed release rate calculator it was determined that about 77 beetles (38.5 females) would need to be released in order to gain complete control in 30 days. This figure may be close to the truth since the treatment with 25 females had only reduced %PM to 21.1% at 30 days post release.

Discussion

The linear consumption model predicted that an average larva would clean 6.32 ± 3.3cm^2 of leaf area of PM spores and hyphae during development (Table 3). Based on this predicted

quantity consumed, it would take four larvae developing from egg to pupa to consume the PM covering an entire Zinnia leaf disc 55mm in diameter (23.7cm^2) if the disc were 100% covered with spores and hyphae. This figure is much lower than the 99.72 cm^2 reported by Liu (1951) for *Halyzia hauseri* feeding on apple PM. Possible explanations for this difference include the larger size of *Halyzia* (~6 mm adult diameter vs. ~3 mm for *Psyllobora*), the inclusion of adult feeding until death in Liu's study, and differences in PM spore density among plant host / powdery mildew combinations as discussed by Takamatsu (2004).

There are several reasons why the constructed simulation model could have predicted a smaller amount of feeding than was actually deduced through the real data collection. All of these reasons involve the assumptions of the model. The glaring possibility is that since adults were not included in the [LAC] feeding model, then the model will always underestimate the actual cleaning being done. Also, there may be physiological differences in the PM species compared here. *Zinnia* PM tends to be very dense and patchy, whereas *Gerbera* PM tends to be more diffuse and uniform. Another possibility is that there was less PM growth in this trial as compared with the laboratory trial which supplied the data for the feeding model, which contained a growth term nested within it. Clearly more real data from more varied systems are required in order to observe the model's behavior, calibrate the model for use in horticultural practice, and to validate the ability of the model to predict consumption and organismal growth.

This was a valuable starting point for a model that, given the proper inclusion of variables, calibration and validation, is simple enough and broad enough in scope to be used in the realm of applied horticultural science.

This quantification of the PM consumption abilities of *P. vigintimaculata* is essential to its evaluation for use as an effective biological control agent against PM fungi. Members of this

genus are known to feed on many genera of PM on many different host plants (Ahmad et al, 2003), and different PM species grow at different densities, rates and patterns, so it is important to realize that varying levels of effectiveness may be seen in different systems and with other *Psyllobora* species.

Literature Cited

Ahmad M., Younis, G., Ali, N., 2003. Biology of the coccinellid *Psyllobora (Thea) bisoctonotata* Muls. (Coleoptera: Coccinellidae) a predator of powdery mildew fungi. Proceedings of the Eighth Arab Congress of Plant Protection, Elbeida City, Libya.

Almeida L.M., Milleo, J., 1998. The immature stages of *Psyllobora gratiosa* Mader, 1958 (Coleoptera: Coccinellidae) with some biological aspects. Journal of the New York Entomological Society 106(4), 170-176.

Amano, K. 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo.

Arnold, C.Y. 1959. The determination and significance of the base temperature in a linear heat unit system. Proceedings of the American Society for Horticultural Science 74: 430-435.

Cruz B., Gonzalez-Avila, M., Soto, L., 1989. *Psyllobora nana* (Coleoptera: Coccinellidae), biological control agent of dusty mildew. Ciencias de la Agricultura 36, 151.

Food and Agricultural Organisation. 1967. Crop losses due to disease and pests. F.A.O., Rome.

Gordon, R. D. 1985. The Coccinellidae (Coleoptera) of America north of Mexico. Journal of the New York Entomological Society 93:1-912.

Gubler W.D., Ypema, H.L., Ouimette, D.G., Bettiga, L.J., 1996. Occurrence and development of resistance in *Uncinula necator* to triadimefon, myclobutanil and fenarimol in California grapevines. Plant Disease 80, 902-909.

Krishnakumar, R., Maheswari, P., 2004. Management of powdery mildew in mulberry using coccinellid beetles, *Illeis cincta* (Fabricius) and *Illeis bistigmosa* (Mulsant). Journal of Entomological Research 28, 241-246.

Lamari L., 2005. ASSESS: Image analysis software for plant disease quantification. APS Press, St. Paul, MN, USA.

Leslie, P.H., 1945. On the use of matrices in certain population mathematics. Biometrika 33, 183-212.

Liu, C.L., 1951. Studies on the feeding capacity and life history of a mycophagous coccinellid, *Halyzia hauseri* Mader, in Kunming. Annales Entomologici Sinici, 1, 1-15.

McGrath, M.T., 2001. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. Plant Disease 85(3), 236-246.

SAS Institute Inc., 2005. JMP® Start Statistics third edition. A guide to statistics and data analysis using JMP and JMP IN software. SAS Institute Inc., Cary, New York.

Sengonca C., Liu, B., 1994. Responses of the different instar predator, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), to the kairomone produced by the prey and non-prey insects as well as the predator itself. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 101, 173-177.

Solomon M.E., 1949. The natural control of animal populations. Journal of Animal Ecology 18, 1-35.

Soylu, S., Yigit, A., 2002. Feeding of mycophagous ladybird, *Psyllobora bisoctonotata* (Muls.) on powdery mildew infested plants. Biological Control of Fungal and Bacterial Plant Pathogens, IOBC-WPRS Bulletin 25, 183-186.

Sutherland A.M., 2005a. Evaluation of *Psyllobora vigintimaculata* (Say) (Coleoptera: Coccinellidae) for Biological Control of Powdery Mildew Fungi (Erysiphales). M.S. thesis, University of California, Davis.

Sutherland A.M., 2005b. Effects of selected fungicides on a mycophagous ladybird (Coleoptera: Coccinellidae): Ramifications for biological control of powdery mildew. IOBC/wprs Bulletin Vol. 28(1), 253-257.

Takamatsu, S., 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. Mycoscience 45, 147-157.

Figure 1. An individual *Psyllobora vigintimaculata* larva feeding on the powdery mildew (PM) *Erysiphe chicoracearum* infecting *Zinnia elegans* "Peter Pan". Leaf area exposed to and fed upon by the larva is visibly discernable from unexposed PM-infected leaf area.

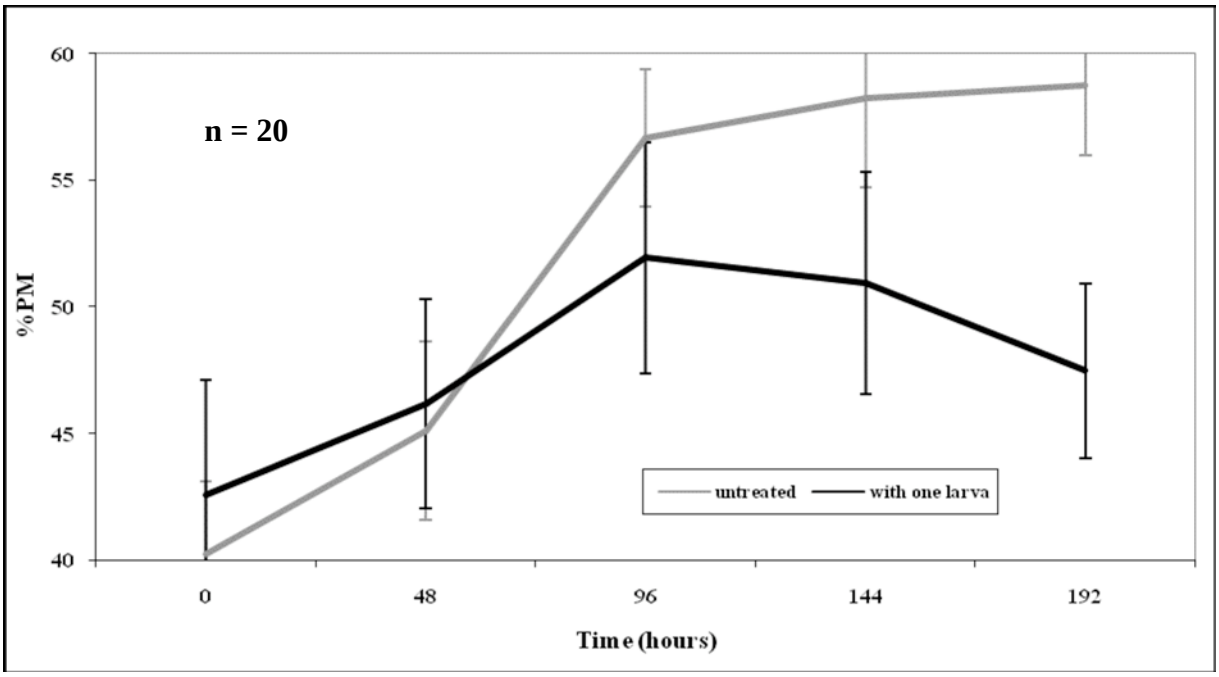

Figure 2. Percentage leaf area affected by powdery mildew on leaf discs (23.7cm^2) over time in the presence of neonate-2nd instar larvae of *Psyllobora vigintimaculata* as compared to untreated.

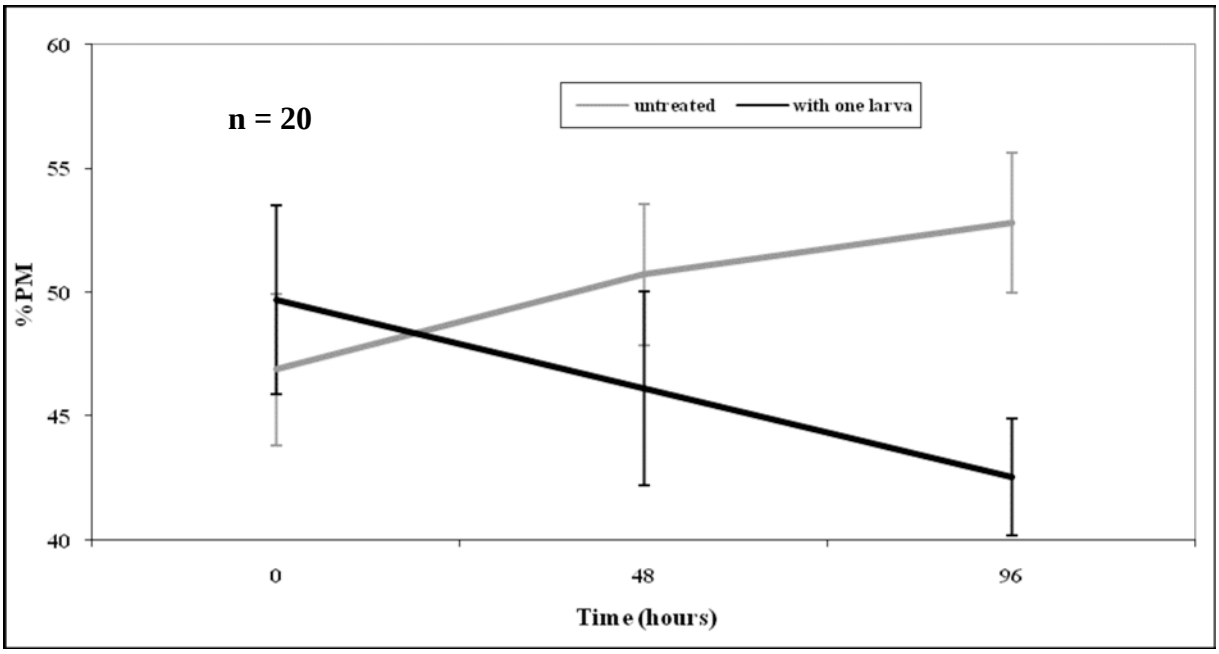

Figure 3. Percentage leaf area affected by powdery mildew on leaf discs (23.7cm^2) over time in the presence of 3rd - 4th instar larvae of *Psyllobora vigintimaculata* as compared to untreated.

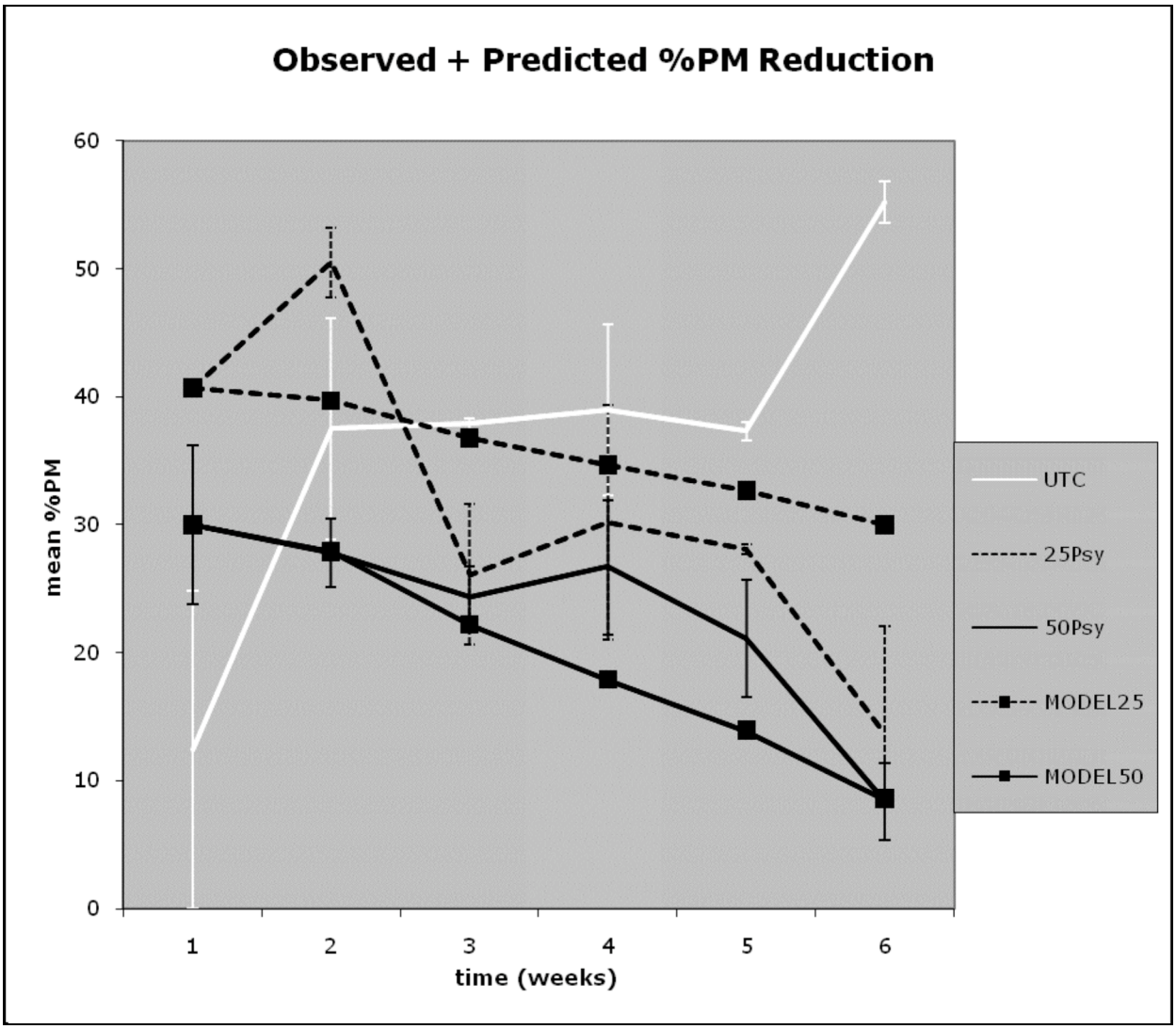

Figure 4. Visible powdery mildew (PM%) removal over time by a cohort of 25 (25Psy) and 50 (50Psy) adult *Psyllobora vigintimaculata* released into cages (~2m^2) containing five *Gerbera jamesonii* plants (8" pots, ~7,152cm^2 total leaf area) moderately infected (~28%) with PM graphically compared to model-generated simulated data under the same conditions and release rates (MODEL25 and MODEL50).

Table 1. Generalized life cycle of *Psyllobora vigintimaculata taedata* with observed stadia durations (mean ± SEM, n = 25) and approximate degree-day requirements . Based on developmental threshold (T_0) of 10.0°C and formula: $DD=\sum(T_{mean}-T_0)_{day}$.

Life Stage	Duration (days) at 20°C	at 25°C	Degree-Days Req.
Egg	6.64 ± 0.25	4.40 ± 0.15	67
1st Instar	4.00 ± 0.18	2.52 ± 0.12	40
2nd Instar	3.80 ± 0.19	2.76 ± 0.10	40
3rd Instar	3.36 ± 0.24	2.28 ± 0.16	33
4th Instar	3.32 ± 0.19	2.16 ± 0.14	33
Pupa	6.68 ± 0.31	4.36 ± 0.17	67
Total (egg-adult)	27.9 ± 0.62	18.5 ± 0.35	280

Table 2. Transformation matrix used to adjust a given age distribution vector for a population of *Psyllobora vigintimaculata* during each time interval (a heat unit period corresponding to three days at mean temperature 23^{o} C, about 40 degree-days). Values in white on a dark field represent age-specific fecundity (F_x). Values on a solid grey field represent the probability of surviving and remaining in the current age class (P_x) during the next time interval, while values on a dashed grey field represent the probability of surviving and moving up to the next age class (G_x) during the next time interval. The sum of the probabilities P_x and G_x must not exceed one, and the difference between their sum and one represents the age-specific mortality (D_x) during the next time interval.

egg	**1st instar**	**2nd instar**	**3rd instar**	**4th instar**	**pupa**	**adult**
0.75	0	0	0	0	0	13
0.2	0.45	0	0	0	0	0
0	0.5	0.65	0	0	0	0
0	0	0.3	0.65	0	0	0
0	0	0	0.3	0.75	0	0
0	0	0	0	0.2	0.75	0
0	0	0	0	0	0.2	0

Table 3. Observed consumption of the powdery mildew (PM) fungus *Erysiphe cichoracearum* by two age groups of the mycophagous coccinellid *Psyllobora vigintimaculata* during development from egg to pupa in terms of the leaf area cleaned (LAC) of visible PM and the naturally-occurring background growth (G) exhibited by PM in the absence of feeding. Based on the linear model: $LAC_{total} = LAC + G$; where LAC = mean ($\%PM_{initial} - \%PM_{final}$) * leaf disc area ± standard error for all larva units, and G = mean ($\%PM_{final} - \%PM_{initial}$) * leaf disc area ± standard error for all untreated units.

Age Group	**LAC**	**G**	**Amount Consumed**
eclosion to 3rd instar	-1.17 cm^2	4.38 cm^2	3.22 ± 1.8 cm^2
3rd instar to pupation	1.69 cm^2	1.41 cm^2	3.10 ± 1.5 cm^2
Total (egg to adult)	0.52 cm^2	5.79 cm^2	6.32 ± 3.3 cm^2

CHAPTER 3

Indication for and Decision Support for PM Management in Vineyards

(Sutherland, A.M., Gubler, W.D., Parrella, M.P. to be submitted to Ecological Applications)

Abstract. Powdery mildew (PM) is the most important pathogen of grapes in California. Management involves use of the UC Davis PM risk assessment index (RAI) model to predict pathogen development so that fungicide applications can be made efficiently. This technique has resulted in decreased frequency and volume of fungicide applications. The only reliable initiation mechanism for this model is based on manual scouting and is therefore subject to human error. The use of a native mycophagous beetle, *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae), as an indicator for PM may offer a solution to this problem. Bioindication, using taxa characteristic of specific environmental conditions, has been used as an effective and inexpensive means of ecosystem assessment and decision support. Vineyard monitoring over three successive seasons utilizing manual leaf inspection and yellow sticky card sampling for beetle presence and density as well as PM presence and severity has revealed several important trends. Adult beetles were adept at locating isolated and low-density PM infections in the large-scale monoculture of commercial vineyards. Also, the observed density of adult beetles trapped in the vineyard was positively and significantly correlated with the severity of PM infecting the grapevines in the immediate vicinity. The possibility of using local beetle density to predict local PM severity and the possibility of using beetle presence as a component of the RAI model are discussed.

Keywords: powdery mildew, commercial viticulture, decision support, bioindication, UC Davis RAI model, *Psyllobora vigintimaculata*, numerical aggregative response

Introduction

Grapes are the second largest agricultural commodity in California in terms of farm receipts. According to the California Association of Winegrape Growers (MKF Research, 2007), sales of winegrapes, which represent about 60% of total grape acreage, exceeded $2.1 billion in 2006. Additionally, this crop was indirectly responsible for $2 billion in tourist expenditures and $16.5 billion in wine sales. Grape powdery mildew (PM), *Erysiphe necator*, is the most serious and widespread grape pathogen in California, and many valuable resources are spent in an attempt to prevent and combat infection (Gubler and Hirschfelt, 1991). As a result of these efforts winegrape vineyards are identified as the largest pesticide user by site for the state as defined by the California Department of Pesticide Regulation (2007). Sulfur, a staple PM prevention material, is the primary pesticide used in commercial winegrape production, with more than 20 million pounds of active ingredient typically applied per annum. Clearly there is a need for pesticide application reduction in California's grape industry, and the implementation of the principles of integrated pest/disease management for grape PM can potentially bring about this reduction.

Integrated disease management for PM in the California winegrape industry involves prevention, resistance management and responsible treatment decisions. Treatment decisions are based on assessments of disease risk through scouting and the use of the UC Davis Gubler-Thomas PM model. There are two portions to the model; the first dealing with the prediction of the release and germination of ascospores, the primary source of spring inoculum, and the second serving as a risk assessment index (RAI) for subsequent conidial infections (Gubler et al, 1999). In the originally-published model, ascospore release is dependant upon at least 2.5mm of spring rainfall or overhead irrigation followed by 13 hours of leaf wetness while temperatures remain

between 10° and 30° C. Recently, in order to reflect field observations, the ascospore release portion of the model has been adjusted so that 10.0mm of rainfall or irrigation is required (W.D. Gubler, personal communication). Ascospore germination and penetration requires three consecutive days with at least six hours between 21° and 30° C. Leaf sampling is sometimes instituted once these conditions have been met in order to confirm the presence of PM and to begin the risk assessment portion of the model. The RAI uses only daily temperature data, with each day with at least six hours between 21° and 30° C adding to the index and each day in which the six hour limit was not attained or with more than 15 minutes above 35° C resulting in subtraction from the index. Index numbers relate directly to the asexual reproductive rate of the pathogen and are used to determine control application necessity and frequency. Thus, while the timing of ascospore release, germination and penetration can be estimated using weather data, the initiation mechanism for the RAI (successful infection and colony establishment) can only be reliably determined through intensive sampling for PM presence. This is inherently subject to human error and inconsistencies. However, use of this model is already responsible for a reduction of two to three fungicide applications over the growing season, and it is possible that with an easier and more reliable trigger for the RAI its impact would be even greater.

Possible solutions to challenges in chemical-based management programs for PM may lie in biological control; however, this is a largely unexplored management tactic. The small ashy gray ladybird, *Psyllobora vigintimaculata* Say (Coleoptera: Coccinellidae), is native to western North America, and is common in California ecosystems (Davidson, 1921). It has been recorded feeding on PM in greenhouses, vineyards and the urban landscape, and its local abundance is directly correlated with increasing PM densities (Sutherland, 2005). If the native *P. vigintimaculata* can be utilized as a naturally-occurring indicator of PM in the local vineyard,

and especially if its vineyard densities correspond to vineyard PM density/severity, then a new decision support tool for PM managers may be realized that reduces pesticide impacts to the environment and increases predictability and effectiveness of currently used management techniques.

Bioindication, or the use of specific taxa to reflect changing environmental conditions and/or biodiversity, has emerged as a powerful tool in the fields of conservation and pollution monitoring. Organisms such as reptiles (Read, 2002) and earthworms (Suthar, 2009) have been used to reflect land-use patterns and resulting biodiversity in agricultural regions. Lichen (Jovan and McCune, 2005) and terrestrial plants (Paoletti et al, 2009) are regarded as accurate indicators of pollutants such as ammonia and ozone. Insects have been suggested as ideal bioindicators, given their ubiquity, habitat specificity, and sensitivity to environmental change (McGeoch, 1998; Hilty and Merenlender, 2000). Dung beetles (Coleoptera: Scarabeidae) in South Africa (McGeoch et al, 2002) and drosophilid assemblages in Brazil (da Mata et al, 2008) served as excellent bioindicators, not only of specific ecosystems, but of ecological change and its direction. Explicit systems involving arthropods used as indicators of pests or disease to aid crop management decision support are unknown, though coccinellid density has been suggested as a tool for aphid population assessment in orchards (Iperti, 1999). Good candidates for bioindicator organisms are relatively abundant, easy to sample for, and, above all, exhibit a strong relationship with some characteristic of their environment (McGeoch, 1998). Intuitively, for a bioindicator to be valuable, its prevalence must also be easier to measure than the environmental information ultimately sought. Therefore, in order for *P. vigintimaculata* to be a good bioindicator of PM, it must be abundant enough to readily sample, it must be reliably associated with PM infection, and measurement of its prevalence must be more efficient than currently-used

measurements of PM presence and severity. Additionally, to serve as a decision support device in an agroecosystem such as winegrapes, *P. vigintimaculata* should respond in a measurable way to fluctuating PM severity in space and time.

Weekly sampling by Sutherland (2005) in Davis, California for PM and *P. vigintimaculata* revealed that beetles were associated with various PM fungi, were active from late February until mid-December, and showed an aggregative numerical response to their mildew food source (Figure 1). Thus, as PM density and severity increased, the density of *P. vigintimaculata* likewise increased (Sutherland, 2005). This phenomenon was also found with winegrapes, *Vitis vinifera*. Additionally, greenhouse observations (Sutherland, unpublished) suggested that adult beetles were attracted to yellow sticky cards used for pest monitoring and were likely caught during flight. These preliminary data and observations suggest that *P. vigintimaculata* is present as a mildew consumer in winegrapes, can be caught and monitored through the use of yellow sticky cards, and might be used as a measure of PM presence and density in the vineyard. Thus, it was hypothesized that grapevines infected with PM should be associated with *P. vigintimaculata* adults caught during flight in the immediate locality, and that increasing numbers of adult beetles will be locally caught as PM severity increases in space and time. Ultimately, the number of beetles caught can be used in PM management decisions to possibly reduce the quantity and frequency of fungicide applications.

Materials and Methods

The cooperation of a large commercial grower of the variety Chardonnay, Herzog Ranch, in Courtland, California (Grape Crush District 17) was obtained for this work through collaboration with a team of UC Davis plant pathologists examining the efficacy of various PM

fungicides. In 2006, sampling began June 1, and in 2007 and 2008 sampling began in April (April 26 and April 16, respectively) -just prior to the typical release of ascospores by the cleistothecia. Experimental units consisted of 50 equally spaced groups of three (2006) or two (2007, 2008) adjacent vines. A sampling unit of five (2006) or three (2007, 2008) randomly-selected fully-expanded leaves within the lower canopy were removed and examined weekly until just prior to harvest (July 20, August 15 and July 31, for 2006, 2007, and 2008, respectively) for presence and severity of PM and for egg density of *P. vigintimaculata*. Severity of PM infection was expressed as an ordinal severity index from zero (no infection visible) to five (entire leaf surface covered by visible PM hyphae) for each sampled leaf. Therefore, in 2006 the index ranged from zero to 25 for each experimental unit, and in 2007 and 2008 from zero to 15. Data from 2007 and 2008 were combined since the experimental units and sampling units were similar. Insect density was expressed as the number of insects per macro-unit (insects / five leaves or three leaves). In addition to this leaf sampling for insect density, double-sided yellow sticky cards (10cm X 16cm) (Seabright Laboratories, Inc.) were hung from the vine cordon within the west-facing canopy and likewise employed as a weekly measure of insect presence and density, expressed as adult beetles per card (both sides). The bivariate relationship between sticky card catches and actual leaf densities of *P. vigintimaculata* egg clusters was described through linear regression in order to determine whether increasing numbers of trapped adults indicated increasing levels of population initiation by the insects on the local vines. To describe the relationship between PM severity and insect density in the vineyard, a direct measure of an aggregative numerical response, the Median test on rank ordered responses was used to determine if increasing levels of PM severity have a significant positive effect on insect density. In order to determine the feasibility of using the presence of *P. vigintimaculata* as an

indicator for PM in the vineyard as opposed to conventional scouting we compared the initial catch or observation of the insect in the vineyard with the first temporal record of PM presence resulting from manual scouting and the first indication of PM conidial infection risk according to the Gubler-Thomas model. Finally, in hopes of developing a treatment threshold based on beetle catch over time which could be used to dictate fungicide applications early in the season, we characterized the synchrony of PM and *P. vigintimaculata* in the vineyard through a graphic representation of insect density, PM severity, and model output over time.

Results

2006

Throughout the seasonal observation period (June 1-July 20) *P. vigintimaculata* adults were never locally trapped without visible presence of PM. In other words, trapped *P. vigintimaculata* adults were always associated with local PM infections. Sticky cards trapped insects in some sampling units when weekly PM severity was very low (rating: 1 out of 25). At least one *P. vigintimaculata* adult was trapped in all cases where weekly PM severity ratings in the associated experimental unit rose to 12 or higher (out of 25). The number of adults caught on sticky cards per week was positively correlated (r^2=0.41, P<0.0001, n=385) to the weekly number of egg clusters found on the spatiotemporally associated experimental unit (Figure 2a). Local PM severity had a significant positive effect (Median test: χ^2=102.7, df=17, P<0.0001) on locally trapped *P. vigintimaculata* adults (Figure 3a). Observations began late, when PM infections were prevalent, and so no attempt was made to compare initial insect presence with initial evidence of PM via manual scouting or the Gubler-Thomas model.

2007 and 2008

During both years the observation period was begun much earlier than in 2006 in order to characterize the onset of PM infection in terms of beetle presence, manual leaf sampling and the Gubler-Thomas model. Therefore, sampling began prior to ascospore release when there was no detectable presence of PM in the vineyard. In both years *P. vigintimaculata* adults were sometimes (twice out of 95 observations in 2007, and 15 times out of 63 observations in 2008) encountered and trapped without being associated with a PM infection. Just as in 2006, sticky cards trapped insects in some sampling units when weekly PM severity was very low (rating: 1 out of 15). In all cases where weekly PM severity ratings in an experimental unit rose to a moderately high level (10 or higher out of 15 in 2007, 11 or higher out of 15 in 2008) there was at least one *P. vigintimaculata* trapped on the weekly sticky card. The number of adults caught on sticky cards per week was positively correlated (r^2=0.43, P<0.0001, n=420) to the weekly number of egg clusters found on the spatiotemporally associated experimental unit (Figure 2b). Local PM severity had a highly significant positive effect (Median test: χ^2=235.5, df=13, P<0.0001) on locally trapped *P. vigintimaculata* adults (Figure 3b). A delayed density-dependent numerical response was exhibited by *P. vigintimaculata* in relation to temporally increasing PM severity, as evident through temporally increasing sticky card catch density (Figure 4 and Figure 5). In 2007 the model predicted moderate PM infection risk (50 out of 100) as early as May 10, and maximum risk (100 out of 100) as early as May 17, based entirely on weather data. Due to some early season applications of paraffinic oil (JMS Stylet Oil; JMS Flower Farms, Inc.) in the study vineyard, however, PM infection was delayed, supplemental inoculation was necessary, and multiple colonies of visible PM hyphae were not encountered

until June 8. Moderate infections (5 out of 15) were not encountered until June 22. Therefore, in 2007 within the study vineyard site, density of beetles caught weekly on sticky cards more closely tracked actual PM severity than did the model due to an inaccurate prediction of ascospore release date (Figure 4). Temporal onset of PM was very similar in 2008 due to another series of early season oil applications and subsequent necessary inoculations. Even though the RAI trigger (ascospore release and infection assumed) occurred April 12 and moderate disease risk was predicted as early as April 26, visible PM colonies were not detected by manual leaf sampling until June 4. Moderate infections were not encountered until June 20. Again, in 2008, given the late onset of disease due to spring fungicide applications, and with possible inaccuracies in the prediction of ascospore release, sticky card catches were a better indicator of site-specific PM severity than the Gubler-Thomas model (Figure 5).

Discussion

Adult *P. vigintimaculata* showed an aggregative numerical response to increasing PM severity in all three years of study. Also, grapevines without PM largely failed to attract *P. vigintimaculata* except in April of 2007 and 2008. Powdery mildew is not always a problem at this time of the year for grapes, but plants in the surrounding agroecosystem may be infected with other PM species. The vineyard study site was adjacent to a riparian area and commercial orchards, both of which may harbor PM early in the spring. Landscape surveys by Sutherland (2005) showed that *P. vigintimaculata* accepts many PM/host complexes as food, and so the vineyard records in April may have been evidence for incidental movement through the vineyard from one PM patch to another. This is a possible disadvantage for the development of a decision support system since early season presence of beetles may falsely indicate the presence of PM in

a commercial vineyard. Perhaps a threshold density of beetles per card per week, rather than simple presence, would be more successful as a positive indication of local PM infection. Also, low severity PM infections sometimes failed to attract adult *P. vigintimaculata* on a weekly basis. This represents another possible shortcoming of decision support based on beetle presence: beetles are absent even though PM infection is underway. Conversely, each year, beetles were always associated with vineyard units once a moderate level of PM severity was reached. Vineyard managers, however, may consider these "moderate" infections unacceptable, and perhaps would initiate fungicide applications much earlier. Confounding this relationship between insect and fungus is evidence that applications of fungicides such as sulfur and strobilurins may repel or negatively impact populations of *P. vigintimaculata* (Sutherland, 2005), resulting in a breakdown of the decision support capabilities of this system.

Sticky card catches of adult *P. vigintimaculata* were better indicators of local PM severity than the Gubler-Thomas model in the early season in both years where this comparison was possible. Early-season grower applications of fungicidal materials, however, created anomalous conditions wherein disease onset was delayed as compared to historical records. Also, these comparisons of beetle catches with model predictions were carried out using the original rainfall requirements of 2.5mm for ascospore release prediction. This requirement has since been changed to 10mm, and such has resulted in a later predicted date of disease onset in 2009. There may be the potential for beetle catches to be used as a supplement to the model, perhaps in triggering the RAI portion, which is quite valuable when asexual reproduction of PM is established (Gubler et al, 1999). It is also possible that beetle catches could be developed into a distinct decision support device, where sticky card catches could dictate spatiotemporal

applications of fungicides and their frequencies. This phenomenon deserves more attention, and a direct comparison with other, established decision support devices is warranted.

Dufrene and Legendre (1997) developed an indicator value method (IndVal), which combines measures of habitat fidelity (frequency of occurrence) and specificity, to quantify the bioindicator value of individual taxa. In the IndVal system, species with both high fidelity and specificity within a habitat are known as "characteristic" indicators, capable of reflecting a specific environmental state or habitat. In order to monitor environmental change, however, so-called "detector" indicators are required (Dufrene and Legendre, 1997). These species show medium specificity to a given habitat, and exhibit fluctuating fidelity based on environmental conditions. Mycophagous coccinellids may serve as such detectors of changing PM infection and severity since many different host-specific PM fungi are accepted as food (Ahmad et al, 2003; Sutherland and Parrella, 2009). Sutherland (2005) in California, and Takeuchi et al (2000) in Japan, both demonstrated that different plant species are infected with PM at different times of the year, and that seasonal utilization of these host complexes by mycophagous coccinellids varied accordingly. Therefore, populations of obligate mycophages such as *P. vigintimaculata* fluctuate greatly over time and space, in response to environmental change (available PM fungi as food), and may indeed prove valuable as "detectors" (Dufrene and Legendre, 1997) of PM infection, transmission and trends in severity.

Literature Cited

Ahmad, M., Younis, G., Ali, N., 2003. Biology of the coccinellid *Psyllobora (Thea) bisoctonotata* Muls. (Coleoptera: Coccinellidae) a predator of powdery mildew fungi. Proceedings of the Eighth Arab Congress of Plant Protection, Elbeida City, Libya.

California Association of Winegrape Growers, 2005. www.cawg.org

California Department of Pesticide Regulation, 2004. Summary of pesticide use report data 2003. www.cdpr.ca.gov

da Mata, R.A., McGeoch, M., Tidon, R., 2008. Drosophilid assemblages as a bioindicator system of human disturbance in the Brazilian Savanna. Biodiversity and Conservation 17, 2899-2916.

Davidson, W.M., 1921. Observations on *Psyllobora taedata* LeConte, a coccinellid attacking mildews. Entomology News 32, 83-89.

Dufrene, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monograms 67, 345-366.

Gubler, W. D., Hirschfelt, D., 1991. Powdery mildew. In: Grape Pest Management, 2nd Ed., University of California IPM Manual. D. L. Flaherty, et al. (eds.).

Gubler, W. D., Rademacher, M.R., Vasquez, S.V., Thomas, C.S., 1999. Control

of powdery mildew using the UC Davis Powdery Mildew Risk Index. APS Net, Plant Pathology On-line. APS Net Feature, Jan. 1999, 8 pp.

Hilty, J., Merenlender, A., 2000. Faunal indicator taxa selection for monitoring ecosystem health. Biological Conservation 92, 185-197.

Iperti, G., 1999. Biodiversity of predaceous coccinellidae in relation to bioindication and economic importance. Agriculture, Ecosystems and Environment 74, 323-342.

Jovan, S., McCune, B., 2005. Air-quality bioindication in the greater Central Valley of California, with epiphytic macrolichen communities. Ecological Applications 15(5), 1712-1726.

McGeoch, M.A., 1998. The selection, testing and application of terrestrial insects as bioindicators. Biological Review of the Cambridge Philosophical Society 73, 181-201.

McGeoch, M.A., Van Rensburg, B.J., Botes, A., 2002. The verification and application of bioindicators: a case study of dung beetles in a savanna ecosystem. Journal of Applied Ecology 39, 661-672.

MFK Research, 2007. Economic impact of California wine 2006. MFK Research, Llc., St. Helena, California.

Paoletti, E., Ferrara, A.M., Calatayud, V., Cervero, J., Giannetti, F., Sanz, M.J., Manning, W.J., 2009. Deciduous shrubs for ozone bioindication: *Hibiscus syriacus* as an example. Environmental Pollution 157, 865-870.

Read, J.L., 2002. Experimental trial of Australian arid zone reptiles as early warning indicators of overgrazing by cattle. Austral Ecology 27, 55-66.

Suthar, S., 2009. Earthworm communities a bioindicator of arable land management practices: A case study in semiarid region of India. Ecological Indicators 9, 588-594.

Sutherland, A.M. 2005. Evaluation of *Psyllobora vigintimaculata* (Say) (Coleoptera:Coccinellidae) for Biological Control of Powdery Mildew Fungi (Erysiphales). M.S. thesis, University of California, Davis.

Sutherland, A.M, Parrella, M.P., 2009: Mycophagy in Coccinellidae: A Review and Synthesis. Biological Control, in press.

Takeuchi, M., Sasaki, Y., Sato, C., Iwakuma, S., Isozaki, A., Tamura, M., 2000. Seasonal host utilization of mycophagous ladybird *Illeis koebelei* (Coleoptera: Coccinellidae). Japanese Journal of Applied Entomology and Zoology 44, 89-94.

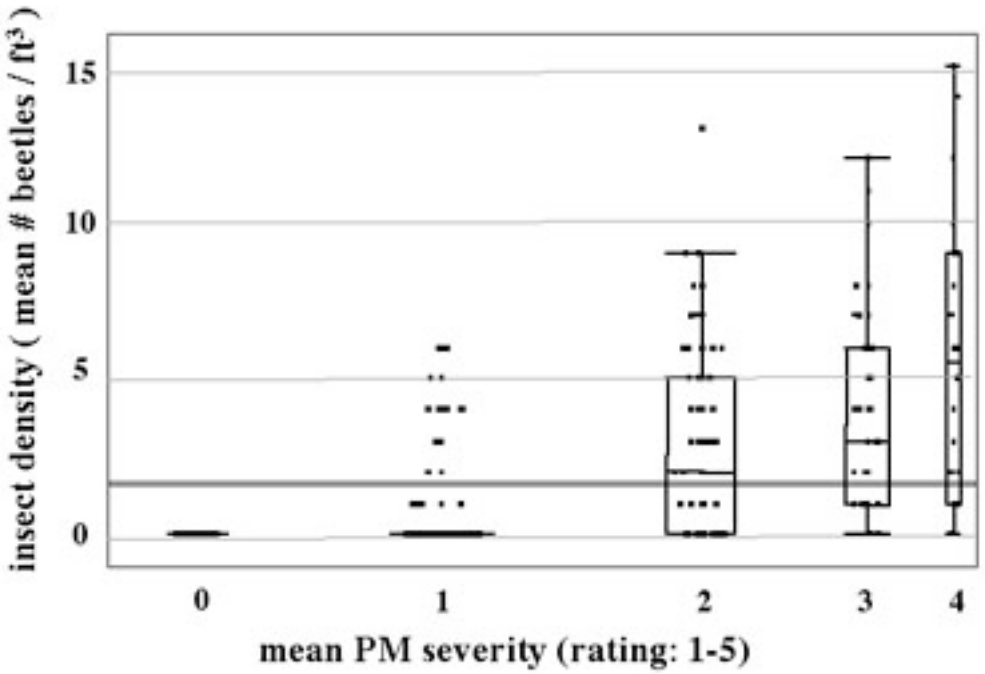

Figure 1. Relationship between powdery mildew severity in Davis, California and the observed density of the native mycophagous coccinellid *Psyllobora vigintimaculata* on 29 different species of urban landscape plants, averaged over time (June 2004-July 2005). Median Test: χ^2 = 83.8, df = 3, P < 0.0001.

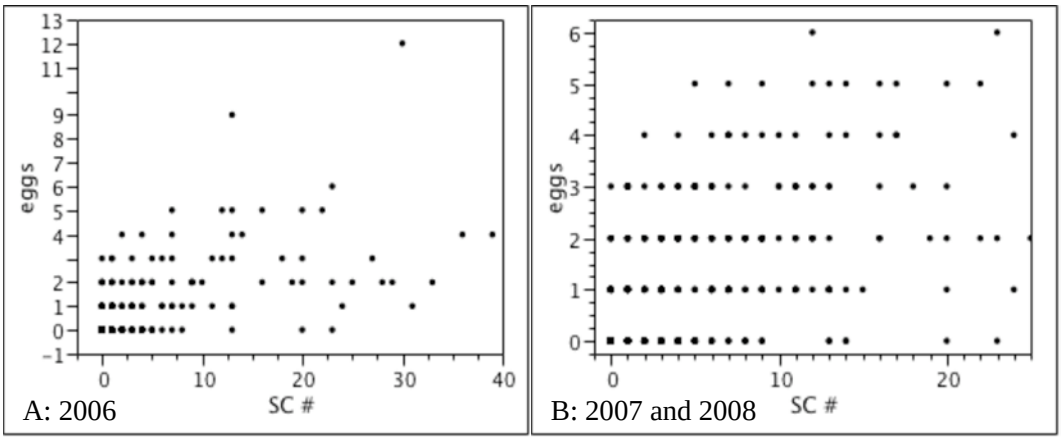

Figure 2. Bivariate relationship between the number of adult *Psyllobora vigintimaculata* caught weekly on yellow sticky cards (SC#) associated with powdery mildew-infected grapevines and the number of *P. vigintimaculata* egg clusters encountered weekly (eggs) on a sampling unit of five (A: 2006) or three (B: 2007 and 2008, combined) fully-expanded leaves. The positive correlations (A: eggs = 0.18 + 0.14SC#, $R^2 = 0.41$, n = 385; B: eggs = 0.14 + 0.18SC#, $R^2 = 0.43$, n = 420) suggest that increasing numbers of trapped adult beetles indicate increasing population density of the insects on the associated grapevines.

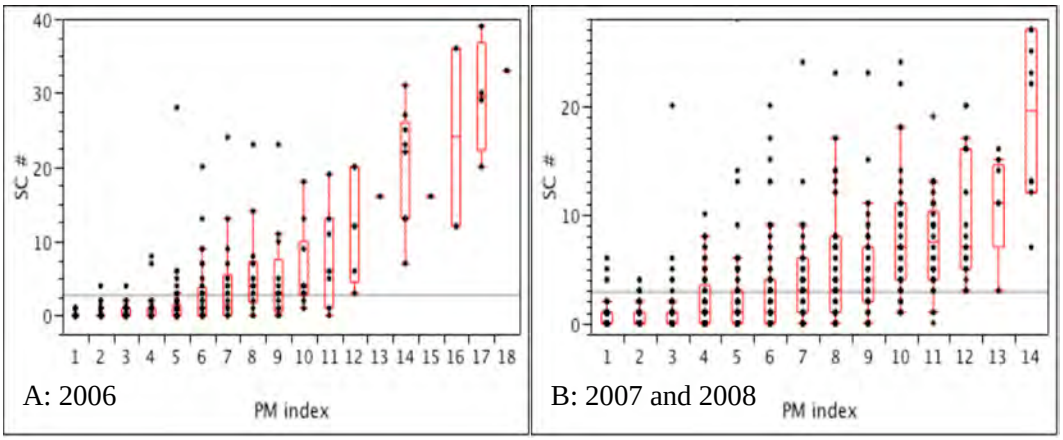

Figure 3. Aggregative numerical response of *Psyllobora vigintimaculata* adults to increasing powdery mildew (PM) severity. The Median Test for nonparametric data illustrates the relationship between PM severity (PM index: scale of zero to 25 in 2006, zero to 15 in 2007 and 2008) and insect density (SC#: number of adult *P. vigintimaculata* caught weekly on yellow sticky cards). In all years there was a significant positive effect of PM severity on insect density (2006: χ^2=102.7, df=17, P<0.0001; 2007 and 2008, combined: χ^2=235.5, df=13, P<0.0001).

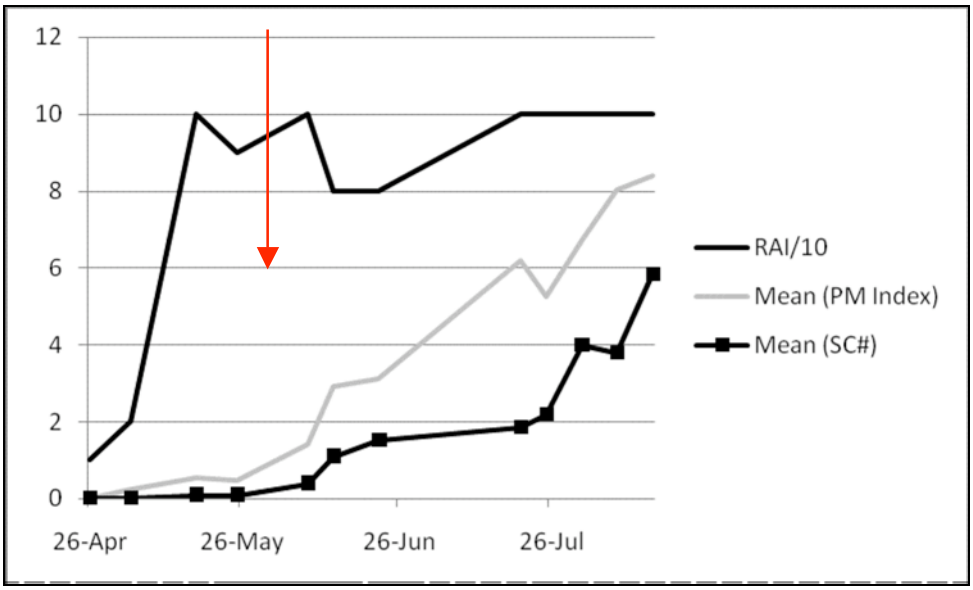

Figure 4. Graphical representation of the seasonal phenology of powdery mildew (PM index: scale of zero to 15) and its consumer *Psyllobora vigintimaculata* (SC#: number of adult beetles caught weekly on yellow sticky cards) on infected grapevines in a commercial vineyard during the 2007 season as compared to predicted disease risk according to the UC Davis powdery mildew risk assessment index model (RAI/10: scale of zero to 100 divided by ten). Due to spring fungicide applications, disease onset was late, and therefore an inoculation with PM conidia (red arrow) was necessary.

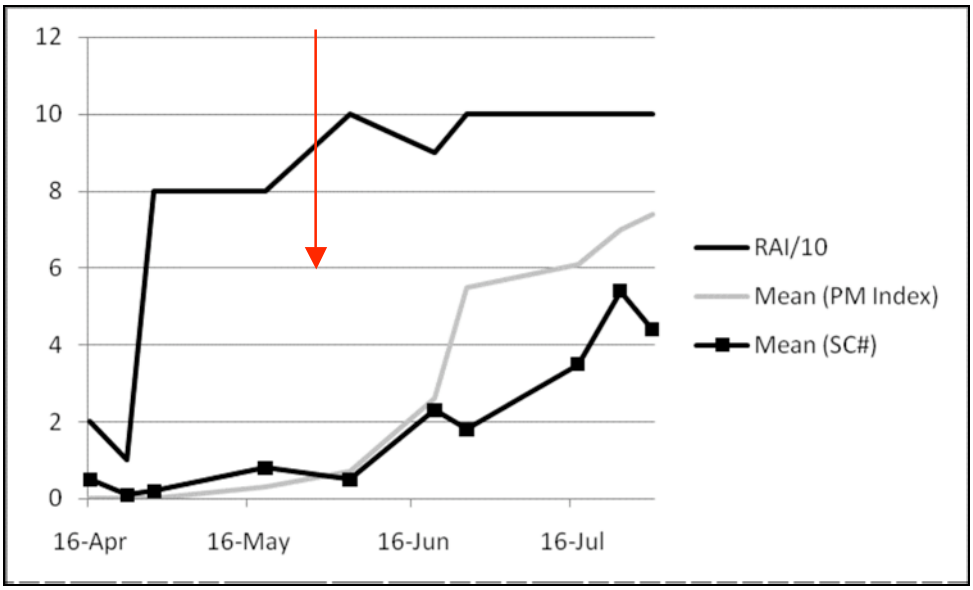

Figure 5. Graphical representation of the seasonal phenology of powdery mildew (PM index: scale of zero to 15) and its consumer *Psyllobora vigintimaculata* (SC#: number of adult beetles caught weekly on yellow sticky cards) on infected grapevines in a commercial vineyard during the 2008 season as compared to predicted disease risk according to the UC Davis powdery mildew risk assessment index model (RAI/10: scale of zero to 100 divided by ten). Due to spring fungicide applications, disease onset was late, and therefore an inoculation with PM conidia (red arrow) was necessary.

CHAPTER 4

Evaluation of the possibility for mechanical transmission of powdery mildew conidia by the obligate mycophage *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae)

(partially published in: Sutherland, A.M., Parrella, M.P., 2009. Mycophagy in Coccinellidae: A Review and Synthesis. Biological Control, in press.)

Abstract. A cosmopolitan coccinellid tribe (Halyziini) is composed of obligate consumers of powdery mildew (PM). A western North American species, *Psyllobora vigintimaculata*, is being evaluated as a biological control agent of PM in greenhouses. An important possible interference with biocontrol in this system may be the possible mechanical transmission of PM conidia from infected to uninfected plants. Many times, fungal pathosystems include arthropods that serve as inadvertant mechanical vectors. Adults and larvae of *P. vigintimaculata* have been observed and photographed with substantial loads of conidia attached to their legs and bodies. Adults are highly mobile, and it is possible that control could be compromised by vectorial activity. A transmission experiment was instituted in growth chambers with directional airflow in which uninfected *Zinnia elegans* were exposed to a small number of infected conspecific plants either in the presence or absence of adult *P. vigintimaculata*. It was hypothesized that if this PM consumer acts as a substantial mechanical vector, then uninfected plants exposed to both PM inoculum and beetles would show a higher and faster infection rate than uninfected plants exposed to inoculum alone. There were no treatment differences in pathogen severity or infection phenology, indicating that any mechanical transmission is overwhelmed by natural aerial transmission. The ramifications of this phenomenon for system ecology and successful control in greenhouses are discussed in detail.

Key words. Powdery mildew, Halyziini, *Psyllobora vigintimaculata*, mechanical transmission

Introduction

The obligate plant parasites known as powdery mildews (PM) (Erysiphales) are collectively considered one of the most important plant-pathogenic fungi in the world in terms of host range, crop damage, and management expense (Amano, 1986; Takamatsu, 2004; Glawe, 2008). Disease phenology is characterized in nature by the release of sexually-produced ascospores from overwintering chasmothecia in the spring, ascospore germination, fungal penetration and infection, the growth of an exterior hyphal mat, asexual reproduction during the growing season, and finally sexual union of hyphal mating types followed by production of new chasmothecia and ascospores in autumn (Glawe, 2008). Sexual union, ascospore production and overwintering many times do not occur in protected systems such as greenhouses, and so transmission and new infections are based solely on asexual reproduction of the fungi (Amano, 1986). The asexual stage of a PM infection involves the abundant production of conidia borne on conidiophores, or spore towers (Glawe, 2008). Transmission in this stage from infected to uninfected plants is primarily mediated by wind, mechanical force (i.e. leaf fluttering) or ambient air movement (Glawe, 2008). After settling upon an appropriate host leaf substrate, and in the presence of specific environmental conditions, the conidia germinate and penetrate the host, initiating a new infection.

The possibility for biological control of PM fungi has been investigated via microbial interference (Chase, 2004), microbial hyperparasitism (Falk et al, 1995), and consumption by arthropods such as tydeid mites (English-Loeb et al, 1999). Recently, coccinellid beetles of the tribe Halyziini, all obligate consumers of PM hyphae and conidia (Sutherland and Parrella, 2009a), have been suggested as augmentative biological control agents in various horticultural systems (Soylu and Yigit, 2002; Krishnakumar and Maheswari, 2004; Cividanes et al, 2007). It

is reasonable to consider that halyziine mycophagous coccinellids, such as *Psyllobora vigintimaculata* Say, foraging directly in asexually-reproducing hyphal mats of PM, may pick up conidial inoculum and serve as mechanical vectors of the pathogen.

A microscopic examination of larvae and adults from a laboratory colony of *P. vigintimaculata* showed conidial chains, individual conidia and hyphal strands adhering to the insects' setae and elytrae (Figure 1). At first, this suggests a possible ecological relationship between insect and pathogen analogous to that of the fungus-farming ambrosia beetles (Curculionidae: Scolytinae and Platypodinae). Typically, these insects inoculate dead wood with xylem-digesting fungal spores and then consume the resulting fungal hyphae. Ambrosia beetles are sometimes considered pests due to their introduction of wood-rotting fungi to living trees (Mueller and Gerardo, 2002). The fungal symbionts are usually saprophytic except for a few phytopathogenic species harbored by phloem-feeding scolytids (Paine et al, 1997). A key characteristic of this system is that the fungi associated with the beetles are true dependent symbionts not found alone in nature. In contrast, viable PM conidia are present in large numbers in the air column, and need no symbiotic vector or "farmer" to initiate new infection.

In a different relationship, larvae of dark-winged fungus gnats (Diptera: Sciaridae) feed on plant-pathogenic fungi, and therefore may contribute to reduction in *Sclerotinia* Fuckel sclerotia (Anas and Reeleder, 1988), but they have also been implicated as important vectors of *Pythium* Pringsh., a facultative parasitic oomycete (Gardiner et al, 1990). In this case, oospores and zoospores remained viable during and after passage through the larva's digestive system while mycelia, the main nutritive source for the insect, were digested. In the Halyziini-PM system the fungal spores are thought to be the nutritive source for the beetles, and therefore should not survive digestion. However, some spores could remain viable; more research is

needed. For instance, Hed et al. (1999) found that the coccinellid *Hippodamia convergens* Guérin-Méneville excreted viable conidia of the fungal pathogen *Discula destructiva* Redlin (dogwood anthracnose) in frass following foraging on infected trees. A similar system involves shore flies (Diptera: Ephydridae) and the transmission of *Thielaviopsis basicola* (Berk. and Broome) Ferraris, a soilborne, root-infecting pathogen. Here there is incidental ingestion of the fungus by flies after feeding on infected plants, passage through the digestive system, and viable, infective chlamydospores recovered in frass (Stanghellini et al, 1999). Insects have also been shown to excrete viable sources of inoculum, thereby serving as inadvertent vectors, in other pathosystems, including verticillium wilt of alfalfa in Canada (Huang and Harper, 1985) and black pod of cocoa in Africa (Evans, 1971).

In the Halyziini-PM system the most logical transmission mechanism to uninfected plants is mechanical via conidia attached to the coccinellid's body. Adults are highly mobile and could conceivably transport spores long distances. Similarly, dogwood anthracnose (see above) conidia are produced within a mucilaginous matrix, and though primarily spread via wind and splashing water, readily adhere to ventral surfaces of *H. convergens*, serving as infective units for up to 16 days under laboratory conditions (Colby et al, 1996). Perhaps the pathogen-vector relationship most analogous to that of the PM-Halyziini system is that of oak wilt, a serious forest and urban disease of oak (*Quercus* spp.) in the eastern and central United States caused by the fungus *Ceratocystis fagacearum* (T.W. Bretz) J. Hunt, and its insect mechanical vectors in the beetle family Nitidulidae. Within infection centers the disease is spread primarily via root grafts between related, adjacent oaks (Appel, 1986), but new infection centers are established through the transport of fungal propagules from fungal mats on oak-wilt-killed trees to fresh wounds on healthy trees by several species of nitidulid sap beetles (Gibbs and Fench, 1980;

Appel et al, 1990; Cease and Juzwik, 2001). This insect-mediated transmission has been shown to be the most important means of new infection in regions where oak assemblages are diverse, and therefore root grafts are rare (Hayslett et al, 2008). Assuming parallels in the PM-Halyziini system, new infection centers could be established as spore-laden mycophagous coccinellids immigrated into healthy (no PM) agricultural or natural ecosystems. A key difference, however, is the aerial transmission of asexual propagules of PM that would occur over long distances regardless of the activity of these insects. Therefore, we hypothesized that PM transmission and infection should progress at a similar rate and to a similar degree with or without the presence of mycophagous coccinellids in the ecosystem. If *P. vigintimaculata* is a mechanical vector of PM, then uninfected plants exposed to both PM inoculum and beetles would show a higher and faster infection rate than uninfected plants exposed to inoculum alone.

Materials and Methods

Four groups of 24 uninfected *Zinnia elegans* Jacquin (cultivar "Peter Pan", Goldsmith Seeds) plants were grown from seed in small containers (4" diameter plastic pots) and irrigated/fertigated via drip emitters in two divided growth chambers (each division ~ $1m^2$) with directional (vertical) airflow in a static environment (25^oC, 80% RH, 18 hour photoperiod). In a separate chamber, a colony of *Erysiphe cichoracearum* Jacz., the PM fungus infecting Asteraceae, was maintained on containerized (4" diameter plastic pots) zinnia plants in an environment conducive to PM germination, infection and growth (sinusoidal daily temperature and humidity curves; from 15^oC coupled with 90% RH to 30^oC coupled with 50% RH, daily ebb-and-flood irrigation, 12 hour photoperiod). A portion of the infected plants was removed weekly from this colony as food for a caged laboratory population of *P. vigintimaculata,* and was

then replaced with newly-germinated uninfected zinnia plants in order to maintain the pathogen in culture via aerial transmission of conidia. Three weeks after germination, five uninfected plants in each chamber division were replaced with conspecific zinnia plants of the same age from the PM colony, uniformly infected with PM. Chamber divisions were then randomly assigned to receive either zero or 25 adult *P. vigintimaculata*; washed with deionized water (beetles stirred as a group with sterile glass rod for two minutes in ~250 ml) to remove PM conidia from the laboratory cage culture and released on hanging platforms suspended 0.5m above the plants. This release rate aimed to introduce a sufficient number of insects as to allow for adequate movement and transmission opportunity within the chamber while minimizing PM consumption. The short duration of the experiment ensured that late instar larvae, which have been shown to be the most effective PM consumers (Sutherland and Parrella, 2006), would not be present. Air movement in the chambers was from floor to ceiling, and was assumed to allow for normal aerial transmission of PM. Insects were allowed to disperse naturally, and plants were retained within the chamber divisions for three weeks.

Software based on image analysis algorithms (Assess Image Analysis Software for Plant Disease Quantification, The American Phytopathological Society, 2002), coupled with digital photography, was used to quantify PM on *Zinnia* plants throughout the study. Beginning with the introduction of inoculum (infected conspecific plants), a digital camera was used to capture weekly images of each plant. The described software was then used to separate visible PM from uninfected leaf tissue based on pixel saturation (Lamari, 2005) within the images, and then to express disease as the percentage of leaf area covered by visible PM colonies (%PM). Response variables included %PM as a measure of PM severity at each sampling event, overall growth ($\%PM_{final}$-$\%PM_{initial}$), and percentage increase ($\%PM_{final}/\%PM_{initial}$). Each chamber division was

viewed statistically as one experimental unit, and each plant as a subsample within that unit. The data were analyzed for a treatment effect using ANOVA and a mixed hierarchical model, with subsample as a random variable nested within the appropriate chamber division, and with two chamber divisions per treatment, as replications in space (Hicks, 1982; Zar, 1996). All analyses were performed with JMP 8 Statistical Software (SAS Institute, 2008).

Results and Discussion

There were no significant differences between the two treatments in terms of %PM at the time of inoculation and insect release ($F=0.70$, $df=1$, $P=0.41$), indicating that the level of disease pressure was similar for all chamber divisions. After three weeks, there were still no significant treatment differences ($F=0.0001$, $df=1$, $P=0.99$), and the mean chamber %PM was almost identical (13.79 for untreated chambers and 13.82 for chambers with 25 beetles). In fact, no treatment differences were detected during the entire three week trial (Figure 2). Likewise, there were no significant differences in terms of disease progression, measured both as overall growth ($F=0.006$, $df=1$, $P=0.94$) and percentage increase ($F=0.175$, $df=1$, $P=0.68$). Subsample data illustrated PM transmission from infected plants to those directly adjacent in the first two sampling events followed by widespread and near uniform infection in the final sampling event. These results suggest that if mechanical transmission did occur in this small experimental system it was overshadowed by natural aerial transmission via airborne conidia. However, since this experiment only addressed very short-range transmission under very specific conditions, more research is needed in this important area. For instance, if spore-laden beetles were introduced as the only source of inoculum onto entirely uninfected plants, the possibility for any mechanical transmission in this system could be assessed. The same number of spores could be introduced

aerially into similar plots of uninfected plants in order to compare the relative efficacy of the two transmission systems. This would mimic the possibility in nature, analogous to pathosystems such as oak wilt (Hayslett et al, 2008) and dogwood anthracnose (Hed et al, 1999), wherein local transmission involves root grafts or splashing water, respectively, but insect vectors serve to initiate new infection centers via mechanical transmission. This relationship seems unlikely in PM pathosystems given that *P. vigintimaculata* was rare in varied urban landscapes without established PM (Sutherland, 2005), and rarely associated with uninfected grapevines in a commercial vineyard (see Chapter 3). There seems to be no biological motivation for such obligate mycophages to visit plants without established PM. Germinating PM conidia require 1-2 weeks to develop into a hyphal mat in California grapevines, depending on temperature (Delp, 1954), and *P. vigintimaculata* eggs should hatch within five days of oviposition (Sutherland and Parrella, 2009b). The hypothetical juxtaposition of these two events creates a situation in which neonate larvae are without substantial food upon eclosion. Appropriately, during two years of landscape sampling, *P. vigintimaculata* eggs were never found in the absence of an established PM infection (Sutherland, 2005). Furthermore, PM species are very host specific and dependent upon specific environmental conditions (Amano, 1986). Mycophagous coccinellids have been shown to have a wide preference for PM fungi (Ahmad et al, 2003; Sutherland and Parrella, 2009a), suggesting that viable conidia on immigrant insects may rarely be crop-specific and therefore pathogenic.

Literature Cited

Ahmad, M., Younis, G., Ali, N., 2003. Biology of the coccinellid *Psyllobora (Thea) bisoctonotata* Muls. (Coleoptera: Coccinellidae) a predator of powdery mildew fungi. Proceedings of the Eighth Arab Congress of Plant Protection, Elbeida City, Libya.

Amano, K., 1986. Host range and geographical distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo.

Anas, O., Reeleder, R.D. 1988. Feeding habits of larvae of *Bradysia coprophila* on fungi and plant tissue. Phytoprotection 69(2): 73-78.

Appel, D.N., 1986. Recognition of oak wilt in live oak. Journal of Arboriculture 12, 213-218.

Appel, D.N., Kurdyla, T., Lewis, R., 1990. Nitidulids as vectors of the oak wilt fungus and other *Ceratocystis* spp. in Texas. European Journal of Forest Pathology 20, 412-417.

Cease, K.R., Juzwik, J., 2001. Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. Canadian Journal of Forest Research 31, 635-643.

Chase, A.R., 2004. Making the grade: New fungicides for ornamentals. Society of American Florists Proceedings for 20th Annual Conference on Pest Management on Ornamentals, 1-5.

Cividanes, T.M., Cividanes, F.J., Matos, B.A., 2007. Biologia de *Psyllobora confluens* alimentada com o fungo *Erysiphe cichoracearum*. Pesquisa Agropecuaria Brasileira, Brasilia 42, 1675-1679.

Colby, D.M., Windham, M.T., Grant, J.F., 1996. Transportation and viability of conidia of *Discula destructive* on *Hippodamia convergens*. Plant Disease 80, 804-805.

Delp, C., 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44, 615-626.

English-Loeb, G., Norton, A., Gadoury, D.M., Seem, R.C., Wilcox, W.F., 1999. Control of powdery mildew in wild and cultivated grapes by a tydeid mite. Biological Control 14, 97-103.

Evans, H.C., 1971. Transmission of *Phytophthora* pod rot of cocoa by invertebrates. Nature 232, 346-347.

Falk, S.P., Gadoury, D.M., Cortesi, P., Pearson, R.C., Seem, R.C., 1995. Partial control of grape powdery mildew by the mycoparasite *Ampelomyces quisqualis*. Plant Disease 79, 483-490.

Gardiner, R.B., Jarvis, W.R., Shipp, J.L. 1990. Ingestion of *Pythium* spp. by larvae of the fungus gnat *Bradysia impatiens* (Diptera: Sciaridae). Annals of Applied Biology 116(2): 205-212.

Gibbs, J.N., French, D.W., 1980. The transmission of oak wilt. United States Department of Agriculture Forest Service Research Paper NC-185.

Glawe, D.A., 2008. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. Annual Review of Phytopathology 46, 27-51.

Hayslett, M., Juzwik, J., Moltzan, B., 2008. Three *Colopterus* beetle species carry the oak wilt fungus to fresh wounds on red oak in Missouri. Plant Disease 92, 270-275.

Hed, B.E., Windham, M.T., Grant, J.F., 1999. Survival of conidia of *Discula destructiva* in frass of the convergent lady beetle. Plant Disease 83: 806-809.

Hicks, C.R., 1982. Fundamental concepts in the design of experiments. CBS College Publishing, New York, NY.

Huang, H.C., Harper, A.M., 1985. Survival of *Verticillium albo-atrum* from alfalfa in feces of leaf-chewing insects. Phytopathology 75, 206-208.

Krishnakumar, R., Maheswari, P., 2004. Management of powdery mildew in mulberry using coccinellid beetles, *Illeis cincta* (Fabricius) and *Illeis bistigmosa* (Mulsant). Journal of Entomological Research 28, 241-246.

Lamari, L., 2005. ASSESS: Image analysis software for plant disease quantification. American Phytopathological Society Press, St. Paul, MN, USA.

Mueller, U.G., Gerardo, N. 2002. Fungus-farming insects: Multiple origins and diverse evolutionary histories. Proceedings of the National Academy of Sciences 99(24): 15247-15249.

Paine, T.D., Raffa, K.F., Harrington, T.C., 1997. Interactions among scolytid bark beetles, their associated fungi, and live host conifers. Annual Review of Entomology 42: 179-206.

Soylu, S., Yigit, A., 2002. Feeding of mycophagous ladybird, *Psyllobora bisoctonotata* (Muls.) on powdery mildew infested plants. Biological Control of Fungal and Bacterial Plant Pathogens, IOBC-WPRS Bulletin 25, 183-186.

Stanghellini, M.E., Rasmussen, S.L., Kim, D.H., 1999. Aerial transmission of *Thielaviopsis basicola*, a pathogen of corn-salad, by adult shore flies. Phytopathology 89, 476-479.

Sutherland, A.M., 2005. Evaluation of *Psyllobora vigintimaculata* (Say) (Coleoptera: Coccinellidae) for Biological Control of Powdery Mildew Fungi (Erysiphales). M.S. thesis, University of California, Davis.

Sutherland, A.M.; Parrella, M.P., 2006. Quantification of Powdery Mildew Consumption by a Native Coccinellid: Implications for Biological Control? Proccedings of the Fifth Annual California Conference on Biological Control (CCBC V), 25-27 July 2006, Riverside, California.

Sutherland, A.M., Parrella, M.P., 2009a. Mycophagy in Coccinellidae: A Review and Synthesis. Biological Control, in press.

Sutherland, A.M., Parrella, M.P., 2009b. Biology and co-occurrence of *Psyllobora vigintimaculata* (Coleoptera: Coccinellidae) and powdery mildews in an urban landscape of California. Annals of the Entomological Society of America, in press.

Takamatsu, S., 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. Mycoscience 45, 147-157.

Zar, J.H., 1996. Biostatistical analysis, 3rd edition. Prentice-Hall Inc., Upper Saddle River, NJ.

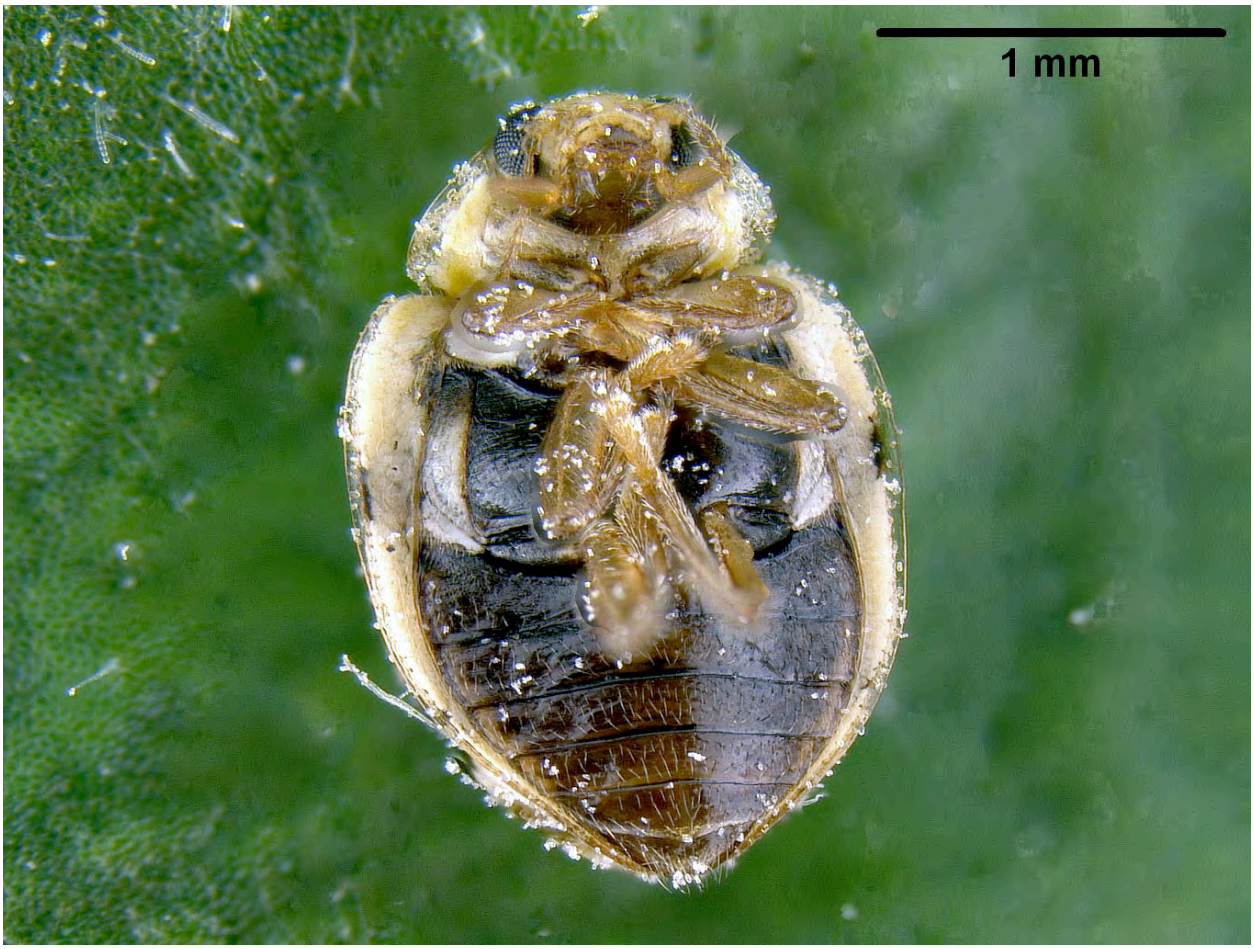

Figure 1. Detailed montage photograph of the ventral aspect of an adult *Psyllobora vigintimaculata*, showing an abundance of powdery mildew conidia and hyphae adhering to the insect after foraging in PM hyphal mats.

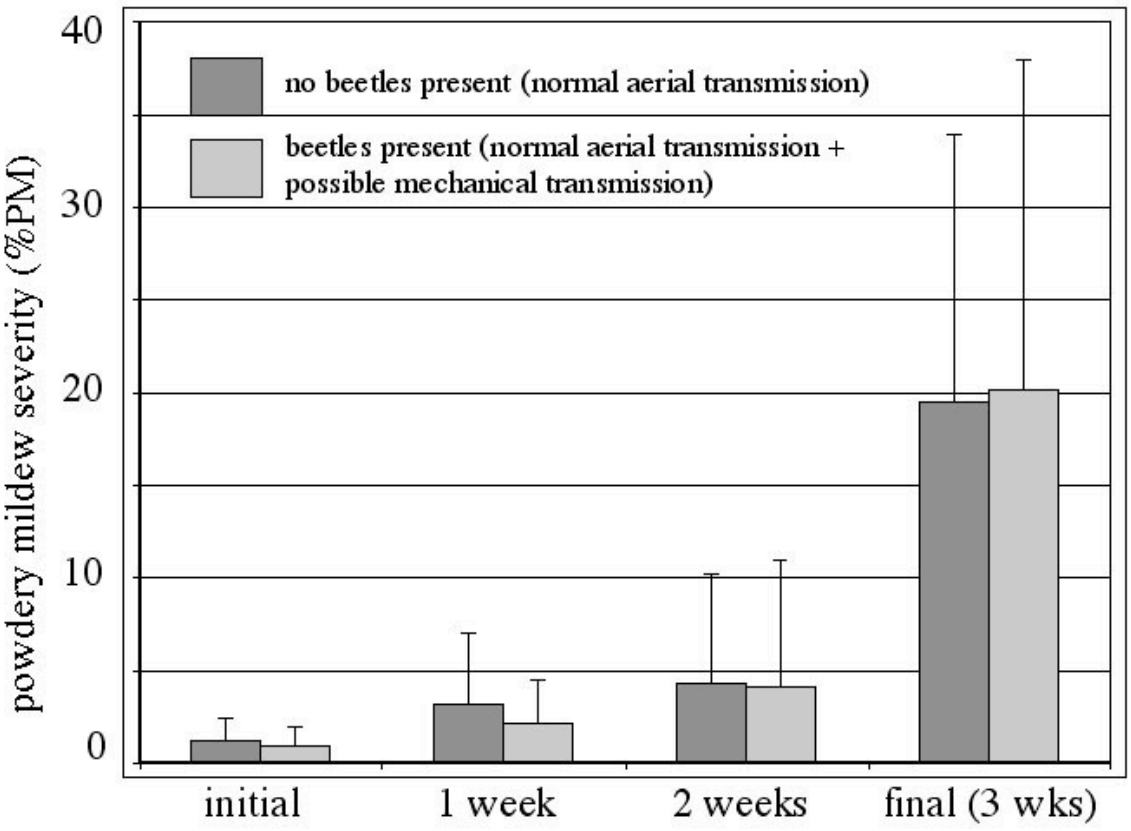

Figure 2. Transmission of powdery mildew (PM) in divided growth chambers, as expressed by severity (%PM) over time in groups of *Zinnia elegans* "Peter Pan" after the introduction of conspecific plants infected with the PM *Erysiphe chicoracearum* either in the presence or absence of adult mycophagous beetles, *Psyllobora vigintimaculata*. No significant treatment effect (F=0.006, df=1, P=0.94) on overall growth was detected through a mixed hierarchical model ANOVA (24 subsamples nested within each treatment).