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Improving Sampling Methods and Biological Control for *Oligonychus perseae* (Acari: Tetranychidae) on 'Hass' Avocados (*Perseae americana*) in Southern California

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by

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DEDICATION

To my parents, Alirio and Elsa, for always being there for me.

ABSTRACT OF THE DISSERTATION

Improving Sampling Methods and Biological Control for *Oligonychus perseae* (Acari: Tetranychidae) on 'Hass' Avocados (*Perseae americana*) in Southern California

by

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Oligonychus perseae Tuttle, Baker and Abattiello (Acari: Tetranchidae) is a foliar spider mite pest of avocados, *Persea americana* Miller (Lauraceae), and both are native to Mexico. In California, *O. perseae* can cause significant premature defoliation to Hass avocados which is most important commercial avocado cultivar in the world market. Recent work form Israel has shown that extensive foliar injury caused by populations of *O. perseae* on Hass foliage can translate into fruit yield reduction. Consequently, there is warranted concern over the damage from *O. peseae* populations in other Hass producing areas such as Spain, Israel, and Costa Rica, but also within its home range in Mexico.

In California, great effort has been placed in developing and implementing an integrated pest management program for *O. perseae*, but improvements are still needed for effective control of this pest in commercial Hass orchards. In particular, the success of releases of commercially-available phytoseiids as part of an inoculation biological control program has been limited, the role of resident phytoseiids for control of *O. perseae* is not well understood, and effective research-based sampling plans to monitor the activity of *O. perseae* and resident phytoseiids are not available. Consequently, the motivation for conducting the work presented in the five chapters of this dissertation was to address these limitations.

The first chapter provides insight into the trophic interactions between *O. perseae* and natural enemies in California Hass avocados. This information can be used to improve the current biological control program of *O. perseae* in California and potentially other avocado systems where this spider mite occurs. The second chapter examines the limitations of the current sampling guidelines recommended for assessing *O. perseae* densities. The third chapter focuses on the development and validation of a binomial sampling plan for *O. perseae* that minimizes the counting effort and could be used by pest control advisers to monitor levels of this pest in commercial orchards. The fourth chapter compares the performance of enumerative and binomial sampling plans to monitor populations of resident phytoseiids that feed on *O. perseae*. Finally, the fifth chapter examines the role of resident phytoseiid populations in controlling field populations of *O. perseae* over a 10-year period.

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Chapter 1

Past, Present, and Future: Biological Control of Spider Mites on California Avocados

ABSTRACT *Oligonychus perseae* Tuttle, Baker and Abatiello (Acari: Tetranychidae) is a foliar pest of avocados, *Persea americana* Miller (Lauraceae), and both species are native to Mexico. Damaging *O. perseae* populations can occur in areas of the world where the Hass avocado cultivar is grown commercially, including California (USA), Costa Rica, Spain, and Israel. In California, the efficacy of biological control agents, including well studied predators such as *Euseius hibisci* (Chant) and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae), for management of *O. perseae* populations in commercial orchards is limited. This present situation differs from the successful biological control of introduced heterospecifc tetranychids on California avocados during previous decades. Using California avocados as a model system, potential key factors responsible for the limitations *O. perseae* biological control are discussed. One key factor that accounts for prevalence of *O. perseae* infestations on California avocados is the increasing distribution of cultivars that are highly susceptible to *O. perseae* feeding. Other attributes contributing to cultivar susceptibility could potentially include the limited searching ability of tetranychid predators on *O. perseae* infested avocado trees. Additionally, some life history traits of phytoseiids that have been used to target *O. perseae* populations likely restrict their utility as effective biological control agents for this pest in the California avocado system. Future research directions for improving *O. perseae* biological control strategies are discussed. Among these is the prospect of using members of the family Stigmaeidae that occur naturally on avocados but whose potential as biological control agents has not been well studied.

KEY WORDS *Oligonychus perseae*, spider mites, avocado, Phytoseiidae, biological control

1.1 Introduction to Spider Mite Biological Control on California Avocados

Avocado, *Persea americana* Miller (Laureaceae) originated in southern Mexico from where it was dispersed and domesticated by humans over thousands of years throughout Mesoamerica (Bost et al. 2013). As a result of avocado domestication by humans in its home range, contemporary cultivars of *P. americana* are grouped among three botanical varieties (i.e., races, or ecotypes) commonly known as Mexican (*P. americana* var. *drymifolia* Blake), Guatemalan (*P. americana* var. *guatemalensis* Williams) and West Indian (*P. americana* var. *americana* Miller) (Bost et al. 2013). These three broad varieties differ not only in growth form, and in fruit nutrient composition traits, but also in their physiological adaptation to different climates. Avocado cultivars associated with Mexican, Guatemalan and West Indian varieties are best suited to semitropical, subtropical and tropical climates, respectively (Wolstenholme 2013). These inherent climatic adaptations have significant bearing on where in the world cultivars associated with these varieties can be grown successfully for commercial production.

However, cultivars from the three botanical varieties can hybridize naturally through cross-pollination and produce hybrid-cultivars with new combinations of 'variety' traits (Lahav and Lavi 2013). Furthermore, new cultivars can also develop as offspring from avocado seeds because avocado seedlings do not breed true-to-type. With modern advances in agricultural science and growing commerce, this natural process of avocado reproduction created incentives for conducting foreign exploration in Mesoamerica and establishing on-going breeding programs for the propagation and distribution of novel marketable cultivars (Crane et al. 2013). In this manner, avocado cultivars have been purposefully introduced and propagated by humans at an accelerated rate in new regions with amenable avocado growing climates where this crop did not previously occur during pre-Columbian times (Bost et al. 2013). For example, during the 19th century, avocados were introduced by humans from its native home range to southern California (USA). With growing demand and economic interest for producing exotic avocado fruit, initial commercial avocado production in southern California began in 1908 (Shepherd and Bender 2002). Since then commercial avocado production in California has expanded to cover approximately 23,406 hectares and is currently valued at $435 million (CAC 2014).

The commercial avocado system in California has been established over an extremely brief period, compared to the evolutionary time frame of avocado domestication in its home range, and various aspects of its history in California have been well documented in the literature. The availability of this information provides an opportunity to track the development and progression of past and present pest problems on California avocados. This investigative process can generate valuable insight as to how pest control strategies might be improved. The primary purpose of this chapter is to understand how specific problems related to foliar feeding spider mite (Acari: Tetranychidea) populations on California avocados progressed over time since this host plant was introduced and how these spider mite problems might be resolved as part of future efforts relying on the sustainable use of predators (i.e., biological control).

There are at least five common foliar-feeding spider mite species (Acari: Tetranychidae) that occur on avocados in the world. These include *Oligonychus punicae*

(Hirst), *O. perseae* Tuttle, Baker and Abattielo, *O. coffeae* Nietner, *O. yothersi* McGregor, and *Eotetranychus sexmaculatus* (Riley) (Peña et al. 2013). Presently, California has three of these exotic species; *O. punicae*, *E. sexmaculatus* and *O. perseae*. Since the 1950s, extensive research efforts were invested in controlling populations of spider mites on California avocados by strategizing the use of candidate beneficial predators under the framework of managed biological control programs. These management strategies stem from three general biological control types known as conservation, augmentation and classical biological control (Van Driesche et al. 2008, Hoy 2011).

Conservation biological control modifies environmental or cultural practices to promote the performance of resident predator populations. One example of conservation biological control includes the provision of supplemental food resources like pollen to boost predator numbers during key periods of the growing season thereby preventing densities of the target pest from exceeding economic thresholds and causing crop damage. Pollen can be deployed mechanically or made accessible by planting cover crops adjacent to avocado trees (Maoz et al. 2011a).

Augmentative biological control involves the supplemental release of predators into the intended crop habitat to boost or augment populations of existing predators thereby increasing their numbers in advance of anticipated pest outbreaks. Suppression results from the short term establishment and population buildup of the released natural enemies. Effective predators of the target pest can be mass-reared and released in great numbers (inundative releases) when target pest densities are elevated and require immediate control. Establishment and subsequent reproduction of released predators is not expected to occur from inundative releases. Alternatively, a small number of predators can be released into the crop habitat early in the season (inoculative releases of biological control agets) so that they can establish temporarily and provide seasonal control for target-pest populations. In general, both conservation and augmentative biological control strategies require input of resources by humans to maintain on-going efficacy at times when pest control is needed.

In contrast, the endpoint of classical biological control, or introduction biological control, is to deliberately establish permanent, self-sustaining control by reuniting the target pest species (often an invasive species causing economic damage) with its key co-evolved natural enemies from its home range (Van Driesche et al. 2008). Key predator species, for example, are identified as those whose populations can maintain, in a density-dependent manner, populations of the target pest in its home range at sub-economic levels. Consequently, the underlying premise of classical biological control is that populations of the target pest have (1) become unmanageable in a new geographic area due to the absence of its co-evolved key predator(s) and (2) effective natural control regulation provided by the available natural enemy community in the invaded area is lacking. One of the essential requirements for designing a successful classical biological control program is to determine the home range of the introduced target pest species (Van Driesche et al. 2008). In previous decades obtaining this information was difficult because there were few objective methods and established theoretical principles by which to determine with confidence the precise geographic origin of exotic pest species found in a new area. As will be discussed, this deficiency in technology became a limiting factor while conducting classical biological control efforts against *O. punicae* on avocados in California. Molecular DNA-based tools have helped overcome this obstacle and this scientific advancement has had tremendous implications in designing classical biological control programs for economically important pests (Van Driesche et al. 2008), including spider mites such as *O. perseae*.

All three types of biological control (i.e., conservation, augmentative, and classical) have been considered or implemented with varying degrees of success for spider mite control in the California avocado system using natural enemies from the mite family Phytoseiidae. The Phytoseiidae contains approximately 1,700 described species among 50 genera (Gerson et al. 2003) and its members display abundant diversity in feeding habits and life history traits (McMurtry and Croft 1997, Gerson et al. 2003, McMurtry et al. 2013). Like spider mites, phytoseiids can inhabit plants, but ecologically phytoseiids function primarily as predators of arthropods, like insects, and some phytoseiid species can feed selectively on spider mite species. For this reason phytoseiids have been used preferentially in spider mite biological control programs. Nevertheless, the compatibility between the life history traits of the target pest and the candidate phytoseiid species on a designated host plant system must be carefully assessed. This implies that the precise application of a phytoseiid species under the framework of biological control will depend on its unique ecology within the intended cropping system at a particular location.

Traditionally, the limited success of phytoseiid-based spider mite control on California avocados has been attributed to the incompatibility between prey-predator life history traits and/or intraguild predation among potential spider mite predators. However, growing evidence suggests that avocado host-plant susceptibility to spider mites like *O. perseae* (Kerguelen and Hoddle 2000) may have also played a significant role in creating the need for effective spider mite control. This complex dynamic, which is affected by avocado cultivar, is not readily apparent and has to be traced through time. To provide insight into this intricate problem in the context of spider mites on California avocados, this chapter has been divided among six related subsections.

First, historical concerns regarding the introduction of exotic avocado pests in California, including insects, are outlined. Second, the historical economic importance of introduced spider mite species (i.e., *O. punicae* and *E. sexmaculatus*) and the success of their biological control programs are reviewed. Third, evidence regarding the introduction of *O. perseae* into the California avocado system from its home range and its general biology are presented. Fourth, the emergence of *O. perseae* as the key foliar pest of avocados in California is discussed. The development of this phenomenon is discussed from two angles: (1) avocado cultivar susceptibility and (2) the ecological limitations of candidate phytoseiids that are either naturally-occurring or have been introduced into avocado orchards for biological control of *O. perseae*. Factors associated with cultivar susceptibility are attributed to acreage expansion and shifts in cultivar acreage. These components are inter-related and their underlying influence has made attaining effective spider mite biological control difficult. Subsequently, the ecological limitations of *O.*

perseae natural enemies is discussed in a fifth section. Finally, the discussion transitions to a sixth section that outlines future prospects for spider mite biological control on California avocados. The previous five sections create a platform on which to discuss future advancements for tetranychid control in California avocados. Among these is the prospective use of understudied predatory mite families such as the Stigmaeidae. Members of this predator group have not been considered as spider mite biological control agents on California avocados, despite their documented natural presence on avocados in California and other avocado growing areas throughout the world.

1.2 Historical Concerns with Avocado Pests in California

California is in close geographic proximity to avocado production areas in Mexico. Therefore, the threat of introductions of avocado-feeding arthropods from their home range into California due to growing commerce (legal and illegal) in fresh fruit and plants has existed ever since this crop was introduced to California. In response, quarantine measures were implemented early on to prevent the unwanted entry of exotic avocado pests into California from the home range of avocado. The main concern was with organisms that feed directly on avocado fruit. In 1914, the United States Department of Agriculture restricted the entry of fresh avocado fruit from Mexico to protect the growing California avocado industry (and Florida which also grows avocados) from the introduction of the injurious avocado seed weevil, *Heilipus lauri* Boheman (Coleoptera: Curculionidae). Later in 1973, the economic justifications for quarantine restrictions on avocado fruit importation from Mexico were expanded to prevent the introduction of other potentially serious avocado fruit pests such as the avocado seed moth, *Stenoma catenifer* Walsingham (Lepidoptera: Elachistidae), and other weevil species in the genus *Conotrachelus* that can attack avocados (Bellamore 2003). Due to changes in international trade agreements (e.g., NAFTA in 1994), the restrictions on fruit importation were gradually lifted during the late 1990s to allow importation of avocado fruit from approved pest-quarantined avocado production areas in Mexico into the United States (Bellamore 2003). After 2007, avocados were allowed into all U.S. states, including California, and this generated concern regarding the potential establishment of exotic species in California such as armored scales (Hemiptera: Diaspididae) which were detected on imported fresh fruit from Mexico (Morse et al. 2009).

Although these quarantine measures may have temporarily reduced the probability for the introduction of avocado feeding arthropods from Mexico, the species diversity of the California avocado arthropod fauna and its geographical distribution within the state grew throughout the 20th century. Early records of arthropods associated with avocado were documented in the periodicals of the California Avocado Society which was established in 1915. This increase in arthropod diversity was most likely the result of three factors. First, new ecological associations were formed between native generalist herbivore species and newly established avocado host plants in California. One example of this is the California-native moth *Amorbia cuneana* (Walsingham) (Lepidoptera: Tortricidae) (Oatman and Platner 1985, Gilligan et al. 2009) whose larvae feed on several plant species from several families including *Umbellularia californica*, a native species in California that, like avocados, is also from the family Laureaceae.

Secondly, generalist herbivores introduced with other crops from other areas of the world may have formed new associations with avocado in various avocado growing regions. One example of this is the polyphagous scale *Hemiberlesia lataniae* (Signoret) (Hemiptera: Diaspididae) which can feed on host plants representative of 78 families and has been recorded on avocados in several countries (Peña et al. 2013). A more recent example that demonstrates the warranted concern for the development of new-host associations in California between exotic avocados and an exotic pest species is provided by the polyphagous shot hole borer, *Euwallacea* sp. (Coleoptera: Curculionidae: Scolytinae), which was detected in California in 2003. This invasive beetle is native to Asia and has symbiotic relationship with a fungus (*Fusarium* sp.) that is transmitted by the beetle to host plants during the construction of galleries inside host-plant woody tissue. Fungal infection of vascular tissue in host-plants can produce branch die-back and eventual tree death. The attack host range of this beetle in California include 253 species (11 are native to California) that are representative of 58 plant families. 54% of host plant species attacked by polyphagous shot hole borer, including avocados, were infected with the fungus carried by this beetle (Eskalen et al. 2013). This pest has also been recorded attacking avocados in Israel. Last but not least, before there were clear organized efforts to prevent the entry of avocado pests and to document their presence, it is possible that more specialized avocado feeding arthropods, including their natural predators, may have been inadvertently introduced from Mesoamerica on avocado breeding material collected from these areas (Moznette 1922). Foliar feeding spider mites and predatory mites associated with avocado in Mexico are some examples of this third factor which will be discussed throughout this chapter.

Most likely, the initial presence of these insect and mites remained undetected or given minor importance on California avocados. This changed as avocado became a popular specialty crop and the expansion of commercial avocado acreage provided a permanent seasonal habitat for populations of native and introduced arthropod species, capable of feeding and reproducing on commercial avocados in California (McLean 1932, Quayle 1933, McKenzie 1934). At the same time, on-going developments on taxonomic, biological and species-inventory research created opportunities to document the presence and understand the role (e.g., beneficial predators/ herbivore pest) of populations of introduced and native species occurring within the California avocado system (Boyce 1947, Ebeling and Pence 1953, Ebeling and Pence 1958). For example, prior to 1990, the two foliar-feeding spider mite species whose presence made commercial California avocado growers wary were *O. punicae* and *E. sexmaculatus* (Riley). Populations of *O. punicae* were first noticed infesting California avocados during the 1920s (McGregor 1942) while *E. sexmaculatus* populations were first noticed during the 1950s (Ebeling and Pence 1953). The detection of these exotic spider mite species on avocados occurred roughly 60-90 years after the earliest recorded presence of avocados in California in 1856 (Shepherd and Bender 2002). The precise origin and manner of introduction of these two species into California has not been confirmed.

1.3 Economic Importance of Early Spider Mites Associated with California Avocados

As spider mites, motile *O. punicae* and *E. sexmaculatus* individuals use cheliceral stylets to feed on the cell contents of tissue from top surface (adaxial) and undersurface (abaxial) of avocado leaves, respectively (Bailey and Olsen 1990). In general, motile stages of these species can also be distinguished by their characteristic body coloration, location of webbing construction on leaf surfaces and visual feeding damage. Motile *E. sexmaculatus* have a yellow-green appearance and produce complicated webbing, defined as three-dimensional and irregular (Saito 1983). Feeding damage caused by populations of this species is detected as brown-purple discoloration on the leaf undersurface. *O. punicae* individuals have a dark red-brown appearance, produce complicated webbing, and feeding damage caused by populations of this species is visible as bronze discoloration on the top leaf surface (UCANR 2008).

Historically, these spider mites have had different levels of economic importance. Low densities of *E. sexmaculatus*, 5-10 mites per leaf, can induce pre-mature leaf drop (Bailey and Olsen 1990), but California populations of this species are mainly confined to coastal areas, particularly Santa Barbara and San Luis Obispo (UCANR 2008). In these areas avocado acreage is not well represented. In addition, warm weather has been thought to prevent populations from becoming elevated in the more arid inland avocado growing areas of southern California (UCANR 2008). Therefore, in California, *E. sexmaculatus* is not considered a key pest of avocados. However, under a different set of avocado growing and climatic conditions like in New Zealand, *E. sexmaculatus* is considered a key pest of avocados (Tomkins 2002, White 2002, Jamieson and Stevens 2007). In contrast, *O. punicae* populations have been recorded throughout the California avocado growing region and densities in this region can naturally build to several hundred mites per leaf (McMurtry and Johnson 1966). On Hass avocado, feeding by *O. punicae* populations on foliage was shown to be negatively correlated with stomatal and mesophyll conductance, photosynthesis, and transpiration rates on leaves (Sances et al. 1982). Continuous feeding from *O. punicae* populations in the approximate range of 50-70 mites per leaf can also cause premature leaf drop but whether this damage influences avocado tree fruit yield has not been determined (Sances et al. 1982). Even so, concern in California about foliar damage caused by *O. punicae* and *E. sexmaculatus* populations declined over decades as new information became available from a large body of biological control studies designed to target spider mite populations using candidate species from families of predatory beetles (Coccinelidae) and mites (Phytoseiidae) that are known to be economically important because they can control pest populations (Flint and Dreistadt 1998).

1.3.1 Success of Early Spider Mite Biological Control Efforts on California Avocados

Avocado field studies conducted during the 1950s suggested that populations of *O. punicae* and *E. sexamaculatus* in California were maintained under natural control by a guild of native predators and/or predators that originated from the home range of avocado (Fleschner et al. 1956a, Fleschner 1958, McMutry and Johnson 1966). Most importantly, these studies revealed that spider mite biological control agents differed in their degree of efficacy. For example, *O. punicae*, which is a pest of avocados in Mexico (Peña et al. 2013), was shown to be under natural control by resident populations of the beetle *Stethorus picipes* Casey (Coleoptera: Coccinellidae) in California. *S. picipes* is a specialized predator of spider mites, as are other *Stethorus* spp. (Chazeau 1985), and *S. picipes* occurs naturally in western North America (McMurtry 1989). Surprisingly, field studies also revealed that natural populations of predatory mites like *Euseius hibisci* (Chant), a species which is native to Mexico (and presumably California) and occurs naturally on avocados, was not consistent in providing effective control of *O. punicae* populations (McMurtry and Johnson 1963, 1966). Instead, *E. hibisci* populations were considered to be better suited for controlling *E. sexmaculatus* populations as the densities of this tetranychid species on California avocados were naturally low in comparison to *O. punicae* (Fleschner et al. 1956a).

With regards to *O. punicae*, which was more abundant and widely distributed, efforts were made to design an augmentative biological control strategy involving field releases of mass-reared *S. picipies* to boost the action of resident conspecific populations. However, this particular approach was shown to be unsustainable because rearing *S. picipes* beetles in great numbers require a lot of spider mites as prey and this was expensive (Scriven and Fleschner 1960). This observation generated the notion that *S. picipes* was more effective in controlling *O. punicae* populations once densities of this spider mite became elevated (McMurtry and Johnson 1968, McMurtry et al. 1972). Additional research was conducted with various phytoseiid species which could be more easily reared as part of a seasonal inoculative biological control strategy. The justification of this work was that introduced populations of select phytoseiid species could potentially control lower population levels of *O. punicae* much earlier in the season than *S. picipes* because their prey requirements were lower, thus preventing *O. punicae* populations from reaching damaging densities (McMurtry et al. 1984). Several candidate phytotseiid species from multiple countries were considered for this strategy because the origin of *O. punicae* populations in California had not been confirmed (this problem still exists today). This uncertainty over the evolutionary area of origin was complicated by the fact that *O. punicae* has been recorded from other host plants from Latin America and Asia (Jeppson et al. 1975).

Nevertheless, field trials were conducted to assess the efficacy of nine candidate phytoseiid species for control of *O. punicae* populations on avocados (McMurtry et al. 1984). Among them was *Neoseiulus californicus* (McGregor), a species that has historically been used successfully for control of spider mites on other crops (Gerson et al. 2003). Three of these predators, including *N. californicus*, are native to California and the remaining six were introduced from South Africa, the Mediterranean, Brazil, and the Cook Islands (McMurtry et al. 1984). Of the three native species, only *Amblyseius limonicus* (Garman & McGregor) was naturally found in avocado. Even so, none of the phytoseiid species provided significant control of *O.punicae* populations (McMurtry et al. 1984).

Subsequently, it was presumed that *O. punicae* populations from California originated from Latin America. In this instance, six additional phytoseiid species were collected and introduced into southern California from Mexico and Central America (i.e., Costa Rica, Guatemala, and El Salvador). None of these predators established (McMurtry 1989). A possible mechanism that explains these unsuccessful outcomes is that well-established resident populations of *E. hibisci* competed with or interfered with the action of introduced phytoseiid species (McMurtry et al. 1984). Climatic mis-match and insufficient release efforts (i.e., low frequency of predator releases and few individuals released) may have also played a role in failed establishment. It might have been possible to have restricted the search area and devoted more time searching for compatible natural enemies in specific locations had clear information (e.g., available from phylogenetic, taxonomic, or host plant use data) on the geographic origin of California's *O. punicae* populations been available at the time.

Despite the limited success with the introduction of several phytoseiid species, the combination of natural predation provided by resident populations of phytoseiid and insect predator species was deemed to be largely responsible for effective spider mite control in California avocado orchards throughout most of the 20th century (McMurtry and Johnson 1966, McMurtry 1992). Similar positive results with the biological control programs of other insect herbivores were also documented on California avocados (Fleschner 1954, McMurtry 1992). However, the success of spider mite biological control was not permanent because of the acquisition of an additional invasive mite pest.

1.4 Introduction of *Oligonychus perseae* into the California Avocado System

Starting in 1990, California avocado production was impacted in an unprecedented manner with an additional series of invasive pests that are economically injurious to avocados and for which effective biological control programs have been difficult to develop. Among the recently introduced pest species is the foliar feeding spider mite *O. perseae* (Fig. 1.1a). This pest was detected on California avocados in 1990 (Bender 1994), prior to the implemented revisions of trade agreements between Mexico and the United States.

The precise invasion pathway of *O. perseae* into California has not been confirmed but evidence from multiple sources has provided clues as to the likely area of origin. The taxonomic description of *O. perseae* is based on specimens collected on avocado foliage originating from San Luis Potosi, Mexico that was interdicted at a quarantine station in El Paso,Texas in 1975 (Tuttle et al. 1976). Given its feeding habit, *O. perseae* was most likely introduced into the United States on infested avocado plant material from Mexico where *O. perseae* has co-evolved with this host plant. It is still not clear whether *O. perseae* made its way from Mexico to California directly on plants or after potentially having been introduced on infested plant material on previous transport attempts through other U.S.-Mexico border states like Texas. Historically, avocados have been grown in Texas (Cooper 1948) but climatic conditions do not support a large commercially viable production region as in California. Furthermore, the direct presence of *O. perseae* populations on avocados in Texas has not been recorded in the literature.

This may make the Texas-California invasion pathway unlikely and hints at direct introduction into California from Mexico.

Most importantly, the previous lack of updated information regarding species-abundance and detailed life-history information on avocado pests in their native home range such as *O. perseae* made it possible for this spider mite species to remain undetected and/or its potential threat as a serious foliar avocado pest to be given minor consideration until established populations of this spider mite started causing problems in a new avocado production area such as California. As a result of these factors, it is possible that *O. perseae* individuals from infested avocado material from Mexico may have been introduced into California at a much earlier period (e.g., 1980s) (Bender 1994), but this seems unlikely given the rapid spread of this pest after its initial discovery in California. Results from molecular studies (Lara, unpublished data) reveal low mitochondrial genetic diversity in California's *O. perseae* populations compared to populations on avocados from Mexico and this is consistent with the hypothesis that a small founding group of *O. perseae* individuals were likely introduced from its home range in Mexico where higher genetic diversity exists. Furthermore, field observations by Hoddle and Lara (unpublished data) indicated that *O. perseae* is distributed naturally over a large geographic region of southern Mexico on backyard avocados in various municipalities from states such as Michoacán, México, Puebla, Morelos, and Guanajuato. *O. perseae* has also been found infesting avocados at the north-eastern (i.e., Baja California) and southernmost (i.e., Chiapas) states of Mexico.

These recent survey results indicate that *O. perseae* is widespread in its home range on avocados, and is often damaging on Hass avocados. If this information, had been known earlier, it could have prompted: (1) initial concern regarding the potential threat posed by *O. perseae* to commercial avocado production outside its home range, and (2) early efforts to document associated biological control agents in its home range, if any, that could effectively regulate *O. perseae* populations in a density-dependent manner on commercial avocado cultivars could have been undertaken. Unfortunately, realizing these scenarios is limited by the scarce availability of research resources and the large number of candidate pest species on a given crop, especially co-evolved herbivores in the crop's area of origin. Consequently, invasive species research for agricultural systems is often driven by post-introduction economic losses experienced in another country rather than interests in pursuing proactive measures to study a native pest species, among many, whose exotic populations may or may not establish and become problematic once introduced from its home range into a new geographic area. For this reason, there was little interest in studying *O. perseae* in Mexico despite documented damage on avocados during the 1970s, even in other minor avocado-growing areas outside of Mexico that are considered to be part of the natural home range of avocados. For example, *O. perseae* was first recorded on avocados in Costa Rica as early as 1974 (Ochoa et al. 1994), but important commercial cultivars like Hass were not introduced into Costa Rica until the mid 1980s (Schroeder 1959, MAG 2007). The full extent of the damage invasive *O. perseae* populations was capable of producing outside of the natural home range of avocado were observed years later in major commercial avocado production areas of California.

Since its detection in California (1990), *O. perseae* populations rapidly established in parts of the southern California avocado growing region extending from San Luis Obispo County to San Diego County and population outbreaks of this spider mite can be extremely damaging. Subsequently, exotic *O. perseae* populations have been found infesting avocados in Israel (2001) Spain (2004), Madeira (2005), and the Canary Islands (2006). As a result, *O. perseae* is considered an international avocado pest of economic importance (Peña et al. 2013). This has fueled substantial interest in understanding the ecology of *O. perseae* on avocados and developing effective pest management strategies for this pest, including the use of natural enemies for use in biological control programs.

1.4.1 General Biology of Oligonychus perseae on Avocados

The biology of *O. perseae* populations in California was studied by Aponte and McMurtry (1997a, 1997b) and reviewed by Hoddle and Morse (2013). As with other tetranychids, the sequential life stages of *O. perseae* are egg, larva, protonymph, deutonymph and adult. Under 15, 20, 25 and 30°C, development from egg to adult on foliage from the Hass avocado cultivar can be completed in approximately 35, 17, 14 and 10 days, respectively (Aponte and McMurtry 1997a). Motile stages of *O. perseae* are capable of constructing webbed nests (Fig. 1.1a) which are made on the leaf undersurface mainly along the midrib and leaf veins (Aponte and McMurtry 1997b). The webbed nests of *O. perseae* demarcate feeding and development arenas and provide mating spaces for adults. Eggs are laid by female adults inside theses nests (Fig. 1.1a) which may provide suitable microclimate conditions for egg development. In addition, webbing functions as a physical protective cover for all life stages against potential predators, such as phytoseiids.

Motile life stages of *O. perseae* use cheliceral stylets to feed on the cell contents of parenchyma tissue from the undersurface of avocado leaves (Aponte and McMurtry 1997b). This gives feeding individuals a greenish-yellow appearance. Continual feeding by *O. perseae* populations on leaf cell contents produces damaged tissue that is visible as brown necrotic circles on the leaf undersurface (Fig. 1.1b). Sometimes this brown discoloration is also visible from the top leaf surface. When feeding areas become necrotic, motile *O. perseae* may abandon the nest in search of new colonization areas on the leaf undersurface and the process of nest construction, reproduction, and communal feeding is repeated. Alternatively, under high population densities on avocado leaves, *O. perseae* individuals can produce fine silken strands which are used for wind-mediated dispersal. In this manner, *O. perseae* individuals might be carried as founders to other avocado trees which may contain un-infested foliage available for colonization.

This type of passive airborne dispersal using silk is referred to as “ballooning” but it is not exclusive to *O.perseae* (*E. sexamaculatus* and *O. punicae* are capable of this as well) or tetranychids (Fleschner et al. 1956b, Kennedy and Smitley 1985). Nevertheless, it represents a natural pathway by which damaging *O. perseae* populations can become distributed within and between avocado orchards in commercial production areas. This dispersal pathway may also explain how, in theory, an initial reproductive group *O. perseae* was to some degree able to increase its own distribution naturally once it was introduced into California. Another contributing factor for the distribution of *O. perseae* within California may have been the human-aided transport of contaminated plant material from area to another, including live infested plants or infested leaves inside packing bins that were moved from orchard to orchard. The underlying factors responsible for the severity of *O. perseae* infestations on California avocados are intricate and will be discussed below.

1.5 Potential Factors that Explain the Economic Importance of *Oligonychus perseae* on Avocados in California

Like *O. punicae* and *E. sexmaculatus*, the accumulation of feeding damage on avocado foliage caused by *O. perseae* populations can induce pre-mature leaf drop (Aponte and McMurtry 1997b) and this occurs when 7.5% of the leaf surface is damaged (Kerguelen and Hoddle 1999). Although *O. perseae, O. punicae,* and *E. sexmaculatus* do not attack avocado fruit directly, mite-induce defoliation can thin out the tree canopy and avocado fruit can become sun-burnt (Fig. 1.1c). Typically in California, *O. perseae* densities begin increasing during later spring months (April-May) when new avocado foliage is available to spider mites for colonization and feeding, followed by a peak in spider mite densities during summer (June-August) and eventual decline during fall (September – November) and winter months (December – February) (Yee et al. 2001a, UCANR 2008, Hoddle and Morse 2013). The main concern with potential damage caused by *O. perseae* populations occurs during the summer period when densities are at peak levels. A custom binomial sampling plan has been developed for *O. perseae* to help avocado pest managers better monitor population densities of this mite on California avocados and to make pest management decisions based on accurate estimates of pest densities (Lara and Hoddle 2013).

The severity of foliar damage resulting from *O. perseae* feeding depends, in part, on the susceptibility of the infested avocado cultivar (Kerguelen and Hoddle 2000). In hindsight, the expansion of avocado acreage and abundance of highly susceptible avocado cultivars to *O. perseae* might explain, in part, why *O. peresea* in comparison to *O. punicae* and *E. sexmaculatus* has become a prevalent pest on California avocados and other Hass avocado growing areas. The mechanisms of how acreage expansion and shifts to susceptible cultivars may have enabled and magnified the extent of *O. perseae* problems in California are discussed below. An understanding of these mechanisms is important for both the design of integrated *O. perseae* management programs and explaining the observed limitations of management programs, including the development of effective biological control strategies.

1.5.1 Acreage Expansion of Hass Avocados

California avocado bearing acreage has increased from approximately 202 ha in 1925 to 24,131 ha in 2012 (USDA 2014). Up until the 1960s, avocado acreage in California was dominated by the Fuerte cultivar (Carman and Craft 1998). Initial Fuerte propagation in California began with budwood collected by Carl Schmidt, during a 1911 avocado expedition, from a backyard avocado tree in the state of Puebla in Mexico. Fuerte was named after the Spanish word for “strong” because trees displayed vigorous growth and survived the severe California winter of 1913 (Rounds 1947). Fuerte produces a green thin-peel fruit and is thought to be a Mexican x Guatemalan hybrid, and molecular data suggests it may have a higher genetic contribution from the Mexican parent (Chen et al. 2009). Due to its frost resistance and good taste compared to other available cultivars during the early 1900s, Fuerte dominated the avocado market in California for several decades and later became one of the dominant commercial cultivars within some areas of its home range in Mexico when it was introduced with other cultivars developed in California during the 1950s-1970s (Sánchez Colín et al. 2001). However, the economic importance of Fuerte was temporary.

The transitional replacement of Fuerte acreage began with the accidental discovery of the hybrid Hass avocado cultivar in 1926 by Rudolph Hass in La Habra, California (Griswold 1946). Molecular evidence supports previous assumptions that the Hass cultivar carries roughly a similar share of alleles from both Mexican and Guatemalan parents (Chen et al. 2009). This hybridization generated a cultivar with a relatively short fruit maturation period (i.e., faster production compared to Fuerte), some level of cold tolerance, and fruit with a thicker but still easy-to-peel pebbly skin whose color turns from green to dark-purple once the fruit ripens. Most importantly, the hybrid Hass fruit is known for its rich nutty and oily taste. This combination of attributes was favorable for commercial production, but during this initial period of discovery the susceptibility of the Hass cultivar to pests was not a research priority to the growing avocado industry. R. Hass patented the Hass cultivar in 1935 and through subsequent marketing and nursery support Hass avocado gradually gained recognition as the preferred commercial cultivar (Griswold 1946), first in California and subsequently abroad in other avocado producing countries.

During the 1960s, the share of total avocado production in California by cultivar was roughly 60% Fuerte, 20% Hass, and the remaining 20% were other cultivars. By the 1990s Hass avocado accounted for roughly 80% of the total avocado production in California (Carman and Craft 1998). Currently, 96% of all avocados produced in California are Hass (CAC 2014). Even so, California is currently only the third largest producer of Hass avocado in the world after Mexico (1st) and Chile (2nd). Mexico has historically been and continues to be the primary producer of avocados in the world with most production occurring in the state of Michoacán. The Hass cultivar was introduced from California into Mexico during the 1960s and since that time Hass acreage in Mexico has increased. During the 1970s, 85% of all commercial avocado trees grown in Michoacán were Hass (Sánchez Colín et al. 2001). In 2011, Mexico produced approximately 1.26 million metric tons of fresh avocado fruit on 126,598 ha (FAO 2014). The state of Michoacán alone devoted approximately 108,683 ha to the production of Hass avocados (Flores and Berman 2012), more than four times the estimated Hass acreage of California. Examples of other countries which have established Hass avocado industries include Australia, Colombia, Costa Rica, Dominican Republic, Guatemala, Israel, New Zealand, Peru, South Africa, and Spain. Overall, it is estimated that the Hass avocado accounts for 90% of global avocado exports (Crane et al. 2013). Despite its economic dominance in the world market, Hass avocado is not known for being a resistant cultivar to pest damage.

1.5.2 Susceptibility of the Hass cultivar to Spider Mites and other Arthropods

A decade after the introduction of *O. persea*e in California, a critical study by Kerguelen and Hoddle (2000) revealed that Hass avocado leaves fed upon by *O. perseae* populations accumulated relatively high leaf area damage (66%) when compared to five other cultivars (Kerguelen and Hoddle 2000). The most resistant cultivar found in that study was Fuerte with only 35% leaf area damage (Kerguelen and Hoddle 2000). Recent work has also demonstrated that the leaf injury *O. perseae* causes to Hass can translate directly into yield reduction and consequently the recommended action threshold for control of *O. perseae* on Hass avocado was determined to be 50-100 mites per leaf (Maoz et al. 2011b).

The combined results from these studies and the gradual increase of Hass acreage lead to the conclusion that the California avocado system had become vulnerable to attack by specialized avocado pest as the industry shifted acreage from pest resistant Fuerte to susceptible Hass avocado production. In addition, the increase in Hass avocado acreage in Mexico over decades, after having been introduced from California, possibly played a role in increasing the probability that herbivores like *O. perseae*, and potentially others (see below), would at some point be introduced from their home range to other susceptible Hass growing regions. The symptoms of this latent vulnerability became apparent in the 1990s when the share of California avocado acreage was 80% Hass and specialist avocado herbivores such as *O. perseae* were introduced. Awareness on this cultivar susceptibility problem has heightened since *O. perseae* was also introduced into the Hass producing regions of Israel and Spain (see Sect. 1.4) where the climate permits the successful production of this cultivar.

It is curious as to why *O. punicae* which was already established in California and had a wider distribution than *E. sexmaculatus*, has not become a major spider mite pest of Hass avocados. Similar to *O. perseae*, the reproduction and development of *O. punicae* is comparatively better while feeding on Hass than other avocado varieties such as Fuerte and Criollo (i.e., plants from the Mexican variety but unknown parentage that were grown from seeds) (Cerna et al. 2009). The answer may lie with an assortment of unstudied biotic factors such as the differential use of leaves by populations of these spider mites (*O. perseae* feeds on the undersurface and *O. punicae* feeds on the upper surfaces of avocado leaves), the outcome of asymmetric competition between populations of these mites on avocado foliage, the differential tolerance of Hass avocados to the degree of damage caused by populations of each spider mite species, or the differential efficacy of resident natural enemies against different spider mite species. This latter factor may be important as *Stethorus* beetles have not been found in great abundance in California orchards infested with *O. perseae* (Yee et al. 2001a) but they have been shown to be important for controlling *O. punicae*. Also no recent work has been conducted to determine if *O. punicae* populations are still under effective natural control by *S. picipes* since the *O. perseae* invasion in California. Future studies will need to confirm the additional influence of these biotic factors as an explanation for the pest status of different spider mite species on avocados in California.

It is also important to recognize that the ecological interactions of spider mites on avocado cultivars like Hass is also mediated by abiotic factors related to climate that operate as part of larger spatial scale processes. As mentioned in Sect. 1.3 of this chapter, *E. sexmaculatus* is not considered a threatening avocado pest in California and previous studies indicate that natural populations of this spider mite mainly occur in some cool foggy areas near the central California coast where avocado acreage is not well-represented (Bailey and Olsen 1990, UCANR 2008). In this case, climate may have also restricted the southward spread of *E. sexmaculatus* populations into the expanding Hass avocado acreage throughout southern California even though Hass has historically been identified as being susceptible to induced premature defoliation caused by *E. sexmaculatus* feeding (Ebeling and Pence 1953). With regards to *O. perseae* and *O. punicae*, inland areas of southern California (e.g., Riverside County and San Bernardino County), where avocado acreage is relatively minimal due high summer heat and sensitivity to potential frost events over winter, do not experience elevated densities of these spider mites during the summer as other coastal regions in southern California where avocado acreage is well represented (e.g., San Diego County and Ventura County). These observations imply that climate influences where cultivars can be grown and also the potential distribution of spider mite infestations within avocado growing regions. For example, within the United States, the presence of *O. perseae* has been reported on avocados in Florida (Peña et al. 2013), but the climate in this region is more suitable for the production of West Indian cultivars which are probably not susceptible to feeding by *O. perseae* to the same degree as Hass. *O. perseae* in Florida is not considered a significant pest and extant populations have not been recently identified despite recent surveys for other invasive pests (e.g., red bay ambrosia beetle [J. Peña pers. comm. 2013]).

Despite the strong influence of climate on *O. perseae* abundance and distribution, the abundance and intrinsic susceptibility of Hass avocado may also directly explain the prevalence of other spider mites outside of the United States such as *Oligonychus yothersi* (McGregor). *O. yothersi* is the primary pest of Hass avocados in Chile and *O. perseae* does not occur there (León Lobos 2003). Also *O. yothersi* is an occasional pest on Florida avocados (Peña and Johnson 1999), but Hass avocado is not heavily grown in Florida. Like *O. punicae*, *O. yothersi* feeds on the upper leaf surfaces and causes bronzing damage on avocado foliage (Jeppson et al. 1975). Research by Léon Lobos (2003) revealed that life table parameters of *O. yothersi* such as net reproduction (R_o), oviposition rates, and survivorship of immature stages were lower on Fuerte than on Hass avocado. These results lead to other important conclusions. First, *O. yothersi* has reproductive advantage feeding on Hass avocado that could translate into increased populations densities. Since Hass is the primary cultivar grown in Chile this explains why *O. yothersi* may have become a prevalent foliar avocado pest there. The second conclusion is that the variable degree of susceptibility between avocado cultivars to herbivory is not unique to *O. perseae*.

Like *O. yothersi* on Hass, a similar prevalence pattern may have developed over time for *E. sexmaculatus* on Hass avocados in New Zealand where *E. sexmaculatus* does not face direct competition from other tetranychids like *O. perseae* (both species damage the leaf undersurface) because *O. perseae* does not occur there. *E. sexmaculatus* has been recorded in New Zealand since the 1950s (on grapes) and was perceived as being of low economic importance on avocados until the 1990s (Jamieson and Stevens 2007). However, similar to California, New Zealand has undergone avocado cultivar shifts and acreage expansion (White 2002). Hass is currently the dominant cultivar and starting in 1998, severe problems with *E. sexmaculatus* were detected on Hass avocados (Jamieson and Stevens 2009). In turn, the growing presence of Hass (White 2002) has likely contributed to the growing severity of *E. sexmaculatus* infestations in New Zealand.

Overall, these patterns establish the relative susceptibility among avocado cultivars to different species of spider mites under varying climatic conditions. There is also growing evidence for avocado cultivars to vary in susceptibility to additional arthropods other than spider mites. One example is the avocado thrips, *Scirtothrips perseae* Nakahara (Thysanoptera: Thripidae). *S. perseae* was a species new to science when it was discovered in California in 1996 (Nakahara 1997) and, like *O. perseae*, post-introduction research has been necessary to understand the dynamics and severity of this invasive species on avocados in California.

S. perseae is a specialized insect that feeds on avocado foliage and small immature fruit to complete its life cycle (Hoddle 2002). Populations of *S. perseae* have established throughout the California avocado growing region but the severity of these problem in commercial orchards are positively correlated with the increasing proximity of sites to the Pacific coast as a result of cooler temperatures that provide an optimal thermal range (15-20°C) that is amenable for development of this species on avocados (Hoddle 2003). The main concern with *S. perseae* is the direct feeding damage it causes to maturing avocado fruit during the spring. Yee et al. (2001b) determined that maturing Hass avocados were especially vulnerable to thrips feeding damage in the size range of 0.42-1.42 cm in length. Fruit that is heavily attacked by *S. perseae* develops scarring and loses economic value as it has to be culled or downgraded. In California, the annual economic losses to commercial avocado production attributed to *S. perseae* populations is approximately $4.45 million (Hoddle et al. 2003). Therefore, this thrips species is the key primary pest of Hass avocado fruit in California even though it is not a specialist on avocado fruit. Molecular studies suggest that California populations of *S. perseae* originated from avocados in Mexico, specifically, the municipality of Coatepec-Harinas in the state of México (Rugman-Jones et al. 2008).

Similar to *O. perseae, O. punicae, E. sexmaculatus,* and *O. yothersi*, the varying susceptibility of avocado cultivars to *S. perseae* feeding has also been observed between Hass and Fuerte (UCANR 2008), but quantitative studies are still needed to compare the extent and underlying nature of these differences for this thrips. Nevertheless, previously documented observations by California avocado researchers also suggested that Fuerte (Hass avocado was also present at the time) was one of the least susceptible cultivars to infestations by greenhouse thrips, *Heliothrips haemorrhoidalis* (Bouché)*,* an exotic generalist herbivore species in California known to attack avocados and other crops as well (Ebeling and Pence 1953).

However, it is important to recognize that Hass is not universally susceptible to all herbivores. For example, Ebeling and Pence (1958) recorded that Fuerte was more susceptible to infestations by insects such as the scale *H. lataniae* and the larval stages of the native herbivore *Sabulodes aegrotata* (Gueneé) (Lepidoptera: Geometridae). The precise nature of reduced Hass susceptibility to infestations of both of these herbivore species has not been studied or quantified. Ebeling and Pence (1958) vaguely indicated that it may have something to do with the "less favorable growing habits" of Hass in comparison to Fuerte. Therefore, to some extent the severity of pest infestations on avocados may be related to the degree of compatibility between the seasonal phenology of cultivars and populations of each herbivore species in California. For example, in the case of S. *perseae* it is likely that Fuerte fruit is less susceptible to thrips feeding in the spring due its early fruit development starting in the preceding year when thrips densities are low. In contrast, development of young Hass fruit occurs later, approximately from March-July (UCANR 2008), and this period overlaps with increasing thrips populations during spring and economic damage results.

Ultimately, these observations on varying cultivar susceptibly to pest infestations may be explained by genetic differences. If evolutionary genetic differences between cultivars explain their inherent morphological, phenological, and physiological traits, it would seem possible that these genetic differences may also confer a suite of herbivory resistance traits to avocado cultivars. In general, the identity of these resistance traits may be associated with structural/morphological or chemical defenses (Mortensen 2013). For example, the resistance of Fuerte avocados to *S. perseae* is also perhaps conferred by chemical profile differences in foliage. The skin of Fuerte fruit is thinner than Hass avocado so fruit morphology does not explain why Hass is more susceptible to feeding *S. perseae* populations. Like *O. perseae*, it is possible that the reproductive potential of *S. perseae* populations is also lower when feeding on Fuerte foliage and this translates into potentially lower populations infesting this cultivar. The value of this hypothesis as an explanation for variable cultivar susceptibility to herbivores requires more study but the underlying logic would explain why the Fuerte cultivar, which is from southern Mexico and whose genetic makeup is close to the Mexican variety, *drymifolia*, (Chen et al. 2009) would be relatively resistant to populations of *S. perseae* and *O. perseae* that are also from the same region. By extension, it is also important to note that chemical profile differences between cultivars may also have significant ecological implications in explaining the limited efficacy of biological control agents against some pest species.

1.5.3 The Implication of Chemical Profiles of Avocado Cultivars in Relation to Herbivory and Biological Control

Research investigations conducted in Mexico, part of the home range of avocado, suggest that characteristic variations in expressed foliar secondary metabolite profiles among and between avocado cultivars may also mediate their susceptibility to avocado herbivores (Bravo-Monzón et al. 2008, Rincón-Hernández et al. 2011, Torres-Gurrola et al. 2011). Rincon-Hernandez et al. (2011) found that chemical profiles of 250 different trees could be used to distinguish trees of Mexican varieties from three other hybrid crosses, Mexican x Guatemalan (Hass avocado belongs to this cross), Mexican x West Indian and Mexican x *P. shiedeana*; *P. shiedeana* is an indigenous species from Mexico and Central America that is related to *P. americana*. Torres-Gurrola et al. 2011 found that the presence of specific foliar chemicals could explain the incidence of galls produced by *Trioza anceps* Tuthill (Hemiptera: Triozidae) on foliage from trees of the Mexican variety, *drymifolia*. Furthermore, a study by Bravo-Monzón et al. (2008) demonstrated that trees of the domesticated Hass cultivar did not produce detectable volatile compounds even when attacked by the specialized avocado stem borer, *Copturus aguacatae* Kissinger (Coleoptera: Curculionidae). Interestingly, macerated leaf extractions showed that Hass avocado had the potential to produce volatiles. In contrast, trees of the indigenous Mexican variety produced less volatile compounds when attacked by *C. aguacatae*.

The ecological implications of these results in relation to the trophic interaction between avocado herbivores, potential natural enemies, and host avocado plants were not studied, but it is known that plants are capable of emitting volatile compounds (kairomones) elicited by herbivory that attract natural enemies of herbivores or in the production of defensive compounds that interfere directly with herbivory (Gatehouse 2002). A quantitative comparison of the foliar chemical profiles between Fuerte and Hass cultivars damaged by either *O. perseae*, *O. yothersi*, or *S. perseae* has not been conducted. Still, based on the study with *C. aguacatae* on the Hass cultivar and the *drymifolia* variety (associated with Fuerte), there is at least indirect evidence to suggest that foliar chemical differences between Hass and Fuerte may exist, and could, in theory, mediate the observed relative susceptibility of these cultivars to avocado herbivores such as *O. perseae*. It is also important to realize that susceptibility within cultivars may also not be uniform and this may generate differences in pest infestations within avocado growing sites. For example, the study by Torres-Gurrola et al. 2011 suggests that levels of chemical protection may vary between trees even under the same growing conditions. Variation of susceptibility between avocado trees to *O. perseae* was also documented from a research population of Gwen avocado trees at South Coast Research & Extension Center in Irvine, CA (V. Ashworth, pers. comm. 2014). Similar variation on *O. perseae* infestations between groups of Hass avocado trees within commercial California orchards have observed by Lara and Hoddle. This important variation needs to be accounted for in future studies that elucidate the influence of host plant chemical profiles on population dynamics of *O. perseae* within avocado orchards.

One possible resistance mechanism could be that Hass avocados trees either do not initiate or mount effective chemical foliar defenses when attacked by feeding *O. perseae* in comparison to Fuerte. This could explain in general why the feeding damage by *O. perseae* is greater on Hass than Fuerte. A second important mechanism could be that the lack of volatile production on Hass avocados attacked by *O. perseae* limits the recruitment of natural enemies to infested foliage that are normally capable of using herbivore induced plant volatiles (HIPVs) to find potential prey. Olfactometer studies demonstrated that phytoseiids and *Stethorus* beetles can sense and respond behaviorally to blends of plant volatiles emitted from spider mite infested foliage from other crops

(Sabelis and Dicke 1985, Nimet et al. 2009). In addition, McMurtry et al. (1992) demonstrated, under laboratory conditions, that *E. hibsici* responded positively to volatiles from extracts of ground *O. punicae* individuals on avocado leaves deposited on filter paper.

Consequently, if resident predators in California avocado orchards such as *E. hibisci* or *S. picipes* are not detecting Hass avocado volatiles from *O.peresae* infested leaves that could positively influence search activity for spider mite patches, this may explain why these predators do not respond to building pest infestations in commercial avocado orchards. For example, *O. perseae* populations display aggregated distributions on avocado leaves (Lara, personal observation). Initially, during the early growing season (spring months) some leaves will contain a few to several hundred *O. perseae* individuals while other leaves remain spider-mite-free. Depending on cultural pruning practices, cultivar growth habits, host plant size and age, an avocado tree canopy can in some cases consist of several hundred leaves and when this number is summed among other trees present in an orchard, the sampling universe of leaves becomes very large. At low *O. perseae* densities a condition is created within each tree such that few infested leaves are "hidden" in the tree canopy. Consequently, the inability of resident predators to effectively find scarce local patches of *O. perseae* among several thousand leaves during early infestation stages could cause populations of resident predators like *E. hibsci* and *S. picipes* to fail to respond numerically to increasing *O. perseae* levels. Ultimately, *O. perseae* populations would escape effective control by the natural predator community and surpass action thresholds because predators were not attracted to infestations.

At the moment, there is no clear field evidence to suggest that HIPVs are used by resident predators in the California avocado system to locate *O. perseae* infestations. Results from other spider mite systems (De Boer and Dicke 2004, Woods et al. 2011, Gadino et al. 2012) suggest that there is potential merit in using synthetic HIPVs (e.g., methyl salicylate) for this purpose. Furthermore, results from a pilot field study by Hoddle (unpublished data) showed that counts of *O. perseae* were significantly higher on sticky cards in close proximity to avocado trees treated with methyl salicylate dispensers in comparison to control plots that lacked this semiochemical. This finding suggested that methyl salicylate may have been repellant and caused *O. perseae* to prematurely disperse from infested leaves. It is also possible that *O. perseae* dispersal was further instigated by higher rates of predation on Hass trees treated with methyl salicylate because of recruitment. However, predator populations were not monitored. Further studies will need to elucidate and quantify the beneficial mechanisms of HIPV deployment (e.g., pest repellency, natural enemy recruitment) on avocados and establish optimal lure release rates for attraction of predators (Woods et al. 2011).

However, this possible lack of kairomone production by susceptible cultivars like Hass is one of several potential factors that may explain the high prevalence of *O. perseae* in California and the limitations of resident biological control agents for suppressing populations of this pest. Additional studies have revealed that life history traits of candidate phytoseiid predators may have also played a direct role in *O. perseae* populations performing well on Hass.

1.6 Present Biological Control Program of *Oligonychus perseae* Using Phytoseiids in California

The mite family Phytoseiidae has a strong historical legacy as being a source of effective spider mite biological control agents in agricultural systems (Gerson et al. 2003, Hoy 2011). Biological control of *O. perseae* populations using phytoseiids has been studied extensively in California and has either relied on natural control provided by resident populations of *E. hibisci* (Chant) and seasonal inoculative releases of a commercial strain of *N. californicus* (McGregor). These same species were previously used with limited success for the biological control *O. punicae* on avocado (McMurtry and Johnson 1966, McMurtry et al. 1984). Not surprisingly, their ability to reduce *O. perseae* populations has also been limited. This outcome stresses that the degree of compatibility of life histories between candidate natural enemy species and the target pest species in relation to the intended release habitat is important. For more than two decades the main problem in implementing a successful phytoseiid-based biological control program for reduction of *O. perseae* populations during the summer, when pest densities increase, has been dealing with the ecological and biological limitations of natural enemies that are easily mass-reared (McMurtry and Scriven 1965a) and readily available in California (McMurtry and Johnson 1966), but either fail to establish and/or do not respond numerically to mite outbreaks and/or require supplementary food resources to promote persistence. A brief review of the ecology of these phytoseiid species in relation to *O. perseae* on California Hass avocados is provided next.

1.6.1 Natural Control Using Euseius hibisci

E. hibisci was originally described from *Hibiscus* sp. leaves from the state of Sonora in Mexico (1959) and this species was re-described by Congdon and McMurtry (1985) from California populations occurring on avocados. Initial monitoring data from 1992 (McMurtry 1993) showed that populations of *E. hibisci* , an abundant and widely distributed native species in California avocado orchards (McMurtry and Johnson 1966), failed to control populations of *O. perseae* which reached densities as high as 1,600 spider mites per leaf during that season. Furthermore, during 1998-2000 Yee et al. (2001a) monitored populations of *O. perseae* and indigenous phytoseiid populations (mainly *E. hibisci*) at three Hass avocado orchards that were representative of coastal, intermediate and warm-inland climates of the avocado growing region in southern California. Similarly, the results from that study indicated that there were no consistent trends between *O. perseae* and natural phytoseiid densities despite the fact that phytoseiids were the most abundant and continually present group of natural enemies on avocado leaves at all three sites. In these studies, *E. hibisci* failed to show any type of density dependent or numerical response to increasing densities of pest mites.

More recent studies have provided supporting evidence for the limited role of *Euseius* spp. as effective biological control agents of *O. perseae* on avocados. During 2009-2010, Lara (unpublished data) studied the cross-sectional spatial dynamics of resident *Euseius* spp. populations (most likely *E. hibisci*) from nine commercial Hass avocado orchards infested with *O. perseae*. In four of these orchards, resident phytoseiids displayed a statistically significant association with infested *O. perseae* leaves but overall populations of these predators failed to display a biologically significant numerical response to high densities of *O. perseae,* which in most cases exceeded the upper action threshold boundary of 100 mites per leaf suggested by Moaz et al. (2011a). This work revealed that *O. perseae* populations readily escaped effective natural control from resident phytoseiid populations. Furthermore, as part of a long-term longitudinal study, Lara and Hoddle (unpublished data) monitored densities of resident *Euseius* spp. and *O. perseae* during 2002-2013 on a group of untreated Hass avocado trees. Cross-correlation analyses revealed a lack of synchrony between *O. perseae* and resident phytoseiids and the inability of predator populations to respond numerically to peak *O. perseae* densities observed during summer, a time when *O. perseae* populations increase, over the 11 year study period.

There are several potential reasons for this outcome. Members of the genus *Euseius* are regarded as pollen specialists (McMurtry and Croft 1997). Furthermore laboratory studies confirmed that *E. hibici* develops faster on a pollen diet than on spider mites alone (McMurtry and Scriven 1964, Zhimo and McMurtry 1990) and imbibing avocado leaf sap may provide nutrients required for optimal reproduction (Porres et al. 1975, McMurtry and Scriven 1965a). Second, behavioral observations revealed that *Euseius* spp. cannot penetrate the webbed nests of *O. perseae* and could only feed on exposed spider mites outside of the nest (Fig. 1.2a) (McMurtry 1993). Together, these results suggested *E. hibisci* is a generalist facultative predator primarily dependent on obtaining plant-based nutrition rather than feeding exclusively on spider mites such as *O. perseae*. Additionally, the leaf feeding habit might explain why populations of *E. hibisci* can subsist year round in avocado orchards in the absence of pollen and when levels of *O. perseae* and other potential prey species are low.

The ecological limitations of *E. hibisci* as an effective predator of *O. perseae* are also compounded when the potential influence of cultivar-susceptibility (see Sect. 1.5.3) is considered as it allows (1) *O. perseae* populations to have an increased reproductive potential, and (2) the potential lack of plant volatiles from infested Hass foliage may protect *O. perseae* populations from detection by these predators early in the season when spider mite populations are increasing and effective predation is needed (this hypothesis requires further study).

1.6.2 Augmentative Biological Control using Neoseiulus californicus

The search for alternative biological control agents for *O. perseae* in California focused on screening commercially available phytoseiid species for deployment in commercial avocado orchards (Hoddle et al. 1999, 2000). The current recommended *O. perseae* biological control strategy depends on inoculative releases of *Neoseiulus californicus* (McGregor), which has characteristics of being a selective spider mite predator and also the capacity to reproduce on non-spider mite food sources (Croft et al.1998). This species was initially described from California lemons (McGregor 1954). On avocados, *N. californicus* can penetrate the webbed nest of *O. perseae* (Fig. 1.2b) and feed on all life stages of this pest (Takano-Lee and Hoddle 2002). However, making the recommended seasonal releases of 2,000 *N. californicus* predators per tree during the growing season to obtain *O. perseae* control is not practical or cost-effective for commercial orchards when large areas need treatment.

Also, despite their generalist feeding habits, *N. californicus* populations cannot establish year-round in California avocado orchards and this is probably the result of at least two non-mutually exclusive mechanisms. Mass reared *N. californicus*, unlike *E. hibisci*, are unable to subsist year-round on specific food resources available within the avocado system (e.g., avocado pollen, supplemental feeding on avocado leaf sap, *O. perseae*, other insect and mite species), and subsequent development and reproduction is insufficient to sustain populations that provide long-term biological control of *O. perseae*. Consequently, *N. californicus* populations may be competitively excluded by populations of *Euseius* spp. which may not only be better adapted for obtaining nutrition from the same available resources in avocado orchards, but they may also have a behavioral advantage in being more effective in detecting and finding these resources on avocados (see McMurtry and Johnson 1966).

The reproductive potential of *N. californicus* and *Euseius* spp. reared on *O. perseae* has not been conducted and results from this type of fundamental study might offer supporting evidence on the overall limited performance of both of these predators in the field when feeding exclusively on *O. perseae* (see Escudero and Ferragut 2005) compared with a diet of this pest that is augmented with avocado pollen or some other readily obtainable plant resource. Finally, a second potential mechanism for the lack of long-term establishment of *N. calfornicus* on avocados could be that asymmetric intraguild predation from native *Euseius* spp. prevent *N. californicus* from permanently establishing and effectively controlling *O. perseae* populations. This mechanism has been shown to occur between populations of *Euseius stipulatus* (Athias-Henriot) and *N. californicus* for control of *Tetranychus urticae* Koch on clementine plants (Abad-Moyano et al. 2010) and could occur in avocados.

1.6.3 Conservation Biological Control using Euseius spp.

Due to the practical and economic limitations of making *N. californicus* releases, rescuing indigenous phytoseiid populations which can subsist year-round in avocado orchards so that they can provide biological control services has been the focus of several studies outside of California where *O. perseae* is also a pest. Research in Israel (Maoz et al. 2011a) and Spain (González-Fernández et al. 2009) demonstrated that supplemental pollen provisioning can boost levels of indigenous *Euseius* spp. populations. However, even with pollen provisioning, field releases of *E. stipulatus* and *N. californicus* were insufficient to control *O. perseae* (Monserrat et al. 2013). Monserrat et al. (2013) posit that this result was most likely due to a disruption of biological control when populations of phytoseiids experienced average temperatures above an estimated optimal value of 24.7°C. This potential influence of abiotic components points to a third mechanism that in some cases might explain why the biological control efficacy of these natural enemies in avocados is limited. However, Monserrat et al. (2013) did not rule out the possibility that intraguild predation between *E. stipulatus* and *N. californicus* may have also been a factor in explaining the lack of *O. perseae* suppression.

1.7 Future Prospects for Improving Biological Control of *Oligonychus perseae* in California

The information reviewed in Sect. 1.1-1.6 lays the foundation on which to discuss future recommendations for improving the spider mite biological control program of the California avocado system. Although this information focuses on *O. perseae*, the underlying principles could be used to study (i.e., identify key crop production vulnerabilities that are conducive to pest problems) and improve biological control programs of tetranychids on other perennial crops.

Avocado plants were introduced into California from Mesoamerica in the mid 19th century (see Sect. 1.1) but problems with avocado-feeding arthropods (i.e., spider mites and insects) developed in the early 20th century as production of commercial avocado cultivars expanded and the avocado system became available as a perennial habitat to populations of avocado feeding pests (see Sect. 1.2). Initial concern with the increased presence of these herbivores prompted investigators, starting in the 1950s, to design and improve natural pest control strategies under the theoretical framework of biological control, a top-down ecological approach for pest management (see Sect. 1.3). Therefore, spider mite control research on commercial avocado host-plants (first trophic level) focused on exploiting the regulation of leaf-feeding populations of *O. punicae* and *E. sexmaculatus* (second trophic level) using natural enemies (third trophic level). Results from field studies indicated that, for the most part, populations of resident predator species such as the coccinellid *S. picipes* and the phytoseiid *E. hibisci* were effective natural enemies of tetranychids in California avocado orchards for at least 40 years (1950s-1990) (see Sect. 1.3.1).

Interestingly, the biological resistance provided by resident predator populations against established tetranychid species in the California avocado system since the 1990's has been absent (e.g., *S. picipes*) or ineffective (e.g., phytoseiids) for control of *O. perseae* (see Sect. 1.4). Research has emphasized top-down control of *O. perseae*, but success with phytoseiid-based biological control strategies has been limited (see Sect. 1.6). Most likely the limited biological resistance of the California avocado system against *O. perseae* has been mediated by the growing availability of susceptible cultivars like Hass that overlap with the phenology of feeding *O. perseae* populations which allow pest populations to escape effective top-down control. Other reasons may include the limited recruitment and searching ability of predators on *O. perseae* infested Hass foliage and other problems having to do with the incompatibility of life-history traits between *O. perseae* and extant candidate phytoseiid species (see Sect. 1.5.3, 1.6). This process may have also occurred with other spider mite species outside of California, such as *O. yothersi* in Chile, where commercial Hass avocados are also produced (see Sect. 1.5.1, 1.5.2). Better understanding of these factors may help restore the biological resistance of the California avocado system against key avocado pests such as *O. perseae* and improve the current pest management program for this spider mite on avocados (UCANR 2008).

Hass is still a major commercial cultivar in California so populations of *O. perseae* are expected to continue to be problematic in the future. In fact, even after two decades since *O. perseae* was introduced into California, economically damaging densities of *O. perseae* populations were observed region-wide in commercial avocado orchards during 2013. This outbreak of *O. perseae* populations resulted in the dissemination of an official area-wide pest alert from the California Avocado Commission (CAC 2013) to warn growers. Due to the lack of effective biological control, the integrated pest management program of *O. perseae* has shifted its reliance almost exclusively to chemical control and this less than ideal situation can lead to other unintended problems such as the development of pesticide resistance among populations (Hoddle and Morse 2013). This increased reliance on chemical control has also been observed with other invasive arthropods that have been introduced into California (Hoddle 2005), such as *S. perseae* (see Sect. 1.5.2), furthering lessening grower reliance on biological control and moving IPM programs from being biologically-based to being pesticide-focused.

To promote judicious pesticide use, a binomial sampling plan for *O. perseae* was developed by Lara and Hoddle (2013). This sampling plan can be used by pest managers to monitor densities of *O. perseae* populations in commercial orchards and is compatible with the long-standing concept of controlling pest populations when densities exceed research-based action thresholds that once crossed will result in economic losses (Stern et al. 1959, Maoz et al. 2011b). Sampling plans are also being developed (Lara and Hoddle, unpublished data) that can be used to monitor densities of resident phytoseiids in commercial orchards. These sampling plans have potential to allow avocado pest managers to concurrently maintain accurate, chronological, and quantitative records on *O. perseae* and phytoseiid populations. Subsequently, these assessments can be used to make informed decisions on pesticide use to target *O. perseae* populations and whether predatory mites are likely to provide some level of control.

Compared to *O. perseae*, these types of custom sampling strategies were not developed for *O. punicae* and *E. sexamuculatus* on California avocados. This reflects the success of natural biological control for these latter species which obviated the pressing need for implementing these types of statistically-based sampling tools. Several alternatives exist for improving the biological control program of *O. perseae* in California. These alternatives are discussed below and include (1) the development of a classical biological control program, (2) the development of augmentation or conservation biological control strategies using largely unstudied resident populations of stigmaeid species present in commercial avocado orchards from California, and (3) addressing the issue of cultivar susceptibility to *O. perseae*. The first two research avenues emphasize top-down control while the third option stresses the idea of exploiting bottom-up (host plant based) strategies to manage of *O. perseae* populations.

1.7.1 Classical Biological Control Using Phytoseiids

Classical biological control is the deliberate practice of prospecting for co-evolved natural enemies in the evolutionary center of origin of the pest. Areas within this native range may be selected preferentially for searching based on climate similarities between the donor (i.e., native range) and receiving (i.e., invaded area) regions. Biological control theory suggests that natural enemies pre-adapted to the prevailing climate in the receiving range are more likely to establish than natural enemies from areas where there is climatic mis-match. Additionally, molecular-based studies may pinpoint areas within a large native range from within which the invading pest population originated. Searching in these areas where there is a close genetic match between the source and invasive population may result in the discovery of natural enemies adapted to the dominant pest genotype in the invaded range. These two things, climatic and genetic matching, may improve the chances of finding natural enemies that could be effective classical biological control agents. Natural enemies released as part of a classical biological control program are expected to establish, spread, and provide permanent suppression of pest populations without ongoing human management. Perennial agro-ecosystems like avocado orchards provide long-term habitat stability that increases the likelihood of natural enemy establishment.

Surveys for mites associated with avocados in Michoacán (Mexico), the largest Hass producing region in the world, resulted in the documentation of 18 species in nine families, of which five in three families were phytophagous and 12 species in four families likely exhibit some level of predatory behavior (see Table 1 in Estrada-Venegas et al. 2002). *O. perseae* and *O. punicae* were commonly encountered during surveys and it was noted that they were of high economic importance in Hass orchards in Michoacán. The three most important phytoseiid predators found in Michoacán were *Amblyseius* (*Typhlodromalus) limonicus*, *E. hibisci*, and *Neoseiulus* sp. *A. limonicus* and *E. hibisci* are residents in California avocado orchards but have provided limited control for tetranychids (McMurtry and Scriven 1965b, McMurtry et al. 1984, Estrada-Venegas et al. 2002). *N. californicus* is a representative of the genus mentioned by Estrada-Venegas et al. (2002) but populations of these predators have not established on avocados for permanent control of *O. punicae* (McMurtry et al. 1984) or *O. perseae* (Hoddle et al. 2000) in California.

In support of work completed by Estrada-Venegas et al. (2002), additional surveys conducted by Hoddle and Lara (unpublished data) in 2012 for predator mites associated with *O. perseae* on Hass and non-Hass avocados were completed in five states in Mexico; Michoacán, Puebla, Morelos, Guanajuato, and México. A total of 21 sites were sampled and a total of 585 natural enemy specimens were collected, of which, 92% of the sampled predators were members of the Stigmaeidae and Phytoseiidae. Further taxonomic and molecular studies are needed to generate a list of unambiguously identified predator species present in Mexico that are associated with *O. perseae*. From this species list, selection of possible biological control candidates for deliberate introduction into California avocado orchards as part of a classical biological control program may be identified. However, these selected agents, if found, would have to demonstrate superior abilities for controlling *O. perseae* when compared to natural enemies already present in California, a finding which could be unlikely. In addition to predator mite species, 2,268 *O. perseae* were collected. These specimens are being used in molecular studies in an attempt to identify the possible source area in Mexico from which the invasive California *O. perseae* population originated. This area of origin, should it be identified, will further refine our interpretation of the importance of natural enemy species associated with *O. perseae* in its native home range.

The consistency across two surveys conducted more than 10 years apart of these two findings, regular outbreaks of *O. perseae* and *O. punicae* on Hass, and a diverse resident predatory guild that is unable to reliably keep these two pest mites below economically damaging densities, supports the suggestion that the susceptibility of the Hass cultivar to spider mites rather than a lack of natural enemies could be the more important factor driving the pest status of these two species, especially *O. perseae*. This observation was striking at times in Mexico when Hass avocados were inter-planted with non-Hass plants; the Hass variety was always noticeably more damaged by *O. perseae* than unidentified non-Hass varieties.

1.7.2 Biological Control Using Stigmaeids

Compared to members of the Phytoseiidae, the use of predatory mites from the family Stigmaeidae as biological control agents for spider mites has not been well studied and this deficiency has been noted previously (Huffaker et al. 1969, Gerson et al. 2003). Nevertheless, there are some examples of stigmaeids that under certain circumstances have been shown to provide effective spider mite biological control agents in agricultural systems. Two well-documented examples include *Zetzellia malli* (Ewing) on apple for control of the spider mite *Panonychus ulmi* (Koch) and *Agistemus longisetus* González-Rodríguez on citrus for control of *Panonychus citri* (McGregor) (Croft and MacRae 1993, Jamieson et al. 2005). Aside from these two examples with spider mites from other orchard crop systems, the use of stigmaeids as effective biological control agents for *O. perseae* in commercial avocado orchards remains to be demonstrated.

1.7.2.1 Field Studies on Stigmaeidae in California Hass avocados

Currently, the work by Yee et al. (2001a) is the only published avocado field study which included seasonal monitoring of *O. perseae*, stigmaeid (identified only to family) and phyotseiid (mainly *E. hibisci*) populations in commercial avocado orchards in California. Results from Yee et al., (2001a) indicated that stigmaeid populations were not as abundant as phytoseiids and overall populations of both of these predators did not respond numerically to increases in *O. perseae* densities. More recently, naturally-occurring stigmaeid and phytoseiid populations were monitored monthly during 2012-2013 (Lara, unpublished data) on an untreated research plot of Hass avocado trees in Irvine, California. Similarly, densities of stigmaeids and phytoseiids (most likely *E. hibisci*) did not reflect a strong numerical response to increases in *O. perseae* during the summer when average densities peaked at more than 50 (2012) and 126 (2013) *O. perseae* per leaf. Still, under field conditions, stigmaeids and phytoseiids were found naturally at other times of the year when *O. perseae* levels were low and this suggests they likely have a generalist feeding habit, are capable of co-existing to some extent (i.e., intraguild predation may not be significant), and are permanent year round resident species in the avocado system.

Progress on stigmaeid research on avocados has not been limited by the unavailability of taxonomic keys to identify this group of organisms to species or the lack of information on the presence of stigmaeids on avocado. These records have been available for several decades since the detailed taxonomic revision on stigmaeids was published by González-Rodríguez (1965). For example, there are at least nine species of stigmaeids that have been historically recorded on avocados throughout the world and these include *A. arcypaurus* González-Rodíguez, *A. ecuadoriensis* González-Rodríguez, *A. fleschneri* Summers, *A. longisetus* González-Rodríguez, *A. simplex* González-Rodríguez, *A. striolatus* González-Rodríguez, *A. tucumanensis* González-Rodríguez, *A. terminalis* (Quayle), and *Z. yusti* Summers.

Among these, *A. arcypaurus*, *A. fleschneri*, *A. longisetus*, and *A. terminalis* have been documented on California avocados (González-Rodríguez 1965) but extensive knowledge on relevant aspects of their ecology within this crop system is unknown (e.g., geographic distribution, phenology, interactions with other mite species, etc.). This information is essential to design experiments and investigate the role of stigmaeids as spider mite natural enemies in avocados. Interestingly, *A. terminalis* and *A. longisetus* have been collected on avocados from Mexico and Central America (González-Rodríguez 1965) and this territory includes the geographic home range of *O. perseae*. This potentially significant ecological connection between *O. perseae* and stigmaeids has received little attention. This observation may reflect confirmation bias in spider mite biological control research that leads to primary experimentation with candidate phytoseiid species because of their (1) documented historical and economic importance and (2) commercial availability. However, as discussed in Sect. 1.6, resident and introduced species of phytoseiids have had limited application for control of *O. perseae* and therefore the development of additional complementary biological control alternatives need to be considered and stigmaeids may be important in this regard.

1.7.2.2 Additional observations on Stigmaeidae on California Hass avocados

A California avocado mite fauna survey was conducted in 2011 by Lara (unpublished data). After Phytoseiidae, Stigmaeidae was the second most abundant group of predatory mites recorded on avocado. A total of 172 phytoseiid females were collected from 700 leaves whereas 102 motile stigmaeid specimens were collected from the same leaves. The natural presence of stigmaeids was detected in several Hass avocado orchards from southern California, including San Diego, Ventura, Santa Barbara and San Luis Obispo Counties. These areas are the principal commercial avocado production counties in California. On sampled leaves, stigmaeids and phytoseiids were found predominantly on the underside of avocado leaves, probably because the abaxial surface provides natural shelter (i.e., domatia) at leaf vein junctions. This ecological interaction between host plant structures and predators has been previously observed for both stigmaeids (*A. longisetus*) and phytoseiids (*E. elinae* [Schicha]) (Walter and O'Dowd 1992).

During the course of this survey, an abundant natural population of stigmaeids was found residing within an organic Hass avocado orchard in Santa Paula, California that was infested with *O. perseae*. An additional larger sample of avocado leaves was subsequently collected from this orchard. Out of 144 randomly selected mature leaves collected (i.e. 1 leaf per tree), 43% contained at least one motile stigmaeid. Phytoseiids (*Euseius* spp.) were also present but as leaves were visually inspected in the field it became apparent that they were not as abundant as stigmaeids. Sampled leaves were processed in the laboratory for the collection of adults. A total of 81 adult stigmaeids were collected, approximately 32 females and 49 males visually identified, whereas only 9 adult female phytoseiids were observed and collected from the same 144 leaves. Unlike stigmaeids, adult male phytoseiids were not sampled because taxonomic keys for the species identification phytoseiids are based on female morphological characters.

Stigmaeids from this orchard in Santa Paula were tentatively identified as *A. longisetus* (Fig. 1.2c) using the taxonomic key prepared by González-Rodríguez (1965) which includes descriptions for both adult sexes. Another species, *A. tucumanensis*, was recorded on avocados from Argentina along with *E. hibisci* (González-Rodríguez 1965). *A. tucumanensis* is morphologically similar to *A. longisetus*, but its presence on avocados from California has not been recorded. Therefore, the sampled stigmaeid population from the orchard in Santa Paula, California is most likely *A. longisetus*. Lines of this natural stigmaeid population were easily reared in the laboratory on excised avocado leaves on a diet of either ice plant pollen (*Mesembryanthemum* sp.) or *O. punicae* to conduct behavioral studies similar to those conducted with candidate phyotseiid species for the control of *O. perseae* (Takano-Lee and Hoddle 2002).

In general, motile stages of *A. longisetus* are slow-moving in contrast to fast-moving *E. hibisci*. Even so, similar to *E. hibisci*, *A. longisetus* cannot penetrate the webbed nest of *O. perseae* and both species are capable of attacking motile *O. perseae* individuals wandering outside of nests (Fig. 1.2c)(Lara, unpublished data). For this reason, *A. longisetus* was reared on *O. punicae* instead of *O. perseae* on the adaxial leaf surface of avocado leaves where this spider mite feeds and constructs it webbing (see Sect. 1.3). *O. punicae* populations consisting of several hundred mites can be easily reared on excised non-Hass avocado leaves and these can sustain feeding *O. punicae* populations for approximately 1-2 weeks. Interestingly, mass-rearing *O. perseae* on a Hass leaf diet was not possible as leaves would deteriorate quickly and populations would not increase as quickly as *O. punicae* in the laboratory

Under laboratory rearing conditions, motile *A. longisetus* have access to all stages of *O. punicae* and can lay eggs on the webbing of this spider mite species, but these predators have some difficulty moving easily on webbing produced by *O. punicae*. On a pollen diet, *A. longisetus* can move easily over the leaf surface, albeit slowly, and will lay eggs on cotton strands. Still, *A. longisetus* did not reproduce in great numbers when reared on ice plant pollen or *O. punicae* alone. In contrast, rearing *E. hibisci* and *N. californicus* on ice plant pollen and *O. punicae*, respectively, produced significantly more progeny. Consequently, food had to be provided more frequently for phytoseiids than stigmaeids. It is also important to note, that in some cases, adults of *A. longisetus* were engaged in intraspecific predation of eggs, but cannibalism did not adversely affect the continuous production of these predators over a two year period in the laboratory because alternate food was provided abundantly.

1.7.2.3 Potential application of Stigmaeidae on California Hass avocados

These laboratory observations reveal potential issues for customizing the use of candidate stigmaeids such as *A. longisetus* for the biological control of *O. perseae* in California. However, drawing tentative conclusions from these observations about the efficacy of stigmaeids for biological control of pest mites in California avocado orchards must be made with caution until quantitative laboratory and field studies similar to those conducted for *Euseius* spp. in California are completed (McMurtry and Scriven 1964, McMurtry and Johnson 1966). Nevertheless, the inability of *A. longisetus* to enter the webbed nest of *O. perseae* indicates that the ecological potential of this predator to control *O. perseae* is likely constrained when pest populations build during spring and are physically protected from predators by webbed nests.

It is also worth noting that part of the biological control success for controlling *P. ulmi* and *P. citri* with stigmaeids as reported previously (Croft and MacRae 1993 , Jamieson et al. 2005) may be due to the fact that *Panonychus* spp. lack complex webbing structures (Saito 1983). This webbing life type perhaps facilitates access of *Panonychus* life stages not only to stigmaeids but also other potential spider mite predators such as *Stethorus* beetles (Jamieson and Stevens 2009). The little webbing life style contrasts significantly with the complicated and webbed-nest structures of *O. punicae* and *O. perseae*, respectively, which have been demonstrated to physically restrict access to predators (McMutry and Johnson 1966, Takano-Lee and Hoddle 2002).

Furthermore, the relatively lower reproductive potential of *A. longisetus* on diets consisting of *O. punicae* and pollen, which have been previously effective for rearing phytoseiids, places economic limitations on mass-rearing *A. longisetus* for the purpose of implementing augmentative biological control strategies against *O. perseae* in avocado orchards. Other suitable rearing diets which might provide improved predator production such as pollen from other host plants, other factitious spider mite species (e.g., *Panonychus* spp.), the selective provisioning of harvested spider mite eggs, or the combination of pollen and spider mites have not been evaluated.

Ideally, it would be expected that the presence of less threatening *O. punicae* populations in avocado orchards might serve as an alternative natural food source for released or natural populations of stigmaeids like *A. longisetus*. Predator populations could build upon this resource and "spill over" onto *O. perseae*. Whether or not this type of apparent competition exists in avocado orchards and would translate into effective *O. perseae* control is unknown. The prospect of promoting this dynamic in avocados is uncertain for *A. longisetus* because this predator has trouble accessing *O. perseae* nests and will not readily reproduce on spider mites like *O. punicae*. These same mobility and reproduction problems were observed with *E. hibisci* in the context of *O. punicae* biological control (McMutry and Johnson 1966). A natural and alternative rearing host on avocados for stigmaeids might be tydeid mites which were also frequently encountered in commercial avocado orchards sampled in 2011 and were found at leaf junctions where stigmaieds and phytoseiids are likely to be found. Again, this potential component of the predator-prey system in California avocados has not been studied.

Since stigmaeids can occur naturally in commercial avocado orchards in California, another approach that could provide some level of *O. perseae* control would be to design a conservation biological control strategy. These efforts may also complement the conservation of beneficial phytoseiids like *E. hibisci*. Resource subsidization such as pollen-provisioning requires significant economic and resource input and is not considered a viable option in California. Instead, conservation might be easily attained by modifying cultural practices for pest management on avocados in the form of selective pesticide use that will not directly harm stigmaeid (or phytoseiid) populations. Due to the introduction of invasive pests into California avocado orchards, pesticide use has increased substantially (Hoddle 2005). In general, the selective use of pesticides has been implicated in preserving stigmaeids in agricultural systems (Gerson et al. 2003). The complementarity between pesticide applications and natural enemies was studied, in part, in the New Zealand citrus system in the context of *P. ulmi*, *A. longisetus*, and a *Stethorus* sp. (Jamieson and Stevens 2009). Pesticide use modification is perhaps a contributing factor to the pronounced presence of *A. longisetus* at the organic orchard in Santa Paula, but surprisingly no *Stethrous* predators were detected in association with *O. perseae*. To begin realizing these conservation efforts for natural enemies on avocados, toxicity studies would need to be conducted to identify registered pesticides that are compatible with stigmaeids and other predators. This type of study was previously conducted for *E. hibisci* in California where the toxicity of field weathered residues was assessed (Zahn 2011).

However, before directing efforts to conserve stigmaeid populations on avocados, the ecological impact of their presence on populations of non-target beneficial heterospecfic predators like phytoseiids requires more study. All of these predators would normally be found in sympatry on the underside of avocado leaves and would most likely compete for food and shelter. Therefore, understanding the outcome of these potential ecological interactions is critical for improving the *O. perseae* biological control program with resident natural enemies and selective pesticides. For example, the generalist predators, *E. hibisci* and *N. californicus*, are capable of intraspecific predation but display a preference for heterospecifics (Schausberger and Croft 2000). Furthermore, the dominant presence of resident *E. hibisci* populations has been implicated in the past for the failed establishment of other phytoseiid species on avocados (see Sect. 1.3.1). The heterospecific feeding habits of *A. longisetus* have not been studied but evidence of reduced levels of cannibalism in the abundance of spider mites and the differential impact of intraguild predation on phytoseiids by stigmaeids has been studied with *A. exsertus* González and *Z. mali*, respectively (MacRae and Croft 1996, Rasmy and Saber 2012). Consequently, the degree of selective predation stigmaeids display with extant confamilial species (even among stigmaeid conspecifics), and other native heterospecific predators (phytoseiids) that occur on avocados, may reveal evidence of asymmetric intraguild predation that hinders effective biological control of *O. perseae*. If detected, evidence of this dynamic could further explain the ecological limitations of *E. hibisci* to control *O. perseae* on avocados. In addition, this information could help identify additional ecological barriers that could diminish the biological control benefits derived from the present introduction of (1) phytoseiid species like *N. californicus* as part of augmentative biological control efforts against *O. perseae* or (2) other predator species introduced from the home range of *O. perseae* in Mexico as part of classical biological control efforts. Consequently, biotic resistance from resident predator guilds mediated via interactions like intraguild predation may simply be too great to manipulatively enhance naturally-occurring biological control.

1.7.3 Cultivar Improvements in California for Pest Control

During the past decades Hass fruit has become the benchmark of fruit excellence in most subtropical commercial avocado growing areas and this has resulted in this cultivar dominating global avocado fruit production (see Sect. 1.5.1). However, optimal production of Hass avocados can be limited by several biological factors such as susceptibility of trees to cold temperatures, inconsistent fruit yield between years (i.e., alternate-bearing), and tree susceptibility to other potential stress factors (e.g., soil salinity, diseases, and herbivore feeding) (Bergh 1985). In the context of foliar-feeding spider mite pests present in the California avocado system, the Hass cultivar is relatively more susceptible to attack by *O. perseae*. Furthermore, the impact of *O. perseae* on California avocado production may have been exacerbated by the region-wide cultivation of Hass trees which produced a monoculture of susceptible host plants. A similar intensified dynamic between foliar-feeding spider mite populations and avocado host plants may be in effect in other areas where Hass is grown (see Sect. 1.5.2, 1.5.3).

This emerging pattern implies that integrated pest management of spider mites, and potentially other arthropods pests (e.g. *S. perseae*), on avocados in areas like California could be improved naturally (without the use of pesticides) by implementing bottom-up (i.e., host plant based) strategies that complement biological control efforts. One potential strategy involves the identification and selective use of novel, marketable cultivars that bolster the biological resistance of the California avocado system against populations of key arthropod pests (e.g., S. *perseae* and *O. perseae*). The development of herbivore-resistant cultivars to target pest populations could be facilitated by identifying and exploiting key host plant mechanism(s) (e.g., physiological, biochemical characteristics) responsible for the reproductive advantage or disadvantage of pests like *O. perseae* on avocado cultivars like Hass and Fuerte, respectively. It is likely that these mechanisms are influenced by the interaction of cultivar genetics and input from the environment (climate) since these components are known to influence cultivar phenotypes and define optimal avocado growing requirements (see Sect 1.1, 1.5.1).

Furthermore, diversifying avocado cultivar acreage is essential to minimize crop losses due to pests. Avocado cultivars with improved levels of herbivore-resistance could be planted selectively by growers in locations with specific climatic conditions that are amenable to producing high quality avocado fruit that are competitive with Hass but offer reduced reproductive advantages to key avocado pests. For example, Hass avocado grows favorably in coastal areas but *S. perseae* and *O. perseae* populations are also capable of doing well in this environment. Therefore, a variety of commercially-acceptable cultivars that are resistant to these pests could be grown in these areas instead of Hass.

With regards to *O. perseae*, the integrated use of herbivore-resistant cultivars may promote the following ecological mechanisms: (1) resistant cultivars will offer a natural buffer against elevated *O. perseae* densities during spring and summer in areas with climates permissive of spider mite outbreaks and (2) a lower baseline of *O. perseae* infestations could allow resident or introduced species of predatory mite populations (see Sect. 1.7.1, 1.7.2) to display improved efficacy because lower densities of pests need control. In addition, these beneficial mechanisms could lower grower concerns over spider mite outbreaks, promote reduced pesticide use, and ultimately restore the natural spider mite balance of the California avocado system as it was known prior to the *O. perseae* invasion (Fleschner et al. 1956a). Reaching this end-point will require a long-term investment on interdisciplinary research that could be adopted as part of future research efforts of the California avocado breeding program.

The long-term goal of the California avocado breeding program is to screen and select new marketable cultivars (scions) and compatible rootstocks. Fuerte is known to be resistant to *O. perseae* populations but this cultivar displays erratic yield and is unlikely to regain acreage dominance in California and replace Hass avocados. Some improved cultivars related to Hass (e.g., GEM and Lamb-Hass) have been identified in California. Lamb-Hass in particular is known to be resistant to *O. perseae*, but its susceptibility to other herbivores in California and the underlying mechanism for this resistant phenotype is unknown. GEM is a promising cultivar (Crane et al. 2013) that has potential to replace Hass acreage in California, but its susceptibility to *O. perseae* has not been evaluated (M.L.Arpaia pers. comm. 2014). Nevertheless, the replacement of Hass acreage with herbivore- resistant cultivars is uncertain and will depend on market factors such as consumer preference. In addition, the California industry will need time to study and optimize the benefits of potential acreage transitions to remain competitive with other major Hass-producing countries that export avocados to the United States (e.g., Mexico and Chile).

A different and possibly more acceptable host plant based approach could be the exploration of rootstock induced herbivore-resistance for Hass scions. For example, rootstocks can be evaluated and selected based on the ability to tolerate challenging edaphic conditions (e.g., high salinity), or their tolerance to *Phytophthora cinnamomi* Rands, which causes avocado root rot. Aside from these selection factors, it is also known that rootstocks can influence the phentotype of commercial cultivars. Clear evidence of this was documented with Hass avocado and the rootstock Martin Grande (G755). Martin Grande confers root rot resistance to Hass scions but it also induces the expression of negative traits like lower tree productivity and alternate bearing on Hass (Crane et al. 2013). Since rootstocks can influence cultivar phenotypes then it might be possible to screen for a Hass-compatible rootstock that (1) increases or at least does not hinder consistent yield, (2) does not negatively alter favorable Hass fruit characteristics, (3) confers tolerance to root rot, and (4) also mediates scion-resistance to key pests of avocados in California like *O. perseae*. The search for rootstock-scion combinations that meet these requirements has not been studied in California but this approach also has potential to restore the biological resistance of the California avocado system against exotic pests and promote competitive Hass avocado production with reduced reliance on pesticides.

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Figure Legends

Fig. 1.1 a) Feeding adult *Oligonychus perseae* (Acari: Tetranychidae) and round-shaped conspecific eggs underneath a webbed-nest (transparent) on avocado leaf undersurface, b) Accumulated necrotic damage on avocado leaf undersurface (concentrated along veins) caused by feeding populations of *O. perseae* and which can lead to pre-mature defoliation on host avocado trees, c) Sunburn (circular black blemish) on maturing avocado fruit due to limited cover protection from foliage

Fig. 1.2 a) Adult *Euseius* sp. (Acari: Phytoseiidae) feeding on a motile *Oligonychus perseae* (Acari: Tetranychidae) individual on avocado leaf undersurface, b) Adult (*black arrow*) *Neoseiulus californicus* (Acari: Phytoseiidae) resting after feeding on spider mites inside transparent *O. perseae* webbed-nest (*white arrow* points to oval-shaped phytoseiid eggs outside of the nest), c) Adult predatory mite (Acari: Stigmaeidae) feeding on a motile *O. perseae* individual

Fig. 1.1.

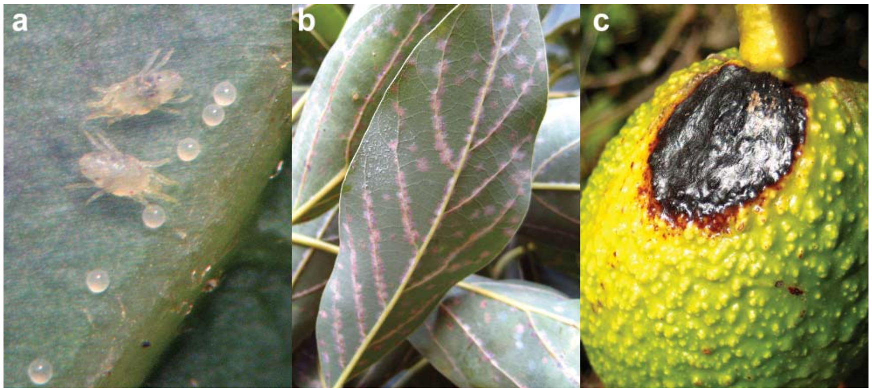

Fig. 1.2.

Chapter 2

Evaluation and empirical model fitting for count data to estimate densities of *Oligonychus perseae* (Acari: Tetranychidae) on 'Hass' avocado leaves in southern California

ABSTRACT *Oligonychus perseae* (Acari: Tetranychidae) is an important foliar spider mite pest of 'Hass' avocados in several commercial production areas of the world. In California, *O. perseae* densities in commercial orchards can exceed more than 100 mites per leaf and this makes enumerative counting a prohibitive strategy for field sampling. In this study, a documented approach to estimate whole leaf densities of *O. perseae* using a partial count of mites along half of a leaf vein on an avocado leaf was evaluated for accuracy. Sampling simulation results revealed that this partial leaf vein count method, in its original form, underestimated mite densities in a range of 15-60%. Consequently, four alternate negative binomial regression models were fit to field-collected data and these models were evaluated for their ability to estimate mite densities on whole leaves within small 30-tree blocks of avocados. Model 3, a revised version of the original half-vein model, showed improvement in providing reliable estimates of *O. perseae* densities for making assessments of general leaf infestation densities across orchards in southern California. The implications of these results for customizing the half-vein method as a potential field sampling tool and for experimental research purposes in the California avocado system are discussed.

KEY WORDS *Oligonychus perseae*, *Persea Americana*, Sampling, Mixed modeling, Negative binomial regression

Oligonychus perseae Tuttle, Baker and Abatiello (Acari: Tetranychidae) is an economically important foliar spider mite pest of 'Hass' avocado (*Persea americana* Miller [Lauracaeae]) in several countries (i.e., Spain, Costa Rica, Mexico, Israel, USA) where this crop is grown commercially. In California, research efforts to design a sustainable pest management program for *O. perseae* that is based on biological and chemical control have centered on screening and understanding the role of commercially-available phytoseiids (Hoddle et al. 2000), expanding the selection of pesticide chemistries to mitigate resistance development (Humeres and Morse 2005), and examining the compatibility between pesticides and naturally-occurring biological control agents, in particular, predatory phytoseiids (e.g., *Euseius* spp.) in commercial avocado orchards (Zahn 2011). However, as with any pest management program designed for an agricultural system, success in implementing control measures depends on reliable sampling methods to estimate pest densities.

Estimating densities of *O. perseae* and spider mites in general, represents a sampling challenge because mites are very small and counting to accurately estimate densities typically requires the use of a microscope or hand-lens. Moreover, populations of *O. perseae* motile stages that cause feeding injury to leaves during the California avocado growing season (March-October) can reach hundreds of mites per leaf and this makes enumerative sampling a time-prohibitive strategy for pest control advisers and growers to adopt for field use. Even for pest management researchers, the process of counting *O. perseae* on leaves can quickly become a time-limiting factor when there are many treatments (e.g., pesticides, natural enemy species) and replicates (i.e., trees per treatment and leaves per tree) to evaluate. This is particularly true when during the course of a sampling interval it is imperative to maintain leaf sample quality as close to the original sample date to avoid obtaining biased counts of living mites that could die in storage thereby causing misleading conclusions about treatments.

Although there have been technological advancements to facilitate the counting process, such as mite brushing machines (Henderson and McBurnie 1943, Morgan et al. 1955), so that mite counts can be conducted without the obstruction of webbing and debris, these approaches are still considered to be too time consuming for field use. Consequently, attempts to facilitate quicker estimates of mite densities have focused on reducing the counting effort by relating densities of mites (y) to some other reliable mite-density indicator (x) that can be readily measured for a fraction of the time and cost.

Previously, Machlitt (1998) developed a custom “half-vein” sampling method for monitoring field densities of *O. perseae* in California avocado orchards. The half-vein method was based on a simple linear regression model with a zero-intercept that estimated densities of *O. perseae* by multiplying the number of motile mites, for each of 10 sampled avocado leaves, that occupied the upper margin of the second major left vein (UML2) on the leaf undersurface by a factor of 12 (i.e., the regression coefficient). The UML2 vein extends from the left side of the midrib to the left leaf margin and is the second prominent vein encountered from the petiole end of the leaf (Machlitt 1998). The statistical correlation between *O. perseae* densities and partial mite counts on UML2 for four age categories of in-season avocado leaves (i.e., less than half-expanded, more than half-expanded, fully-expanded, and combined leaves of all ages) was found to be greater than 0.85 in all cases and statistical modeling was based on mean values (n=62) for sample batches consisting of 10 avocado leaves instead of individuals leaves.

Since the development of the half-vein method, the recommendation made to California pest control advisers interested in using the half-vein method to make assessments of *O. perseae* infestations in commercial avocado orchards was to collect at least one in-season leaf, of mixed-age, for each of ten trees (UC IPM 2011). However, the optimal sample size and the manner of leaf selection to obtain reliable mite density estimates for a block of avocado trees were not determined and a robust performance evaluation of this sampling method for field use has not been conducted since it was developed. Despite these evaluative shortcomings, the half-vein method has been used in California in pesticide efficacy field trials for control of *O. perseae* (Morse 2008, Morse 2011). Outside of California, the half-vein method was adopted to estimate an action threshold range for *O. perseae* in Israel (Maoz et al. 2011), and other mite density estimation properties associated with vein UML2 (i.e., the number of *O. perseae* occupied nests and necrotic spots caused by mite feeding damage) were used to assess the efficacy of conservation biological control practices (i.e., pollen provision for phytoseiids) for *O. perseae* suppression (González-Fernández et al. 2009).

More recently, Lara and Hoddle (in press) developed a new sampling method based on presence-absence sampling for a fixed sample size of 30 avocado leaves that can be used by pest control advisers to assess densities of *O. perseae* in commercial California avocado orchards. This presence-absence sampling method relates mean densities of *O. perseae* to the proportion of mature avocado leaves infested with at least one mite and this approach reduces the counting burden more than the half-vein method. Additional statistical research and field validation has been conducted to determine the optimal manner of leaf selection within and between trees (DePalma et al. 2012) over large areas (i.e., tree blocks with 200x200 tree dimensions) based on an understanding of the spatial dynamics of *O. perseae* in commercial avocado orchards (Li et. al. 2012). The work necessary to customize the presence-absence sampling strategy for the California avocado system required enumerative counting of *O. perseae* on the entire leaf undersurface of avocado leaves. During this data collection process, we used this counting exercise as an opportunity to conduct a performance evaluation of the half vein method in its original form and to improve assessments of the relationship, if necessary, between *O. perseae* densities and the number of mites found along vein UML2 using statistical analyses of count data. The results of the original half-vein method evaluation and statistical modeling of mite counts from UML2 for estimating densities of *O. perseae* on avocado leaves are reported here.

Materials and Methods

Leaf Collection and Enumerative Mite Counts. In-season, mature ‘Hass’ avocado leaves were collected cross-sectionally during the peak summer season of *O. perseae,* June-September (Yee et al. 2001), from 10 commercial avocado orchard sites in southern California were processed in the laboratory (Table 2.1). In California, avocado trees typically have a spring and summer growth flush period during the year. Typically, the flushing period extends from April to July (UC IPM 2008) and recognition of each type of flush from current and previous seasons can be detected by looking at the visible bud scars on shoots (see Cutting 2003).

A selection preference for mature leaves was based on the availability of these leaves during summer and an understanding that *O. perseae* populations on mature leaves had a longer time to build up since the spring leaf flush period, reflecting the current severity of mite infestations within the sampled orchard. Consequently, these data are of primary importance to pest managers monitoring levels of *O. perseae* at these sites. At each site, eight avocado leaves per tree (i.e., two leaves randomly selected per cardinal point) were collected from trees arranged in 5x6 grids that were situated within larger blocks of avocado trees that were infested with *O. perseae*.

An 11th orchard located in Irvine, California was sampled monthly in 2012 and 2013 during the *O. perseae* season, approximately May-September, when monitoring for this pest is recommended (UC IPM 2011). The longitudinal data collected from orchard 11 was reserved for validation of the updated half-vein method (see below) over two field seasons. At this site, ten 'Hass' avocado leaves from spring flush were collected per tree from two groups of untreated trees, 1 (n=9 trees) and 2 (n=8 trees), planted on an 18x14 grid. The only difference between these groups was that trees from group 2 were pesticide treated in 2003 (9 yr before data for this evaluation was collected). In this study, a selection preference for spring leaves over summer leaves was made to widen the observation window of *O. perseae* densities on aging leaves (i.e., less than half-expanded to fully-expanded leaves).

For each sampled leaf across all eleven sites, information was recorded for three variables: 1) y_{obs}, the total number of motile *O. perseae* mites (all stages except eggs) on the entire leaf undersurface, 2) x_1, the number of motile *O. perseae* mites situated along the upper margin of vein UML2 as discussed previously and 3) x_2, leaf length (cm) which was measured as the direct distance along the midrib between the leaf tip and base proximal of the petiole. Leaf length was recorded to test the null hypothesis that higher mite counts were not correlated with larger leaves.

Evaluation of the Original Half-Vein Method. The mite count database comprised of sites 1-9 was divided into two subset databases, A (n=1212 leaves) and B (n=1210 leaves). Because orchards 1-9 had two replicates per cardinal direction per tree (i.e., 8 leaves per tree), the data from the first and second replicates were assigned to databases A and B, respectively. Database B had partial data missing for two leaf sample units and these were not included in the analyses, whereas database A was complete with all four leaves available per tree. For orchard 10, mite count data from two adjacent 5x6 blocks were assigned to each database because there was only 1 replicate set of leaves (i.e., 4 leaves) for each sampled tree. Database A was first used to evaluate the performance of the original half-vein method for each orchard and then was used as a training set to generate a new model that redefined the relationship between mite counts on the entire leaf undersurface and partial mite counts on vein UML2. Database B, and the data collected from site 11, were used to cross-validate the newly described relationship.

For each orchard within database A, the mean of mite counts for vein UML2 was multiplied by 12 to predict the average number *O. perseae* per leaf. This general multiplication factor, as it appears in published pest management guidelines (UC IPM 2011), accommodates leaves of all age classes and is close to the original regression coefficient (11.35) reported for fully expanded avocado leaves (Machlitt 1998). The predicted mite densities were compared to the observed densities for each orchard site using the percent error formula as a measure of accuracy: 100 x $[(y_{pred} - y_{obs}) / y_{obs}]$. Negative percent error values indicated that the expected mite density has been underestimated and positive values indicated that the expected mite density has been overestimated. Ideally, the percent errors for a model should be close to zero.

Correlation Between Response and Predictor Variables. For each site, the degree of association between observed total mite counts, x_1 and x_2 was evaluated by determining Spearman's correlation coefficient for each variable pairing using PROC CORR in SAS Software 9.3 (SAS Institute 2011). Unlike Pearson's product moment correlation statistic, a valid interpretation of Spearman's rank correlation does not depend on the assumptions that the data being analyzed follow a normal distribution and for pairs of variables to be linearly correlated, only that the relationship be monotonic (McDonald 2009).

Relationship Between Total Mite Counts and Vein UML2. The relationship between observed mite counts and x_1 was defined using negative binomial regression under the framework of generalized linear mixed models (GLMMs) to account for the non-normal distribution of the count data, its overdispersion behavior, and the potential contribution of random effects from orchards and avocado trees in explaining the observed response variation. Under the GLMM framework, the canonical log link function was used to restrict the expected mean response to positive values, a scale parameter (k) was used to model the variance as function of the mean and, when specified, random effects were accounted through the linear predictor (i.e., as G-side random effects). Although the response variable did not need to be transformed, transformation of the predictor variable x_1 (Faraway 2006) was needed to linearize the relationship on the link scale. Initially, linear (i.e., 1st and 2nd order polynomials) and nonlinear model equations with a single fixed effect were selected from a screening of an extensive compilation of functions reviewed by Ratkowsky (1990) and subsequently re-parameterized to obtain linear predictors for final model fitting. Below are selected candidate models on the response scale using the inverse exponential link,

$$y = \exp[\alpha + \beta \ln(x_1 + 0.31)] \tag{1}$$

$$y = \exp[\alpha + \beta \ln(x_1 + 0.31) + u] \tag{2}$$

$$y = \exp[\alpha + \beta \ln(x_1 + 0.31) + v] \tag{3}$$

$$y = \exp[\alpha + \beta \ln(x_1 + 0.31) + u + uv] \tag{4}$$

where y refers to the expected mean of total *O. perseae* counts on a leaf given the presence of random effects; x_1 is the partial count of *O. perseae* along vein UML2 ; α and β are estimated parameters for the fixed effect and μ and ν represent random effects attributed to avocado orchards and trees, respectively. The term uv represents the interaction between orchard (block) and individual avocado trees at each site. When specified in the models, these random effects can account for the clustered data collection process given that a subset of orchards and nested levels of individual trees were sampled from a large population in southern California: there are approximately 5,000 commercial California avocado growers and the average orchard size is four hectares (CAC 2013).

Because x_1 can assume a value of zero, an estimated constant γ=0.31 was added to the equations to avoid calculation errors (i.e., natural logarithm of zero) and obtain a linear predictor as expected for GLMMs. An estimate of γ was obtained by first fitting the template of model 2 using the flexible NLMIXED procedure and TruReg estimation method in SAS 9.3 (SAS Institute 2011) and then applying this adjustment constant to all other models. The TruReg method was selected because it generated the least algorithm convergence problems during the exploratory analyses of model selection. Once γ was fixed at 0.31, all models were refitted with the GLIMMIX procedure and Laplace estimation method in SAS 9.3 (SAS Institute 2011). The Laplace estimation method was selected because it represents an actual likelihood to fit probability distributions for counts (i.e., Poisson, negative binomial) and allows Pearson chi-square/df values to be calculated for model-fit diagnosis (Gbur et al. 2003).

Competing models were evaluated using the Akaike information criterion (AIC), Bayesian information criterion (BIC), Pearson chi-square/df values, graphical fit of the models for the observed count data, hypothesis testing for the statistical significance of the covariance parameters and results from sampling simulations described in the next subsection. Smaller values of AIC and BIC generally indicated improved fit of the models. Pearson chi-square/df values (ideally close to one) can be used to assess correct specification of the conditional distribution of the response.

Model Validation. The utility of the three new models to estimate densities of *O. perseae* within blocks of avocado trees was evaluated with sampling simulations using PROC SURVEYSELECT in SAS 9.3 (SAS Institute 2011). For each model, 500 simple random sample iterations, without replacement, were conducted for each of twelve stratified sampling combinations using database B. With some exceptions, these combinations consisted of a total sample size of 10, 20, and 30 avocado leaves with a stratified specification of 1, 2, 3, and 4 leaves sampled per tree. For the scenario of 3 leaves per tree, a total of 9 and 21 leaves were sampled when the target total sample size was 10 and 20 leaves, respectively, to maintain a balanced sample size between trees. For the same reason, a total of 12 and 32 leaves were sampled when the target sample size of 10 and 30 leaves was specified with a 4 leaf per tree combination. For each leaf sample batch, the mean number of *O. perseae* per leaf was estimated using only the fixed effects of the four negative binomial regression models listed above (i.e., the marginal estimates of the model), the original half vein model and the recorded enumerative mite counts. These estimates were compared against the observed densities, calculated from enumerative *O. perseae* counts on all leaves, using the percentage error formula values averaged over all iterations. These results were used to determine how to sample within a block of avocado trees.

Validation over time was possible using data collected from the two groups of trees at orchard 11 across two field seasons. For each sample date and group combination (n=19), a simple random sample of 1 leaf per tree was conducted for 500 iterations. This sampling structure was determined from the validation results of database B and was considered appropriate due to the small number of trees (<10) within both groups. For each leaf sample batch, the number of *O. perseae* per leaf was estimated using models 1-4, the original half-vein model, and recorded enumerative counts. Similar to the cross-sectional validation analyses, the estimated *O. perseae* densities from all selected models were compared against the expected *O. perseae* densities on all leaves using the percent error formula.

Results

Evaluation of the Original Half Vein Method and Correlations Between Variables. Table 2.2 lists the estimated *O. perseae* densities based on original half-vein method using vein UML2. When compared to the enumerative mite counts, the percent errors revealed that the half vein method underestimated *O. perseae* densities at these orchards in a range of 41-60%; a notable exception was site four with a 15% error. Overall, these results indicated that the half-vein method in its original form was not reliable for estimating *O. perseae* densities at these commercial avocado orchards (Table 2.2). Nevertheless, the results of correlation analyses for orchard sites 1-10 (Table 2.3) indicated that there was a consistent and statistically significant association between total *O. perseae* mite counts on the undersurface of 'Hass' avocado leaves and partial counts of this mite along vein UML2 across avocado orchards. These results provided the justification for improving the original half-vein method using the partial *O. perseae* counts along UML2 as a predictor variable (x_1). At orchards 5, 8, and 9 there was a statistically significant association between total *O. perseae* mite counts and leaf length but correlation values were not consistently high and significant at all sites to warrant the addition of a second predictor variable (i.e., leaf length) to the selected empirical regression models.

Similarly, correlation analyses for orchard 11 (Table 2.4) indicated that there was a consistent and statistically significant association between total *O. perseae* mite counts and partial counts along vein UML2 over time at this orchard but there was a pattern of encountering lower correlation coefficients in comparison to orchards 1-9. In general, correlations were higher in later summer months (i.e., July, August). Leaf length was found to be correlated with total mite counts and partial counts in only 5 and 2 sample dates for the first and second group of trees, respectively, and consequently leaf length was not considered to be a consistent indicator of mite densities during the growing season and not included as a model variable.

Model fitting. Table 2.5 lists the fixed effect parameter estimates, fitting criteria and covariance parameter estimates of random effects, when specified, for each candidate model. A graphical comparison of the estimated means from fixed effects of each fitted model, the original half-vein method and observed mite counts for database A is shown on the response scale in Fig. 2.1 and the log link scale in Fig. 2.2. For models 1 and 2, Pearson Chi-Square/DF values were greater than one and this result was interpreted as a sign overdispersion, whereas the values for models 3 and 4 were closer to the ideal value of 1 and these results implied improved model fit. Model 4 had the lowest AIC and BIC values which suggested a better fit than competing models (Table 2.5). Graphically, however, model 4 was conservative in estimating the counts of *O. perseae* on avocado leaves (Fig. 2.1) and would be prone to underestimating mite densities (see validation results). The opposite problem was detected for model 1 which appeared to overestimate counts of *O. perseae* graphically (Fig. 2.1).

Model 3 generated an intermediate fit for the observed count data and lower AIC and BIC values than models 1 and 2 and the combination of these results implied an overall better fit for model 3 (Table 2.5). A potential explanation for these results was revealed by inspection of the covariance parameters. Formal hypothesis testing of the covariance parameters indicated that they were significantly different from zero and this implied that incorporation of random effects were needed for improved interpretation of the observed data. Model 4 distinguished between the random effect associated with orchards as a block effect and interaction with individual trees but because all trees were not subjected to pest management (i.e., there was only one type of "treatment", this being nothing) the specification of an interaction term may introduce some level of redundancy. The structure of model 3 represented a possible solution to this problem. Even though model 3 specified only a tree effect, this model implicitly accounted for effects at the orchard level and this was reflected in the increased value for the covariance parameter estimate (Table 2.5).

Cross Sectional Validation. Cross sectional performance evaluation of fitted models was based on sampling simulations for twelve stratified avocado leaf sampling combinations using database B. An assessment of all combinations for the enumerative counts indicated that an overall sample of 30 leaves, with one leaf sampled per tree, generated lower percent errors in comparison to a 20 and 10 leaf sample (results not shown). The results of the percent errors for all models based on a 30 leaf sample with 1, 2, 3, or 4 leaves per tree are listed in Tables 2.6- 2.9. In these analyses, lower percent errors were achieved with a stratified sample of 1 leaf per tree and this would be the recommended sampling structure within a 5x6 sampling block. The lowest percent errors across all sites, ranging from -4 to 4%, were associated with enumerative mite counts. Percent errors for the original half-vein method ranged from -1 to -72% across all sites and sample sizes. The original half-vein model consistently underestimated densities and this pattern was detected graphically (Fig. 2.1).

Models 1-3 displayed improved performances in reducing percent errors and mitigating underestimation compared to model 4 and the original half-vein method under evaluation, but significant patterns were detected across sampling combinations (Tables 2.6-2.9). Estimates of percent errors for model 1 revealed a strong pattern of overestimating mite densities and in some cases, such as in orchards 2, 4, and 6, the absolute percent error values exceeded those of the original half-vein model (Tables 2.6-2.9). With the exception of orchard 4, the absolute percent error values for models 2 and 3 indicated that these models performed better than model 1 in estimating mite densities (Tables 2.6-2.9). While model 3 revealed a slight pattern of underestimation, model fit criteria reported in Table 2.5 supported the statistical validity of model 3 over model 2.

Longitudinal Validation. A graphical comparison of mite density estimates from models 1-4, the original half-vein method, and observed mite counts for the two groups of trees sampled over time are shown in Figs. 2.2 (group 1) and 2.3 (group 2).

Enumerative mite counts on the leaf undersurface and vein UML2 at this site were not as high as in database A and B, but the estimated percent errors and mite density values from sampling simulations (Table 2.10) offered an objective approach for model evaluation. *O. perseae* densities predicted from total enumerative counts generated the lowest percent errors and consequently, this continued to be the most accurate sampling approach. The half-vein method underestimated *O. perseae* densities in an absolute range of 29-60% at densities greater than 16 mites per leaf. By comparison, lower percent errors in an absolute range of 1-29% were generated with model 3 at mite densities greater than 16 mites per leaf during summer months. Therefore, model 3 would be a better alternative than the original half-vein model and models 1, 2, and 4 for the purposes of estimating per leaf densities of *O. perseae* in an orchard. At densities lower than 16 mites per leaf, the original half-vein method performed better than the new fitted models but the predicted mite densities from models 3 were still within an acceptable range that would have indicated that *O. perseae* population densities at this site were relatively low; checking only the percent errors gave a misleading impression that model 3 overestimated densities by several orders of magnitude.

Discussion

Populations of *O. perseae* were first detected in California in 1990 (Bender 1993). Since that time, this non-native species has become a key foliar pest of 'Hass' avocados throughout the commercial growing region in southern California and this has created the need for developing effective control and sampling tools (UC IPM 2008, 2011). The motivation for this study was to evaluate the performance of the original half-vein model (Machlitt 1998) for the purpose of estimating *O. perseae* densities using partial counts of this pest along the upper margin of leaf vein UML2 (a single predictor, x_1) and, if necessary, to update the structure of the original model and provide validated sampling guidelines that would facilitate its potential application in commercial avocado orchards. The initial evaluation based on all leaf samples provided strong evidence that the original half-vein method underestimated mite densities in a range of 15-60%. The consistent underestimation pattern implied that the relationship of *O. perseae* counts and vein UML2 had potentially changed since the time the original analyses were conducted (Machlitt 1998), possibly because of lower population densities of naturally-occurring *O. perseae* in untreated orchards. Unfortunately, access to the original datasets from Machlitt (1998) for further inspection was not possible. There is evidence for differences in cultivar susceptibility (Kerguelen and Hoddle 1999) but this potential change in host plant tolerance to mite populations over the years for the economically important 'Hass' variety needs further study. Nevertheless, the consistent significant correlation across sites (Table 2.3-2.4) detected between the response and predictor variable for UML2 implied that an updated model for estimating *O. perseae* densities using partial counts might be useful. A simple solution would have been to introduce a correction factor into the model equation, but the error range was too wide for an optimal adjustment to the original model to be recommended.

A preliminary assessment of pooled data from database A for sites 1-10 suggested that there could be a curvilinear relationship between total *O. perseae* counts and x_1 that was not originally captured (Machlitt 1998). A possible biological explanation for this result is that the total number of *O. perseae* mites cannot be expected to increase linearly on avocado leaves without reaching some type of carrying capacity. Hypothetically, as mite densities increase, negative feedback mechanisms such as intraspecific competition for nesting sites and a reduction in available feeding surface area function to counter population growth. However, *O. perseae* count data for training data sets (database A) was not collected longitudinally and therefore, fitting classical biological growth curve models (e.g., logistic, Gompertz, Chapman-Richards, Bertalanffy) (Kaufman 1981) were not appropriate.

Instead, an empirical modeling process independent of explanatory biological mechanisms and based solely on the observed relationship between total mite counts and x_1 was adopted. One generalized linear model (i.e., model 1) and three generalized linear mixed models (GLMMs) (i.e., models 2-4), which were better adapted for handling non-normal data and accounting for potential random effects (Bolker et al. 2009), were evaluated and their performance compared with the original half-vein model and enumerative counting approach through simulated sampling of additional cross-sectional and longitudinal field data not used in the modeling process. As expected, the enumerative counting strategy consistently provided the highest level of accuracy (Tables 2.6-2.10) but this approach is not suitable for field use in commercial avocado orchards because it is overly time consuming. The overall results from the model-fit statistics

(Table 2.5) and sampling simulations (Table 2.6-2.10) suggested that model 3 with a random effect from avocado trees performed better than competing models and could potentially be customized as an alternative time-saving sampling tool to estimate per leaf densities of *O. perseae* in California.

As a GLMM, the fixed effect components of model 3 inherently provide an estimate of *O. perseae* counts on a leaf for the average tree within an orchard for values of x_1. In this study, more than 300 commercial trees were sampled and these were representative of the types of trees that might be sampled by Pest Control Advisers (PCAs) or growers to assess levels of *O. perseae* in orchards. Due to the design of the data collection process, the practical use of model 3 is compatible with making a general assessment of *O. perseae* densities over 5x6 tree blocks with 1 in-season mature leaf randomly sampled per tree (Tables 2.6-2.10) during the summer (e.g. July-August) when elevated densities, as indicated by the presence of these mites on the leaf undersurface and the prominence of necrotic spots (feeding damage), are suspected in relatively small sections of the orchard. The use of model 3 early in the season during spring months when *O. perseae* populations may be low (e.g., April-May) is not recommended because this model is likely to overestimate densities as was shown from the validation results of orchard 11 sampled over time (Table 2.10).

For each collected leaf within the block of interest, vein UML2 can be examined with a 20x hand lens to count the number of *O. perseae* occupying the upper margin of this vein. Individual values of x_1, along with parameters values α and β for model 3 (Table 2.5), are entered into the respective model equation (3) to produce estimates of *O.*

persea at the leaf level. These estimates are averaged over the number of leaves sampled (e.g., 30 leaves) and a final mite density assessment for the block is made. In this study, 95% of the x_1 values across all datasets (n=3,849 leaves) were less than or equal to 26 *O. perseae* mites. With training, it can take approximately 30 seconds to 1 minute per leaf to obtain values for x_1 and therefore the density assessment for these smaller blocks should take approximately 15-30 minutes. When the objective is to characterize the severity of mite infestation over larger spatial areas (e.g, 200 x 200 tree blocks or smaller) relatively quickly, our recommendation is to use a binomial sampling structure with a minimum sample size of 30 leaves (Lara and Hoddle, in press). Binomial sampling can be used to effectively classify densities of *O. perseae* as being above or below a working action threshold of 50 mites per leaf throughout the period of the growing season that generates concern for this mite (e.g., April-Sept.). The binomial sampling plan accounts for potential spatial correlation and reduces the sampling effort even further because no mite counting is required, only the proportion of leaves that are infested with at least one mite is recorded and used to assess per leaf densities of *O. perseae* (Lara and Hoddle, in press).

Growers can combine the information from the sampling tools described above with information from visual inspection of the feeding damage, current and anticipated weather conditions (observations have been made that *O. perseae* populations decline under periods of high summer temperatures [UC IPM 2011]), time of year (mite populations usually peak during the summer and decline as the weather get cooler), known information on the history of mite infestations in other parts of the orchard, and availability and potential application timing of pest control materials (e.g., commercially available phytoseiids or pesticides) to make an informed decision on the appropriate control strategy at the orchard level. Although improvements have been made to the *O. perseae* sampling program in California since populations of this mite were first detected in 1990 (Bender 1993), work is still needed to expedite sampling data collection and processing through the use of a customized agricultural software application designed for use on smartphones or tablets. The potential benefits for implementing this paperless technology in forest systems is discussed by Kennedy et al. (2013). If developed for the avocado system, this type of technology would enable PCAs to readily enter and store sampling data (e.g., presence-absence or x_1 values) directly into handheld devices and software would perform numerical model calculations and provide an estimate of mite densities. This information could be relayed directly to growers electronically with information on the recommended treatment, if necessary. Furthermore, the readily accessible electronic records of these assessments and any other type of complementary data collected at the time of sampling (e.g. pictures of feeding damage, GPS coordinates of trees sampled) could be used to build a spatial and temporal pest profile for the orchard that could be use to identify and target areas with a known history of pest population flares for monitoring during the summer.

Finally, for research experiments where highly accurate mite density estimates are required to compare the pest-control effectiveness of treatment applications such as different pesticide materials or natural enemy species, enumerative counting should always be considered as the first option and this procedure can be facilitated with the aid of a microscope in the laboratory and leaves can be cool-stored to preserve quality until counting. The original half-vein model (Machlitt 1998) was specifically designed with the intention of assisting growers and PCAs with monitoring *O. perseae* populations in commercial avocado orchards and model 3 was customized to meet this objective. In this observational study we did not evaluate the performance of the original half-vein method under an experiment research design with a series of replicated treatments (e.g., a pesticide trial), but the consistent underestimating behavior pattern of the UML2 model suggests that additional error may be introduced into the estimates of mite densities that could potentially affect the interpretation of treatment comparisons. Future research should determine if different types of treatment applications (e.g., pesticides vs. natural enemies) will affect the empirical relationship between total mite counts and partial counts along UML2 as seen on untreated trees in this study and give indication of the appropriate sample size needed to maintain reliable interpretation of the results.

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Table 2.1. Avocado leaf collection summary and observed *Oligonychus perseae* densities for each experimental orchard

Orchard	Sample date	No. of leaves	County	Mean no. of mites per leaf	Range of mite counts per leaf
1	July 2009	247	San Diego	77	0 - 607
2	Aug. 2009	240	Santa Barbara	42	0 - 1088
3	Aug. 2009	240	Santa Barbara	342	0 - 3016
4	Aug. 2009	240	Santa Barbara	37	0 - 1411
5[a]	Aug. 2009	240	Santa Barbara	307	1 - 2039
6	Sept. 2009	240	Santa Barbara	49	0 - 567
7	June 2010	239	Santa Barbara	205	0 - 1633
8	July 2010	240	Ventura	208	0 - 1060
9	July 2010	256	Orange	519	2 - 2850
10	Aug. 2010	240	Santa Barbara	18	0 - 475

[a] Leaves were collected from an adjacent plot of ‘Hass’ avocado trees eight days after having been collected at Orchard 3.

Table 2.2. Percent errors for *Oligonychus perseae* densities estimated using enumerative counts and the original half vein method (Machlitt 1998) for database A

Orchard	No. of leaves	Observed mite density	Half-vein estimate	Percent error
1	124	93	37	-60
2	120	47	19	-60
3	120	358	216	-40
4	120	36	30	-15
5	120	339	182	-46
6	120	48	28	-41
7	120	213	103	-51
8	120	227	124	-45
9	128	564	236	-58
10	120	17	7	-60

Table 2.3. Spearman correlation coefficients between total counts of *Oligonychus perseae*, partial counts along vein UML2 and avocado leaf length

Orchard	Total mites vs. UML2	Total mites vs. Leaf length	UML2 vs. Leaf length
1	0.77***	0.04	0.04
2	0.74***	0.08	0.10
3	0.86***	-0.03	0.03
4	0.70***	0.10	0.11
5	0.85***	0.20*	0.23*
6	0.83***	-0.01	0.02
7	0.89***	0.10	0.01
8	0.84***	0.23*	0.26**
9	0.72***	0.19*	-0.005
10	0.50***	-0.12	-0.12

* Significant at $p<0.05$; ** significant at $p < 0.005$; *** significant at $p<0.0001$.

Table 2.4. Spearman correlation coefficients between total counts of *Oligonychus perseae*, partial counts along vein UML2 and avocado leaf length at orchard 11 for spring flush leaves starting in April (2012 and 2013) in Irvine, California

Set	Sample date	No. of leaves	Total mites vs. UML2	Total mites vs. Leaf length	UML2 vs. Leaf length
1	June 2012	90	0.43***	0.22*	0.17
	July 2012	49	0.48**	0.18	0.12
	Aug. 2012	89	0.67***	0.53***	0.22*
	April 2013	90	0.56***	-0.03	0.002
	May 2013	90	0.46***	-0.22*	-0.15
	June 2013	90	0.56***	0.11	0.02
	July 2013	89	0.77***	0	-0.07
	Aug. 2013	89	0.65***	0.31**	0.21*
2	July 2012	80	0.34**	0.15	0.05
	Aug. 2012	80	0.57***	0.31*	0.01
	April 2013	80	0.62***	0.11	-0.01
	May 2013	80	0.48***	0.17	0.09
	June 2013	80	0.60***	-0.04	0.72
	July 2013	80	0.75***	0.16	0.04
	Aug. 2013	80	0.69***	0.20	0.17

* Significant at $p<0.05$; ** significant at $p < 0.005$; *** significant at $p<0.0001$.

Table 2.5. Parameter estimates for the four nonlinear negative binomial models tested

Model	α	β	k	Covariance parameters Orchard	 Trees	AIC	BIC	Pearson Chi-Square/DF
1	3.41	0.91	1.38			12475	12490	1.83
2	3.36	0.84	1.20	0.23*		12343	12344	1.96
3	3.08	0.87	0.71		1.03*	12232	12247	0.78
4	3.07	0.81	0.69	0.53*	0.64*	12126	12127	0.87

* Significant at $p < 0.0001$.

Table 2.6. Comparison of estimated *Oligonychus perseae* densities and percent errors for sampling methods based on 500 simple random sampling (SRS) iterations and a sample size of 30 avocado leaves with 1 leaf sampled per 30 trees

		Estimated density for models[b]						Mean percent errors for models[c]					
Site	E(y)[a]	EM	HV	1	2	3	4	EM	HV	1	2	3	4
1	61	61	23	61	52	42	38	-1	-62	-1	-15	-32	-38
2	37	37	23	57	48	39	35	-1	-38	55	29	5	-6
3	327	326	208	387	280	243	195	0	-36	18	-14	-26	-40
4	38	36	37	83	66	55	47	-4	-2	118	73	44	24
5	275	273	158	312	236	201	166	0	-42	14	-14	-27	-39
6	50	51	31	71	58	48	42	0	-39	42	15	-6	-17
7	196	198	97	199	153	129	108	1	-50	1	-22	-34	-45
8	189	190	96	202	158	132	112	0	-49	7	-17	-30	-41
9	474	476	192	374	281	240	197	1	-60	-21	-41	-49	-58
10	19	19	5	22	20	16	15	-1	-72	16	8	-17	-19

[a] Expected density, E(y), on the response scale was determined from enumerative counts of all sampled leaves for each orchard within database B.

[b] Estimated densities based on 10 leaves for enumerative counts (EM), the original half-vein method (HV) and negative binomial regression models (1-4).

[c] Mean percent errors were calculated between the expected and estimated mite densities across all sampling iterations.

Table 2.7. Comparison of estimated *Oligonychus perseae* densities and percent errors for sampling methods based on 500 simple random sampling (SRS) iterations and a sample size of 30 avocado leaves, 2 leaves per 15 trees

		Estimated density for models[b]						Mean percent errors for models[c]					
Site	E(y)a	EM	HV	1	2	3	4	EM	HV	1	2	3	4
1	61	62	24	62	54	43	39	0	-61	2	-13	-30	-36
2	37	37	23	58	48	39	35	1	-37	57	30	6	-5
3	327	330	211	392	283	246	197	1	-35	20	-13	-25	-40
4	38	40	38	84	67	56	48	4	0	122	77	46	26
5	275	277	159	314	238	202	167	1	-42	14	-13	-26	-39
6	50	51	31	73	59	48	42	1	-38	44	17	-4	-16
7	196	194	96	196	151	127	107	-1	-51	0	-23	-35	-46
8	189	191	97	203	159	133	113	1	-49	7	-16	-30	-40
9	474	474	191	374	280	239	196	0	-60	-21	-41	-50	-59
10	19	19	5	22	20	16	15	-1	-72	17	8	-17	-19

[a] Expected density, E(y), on the response scale was determined from enumerative counts of all sampled leaves for each orchard within database B.

[b] Estimated densities based on 10 leaves for enumerative counts (EM), the original half-vein method (HV) and negative binomial regression models (1-4).

[c] Mean percent errors were calculated between the expected and estimated mite densities across all sampling iterations.

Table 2.8. Comparison of estimated *Oligonychus perseae* densities and percent errors for sampling methods based on 500 simple random sampling (SRS) iterations and a sample size of 30 avocado leaves, 3 leaves per 10 trees

		Estimated density for models[b]						Mean percent errors for models[c]					
Site	E(y)[a]	EM	HV	1	2	3	4	EM	HV	1	2	3	4
1	61	62	24	61	53	42	39	1	-61	0	-14	-31	-37
2	37	36	23	57	47	38	34	-2	-38	54	28	4	-7
3	327	328	210	390	282	245	196	0	-36	19	-14	-25	-40
4	38	35	36	81	65	54	46	-7	-4	114	71	41	22
5	275	275	159	313	237	201	167	0	-42	14	-14	-27	-39
6	50	52	32	73	59	49	43	3	-37	46	18	-3	-15
7	196	196	96	197	151	128	107	0	-51	0	-23	-35	-45
8	189	191	97	204	160	134	114	1	-49	8	-16	-29	-40
9	474	473	192	375	281	240	197	0	-59	-21	-41	-49	-58
10	19	20	6	23	21	16	16	5	-71	20	10	-15	-17

[a] Expected density, E(y), on the response scale was determined from enumerative counts of all sampled leaves for each orchard within database B.

[b] Estimated densities based on 10 leaves for enumerative counts (EM), the original half-vein method (HV) and negative binomial regression models (1-4).

[c] Mean percent errors were calculated between the expected and estimated mite densities across all sampling iterations.

Table 2.9. Comparison of estimated *Oligonychus perseae* densities and percent errors for sampling methods based on 500 simple random sampling (SRS) iterations and a sample size of 32 avocado leaves, 4 leaves per 8 trees

		Estimated density for models[b]						Mean percent errors for models[c]					
Site	E(y)[a]	EM	HV	1	2	3	4	EM	HV	1	2	3	4
1	61	62	23	61	52	42	38	1	-62	-1	-15	-32	-38
2	37	37	23	57	48	39	35	-1	-38	55	29	5	-6
3	327	321	205	382	277	241	193	-2	-37	17	-15	-26	-41
4	38	40	40	87	69	57	49	5	4	130	81	51	30
5	275	272	157	310	235	200	165	-1	-43	13	-15	-27	-40
6	50	52	31	73	59	48	42	3	-38	45	17	-4	-16
7	196	203	99	202	155	131	110	3	-49	3	-21	-33	-44
8	189	191	98	204	160	134	114	1	-49	8	-16	-29	-40
9	474	471	191	373	280	239	196	-1	-60	-21	-41	-50	-59
10	19	19	5	22	21	16	15	-1	-72	17	9	-17	-19

[a] Expected density, E(y), on the response scale was determined from enumerative counts of all sampled leaves for each orchard within database B.

[b] Estimated densities based on 10 leaves for enumerative counts (EM), the original half-vein method (HV) and negative binomial regression models (1-4).

[c] Mean percent errors were calculated between the expected and estimated mite densities across all sampling iterations.

Table 2.10. Comparison of estimated *Oligonychus perseae* mean densities and percent errors for sampling methods based on 500 simple random sampling (SRS) iterations and a sample size of 1 avocado leaf per tree at orchard 11

			Estimated mean density						Mean percent errors for models					
Set	Sample date[a]	E(Y)	EM	HV	1	2	3	4	EM	HV	1	2	3	4
1	June 2012	0.60	0.58	0.37	11	12	8	9	-4	-39	1790	1827	1310	1382
	Aug. 2012	51	52	20	54	46	37	34	3	-60	6	-9	-27	-34
	April 2013	7	7	9	29	26	20	19	-2	23	308	264	185	170
	May 2013	0.16	0.17	0.50	12	12	9	9	7	222	7343	7439	5432	5686
	June 2013	6	6	11	34	31	24	23	2	82	481	423	307	288
	July 2013	87	86	48	111	92	75	66	-2	-45	27	5	-14	-24
	Aug. 2013	126	127	89	190	151	125	108	1	-29	51	20	-1	-15
2	May 2012	0.18	0.12	0.69	12	12	9	9	-34	296	6721	6748	4946	5144
	June 2012	0.74	0.80	0	10	11	8	8	8	-100	1322	1368	967	1032
	July 2012	1	0.95	0.82	12	12	9	10	-2	-15	1185	1193	852	891
	Aug. 2012	17	17	9	30	28	21	20	0	-45	83	66	29	23
	Sept. 2012	0.33	0.29	0	10	11	8	8	-11	-100	3127	3230	2322	2469
	April 2013	6	6	7	25	22	17	16	-2	11	291	249	172	158
	May 2013	1	0.95	1	13	13	10	10	-6	30	1222	1207	871	897
	June 2013	6	6	8	29	26	20	19	0	50	413	365	261	246
	July 2013	112	111	72	155	124	102	89	-1	-36	39	11	-8	-21
	Aug. 2013	102	103	58	131	106	87	76	1	-43	28	4	-15	-25

[a] The following sample dates with small sample sizes (<50 leaves) and/or no mite infestations were not included in the validation analyses: March, May and July 2012 for set 1; March 2012 for set 2.

Figure Legends

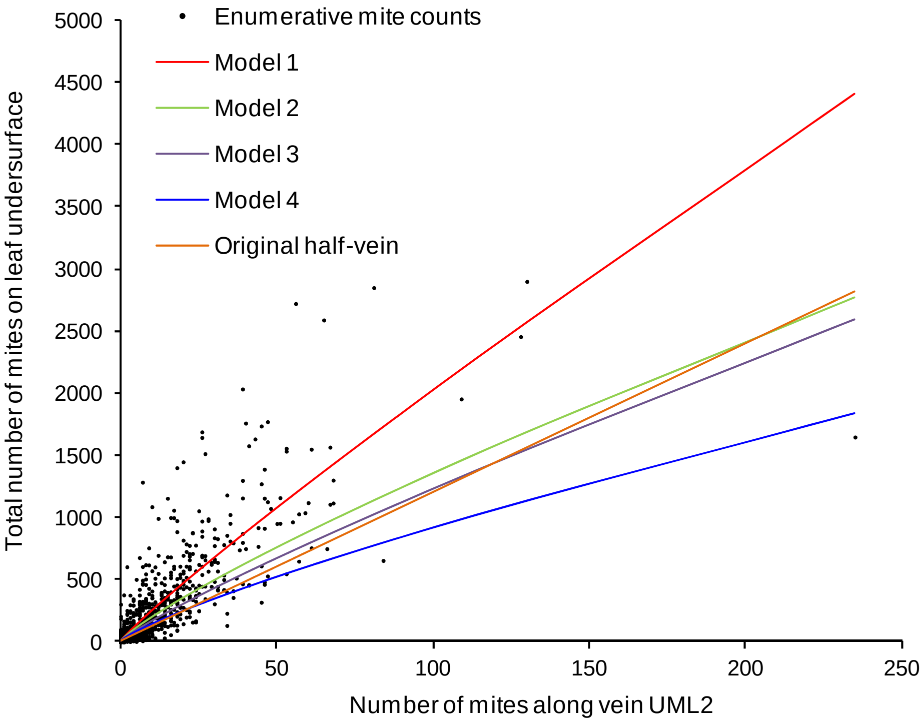
Fig. 2.1. Comparison of fitted models, original half-vein method and observed *Oligonychus perseae* counts on avocado leaves across ten sites from validation database B based on the response scale.

Fig. 2.2. Comparison of fitted models, original half-vein method and observed *Oligonychus perseae* counts on avocado leaves across ten sites from validation database B based on the log link scale.

Fig. 2.3. Comparison of fitted models, original half-vein method and observed *Oligonychus perseae* counts on avocado spring flush leaves from dataset 1 of orchard 11 based on the response scale.

Fig. 2.4. Comparison of fitted models, original half-vein method and observed *Oligonychus perseae* counts on avocado spring flush leaves from dataset 2 of orchard 11 based on the response scale.

Fig. 2.1.



Enumerative mite counts
Model 1
Model 2
Model 3
Model 4
Original half-vein
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Total number of mites on leaf undersurface
0
50
100
150
200
250
Number of mites along vein UML2

Fig. 2.2.

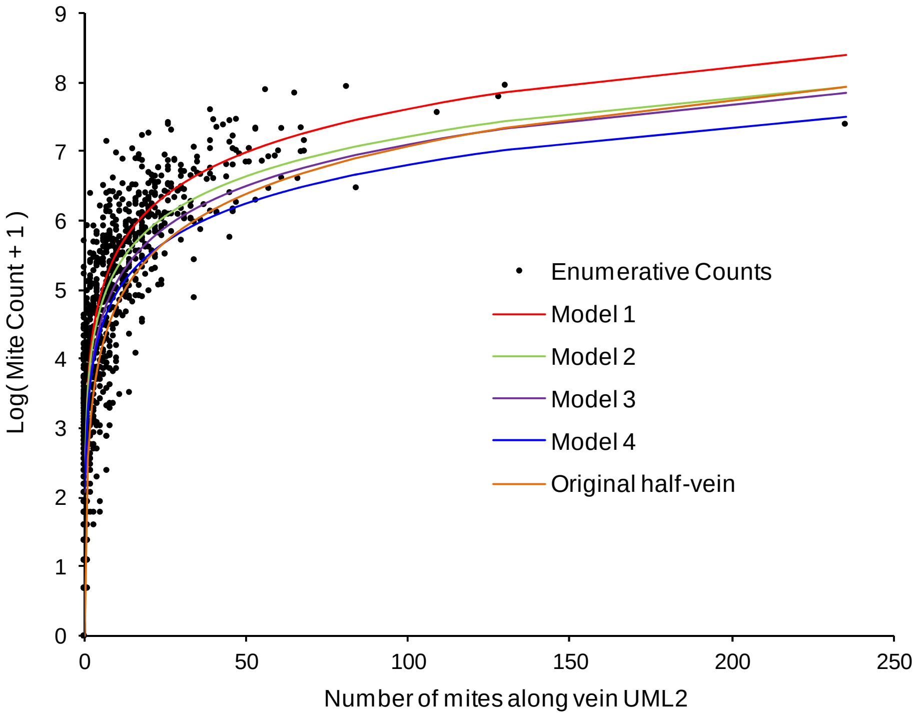
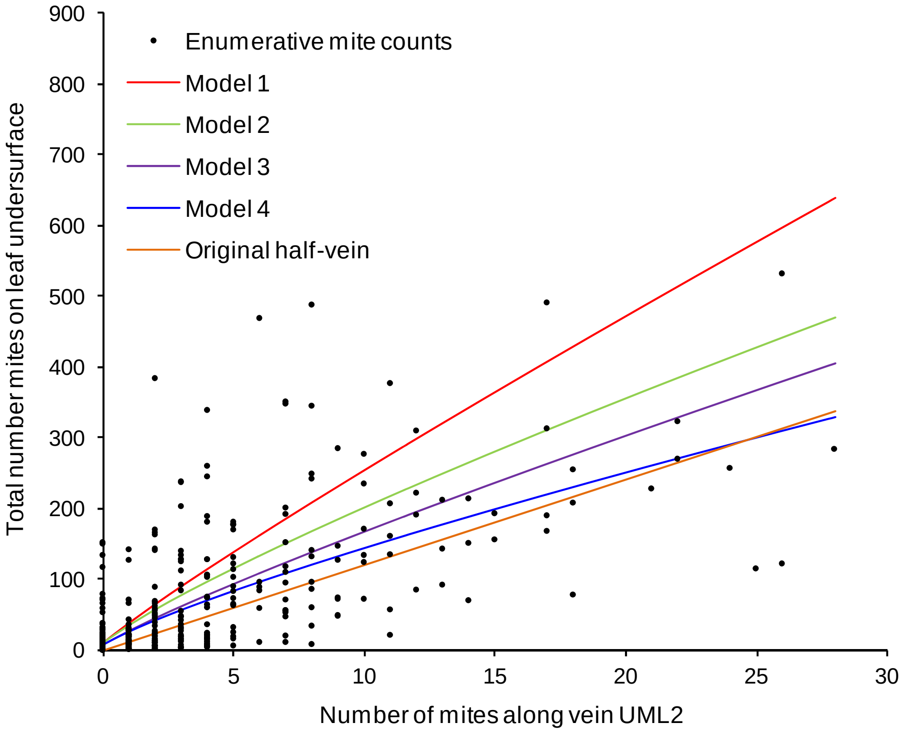
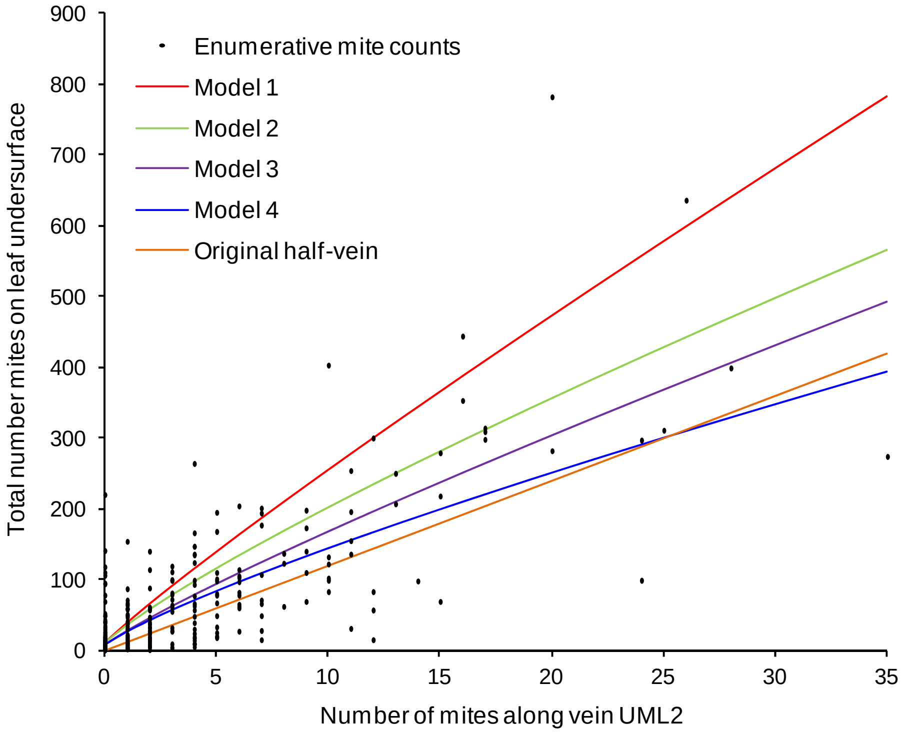

9
8
7
6
5
4
3
2
1
0
Log(Mite Count + 1)
0
50
100
150
200
250
Number of mites along vein UML2
● Enumerative Counts
Model 1
Model 2
Model 3
Model 4
Original half-vein

Fig. 2.3.



Enumerative mite counts
Model 1
Model 2
Model 3
Model 4
Original half-vein
Total number mites on leaf undersurface
Number of mites along vein UML2
0
100
200
300
400
500
600
700
800
900
0
5
10
15
20
25
30

Fig. 2.4.



Total number mites on leaf undersurface
900
800
700
600
500
400
300
200
100
0
0
5
10
15
20
25
30
35
Number of mites along vein UML2
Enumerative mite counts
Model 1
Model 2
Model 3
Model 4
Original half-vein

Chapter 3

Comparison and Field Validation of Binomial Sampling Plans for *Oligonychus perseae* (Acari: Tetranychidae) on Hass Avocado in Southern California

ABSTRACT Enumerative sampling plans that require full mite counts are not a viable option for making treatment decisions for the spider mite *Oligonychus perseae* Tuttle, Baker, and Abatiello, an avocado leaf pest, because of its high action threshold of 50-100 motile mites per leaf. Therefore, binomial sampling models that classify densities of *O. perseae* were developed and their performance evaluated using a mite-count database with more than 20,000 'Hass' avocado leaves infested with varying densities of *O. perseae*. Under this framework, a 50 mite per leaf action threshold was adopted in combination with three modeling techniques (i.e., Taylor's power law, maximum likelihood, and an empirical model) to characterize the mean-proportion relationship for two leaf infestation tally thresholds of either one or two mites. Operating characteristic and average sample number results for sequential binomial models were used as the basis to develop and validate a fixed-size binomial sampling plan that remains sensitive to relevant *O. perseae* densities, requires a minimum leaf sampling cost of 30 leaves, and takes into account the spatial dynamics of *O. perseae* to make reliable mite density classifications. Recommendations for implementing this fixed-size binomial sampling plan to assess densities of *O. perseae* in commercial California avocado orchards are discussed.

KEY WORDS persea mite, avocado, fixed-size binomial sampling, sequential sampling

Oligonychus perseae Tuttle, Baker, and Abatiello (Acari: Tetranychidae) is a widely distributed foliar spider mite pest of avocados (*Persea americana* Miller [Lauraceae]) in many areas of the world (i.e., California, Costa Rica, Israel, and Spain), including its native home range of Mexico. Motile stages of *O. perseae* (i.e., larva, protonymph, deutonymph, and adult) feed on cell contents on the undersurface of avocado leaves under webbed nests, where they also complete their development and reproduce (Aponte and McMurtry 1997a, 1997b). *O. perseae* feeding damage can be detected visually on foliage as necrotic spots and percent leaf area feeding damage greater than 7.5% increases the probability for premature leaf drop (Kerguelen and Hoddle 1999). Mite-induced defoliation may result in sunburnt avocado fruit and translate to yield losses (Maoz et al. 2011, UC IPM 2011).

Since its introduction to California in 1990 (Bender 1994), *O. perseae* has established throughout approximately 90% of the avocado growing region and is most damaging to foliage of the susceptible Hass cultivar (Kerguelen and Hoddle 2000). The Hass cultivar was discovered in California in the early 19th century and over the span of several decades it has become the most important commercial avocado cultivar in the world market (Crane et al. 2013). California avocado production is valued at $435 million and the Hass cultivar accounts for 94% percent of the total production area (23,406 hectares) (CAC 2012, 2014). Therefore, concerns in California and other commercial Hass-producing countries (e.g., Mexico, Israel, Spain, Costa Rica) over the potential economic damage caused by feeding populations of *O. perseae* is warranted.

In California, *O. perseae* infestations typically occur during late spring, summer and early fall (UC IPM 2011) when the combination of warm temperatures and the availability of foliage is conducive to increases in mite population growth (Yee et al. 2001). Consequently, monitoring *O. perseae* population activity in commercial orchards is recommended every 7-10 days (UC IPM 2011) starting in the spring as this coincides with the first growth flush period of avocado trees; a second flush period occurs in summer (Mickelbart et al. 2012). Pest monitoring efforts in California avocado orchards for target species like *O. perseae* can be conducted by state-licensed agricultural pest control advisers who can be contracted by avocado growers for this purpose. To standardize pest management efforts of *O. perseae* populations, the California avocado industry adopted a nominal action threshold of 100 mites per leaf (Hoddle and Morse 2013). There is no scientific basis for this action threshold in California but supporting evidence is available from the Israeli avocado system. Results from a field study conducted by Moaz et al. (2011) in Israel suggested that a working action threshold of 50-100 motile *O. perseae* per leaf in Hass avocados is adequate for timing management decisions. These results have potential to be implemented in the California avocado system which is predominantly Hass-based and relies on chemical control of *O. perseae*. Chemical control is the principal pest management strategy for reducing *O. perseae* populations in California because the success of the current inoculation biological control program for this pest involving seasonal releases of *Neoseiulus californicus* (McGregor) (Acar: Phytoseiidae) has been limited (Hoddle et al. 2000, Hoddle and Morse 2013).

Despite the availability of different control options and an action threshold range, making reliable inferences on *O. perseae* densities as part of pest monitoring efforts is still a technical challenge for pest control advisers because a practical sampling plan that can be used to accurately assess the severity of *O. perseae* infestations in commercial orchards is not available. Three ecological factors associated with *O. perseae* infestations have created barriers for developing a reliable sampling plan for this mite. First, inspection of *O.perseae* feeding-damage alone is not a reliable quantitative indicator of mite densities present at the time of sampling because *O. perseae* population densities can change due to environmental factors such as heat waves that have the potential to reduce mite populations (UCANR 2008). Therefore, examining only *O. perseae* feeding damage on the leaf undersurface, which was accumulated over the extent of previous weeks or months of the current growing season, may lead to misleading interpretations on the severity of current *O. perseae* infestation levels which could be below a specified action threshold (e.g. 50-100 mites per leaf) and do not require control. Second, *O. perseae* is small and conspecific populations can build rapidly to several hundred mites per leaf. Therefore, estimating *O. perseae* densities by counting microscopic mites with a hand lens in the field over the entire leaf surface is not practical.

In response to these two factors, Machlitt (1998) developed a sampling approach that involves counting the total number of motile *O. perseae* along a specific vein on avocado leaves to estimate the total number of mites on a leaf on neighboring trees (see UC IPM 2011). This method reduces the counting effort to some extent but the major drawback is that this method can underestimate *O. perseae* densities by up to 70% (Lara and Hoddle, unpublished data). In addition, this method does account for a third factor which refers to the non-uniform spatial distribution of *O. perseae* populations among leaves in a tree and between trees within an orchard (DePalma et al. 2012, Li et al. 2012). This implies that the spatial ecology of *O. perseae* on Hass avocados should be addressed as part of the development of effective sampling plan guidelines for this pest in commercial orchards.

The unavailability of a reliable sampling plan for *O. perseae* can hinder effective management *O. perseae* populations in commercial Hass avocado orchards because there are no standard recommendations based on action thresholds to readily guide the implementation of pest control measures. Furthermore, over the past two decades, pesticide use in California avocado system has increased significantly due to the introduction of invasive avocado herbivore species like *O. perseae* (Hoddle 2005) and this can lead to the development of pesticide resistance (Humeres and Morse 2005), which prompted further research in screening and registering additional products to manage pesticide resistance development by *O. perseae* in California (Hoddle and Morse 2013). A complementary management tool that could promote judicious pesticide use would be the development of a binomial sampling plan for the California avocado system that reduces mite counting efforts and allows pest control advisers to reliably determine whether *O. perseae* densities have exceeded a pre-determined action threshold.

Binomial sampling operates on the premise that the proportion of sample units infested (e.g. leaves) is related to mean pest density (Binns et al. 2000). Under this premise, the mean-proportion relationship can be used to set up a sampling strategy whereby pest density levels as indicated by the proportion of infested leaves are classified as being above or below an action threshold that requires treatment. A binomial sampling strategy can be combined with further sampling guidelines to design a custom sampling plan for a target pest under field conditions. A custom binomial sampling plan for *O. perseae* in the California avocado system has not been developed but several binomial sampling plans founded on the mean-proportion relationship have been used for other economically important spider mites on perennial host plants, including *Panonychus citri* infesting oranges in California (Zalom et al. 1985), *Panonychus ulmi* on apples in New York (Nyrop et al. 1989), *Mononychellus progresivus* and *Oligonychus gossypii* on cassava in Africa (Bonato et al. 1995), *Tetranychus urticae* on mandarins in Spain (Martínez-Ferrer et al. 2006), and *Oligonychus yothersi* on mate-tea in Brazil (de Gouvea et al. 2007).

It is important to understand that binomial sampling plans are composite statistical models defined by a set of estimated parameters. These parameters are informed by the unique biology and ecology of the target species and user-specified sampling criteria (e.g., a fixed or a sequential sample size) that facilitate practical pest management in the crop system of interest. Consequently, several candidate sampling models (plans) for a target pest may be generated from the various combinations of modeling techniques and the final combination is determined by the researcher. Ultimately, the recommended sampling plan should reflect a compromise between statistical reliability and practical utility. The combination of these two factors provides a comprehensive measure of overall sampling performance. To optimize binomial sampling plan performance careful consideration should be given to integral components of the modeling process. In general, these components refer to: (1) the selection of a modeling approach to describe the relationship between the mean pest densities and pest incidence on sampling units (i.e., proportion of leaves infested), (2) the selection of a sample size framework (i.e., sequential vs. fixed-size sampling) that is combined with a mean-proportion model, (3) validation of a mean-proportion model based on the selected sample-size framework, and (4) validating recommendations for the spatial selection of sample units (e.g. leaves) that will allow for practical implementation of a binomial sampling plan under field conditions unique to each crop system in a particular region. A theoretical understanding of these binomial sampling components is provided by Jones (1994) and Binns et al. (2000).

In this study, the primary objective was to develop and validate a custom fixed-size binomial sampling plan with practical application for standardizing pest management of *O. perseae* in California Hass avocado orchards. Overall, sequential sampling is statistically and practically efficient for minimizing the overall number of sample units (e.g., leaves) needed to make a treatment decision in comparison to sampling plans with a fixed sample size. However, the sample size and thereby the time needed to terminate sequential sampling can vary depending on the levels of mite infestations. In some cases, as will be discussed for *O. perseae* in this study, this dynamic can lead to impractical sample sizes (e.g. hundreds of leaves) needed to terminate sequential sampling to assess infestations levels of a target pest. For this reason, validations of sequential sampling models based on field-collected data were used to determine a research-based sample size amenable for developing a fixed-size sampling plan that addressed four sampling criteria: (1) sampling guidelines that indicate how sample units (i.e. leaves) are to be selected within avocado orchards; (2) the type of information related to presence of *O. perseae* that is collected from each leaf; (3) how the pooled information from a fixed batch of leaf samples can be used by pest control advisers to accurately assess the severity of spider mite infestations within an orchard in reference to an action threshold, and (4) how to make an informed decision regarding control measures such as pesticide sprays to reduce spider mite populations. This extensive combination of criteria as part of developing sampling plans for field application is infrequently presented in the literature because the methodology needed to develop and validate each component is complex.

Consequently, to optimize the informed selection of a statistically-reliable and practical binomial sampling plan with spatial components for *O. perseae*, a non-traditional four-phase modeling approach was adopted. This modeling complexity stems from having to address the spatial ecology of *O. perseae* infestations in Hass avocados (Depalma et al. 2012, Li et al. 2012), analyzing field data collected over time and across multiple locations, and the series of research-based decisions that needed to be made as part of the four modeling components of the binomial sampling plans listed above. Specifically, in the first phase, various modeling techniques were used to construct candidate mean-proportion models. In the second phase, these mean-proportion models were used to design and validate sequential binomial sampling strategies. In the third phase, the validation results from phase 2 were used to design fixed-size binomial sampling strategies. Finally, in phase 4, spatial guidelines were combined with candidate models from phase 3 and validated to produce the final binomial sampling plan.

Combining these phases into a comprehensive linear modeling process became a secondary, but still important, objective because there are no user-friendly statistical programs that readily guide comparative binomial sampling analyses. The modeling procedures used address the first and second major objectives are presented here to guide pest control advisers with *O. perseae* sampling and other potential researchers who are interested in the methodology of designing and validating binomial sampling plans with spatial guidelines.

Materials and Methods

Phase One: Determining the Mean-Proportion Relationship. Fully-expanded “Hass” avocado leaves from nine commercial orchards in southern California were collected across 64 dates in the period of 1997-2010. Leaves were brought to the laboratory and the total number of motile *O. perseae* on each leaf was recorded. For each sample date, the mean density and proportion of infested leaves with motile *O. perseae* was calculated. A final set of 4,263 leaves were processed and more than 180,000 mites were counted (Table 3.1).

Using this mite count database, three techniques were used to model the mean-proportion relationship of *O. perseae* on avocado in combination with two tally thresholds, T1 and T2, representing 1 or 2 motile mites per leaf, respectively. Under the simplest mean-proportion model for T1 a leaf is scored as infested when there is at least one motile mite present. Increasing the infestation threshold, Tn, can mitigate curve saturation around a higher range of densities that are of interest for pest management but at the expense of spending additional time looking for more mites on a leaf before considering a leaf sample to be infested (Binns et al. 2000). Nevertheless, this time cost per sample unit can be offset by an overall reduction in the average number of leaves required for making a treatment decision. Consequently, the three mean-proportion techniques were adapted to an additional tally threshold of T2 for a comparison of model performance. Mean-proportion models based on T3, T4, T5 and T9 were considered but were determined to be impractical for field application and were not developed further.

Empirical Model. The first modeling technique relied on linear regression to describe the relationship between log transformed mean densities and proportions of mite infested leaves. The approach was based on the empirical equation (Kono and Sugino 1958, Binns et al. 2000) and resulted in a single model:

$$ln[-ln(1 - p_{Tn})] = a + b(ln(m)) \tag{1}$$

where m refers to the mean density of *O. perseae*, a and b are estimated parameter coefficients and p_{Tn} refers to the proportion of infested leaves according to a predetermined infestation threshold. Once coefficients a and b were determined, equation (1) was re-arranged to solve for the proportion of infested leaves for any Tn as,

$$p_{Tn} = 1 - \exp\{-\exp[a + b(ln(m))]\} \tag{2}$$

Models Based on the Negative Binomial Distribution. Two alternative techniques fit the negative binomial distribution to untransformed mite count data. The negative binomial approach requires an estimate of nuisance parameter k which can be derived through either maximum likelihood or Taylor's power law (TPL) (Taylor 1961). Maximum likelihood estimates of k for each sample date were obtained using PROC GENMOD in SAS (SAS Institute 2008). Alternatively, estimating k under TPL required modeling the relationship of observed mean and variance values from all sampled dates combined as follows:

$$s^2 = cm^d \tag{3}$$

where s^2 is the estimated variance of a population, m is the sample mean density and c and d are parameters estimated from linear regression using PROC REG in SAS (SAS Institute 2008). Once coefficients c and d from TPL were estimated, they were used to solve for k as function of m:

$$k = m/(cm^{(d-1)} - 1) \tag{4}$$

Consequently, maximum likelihood and TPL both generated a unique series of k values. The percentiles of each series were calculated using PROC UNIVARIATE in SAS (SAS Institute 2008). Screening several values led to the selection of the 75th, 85th and 90th k percentiles to fit mean-proportion models to the observed data. For each selected k, the expected probability of finding an infested leaf with one or more mites across a range of *O. perseae* densities, p_{T1}, was determined by using the following negative binomial formulas (Bliss and Fisher 1953, Jones 1994):

$$\text{Maximum likelihood: } p_{TI} = 1 - (1 + m/k)^{-k} \tag{5}$$

$$\text{TPL: } p_{TI} = 1 - \exp\left\{\frac{-m[ln(cm^{(d-1)})]}{cm^{(d-1)} - 1}\right\} \tag{6}$$

The expansion of the general negative binomial formula that deals with probability calculations for tally thresholds greater than T1 was described by Jones (1994). This combination of two tally thresholds, two modeling techniques and parameter estimates based on the negative binomial distribution (*k* percentiles for MLK and TPL) generated a set of 11 mean-proportion models, six based on MLK and four based on TPL. With the addition of a single empirical model described above, a total of 12 mean-proportion models were developed.

Phase 2: Determining an Average Sample Number from Sequential Sampling Models. The 12 mean-proportion models from phase 1 were used to set up custom sequential binomial sampling plans defined by an infestation proportion, θ_{50}, corresponding to an action threshold of 50 mites per leaf. This critical proportion threshold is surrounded by lower and upper sampling boundaries set at 25 (θ_{25}) and 75 (θ_{75}) mites per leaf, respectively. The exact values of θ_{25}, θ_{50} and θ_{75} are unique for each mean-proportion model. The lower range of a working action threshold, 50-100 mites per leaf (Moaz et al. 2011), was selected to minimize the effect of curve saturation which would require a higher average number of leaf samples to terminate sequential sampling as indicated by preliminary analyses (results not shown).

Resampling Validation Data. The performance of the 12 sequential sampling plan models was evaluated by re-sampling simulations of 245 additional data sets from ten orchards (Table 3.2) using the program RVSP (Naranjo and Hutchinson 1997). An copy of this software is available from Naranjo and Hutchinson (2014). Here, 95% of data sets are from orchard number 11 and consisted of in-season spring (n = 90) and summer (n =

90) flush leaves sampled monthly from nine pesticide-free trees during the period from 2002-2012. All other data sets were obtained from orchards with elevated mite infestations during the summer to extend the range of locations and included higher mite densities for model evaluations. Data sets with no mites present or missing samples were omitted from analyses. When taken together, this validation database of 20,403 leaves covered a range of 0-579 mites per leaf. These additional data sets used for validation were not used to build the mean-proportion models and no assumptions were made concerning the underlying mite distributions. Using these field collected data provided a realistic evaluation of the performance of candidate binomial sampling models for a wide range of mite densities across years, leaf age, tree age, and locations. However, to evaluate the robustness of the mean-proportion relationship and the integrity of these validation analyses, the observed mean-proportion values from the validation database were compared with those of the model database.

Resampling Validation Procedures. The sampling simulation of each validation data set was set at 10,000 runs, with leaf replacement, and a minimum sample size of 20 leaves. The minimum sampling requirement of 20 leaves was determined from preliminary validation simulations without any restrictions on leaf sample size and encourages pest control advisers to walk within a tree block of interest before making a treatment decision. The type I and type II error rates, α and β, were set at 0.10. After a minimum sample of 20 leaves were randomly selected, two hypotheses regarding the observed proportion of leaves infested (θ_{obs}) were tested using Wald's sequential probability ratio test (Wald 1947, Jones 1994),

$$H1: \theta_{obs} \leq \theta_{25}$$

$$H2: \theta_{obs} \geq \theta_{75}$$

Selection of H1 terminates the sequential sampling process and implies that mite densities are below 50 mites per leaf and that control for *O. perseae* is not recommended. Selection of H2 terminates the sequential sampling process and implies that mite densities are above 50 mites per leaf and that *O. perseae* control should be considered.

From these simulations we obtained two values, average sample number (ASN) and operating characteristic (OC), for each of the 245 data sets evaluated. ASN was the average sample number over all simulations needed to terminate sequential sampling. The OC curve was interpreted as the probability of deciding not to treat for *O. perseae* over a range of mite densities. For practical sampling plans, the probability of "deciding not to treat" should be high at densities below the action threshold (θ_{50}) and low at densities above θ_{50}. To indicate the trend of the OC values from the validation datasets around the action threshold of 50 mites, we used a logistic model from Martínez-Ferrer et al. (2006):

$$OC = [\exp(q - rx)]/[1 + \exp(q - rx)] \qquad (7)$$

where x is the observed mean density of each validation data set and q and r are parameters estimated using PROC NLIN in SAS (SAS Institute 2008).

Phase 3: Development and Validation of Fixed-Size Binomial Models. Based on the ASN results for the validation of sequential binomial sampling models (see results section), the empirical mean-proportion model was considered for further development of a binomial sampling model with a fixed sample size of 30 leaves, an action threshold of

50 mites per leaf, and tally thresholds T1 and T2. Both of these fixed-sample binomial sampling models were validated with field data in two phases. First, the validation database described in the previous section was re-evaluated with the RVSP program (Naranjo and Hutchinson 1997) to generate OC values for 10,000 runs of each dataset, without leaf replacement, and a treatment recommendation was made when 30 leaves had been selected. For each simulation run, the recommendation to initiate control measures was made if the observed mite infested proportion of each 30-leaf batch was equal to or greater than the corresponding action threshold of θ_{50} (i.e., 0.91 for T1, 0.86 for T2).

Phase 4: Development and Validation of Spatial Sampling Guidelines for Fixed-Size Binomial Models. A second set of field validations were conducted to generate research-based recommendations regarding the number of leaves per tree and tree selection patterns for 30 leaves. These recommendations would facilitate the implementation of this binomial sampling strategy in the field for making reliable classifications of *O. perseae* densities.

The database used for the second field-validation phase consisted of infestation leaf scores sampled from each cardinal direction (i.e., N, E, S, W) on 400 trees from two commercial orchards located in Santa Paula (orchard A) and Carpinteria (orchard B), California. Each randomly selected leaf was scored 0 if it was not infested with *O. perseae* or 1 if infested. In-season mature avocado leaves (n = 1,600) were sampled once at each orchard during July (orchard A) and September 2010 (orchard B). *O. perseae* densities over the entire block of 400 trees at orchards A and B were estimated from enumerative counts as 208 (n = 240 leaves) and 8 (n = 1,600 leaves) mites per leaf, respectively. Leaf scoring at orchard A was only possible for tally threshold T1 and the scoring data were collected on-site. However, leaves scored zero (n = 107) were brought back to the laboratory for thorough inspection under a microscope and the scores for 58% of these selected leaves (n = 62) were corrected to a score of 1. All leaves from orchard B were brought back to the laboratory and all mites on the entire leaf undersurface were counted using stereomicroscopes; this made it possible to score leaves using tally thresholds T1 and T2 at this site. Orchards A and B were selected because 1) they contained contrasting levels of *O. perseae* infestations below and above the action threshold of 50 mites per leaf and, 2) the 400 trees were planted on a 20 x 20 grid that permitted a systematic performance evaluation of five tree-selection patterns that pest control advisers could consider adopting for selecting 30 leaves under the binomial sampling approach.

The five sampling patterns considered required the selection of trees: (1) on a grid, (2) around the perimeter of a block, (3) walking in the shape of “w”, (4) simple random sampling (SRS) and (5) maximin sampling (Fig. 3.1). The maximin sampling approach involved selecting trees in a manner consistent with maintaining a maximum distance between all other trees sampled (see DePalma et al. 2012). Furthermore, except for SRS, all of the sampling patterns were structured to maintain a minimum separation distance of 4 trees (i.e., every fifth tree was sampled) between sampled trees to avoid obtaining biased estimates of *O. perseae* infestation levels; see Li et al. (2012) for a discussion on the implications of spatial correlation of *O. perseae* counts). All five sampling patterns were evaluated at orchards A and B under three simulated stratified sampling scenarios where 2, 3, or 4 leaves per tree were randomly selected and scored until a maximum number of 30 leaves had been collected. With some exceptions, these sampling simulations were conducted with 500 runs using PROC SURVEYSELECT in SAS (SAS Institute 2008).

Similar to the first validation phase of the fixed-size binomial sampling model, a treatment decision was made if the observed mite infested proportion for each batch of 30 leaves was equal to or greater than the corresponding action threshold of θ_{50} (i.e., 0.91 for T1, 0.86 for T2). For practical purposes, each simulation run was scored 1 when the mite assessment evaluation was "not recommend treating" and 0 if "treatment recommended" resulted. The OC value under each sampling scenario at each site was computed as the average score to "not recommend treating" over all simulation runs. Finally, the OC results were used to compare the performance of all sampling patterns and leaf-per-tree criteria against the scenario where the treatment recommendation was based on all 400 trees being sampled at orchards A and B.

Results

Phase One: Determining the Mean-Proportion Relationship. The linear regression parameter estimates for the empirical model and TPL are listed in Table 3.3. TPL parameter estimates were within the range of what has been reported for various spider mites on other crops (Jones 1990). The range of *k* estimates (n = 64) generated under the TPL and maximum likelihood had a positive curvilinear relationship with mean density (Fig. 3.2) and this prevented the use of linear regression to fit a common *k* to the data sets (Bliss and Owen 1958). To resolve this problem, Nyrop and Binns (1992) suggested using *k* values that corresponded to relevant densities. The 75th and 85th *k* percentiles (Table 3.4) estimated through maximum likelihood and TPL, respectively, corresponded to the average values around a range of mite densities, 45-54, surrounding the 50 mite per leaf action threshold for *O. perseae*. The 90th *k* percentile was chosen as an out-of-range estimate for performance comparison.

Using the combined parameter estimates above, a total of 12 mean-proportion curves were generated, five for tally threshold T1 and seven for T2. For both tally thresholds, the curves generated by maximum likelihood (Figs. 3.3 and 3.5) were characterized with early saturation at lower mean densities in comparison to the curves generated by the empirical equation and those constrained by TPL (Figs. 3.4 and 3.6). The curves generated by TPL and the empirical equation retained sensitivity around the 50 mite action threshold and this implied that these models would be better suited for developing binomial sampling plans for *O. perseae* (see validation results below).

Phase 2: Determining an Average Sample Number from Sequential Sampling Models. A graphical comparison indicated the fundamental mean-proportion relationship from the model database was representative of the validation database under both thresholds, T1 and T2 (Figs. 3.7 and 3.8). Furthermore, fitting the empirical equation (1) independently to the validation database generated parameter estimates $a = -1.78$, $b = 0.59$ for T1 and $a = -1.76$, $b = 0.57$ for T2. These sets of parameter estimates are within the 95% confidence interval for the model database parameters calculated from Table 3.3.

Consequently, these results suggested that the mean-proportion relationship for *O. perseae* in southern California is robust and is expected to remain consistent across sites and over years. However, two validation datasets from June-July 2005 have *O. perseae* densities below 50 but with relatively high infestation percentages, 24 mites per leaf (96% leaf infestation) and 17 mites per leaf (85% leaf infestation) and therefore resulted with a high probability of treating for *O. perseae* (Figs. 3.9-3.13, OC).

Relative to the 85th and 90th percentile *k* estimate, sampling plans with a 75th *k* percentile determined from maximum likelihood were characterized with a higher probability of making a correct treatment diagnosis below and above the 50 mite per leaf action threshold for both tally thresholds (Figs. 3.9 and 3.11, OC). Across all simulations for T1, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-150 and 1722, respectively (Fig. 3.9A, ASN). Across all simulations for T2, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-83 and 701, respectively (Fig. 3.11A, ASN).

The combined OC and ASN characteristics for sampling plans designed with TPL and the empirical model retained a high probability of correctly classifying densities that were above or below the 50 mite per leaf action threshold, and had a lower average sample size. For TPL, optimal OC results were achieved with the 75th *k* percentile (Figs. 3.10 and 3.12). Across all simulations for T1, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-37 and 251, respectively (Fig. 3.10A, ASN). Across all simulations for T2, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-30 and 196, respectively (Fig. 3.12A, ASN).

The empirical model operates independently of nuisance parameter *k* and therefore only generated two mean-proportion curves. Across all simulations for T1, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-32 and 258, respectively (Fig. 3.10B, ASN). Across all simulations for T2, the average range and maximum number of sequential leaf samples needed to reach a treatment decision were 20-28 and 170, respectively (Fig. 3.13, ASN). Because the empirical model had a lower and narrower sampling range, compared to TPL and MLK, the results from this modeling technique were used to develop and validate a fixed-size binomial sampling model.

Phase 3: Development and Validation of Fixed-Size Binomial Models. The OC results of the first validation phase for the empirical mean-proportion model under a fixed-size (30 leaves) binomial sampling framework for tally thresholds T1 and T2 are shown in Fig. 3.14. Similar OC trend-lines were observed with a high probability to "not treat" at low densities and this probability approached zero as mite densities neared and exceeded the action threshold of 50 mites per leaf. However, at higher mite densities a lower probability to "not treat" was observed for T2 (Fig. 3.14b) and this indicated that this tally threshold performed slightly better.

Phase 4: Development and Validation of Spatial Sampling Guidelines for Fixed-Size Binomial Models. The second validation phase focused on evaluating the performance of five tree-selection patterns and leaf-per-tree criteria for 30 leaves on a

20x20 grid at orchards A and B. The OC results (Tables 3.5 and 3.6) indicated that the maximin tree-selection approach with either 2, 3 or 4 leaves per tree generated treatment recommendations with 100% accuracy at both orchards across all simulations under a tally threshold T1: “to treat” at orchard A and “not to treat” at orchard B. A similar conclusion was reached for T2 at orchard B. However, an enumerative mite density estimate for 4 leaves per tree selected under the maximin pattern at orchard B generated the lowest maximum estimate of 15 mites per leaf in comparison to 2 or 3 leaves selected in the same manner (Table 3.6). The other sampling patterns and leaf-per-tree criteria generated estimated maximum densities in a range of 35-54 mites per leaf at orchard B. Based on the OC results, the next best sampling approaches were the “w” sampling pattern and SRS. While the probability to “not treat” was 100% at orchard B, this probability ranged between 10-15% for the highly infested block of trees at orchard A. However, SRS does not take into account a minimum spatial separation of selected trees and therefore is not recommended for sampling. Grid sampling and perimeter sampling generated accurate treatment recommendation across all simulations at orchard B but evidence of reduced performance was revealed in the OC results for orchard A. At orchard A, the probability of deciding not to treat under grid and perimeter sampling ranged between 29-35%. The results for these sampling patterns generated concern because the level of infested leaves (96%) over the entire set of 400 trees at orchard A had indicated that mite densities exceeded 200 mites per leaf, four times the action threshold of 50 mites per leaf.

Discussion

Among the three techniques used to set up sequential binomial sampling models, practical OC and ASN validation results from field-collected data were obtained with parameter estimates from TPL (75^{th} *k* percentile) and the empirical model for tally thresholds T1 and T2. A similar conclusion on the parallel performance of these techniques was reached by Nyrop and Binns (1992) across various thresholds for the economically important spider mite, *Panonychus ulmi*, on apples. In contrast, the wide ASN range obtained using binomial sampling models based on maximum likelihood (MLK) estimates of *k* implied that more samples were required to terminate sampling. Nevertheless, this additional sampling burden for MLK translated into better OC performance. Not surprisingly, the lower ASN range for binomial models based on TPL and the empirical mean-proportion relationship translated into a trade-off in reduced OC performance. However, the OC values for these binomial models displayed an ideal trend in discerning between mite densities above and below the action threshold of 50 mites per leaf. Between TPL and the empirical model, the latter performed slightly better with a lower ASN range of 20-32 and 20-28 for T1 and T2, respectively. Because only slight improvements in performance were obtained with T2, adopting the more basic infestation criteria of T1 is more convenient for field application as it only requires finding one mite on a leaf before the next sample is collected.

The empirical mean-proportion model validated for sequential sampling was used to establish a fixed-size binomial sampling plan for 30 avocado leaves on a 20 x 20 grid. The first phase validation results indicated that this alternate sampling strategy retained favorable OC properties for determining levels of mite densities that exceeded the 50 mite per leaf action threshold. The OC results of the second validation phase conducted in two commercial avocado orchards indicated that targeting trees that were maximally spaced from each other (i.e., maximin selection) was a superior sampling strategy to all other evaluated tree-selection patterns (i.e., SRS, "w", perimeter, and grid sampling). Within the maximin selection framework, selecting four leaves per tree generated the lowest maximum estimate of mite densities at orchard B and this sets a precedent for setting a minimum requirement of sampling all four cardinal points from a selected tree. Although blocks of avocado trees can come in different tree-density sizes, the recommendations stemming from our field-data validations can accommodate smaller sized blocks (<400 trees available for selection). Blocks with a larger density of 400 trees (e.g., 800 x 200) can be divided up into smaller sampling units accordingly.

Previously, DePalma et al. (2012) evaluated similar tree-selection patterns (i.e., perimeter, border, zigzag, diagonal, SRS) using simulated *O. perseae* data for a sequential sampling framework on a 20 x 20 grid and an earlier version of the empirical mean-proportion relationship for T1 used in this study. Similarly, OC and ASN results from that study indicated that maximin tree selection was superior to all other evaluated sampling patterns and that an optimal number of six leaves per tree for an average of five to ten trees were required for terminating sequential sampling and making a reliable chemical treatment decision ("spray" vs. "no spray"). We were not able to evaluate the fixed selection of more than four leaves per tree for a lot of 400 trees but the two studies concur in accommodating the sampling of all cardinal points on a tree. This recommendation takes into account the fact that significant differences in *O. perseae* levels can exist between canopy faces on a tree but there is no evidence for a consistent pattern across avocado orchards that would warrant targeting a specific cardinal point (see Li et al. 2012). At orchard B for example, chi-square tests indicated that there was a statistically significant relationship between infestation leaf score (T1 and T2) and cardinal point (T1: $\chi^2_3 = 30.09$ (n = 1,600), $P < 0.0001$; T2: $\chi^2_3 = 33.58$ (n = 1,600), $P < 0.0001$), with a trend of lower mite infestation levels associated with north-facing avocado leaves; this conclusion was further corroborated with heat maps of mite densities (results not shown). In contrast, at orchard A, a chi-square test revealed that there was a marginal statistically significant relationship between leaf infestation score for T1 and cardinal direction ($\chi^2_3 = 7.85$ (n = 1,600), $P = 0.05$), with a slightly higher frequency of infested leaf scores on leaves facing South and West. Due to the inconsistency of leaf infestation patterns within trees across sites, we recommend sampling all points of selected trees to obtain a representative snapshot of *O. perseae* levels.

To maximize efficiency, sampling efforts could first be directed towards individual blocks of avocado trees with a history of *O. perseae* problems. The identification of these areas will be based on the experience of the pest control adviser at the orchard and any information provided by the grower. Subsequent sampling efforts can be directed to blocks of an orchard as part of routine monitoring of *O. perseae* densities. As the pest control adviser walks/drives through the orchard, observed characteristic foliar damage (i.e., necrotic spots) caused by *O. perseae* could signal to the pest control adviser that assessing *O. perseae* levels could be important. The extent of sampling efforts will vary according to the standard practices of each pest control adviser and the history of *O. perseae* problems at the orchard. In any case, for each selected block, the validation results from field collected data suggest that the binomial sampling strategy based on the empirical model can accommodate a selection of differently aged leaves that result from either spring or summer flush, but we recommend that sampling be directed towards fully-expanded leaves from spring flush as these tend to have higher mite densities than summer flush. This recommendation is based on the observed range of mite densities covered by the validation database for orchard number 11. Over the 10 year period this orchard was sampled, *O. perseae* densities on summer flush leaves ranged from 0-36 while those on spring flush from the same untreated trees ranged from 0-139. An intuitive reason for this difference is that *O. perseae* populations on spring flush leaves have a relatively earlier and longer window for growth (e.g., April-September) in comparison to the later summer flush (e.g., June-September) when mite sampling would be conducted. In the field, however, carefully selecting spring flush leaves would add another time-consuming element and it may not be readily obvious as to which flush cohort sampled leaves belong. For this reason we recommend general selection of in-season fully-expanded leaves (when available) that will likely include samples from spring and summer flush. The validity of this recommendation stems from our OC and ASN results for datasets with fully-expanded leaves that did not distinguish between spring or summer flush that were collected in the summer among nine orchards (3-10) throughout southern California with *O. perseae* densities ranging from 36-580 mites per leaf (Fig. 3.9B).

Practical Application for Commercial Avocado Orchards. Implementing the fixed-size binomial sampling plan in commercial avocado orchards for blocks with 400 trees or less requires the selection of 30 in-season, fully-expanded ‘Hass’ avocado leaves. An average of four leaves is collected randomly around the perimeter from each of 8 selected trees that are separated by at least four trees to reduce bias from spatial correlation. Two leaves can be randomly omitted from sampling among selected trees to reach the recommended 30 leaf sample size and this reduced sampling structure was validated using field collected data (Table 3.5). Recommendations are not based on a per acre basis because tree planting densities may vary across orchards. Although the sample size of 30 leaves is economical, reliable assessments depend on maximally spacing trees from all other available trees within a block of interest with a minimum separation requirement of four trees. For this reason, it is expected that the pest control adviser or grower is familiar with the tree-layout of the orchard so as to pre-select eight trees. In our experience, some commercial orchards keep electronic maps that can be readily used for this purpose. In some cases, trees located on hilly terrain are not accessible for safety reasons and only access to trees along the perimeter or via access roads is possible. Pest control advisers can use perimeter sampling to mitigate this sampling constraint as a last resort, but whenever possible, maximin tree-selection criteria should be used.

Each selected leaf is inspected with a hand lens on-site and scored 1 if there is one or more motile *O. perseae* mites present or 0 if no motile mites are observed. Based on our field experience, processing individual leaves for tally threshold T1 takes approximately 30 seconds and this translates into 15 minutes for inspecting a batch of 30 leaves. This time estimate excludes walking time between trees which will depend on tree spacing and the duration of other pest inspection activity (e.g., recording notes, taking pictures of foliar damage) conducted by the person sampling leaves. An average of all leaf scores is calculated manually to determine the proportion of infested leaves. There is future potential to develop a smart-phone or tablet application that will automatically perform these calculations and allow for GPS-identified photos to be stored for fast delivery of *O. perseae* monitoring results and recommendations to avocado growers. Leaf infestation proportions greater than 0.91 indicate that mite levels are above 50 mites per leaf and that initiating control measures is warranted. This procedure can be repeated as needed on multiple blocks in the orchard to gauge the severity of mite infestations across the entire orchard. This approach may reduce unnecessary pesticide applications as only blocks requiring treatments will be identified. The frequency of *O. perseae* population density monitoring depends on the management dynamics of each orchard but we have provided field-validated sampling guidelines that can be easily implemented throughout southern California. Previous published pest management guidelines (UC IPM 2011) recommended initiating treatment when 2 out of 5 leaves were infested (i.e., 40% foliage infestation) with motile stages of *O. perseae*. There is no scientific foundation for this recommendation and this 40% infestation level corresponds to an estimated density of four mites per leaf based on our empirical model, which is 12 times below the recommended 50 mite per leaf action threshold.

Future directions for the extension of *O. perseae* binomial sampling involve customizing this binomial approach for other regions where this mite is a pest (e.g., Mexico, Costa Rica, Israel, and Spain). Using the methods presented in this study, research devoted to improving field sampling approaches for *O. perseae* in these other regions can focus on validating the empirical mean-proportion relationship and sampling patterns with field collected data. Overall, a statistically sound sampling plan for *O. perseae* that is easily implemented in commercial 'Hass' avocado orchards will assist greatly with pest management decision making.

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Table 3.1. Summary data for *Oligonychus perseae* counts on avocado leaves collected from different counties in California that were used to generate mean-proportion models

Orchard	Sampling Date	County	No. of data sets[a]	No. Leaves	Total Mites Counted
1	Mar. 97 - Oct. 97	Ventura	14	839	11,119
2	Aug. 99 - Oct. 99	Orange	8	480	33,497
2	May 2000 - April 2001	Orange	35	2,100	13,004
3	July 2009	San Diego	1	124	11,483
4	Aug. 2009	Santa Barbara	1	120	5,594
5	Aug. 2009	Santa Barbara	1	120	42,950
6	Aug. 2009	Santa Barbara	1	120	4,313
7	Sept. 2009	Santa Barbara	1	120	5,805
8	June 2010	Ventura	1	120	25,519
9	July 2010	Ventura	1	120	27,180

[a] Each data set corresponds to an independent batch of leaves collected at each site during the indicated sampling period.

Table 3.2. Summary of *Oligonychus perseae* avocado leaf infestation data from different counties in California that were used to validate sampling plans based on mean-proportion models

Orchard	Sampling Date	County	No. Data Sets[a]	No. Leaves	Total Mites Counted
3	July 2009	San Diego	1	123	7,546
4	Aug. 2009	Santa Barbara	1	120	4,423
5	Aug. 2009	Santa Barbara	3	360	112,868
6	Aug. 2009	Santa Barbara	1	120	4,557
7	Sept. 2009	Santa Barbara	1	120	6,045
8	June 2010	Ventura	1	120	23,396
9	July 2010	Ventura	1	120	22,723
10	July 2010	Orange	2	260	137,257
11	July 2002 - June 2012	Orange	111	9,990	92,918
11	Sept. 2002 - Apr. 2012	Orange	123	11,070	37,025

[a] Each data set corresponds to an independent batch of leaves collected at each site during the indicated sampling period.

Table 3.3. Linear regression parameter estimates for the empirical and Taylor's power law (TPL) mean-proportion models for tally thresholds of one (T1)and two mites (T2)

		T1	T2
Technique	Parameter	Estimate ± SE	Estimate ± SE
Empirical	*a*	-1.56 ± 0.11	-1.92 ± 0.10
	b	0.62 ± 0.04	0.67 ± 0.04
TPL	*c*	9.78 ± 1.15	9.78 ± 1.15
	d	1.6 ± 0.05	1.6 ± 0.05

Table 3.4. Percentile values for parameter *k* estimated using Taylor's power law (TPL) and maximum likelihood (MLK)

	k percentiles		
Model	75th	85th	90th
TPL	0.38	0.48	0.63
MLK	0.48	0.62	0.70

Table 3.5. Operating characteristic (OC) and proportion of infested leaves (P_{T1}) results for five simulated tree-selection patterns for 30 leaves at orchard A under a tally threshold of one mite (T1)

Sampling Pattern	No. Simulations	No. Leaves[a]	No. Trees	OC_{T1}	P_{T1}
Grid	500	2	15	0.29	0.93
		3	10	0.36	0.93
		4	8	0.35	0.93
Perimeter	500	2	15	0.32	0.93
		3	10	0.31	0.93
		4	8	0.29	0.94
W	500	2	15	0.1	0.96
		3	10	0.1	0.96
		4	8	0.15	0.96
SRS	500	2	15	0.12	0.96
		3	10	0.12	0.96
		4	8	0.12	0.96
MaxiMin	500	2	15	0	0.98
		3	10	0	0.97
	100[b]	4	8	0	0.96
All Trees	-	4	400	0	0.96

[a] The selection of 30 leaves was stratified to 2, 3 or 4 leaves per tree. In the case of 4 leaves, the samprate option in PROC SURVEYSELECT was set to 0.9375 so that only 30 out the possible 32 leaves available from 8 trees were selected.

[b] The number of simulations were conservatively set to 100 because all available leaves for each selected tree were selected

Table 3.6. Operating characteristic (OC) and proportion of infested leaves (P_T) results for five simulated tree-selection patterns for 30 leaves at orchard B under tally thresholds of one (T1) and two mites (T2)

Sampling Pattern	No. Simulations	No. Leaves[a]	No. Trees	OC_{T1}	P_{T1}	OC_{T2}	P_{T2}	Mean Mite Density ± SD	Max. Density[b]
Grid	500	2	15	1	0.32	1	0.21	11.1 ± 6.7	34
		3	10	1	0.33	1	0.20	11.0 ± 6.5	37
		4	8	1	0.32	1	0.20	10.7 ± 6.7	34
Perimeter	500	2	15	1	0.29	1	0.22	9.2 ± 6.0	33
		3	10	1	0.29	1	0.21	8.3 ± 6.7	33
		4	8	1	0.29	1	0.22	8.8 ± 7.0	34
W	500	2	15	1	0.33	1	0.23	11.4 ± 9.2	49
		3	10	1	0.32	1	0.22	10.8 ± 8.7	44
		4	8	1	0.33	1	0.22	11.1 ± 8.7	45
SRS	500	2	15	1	0.33	1	0.22	8.9 ± 8.5	54
		3	10	1	0.33	1	0.22	8.2 ± 8.4	50
		4	8	1	0.33	1	0.23	8.5 ± 8.9	53
MaxiMin	500	2	15	1	0.35	1	0.26	9.1 ± 5.4	22
		3	10	1	0.37	1	0.25	12.2 ± 4.7	19
	100[c]	4	8	1	0.38	1	0.24	13.5 ± 1.2	15
All Trees	-	4	400	1	0.32	1	0.22	8	-

[a] The selection of 30 leaves was stratified to 2, 3 or 4 leaves per tree. In the case of 4 leaves, the samprate option in PROC SURVEYSELECT was set to 0.9375 so that only 30 out the possible 32 leaves available from 8 trees were selected.

[b] Maximum density of *Oligonychus perseae* was determined from enumerative mites counts across all 500 simulations with a sample size of 30 selected leaves.

[c] The number of simulations were conservatively set to 100 because all leaves for each selected tree were selected.

Figure Legends

Fig. 3.1. Schematic representation of the five evaluated tree-selection patterns with a minimum spatial separation requirement of four trees between selected trees on a 20 x 20 grid: A) grid sampling, B) perimeter sampling, C) sampling in the shape of “w”, D) maximin sampling, and D) a simple random sample (SRS) with no spatial separation requirements.

Fig. 3.2. Estimates of *k* based on Taylor’s power law (TPL) and maximum likelihood (MLK) across mean *Oligonychus perseae* densities.

Fig. 3.3. Comparison of the mean-proportion curve for the empirical model with the 75th, 85th and 90th *k* percentile curves under maximum likelihood (MLK) for a tally threshold of one mite (T1).

Fig. 3.4. Comparison of the mean-proportion curve for the empirical model with the 75th *k* percentile curve under Taylor’s power law (TPL) and a tally threshold of one mite (T1).

Fig. 3.5. Comparison of the mean-proportion curve for the empirical model with the 75th, 85th and 90th *k* percentile curves under maximum likelihood (MLK) and a tally threshold of two mites (T2).

Fig. 3.6. Comparison of the mean-proportion curve for the empirical model with the 75th, 85th and 90th *k* percentile curves under Taylor’s power law (TPL) and tally threshold of two mites (T2).

Fig. 3.7. Mean-proportion values for the model database and validation database for a tally threshold of one mite (T1).

Fig. 3.8. Mean-proportion values for the model database and validation database for tally threshold of two mites (T2).

Fig. 3.9. Operating characteristic (OC) and average sample number (ASN) for validation of sequential sampling plans with a tally threshold of one mite (T1) and three maximum likelihood estimates of parameter *k*, (A) 75th percentile, (B) 85th percentile, and (C) the 90th percentile.

Fig. 3.10. Operating characteristic (OC) and average sample number (ASN) for validation of sequential sampling plans for a tally threshold of one mite (T1) for (A) Taylor's power law with 75th *k* percentile, and (B) the empirical model.

Fig. 3.11. Operating characteristic (OC) and average sample number (ASN) for validation of sequential sampling plans for a tally threshold of two mites (T2) with three maximum likelihood estimates of parameter *k* for (A) 75th percentile,(B) 85th percentile, and (C) the 90th percentile.

Fig. 3.12. Operating characteristic (OC) and average sample number (ASN) for validation of sequential sampling plans for a tally threshold of two mites (T2) with three Taylor's power law estimates of parameter *k* for the (A) 75th percentile, (B) 85th percentile, and (C) the 90th percentile.

Fig. 3.13. Operating characteristic (OC) and average sample number (ASN) for validation of the sequential sampling plan under the empirical model and a tally threshold of two mites (T2).

Fig. 3.14. Operating characteristic (OC) for validation fixed-size binomial sampling plans under the empirical model and tally thresholds of (A) one mite, T1 and B) two mites, T2.

Fig. 3.1.

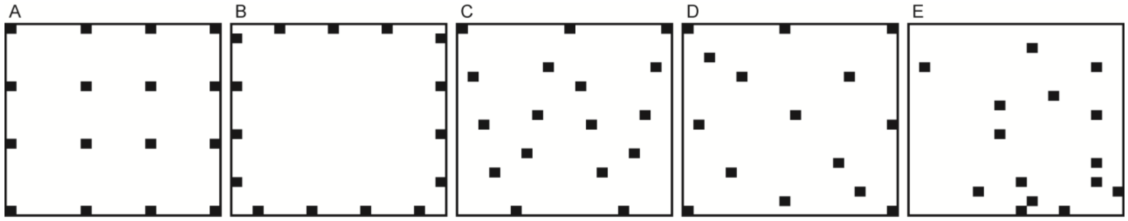
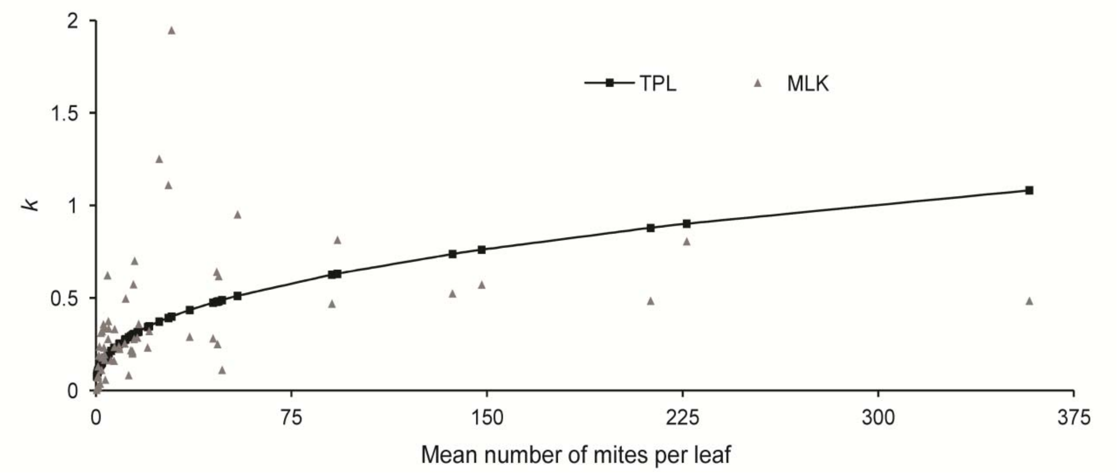


Fig. 3.2.



2
1.5
1
0.5
0
k
TPL
MLK
0
75
150
225
300
375
Mean number of mites per leaf

Fig. 3.3.

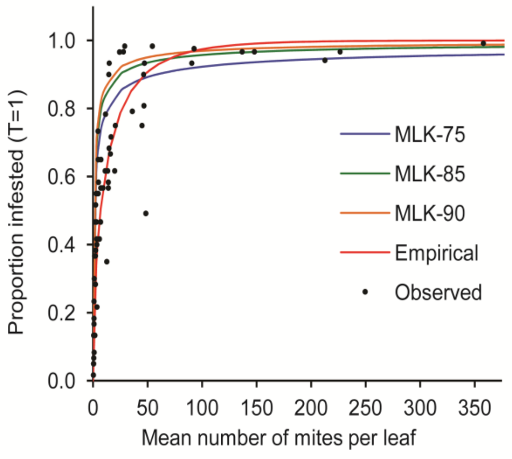

Proportion infested (T=1)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
Mean number of mites per leaf
MLK-75
MLK-85
MLK-90
Empirical
Observed

Fig. 3.4.

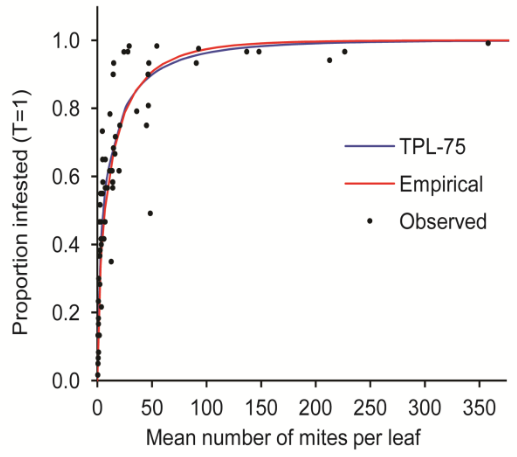

Proportion infested (T=1)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
Mean number of mites per leaf
TPL-75
Empirical
Observed

Fig. 3.5.

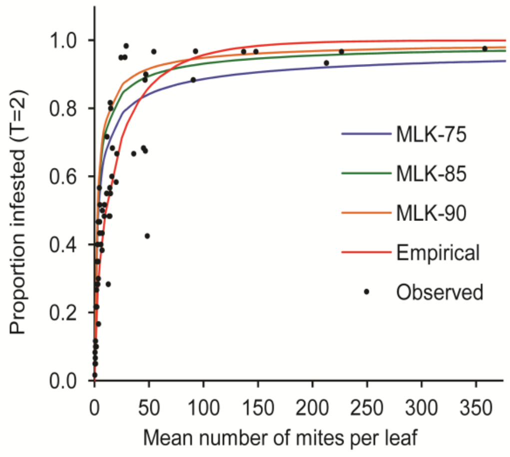

Proportion infested (T=2)
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
250
300
350
Mean number of mites per leaf
MLK-75
MLK-85
MLK-90
Empirical
Observed

Fig. 3.6.

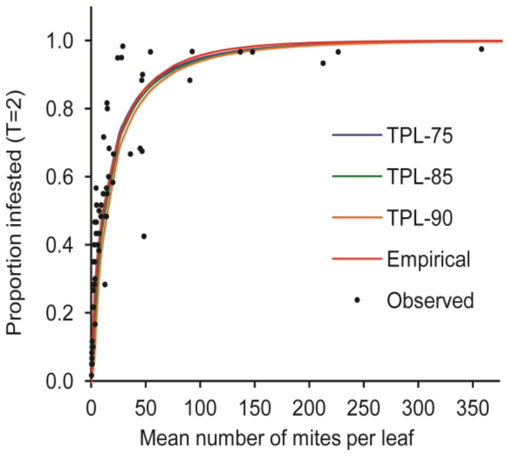

Proportion infested (T=2)
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Mean number of mites per leaf
TPL-75
TPL-85
TPL-90
Empirical
Observed

Fig. 3.7.

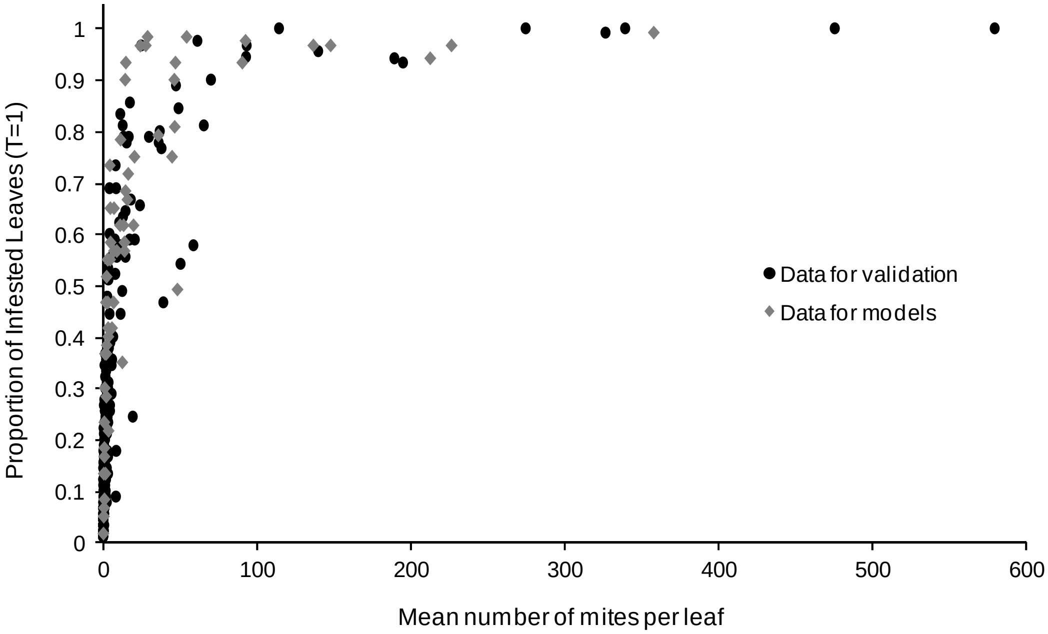

Proportion of Infested Leaves (T=1)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Mean number of mites per leaf
● Data for validation
◆ Data for models

Fig. 3.8.

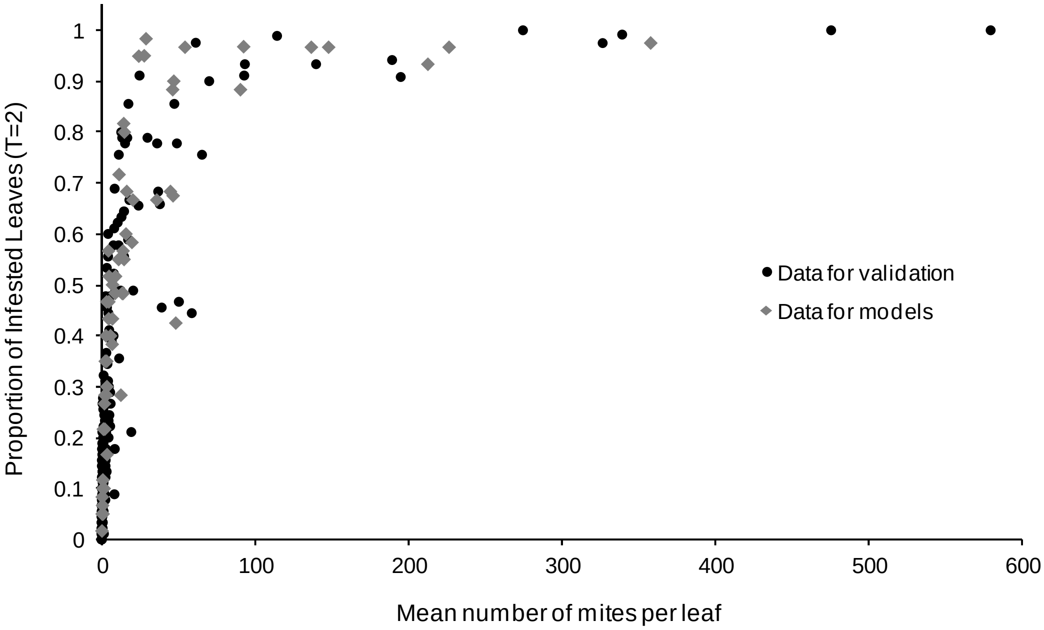

Proportion of Infested Leaves (T=2)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Mean number of mites per leaf
Data for validation
Data for models

Fig. 3.9.

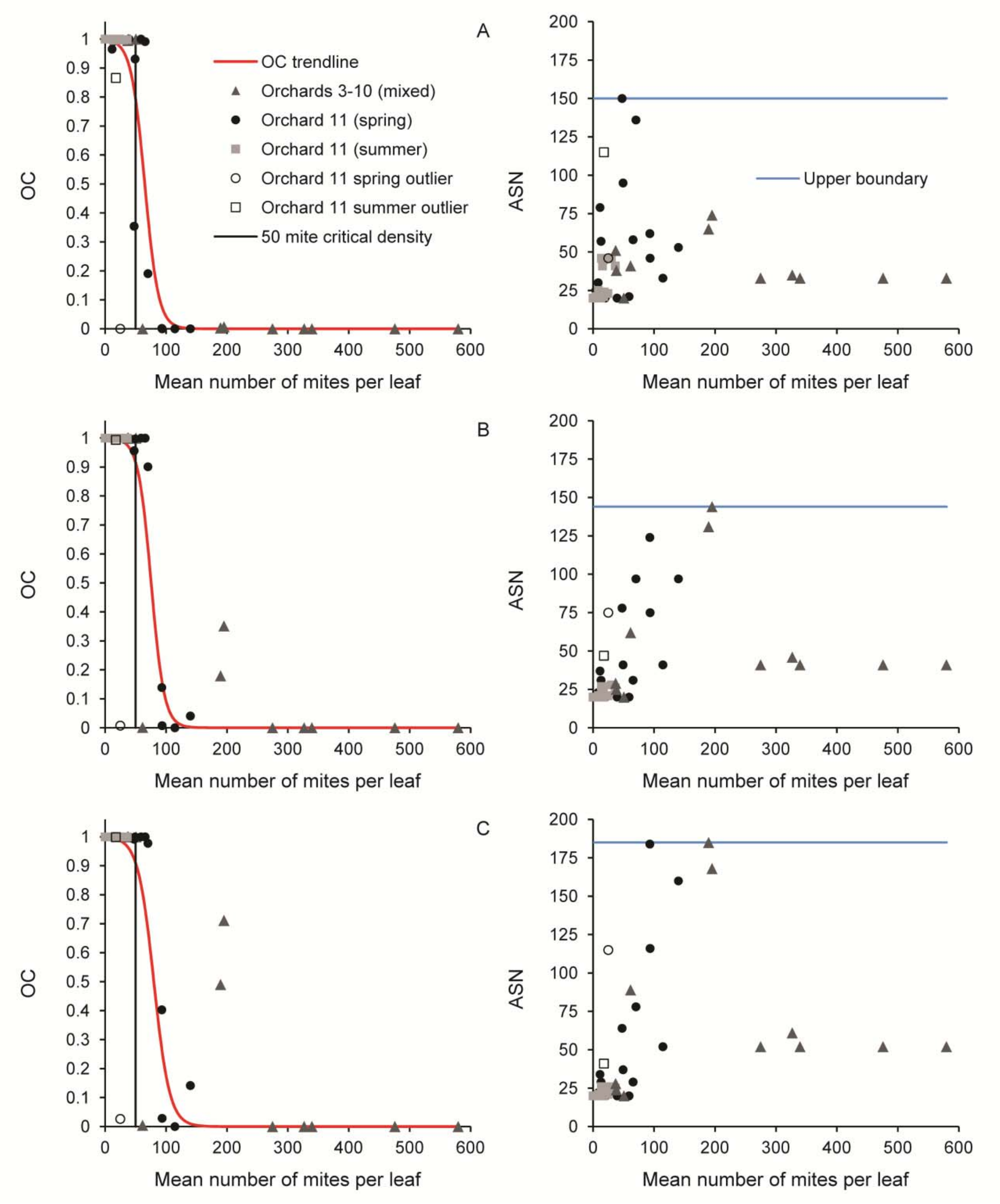

A
B
C
OC
ASN
Mean number of mites per leaf
0
100
200
300
400
500
600
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
25
50
75
100
125
150
175
200
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density
Upper boundary

Fig. 3.10.

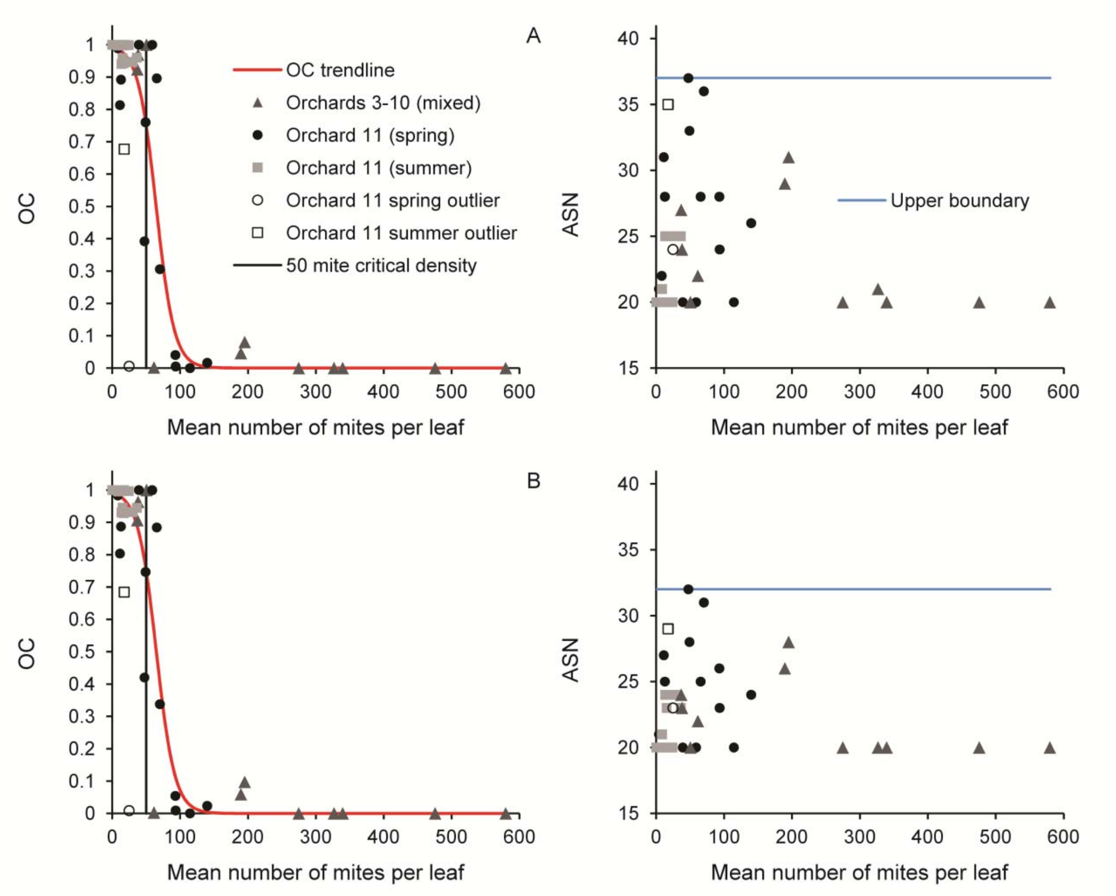

A
B
OC
ASN
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density
Upper boundary
Mean number of mites per leaf
0
100
200
300
400
500
600
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
15
20
25
30
35
40

Fig. 3.11.

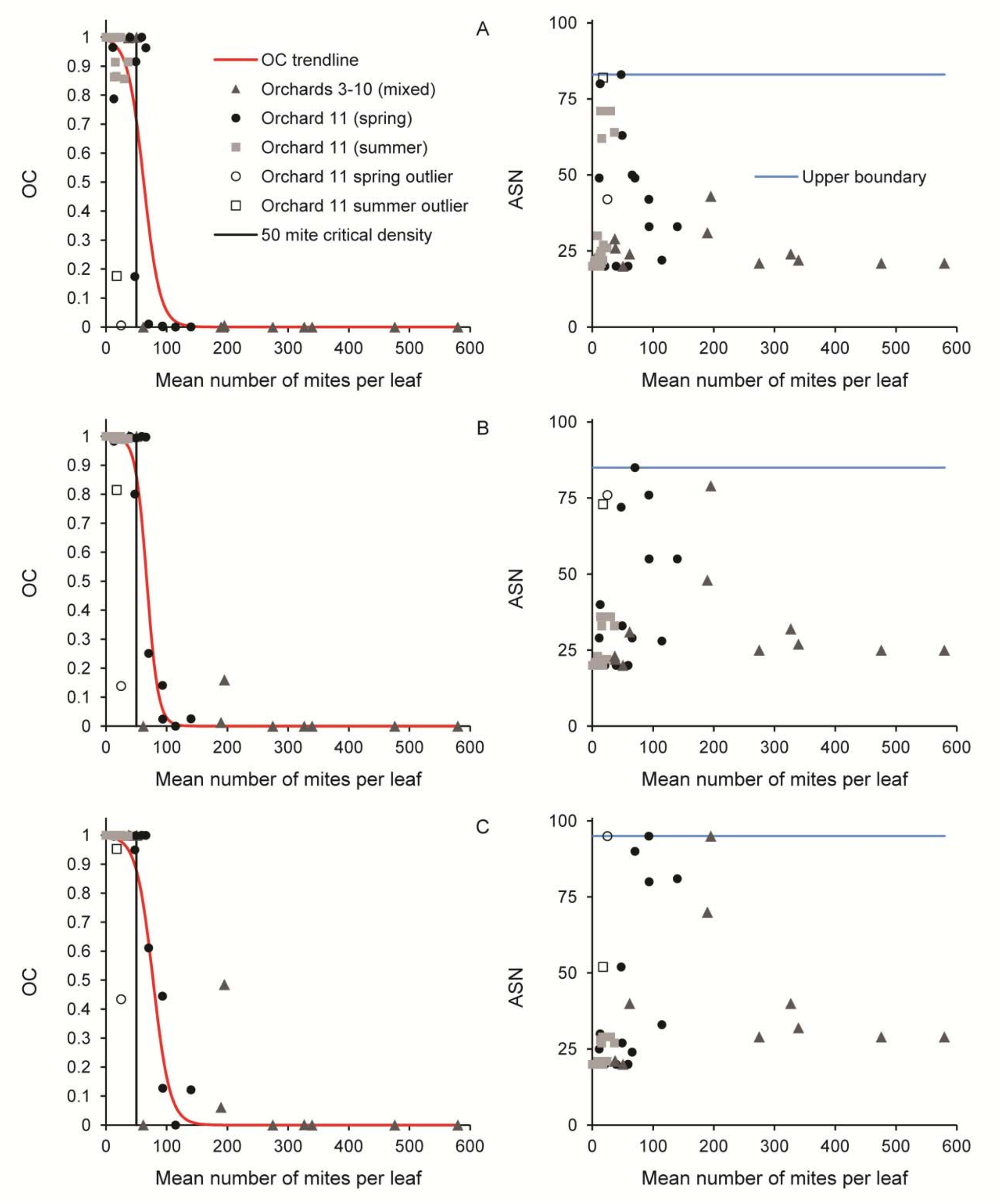

A
B
C
OC
ASN
Mean number of mites per leaf
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density
Upper boundary
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
25
50
75
100
0
100
200
300
400
500
600

Fig. 3.12.

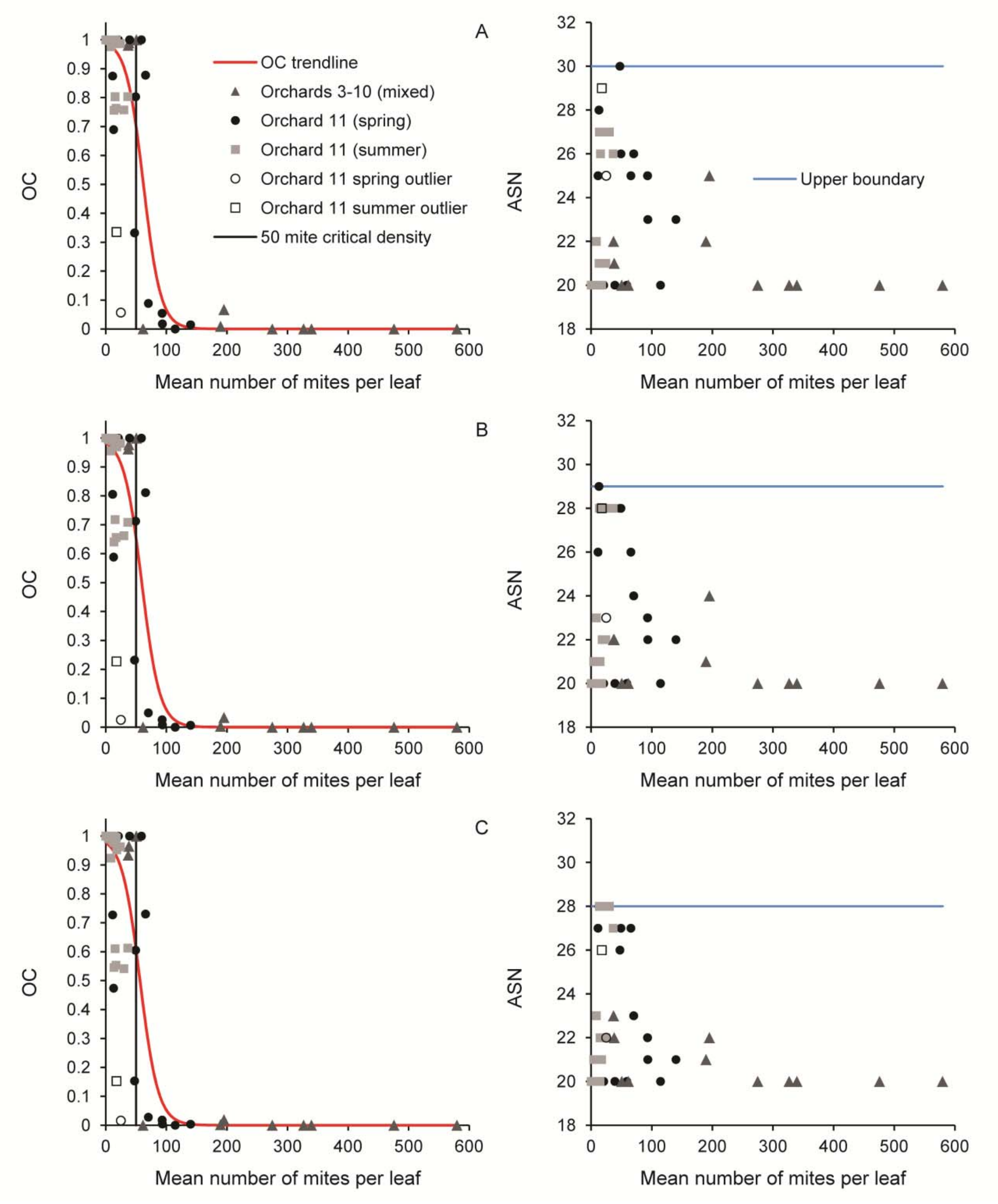
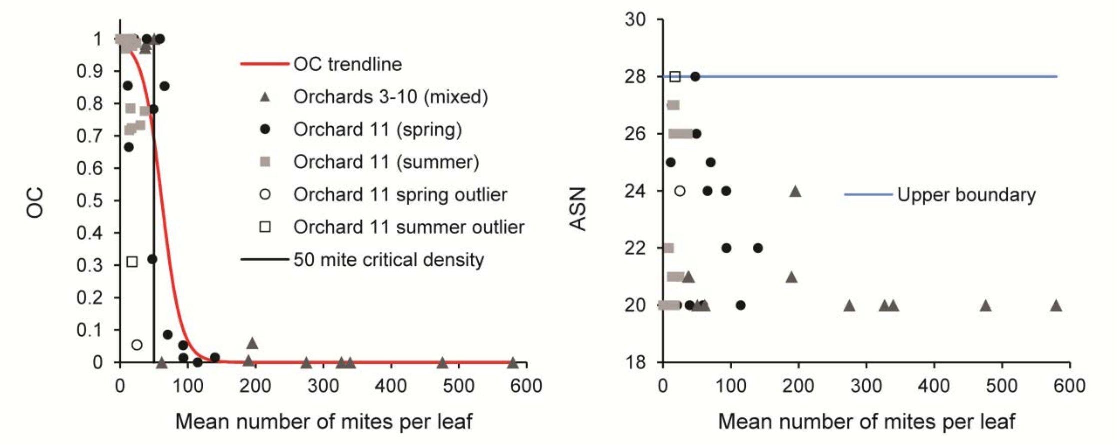
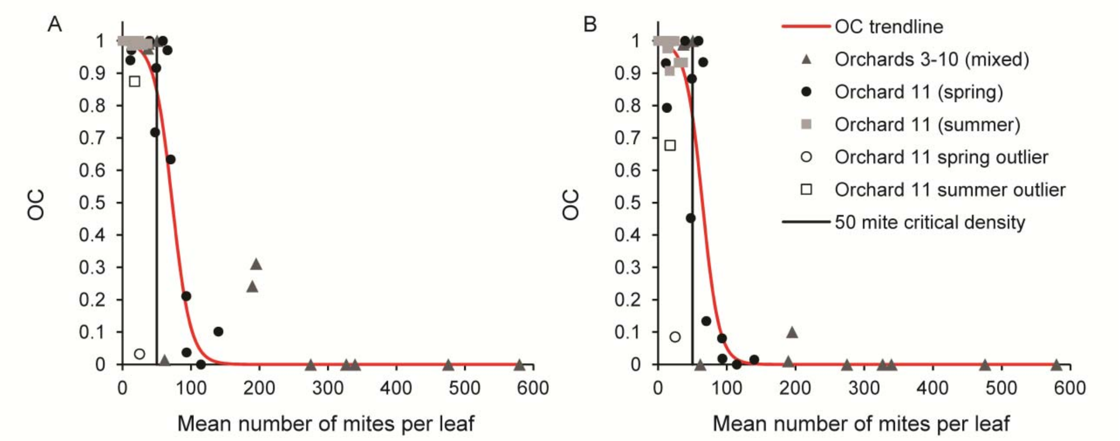

A
B
C
OC
ASN
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
32
30
28
26
24
22
20
18
0
100
200
300
400
500
600
Mean number of mites per leaf
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density
Upper boundary

Fig. 3.13.



OC
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
100
200
300
400
500
600
Mean number of mites per leaf
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density
ASN
30
28
26
24
22
20
18
0
100
200
300
400
500
600
Mean number of mites per leaf
Upper boundary

Fig. 3.14.


A
B
OC
Mean number of mites per leaf
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
100
200
300
400
500
600
OC trendline
Orchards 3-10 (mixed)
Orchard 11 (spring)
Orchard 11 (summer)
Orchard 11 spring outlier
Orchard 11 summer outlier
50 mite critical density

Chapter 4

Spatial Dependence and Sampling of Phytoseiid Populations on Hass Avocados in Southern California

ABSTRACT Phytoseiid research has been critical for understanding the complexity of developing an effective biocontrol strategy for suppressing *Oligonchus perseae* Tuttle, Baker, and Abatiello (Acari: Tetranychidae) in California avocado orchards. However, basic understanding of the spatial ecology of natural populations of phytoseiids in the context of *O. perseae* infestations and the validation of research-based strategies for assessing densities of these predators has been limited. To address these shortcomings, cross-sectional and longitudinal observations on the densities of phytoseiids and *O. perseae* were taken across nine commercial avocado orchards and one research plot. Subsets of these data were analyzed statistically to describe the spatial distribution of phytoseiids in avocado orchards and to evaluate the merits of developing binomial and enumerative sampling strategies for these predators. Spatial correlation of phytoseiids between trees was detected at one site and a strong association of phytoseiids with *O. perseae* was detected at four sites. Sampling simulations revealed that enumeration-based sampling performed better than binomial sampling for estimating phytoseiid densities. The ecological implications of these findings and potential for developing a custom sampling plan for phytoseiids inhabiting avocado orchards in California are discussed.

KEY WORDS Phytoseiidae, *Oligonychus perseae*, binomial sampling, fixed-precision sampling, geostatistics

Oligonychus perseae Tuttle, Baker, and Abatiello (Acari: Tetranychidae) is native to Mexico and is a key foliar spider mite pest of avocados (*Persea americana* [Laureaceae]). Invasive populations of *O. perseae* were detected in southern California in 1990 (Bender 1994) and this pest has invaded Spain and Israel (Vela et al. 2007, Maoz et al. 2011a). Extensive feeding damage by *O. perseae* on Hass avocado leaves causes necrotic spots leading to premature defoliation, which can result in yield losses (Maoz et al. 2011b). The action threshold for controlling this pest is 50-100 mites per leaf (Aponte and McMurtry 1997, Kerguelen and Hoddle 2000, Maoz et al. 2011b). Effective *O. perseae* control is warranted because Hass fruit accounts for more than 90% of avocados produced in California and the crop is valued at more than $300 million per year (CAC 2012). To facilitate cost effective pest management of *O. perseae* in California avocado orchards, a binomial sampling plan that accounts for the spatial distribution of this pest mite was developed (DePalma et al. 2012, Li et al. 2012, Lara and Hoddle 2013). This sampling plan allows pest managers to classify *O. perseae* densities and make an informed decision as to whether or not control is needed.

The biological control program for *O. perseae* in California needs further development and currently pesticides are the primary control strategy for suppressing populations of this pest in commercial avocado orchards (UC IPM 2011, Hoddle and Morse 2013). Inoculative biological control (Van Driesche et al. 2008) guidelines were developed using commercially available phytoseiids (Hoddle et al. 1999). However, effective control of *O. perseae* with the optimal agent, *Neoseiulus californicus* (McGregor), requires seasonal releases of 2,000 phytoseiids per tree (Hoddle et al. 2000).

Implementing this release strategy is not practical or cost effective for large areas that need to be treated. The prospect of implementing conservation biological control of *O. perseae* in California using natural populations of phytoseiids is limited by key ecological characteristics of these natural enemies. For example, *Euseius hibisci* (Chant) is a native and widely distributed phytoseiid in the California avocado system (McMurtry and Johnson 1966, Yee et al. 2001, Takano-Lee and Hoddle 2002), but this species, like other members of its genus, is regarded as a pollen specialist (McMurtry 1997) and has faster development on a pollen diet than on spider mites alone (McMurtry and Scriven 1964, Zhimo and McMurtry 1990). Furthermore, *E. hibisci* cannot penetrate the webbed nests made by *O. perseae* and this further constrains the biological control potential of this naturally-occurring predator in the California avocado system (McMurtry 1993).

Recent conservation biological control research on *O. perseae* has attempted to rescue the limited numerical response of resident phytoseiid populations in Hass avocado orchards. The results from those studies suggest that provisioning indigenous populations of *Euseius scutalis* (Athias-Henriot) in Israel (Maoz et al. 2011a) and *Euseius stipulatus* (Athias-Henriot) in Spain (González-Fernández et al. 2009) with pollen resources can enhance populations and their subsequent efficacy for suppressing *O. perseae* populations in avocado orchards. In California, the success of provisioning *E. hibisci* populations for controlling a secondarily important avocado spider mite pest, *Oligonychus punicae* (Hirst), was demonstrated under greenhouse conditions (McMurtry and Scriven 1966), but the implementation of this strategy in commercial California avocado orchards would be a technical challenge and currently remains an undeveloped alternative for controlling *O. perseae*. However, the use pollen-provisioning for improving phytoseiid-based biological control has been evaluated in another economically important perennial crop system in California. Grafton-Cardwell and Ouyang (1995) demonstrated that provisioning resident and released populations (under the framework of augmentative biological control) of the generalist phytoseiid *E. tularensis* Congdon with a suitable pollen diet (apples) was not effective for improving biological control of *Scirtothrips citri* (Moulton) (Thysanoptera: Thripidae) in citrus orchards located in the San Joaquin Valley. Nevertheless natural populations of *E. tularensis* can be found in commercial citrus orchards and this phytoseiid has a beneficial role as a generalist predator of citrus pests, including *S. citri* and spider mites. Similarly, natural populations of *E. hibisci* can subsist in California avocado orchards throughout the year (Yee et al. 2001; Lara unpublished data) and this predator can feed on *O. perseae* that are encountered outside of nests (McMurtry 1993; Lara, unpublished data). For these reasons *E. hibisci* and its impact on *O. perseae* requires further investigation in California.

To better understand the ecology of natural phytoseiid populations in California avocado orchards, the objectives of this work were to investigate their spatial dynamics and explore the possibility of developing sampling guidelines for these predators. Previously, a field method for estimating densities of phytoseiids in California avocado orchards was developed (Machlitt 1998) but an optimal sample size and leaf selection guidelines were not validated. Interest in sampling predators in avocado orchards is more than academic; avocado pest control specialists have repeatedly expressed high interest in being able to do this. An easy-to-use and statistically reliable sampling plan would be convenient for studying the population dynamics of phytoseiids as part of field-based research projects and for orchard managers controlling pest outbreaks; both require robust techniques for accurately and quickly estimating densities of phytoseiids and *O. perseae* in orchards.

There are various types of sampling strategies for arthropods, including mites, which can be customized for agricultural systems (Pedigo and Buntin 1994, Binns et al. 2000). In this study, three general sampling strategies for phytoseiids were considered. Fixed-size binomial sampling for estimating phytoseiid densities involves collecting a fixed number of avocado leaves and using the observed proportion of leaves containing at least one phytoseiid to estimate mean densities; no counting of predators is required and for this reason, binomial sampling is an attractive sampling strategy. In contrast, sequential fixed-precision sampling involves counting mites on a sequential collection of leaves until a fixed level of precision is achieved. Fixed-precision in these types of sampling plans refers to setting a preferred constant ratio between the standard error and mean density and this requirement can be visualized as a sampling stopline. Similar fixed-precision and fixed-size binomial sampling strategies were previously developed for the phytoseiid *E. tularensis* Congdon on citrus in California (Grout 1985). The third sampling strategy considered was fixed-size enumerative sampling which requires counting mites on a pre-determined number of leaves; precision with this type of sampling strategy is not controlled but it provides practical consistency and was included for comparison with binomial and fixed-precision sampling as a direct measure of accuracy. Accuracy was evaluated by how close predicted mean estimates from leaf subsamples across these three sampling strategies were to mean phytoseiid densities determined from complete counts of the full set of leaves evaluated.

For any sampling strategy, consideration of spatial autocorrelation is important for establishing research-based sampling guidelines that would safeguard against obtaining biased density estimates such as sampling only within hotspots (i.e., a set of neighboring trees that are infested because of their close proximity to one another) of elevated spider mite activity which may not be representative of mite activity over the whole spatial area of interest (i.e., a block of trees) in which that hotspot is located. Spatial correlation for organisms can be detected using geostatiscal methods (e.g., semivariograms) and these have been used to describe the spatial distribution of insect populations (see Schotzko and O'Keeffe 1982, Rossi et al. 1992, Gilbert and Grégorie 2003, Park and Obrycki 2004, Reisig 2011, Rhodes et al. 2011).

Consequently, this article documents the evaluation of three specific sampling components for phytoseiids using field collected data for the California avocado system: (1) a comparison of binomial and enumeration-based sampling plans for estimating phytoseiid densities, (2) evaluating the degree of association of natural phytoseiid populations with pest infested leaves across a range of *O. perseae* densities, and (3) quantifying the level of spatial autocorrelation within resident phytoseiid populations which should be accounted for in a sampling protocol.

Materials and Methods

Site Selection and Data Collection. Nine commercial Hass avocado orchards were selected in 2009-2010 based on the criteria of being infested with *O. perseae* and containing natural populations of phytoseiids (Table 4.1). With the exception of orchard 1, a section within each orchard with approximately thirty trees planted on approximately a 5x6 grid was sampled. At orchard 1, sampled trees were arranged irregularly. For each tree, two mature leaves were randomly collected from each cardinal point (i.e., eight leaves per tree). Sites 3A and 3B were adjacent sections of the same orchard and four leaves were sampled from each tree. Similarly, sites 9A and 9B were different sections of the same orchard but a full set of eight leaves was sampled from each tree. Data collected from these orchards were compiled to form databases A and B and were used in spatial and sampling plan analyses.

A tenth orchard (a research plot with >200 avocado trees planted on an 18x14 grid) located in Irvine, California was sampled bimonthly from 2002-2005 and monthly from 2006-2012 with ten leaves randomly sampled from each of nine Hass trees. Data collected from this tenth orchard were primarily collected for time series analyses of avocado mite populations but were also used in this study to validate sampling strategies for phytoseiid populations. The final database compiled from this tenth orchard, henceforth referred to as database C, was prepared by including only leaf sample batches with mean densities > 0 and this generated 100 individual datasets (a total of 8, 889 leaves of various ages) used for validation purposes.

For all orchards, avocado leaves were returned to the laboratory and the total number of *O. perseae* and phytoseiids of all stages except eggs were counted under stereomicroscopes with user-adjusted magnification. This allowed mites that were inside or outside the semi-transparent webbed-nests constructed by *O. perseae* to be readily detected and identified. For orchards 1-9 leaf length was measured as the distance along the midrib from the leaf tip to the petiole end. Individual phytoseiids were not identified to species level but previous studies have demonstrated that *Euseius hibisci* is a dominant species in California avocado orchards (McMurtry and Scriven 1966, Yee et al. 2001). The phytoseiids detected on avocado leaves have a very distinct morphology (e.g., a pear-shaped idiosoma) and move relatively quickly in comparison to other mites (e.g., stigmaeids). These two characteristics were consistent with the behavioral identification of native phytoseiid species (*Euseius* spp.) on avocado; colonies of these predators have been maintained in the laboratory for behavioral studies and were used to train assistants (Lara, unpublished data).

Fixed-size Binomial and Enumerative Sampling. For orchards 1-9, the first set of replicates sampled per cardinal direction from each tree were each assigned to database A. The second set of replicates were assigned to database B. Database A was used as a training dataset to model the relationship between the mean number of phytoseiids per leaf, m, and proportion of leaves containing at least one phytoseiid, p, using the Kono-Sugino equation (Jones 1994):

$$\ln(m) = c' + d' \cdot \ln(-\ln(p)) \quad (1)$$

where m and p are paired data point values observed at each site, as previously described, and c and d are parameters estimated from simple linear regression using PROC REG in SAS 9.3 (SAS Institute 2011). Database A consists of ten datasets and for this reason, equation 1 was re-fit to the 100 datasets from database C for a comparison of model parameters.

Database B was used to validate the fundamental mean-proportion relationship using sampling simulations with PROC SURVEYSELECT in SAS 9.3 (SAS Institute 2011). For each dataset, 500 sampling simulations consisting of a simple random selection of 30 leaves were conducted. Thirty leaves is the minimum practical sample size that has been previously validated for *O. perseae* (DePalma et al. 2012, Lara and Hoddle 2013). Data from orchard 1 were not included in the validation phase because enumerative counts of phytoseiids were not conducted for some leaves and this would have prevented a balanced validation design. For each simulation, densities were first predicted using the binomial equation (1) and enumerative counts and then compared with observed phytoseiids densities at each site using the general percent error (PE) formula as a measure of accuracy:

$$\mathrm{PE} = [(\text{predicted - observed})/\text{observed}] \cdot 100 \quad (2)$$

Lower PE values indicate higher accuracy. For predictions based on enumerative counts, a measure of precision (PR) was calculated as the standard error for each simulation divided by the sample mean (Pedigo and Buntin 1994). Similarly, lower PR values indicated higher precision.

Enumerative counting is time-consuming and for this reason, sampling simulations were repeated for reduced fixed-sample sizes consisting of 20 and 10 leaves using pooled data from both databases B and C. Similarly, PE and PR were calculated for these samples sizes at each orchard. However, an inherent problem with these additional simulations was that precision was not directly controlled (this sampling structure only controls the sample size). Consequently, the merits of developing a sequential fixed-precision enumerative sampling plan were evaluated.

Fixed-Precision Sequential Sampling. Fixed-precision sampling entails the sequential collection of leaves and counting the number of phytoseiids on each leaf until a stopline is reached. The stopline is defined by a pre-determined level of precision and was calculated based on the formula described by Green (1970):

$$\log(T_n) = \frac{\log(\mathrm{D}^2/a)}{b-2} + \frac{b-1}{b-2} \cdot \log(n) \quad (3)$$

where T_n is the cumulative number of phytoseiids that have been counted on n leaves, D is the fixed level of desired precision, and a and b are estimated parameters from Taylor's power to describe the mean-variance relationship (Taylor 1961). Setting precision at D=0.25 and D=0.10 has been deemed acceptable for sampling in agricultural systems and studying population dynamics, respectively (Southwood 1978). In this study, stoplines for Green's fixed-precision sampling were generated for D=0.10, 0.25 and 0.30 using database A and the expected number of samples required for target densities (1, 2, and 3 phytoseiids per leaf) were compared.

Validation of sampling plans for each precision level were performed with databases B and C using the RVSP simulation program developed by Naranjo and

Hutchinson (1997). Validation with D=0.10 was only possible with 46 datasets which had densities greater than or equal to 0.1 (e.g., 10 predators per 100 leaves). Validation of all sequential sampling plans was conducted with a starting sample size of 10 leaves and leaves were selected with replacement. Final evaluation of performance was based on expected (D) and observed levels of precision (PR).

Spatial Analyses. The complete set of count data from six sites (2, 3A, 5, 6, 7, and 9A) with relatively higher densities of phytoseiids (Table 4.1) were selected for spatial analyses. Initial effort was placed in working with potential distributions for count data (i.e., negative binomial, Poisson) but this generated model fitting problems when attempting to run diagnostics on spatial correlation. Consequently, counts of phytoseiids from selected orchards were transformed based on natural log transformation or optimal Box-Cox transformation lambda values obtained with PROC TRANSREG in SAS 9.3 (SAS Institute 2011) in order to meet normality assumptions and to facilitate analyses with less complex modeling frameworks across sites.

For each site, detection of statistically significant spatial autocorrelation in transformed counts of phytoseiids was evaluated in three different ways. These evaluations involved (1) comparing pairs of spatial and non-spatial linear mixed models (LMMs) specified with PROC GLIMMIX in SAS 9.3. (SAS Institute 2011) using likelihood ratio tests (LRTs) (West et al. 2007), (2) calculating Moran's *I* (Moran 1950) using residuals from each non-spatial model, and (3) using semivariograms (Littell et al. 2006) to detect spatial dependence in the residuals of non-spatial LMMs.

In this study, LRTs were used to test the null hypothesis that the spatial LMMs did not explain more of the observed variation in transformed phytoseiid counts than non-spatial LMMs; a complete description of LMM components is provided below. The LRT statistic was calculated as the difference in -2log likelihood values between non-spatial and spatial LMMs. P-values were based on one degree of freedom (spatial LMMs have an additional covariance parameter α) and the assumption that the LRT statistic follows a χ^2 distribution (West et al. 2007).

Moran's *I* is a standard geostatistic used to measure and test for spatial correlation (Getis 2010); values range from -1 to 1 (negative to positive correlation) and values near zero are indicative of random spatial processes (the null hypothesis). To maintain flexibility and simplicity, Moran's *I* for each site was calculated under the randomization assumption with binary weights. The randomization assumption implied that the observed data points (n) from each site, in this case model residual values, were one of $n!$ possible spatial arrangements for cell values on a grid. With binary weights, neighboring pairs of residuals on a grid were scored 1 while other pairs separated by a distance greater than one tree away were scored 0. It is important to note that Cliff and Ord (1972) proposed a modified equation for deriving Moran's *I* among regression residuals but this option is not available within SAS 9.3 software. Despite this limitation, a robust interpretation on the degree and statistical significance of spatial correlation from each site was conducted using a third diagnostic tool, the semivariogram.

The semivariogram is a plot used in geostatistical analyses to depict the relationship of half the variance value between paired observations as a function of distance (h) on spatial surface. The shape of the semivariogram is characterized by the nugget (c_n), partial sill (σ_0^2), and range (a_0). In practical terms, the nugget represents potential measurement error and microscale variation (i.e., variation at smaller distances not measured). The partial sill is the upper bound of the semivariance values minus the nugget, and the range is the distance after which transformed counts of phytoseiids would no longer be considered spatially correlated (Rossi et al. 1992, SAS Institute 2011).

For all spatial and non-spatial LMMs, leaf length, cardinal direction, and scored presence of *O. perseae* on sampled leaves (i.e., 1 if present, 0 if absent) were included as fixed effects and the corresponding parameters were estimated using restricted maximum likelihood (West et al. 2007). Fixed effects were removed from the final model if they were not statistically significant as indicated by Type III tests with *alpha*=0.05 and Kenward-Roger adjusted degrees of freedom (Kenward and Roger 1997, Gbur et al. 2012). When the effect of cardinal direction was significant, pairwise comparisons of least square means were conducted to detect any patterns across sites. For orchards 7 and 9A, leaf length was log transformed to improve model fit based on lower values for Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). With the exception of orchard 3A, enumerative counts of *O. perseae* were not used because exploratory data analyses revealed there was no consistent linear relationship between this variable and the response variable across sites.

The distinction between spatial and non-spatial LMMs was reflected in the manner in which random effects were specified for these model types. In this study, experimental avocado trees were a representative group sampled from a larger population of avocado trees available within each orchard and, by extension, the entire California avocado system as a whole. Therefore, in non-spatial LMMs the tree effect was included as a G-side random effect with a variance component (VC) covariance structure, which assumes no spatial correlation for observations between trees. For spatial LMMs, the effect of trees was included as a G-side random effect with either a spherical, exponential, or Gaussian spatial covariance structure. Relative orientation of individual trees for spatial LMMs was specified in terms of tree location on a grid framework (i.e., rows and columns), but records of the average physical distance between trees were also collected at each site (Table 4.1).

Fundamentally, the spatial covariance structures listed above assume correlation between observations diminishes with distance. The practical range or tree distance, r, at which spatial correlation falls below 0.05 was determined using the estimated spatial covariance parameter α listed by PROC GLIMMIX in SAS 9.3 (SAS Institute 2011). For the spherical and exponential model, r was calculated as 3α and for the Gaussian model as $\sqrt{3}\alpha$ (SAS Institute 2011). Final selection of the spatial covariance structure for spatial LMMs at each site was based on successful algorithm convergence, lower AIC/BIC values, and a comparative fitting of theoretical spatial models to residuals from non-spatial LMMs using semivariograms.

Semivariograms were generated by calculating the empirical semivariance values using the 'robust' option with PROC VARIOGRAM in SAS 9.3 (SAS Institute 2011). The 'robust' option was used to diminish the influence of any potential outliers (Cressie and Hawkins 1980). Estimates of c_n, σ_0^2, and a_0 were derived by fitting theoretical

Gaussian, spherical, and exponential spatial models to empirical semivariance values. In preliminary analyses, these theoretical spatial models were fit directly to untransformed count data, but the structure of those models, unlike LMMs, did not account for the influence from fixed effects and are not discussed further. Thus, the final combination of analytical methods (LMMs, Moran's *I*, and semivariograms) was employed to reach a reliable and comprehensive conclusion on the degree of spatial autocorrelation among phytoseiid populations within selected orchards in the context of other explanatory variables.

Results

Fixed-Size Binomial and Enumerative Sampling. A curvilinear relationship between observed mean-proportion values from database A was detected and this was accounted for with the fitted Kuno-Sugino model (Fig 4.1.). The estimate for model parameter c' was 0.54 and its 95% confidence limits were 0.34 and 0.74. The estimate for model parameter d' was 1.01 and its 95% confidence limits were 0.83 and 1.20. For database C, c' and d' were estimated as 0.35 and 1.07, respectively, and these values were within the 95% confidence limits determined from database A. Fig. 4.2 shows a plot of observed data points from database C against predictions from the empirical model based on database B. Observed phytoseiid densities for database C ranged between 0.01 to 0.9 (i.e., 1 to 90 per 100 leaves).

The validation results based on database B with a fixed sample size of 30 leaves are listed in Table 4.2. PE values based on binomial sampling were higher for sites 4, 6, and 8, and at these sites, observed phytoseiid densities were relatively lower (Table 4.1). In contrast, PE values based on enumerative counting with a fixed sample size of 30 leaves (Table 4.2) were consistently lower than binomial sampling and this reflects the higher accuracy of counting all phytoseiids on a leaf. However, precision for enumerative-based estimates was not controlled and both PE and PR values increased (i.e., lower accuracy and precision) for smaller sample sizes based on enumerative sampling (Table 4.3). A similar pattern related to the range of phytoseiid densities was detected with a fixed enumerative sample size of 20 leaves using database C (Fig. 4.3). For phytoseiid densities equal to or greater than 0.10 (e.g., 1 phytoseiid per 10 leaves) PE values ranged from -3.5% to 24% while for densities less than 0.10 (e.g., 1 phytoseiid per 20 leaves) PE values ranged from 26% to 350% (Fig. 4.3A). Precision values based on a fixed sample size of 20 leaves ranged from 0.30 to 1 and this indicates that precision was not directly controlled (Fig. 4.3B).

Fixed-Precision Sequential Sampling. Three stoplines based on Green's sequential sampling plan were generated, one for each fixed level of desired precision (D = 0.10, 0.25, and 0.30) (Fig. 4.4). In general, for a precision of D=0.25 and 0.30, a sequential sample size of approximately 10 leaves would be necessary to detect densities greater than 1 predator per leaf (Fig. 4.4). However, the observed average precision of sequential simulations based on D = 0.30 was 0.25 (Fig 4.5) and an average sequential sample size of 27 and 400 leaves was necessary for detecting densities greater or equal to

1 predator per leaf and densities less than 1 predator per leaf, respectively (Fig. 4.6). The average PE value was 6% and ranged between 2% and 17% (results not shown).

For D = 0.25, the observed average precision across all simulations was 0.20 and an average sequential sample size of 54 and 1,032 leaves was necessary to detect densities greater than or equal to 1 predator per leaf and densities less than 1 predator per leaf (results not shown). The sampling plan based on D = 0.30 provided a better approximation for the desired precision of 0.25 that is recommended for pest management applications (see Discussion below, Southwood 1978). The average PE value was 4% and ranged between 1% and 10% (results not shown).

For D=0.10, a sequential sample size of approximately 20 leaves would be necessary for detecting densities greater than 1 predator per leaf (Fig. 4.4). However, the observed average precision of sequential simulations based on D=0.10 was 0.08 (Fig. 4.7) and an average sequential sample size of 55 and 634 leaves was necessary for detecting densities greater or equal to 1 predator per leaf and densities less than 1 predator per leaf, respectively (Fig. 4.8). The average PE value was 0.6% and ranged between -0.02% and 2% (results not shown).

Spatial Analyses. The model fit statistics for spatial and non-spatial LMMs are provided in Table 4.4. The objective of testing the significance of fixed effects (i.e., leaf length, cardinal direction, and *O. perseae* levels) was not to use their parameter estimates to predict phytoseiid densities in the field but rather to account for the influence of these effects when detecting spatial correlation and to obtain further insight into the ecology of natural phytoseiid populations (e.g., the possible role of varying leaf area as would occur when counts are made on smaller or large leaves). None of the evaluated fixed effects were statistically significant (alpha=0.05) across all sampled orchards (Table 4.4). For orchard 2, pairwise comparisons (results not shown) revealed that counts of phytoseiids were significantly lower on north-facing leaves while at orchard 9A, counts of phytoseiids were significantly higher on west-facing leaves (results not shown). Because there were no consistent trends for cardinal direction, these results suggested that sampling within a tree can accommodate the random selection of leaves. Similarly, the positive influence of leaf length was only statistically significant (results not shown) for orchards 2, 7, and 9A and this indicated that, in some cases, larger leaves may contain a higher number of predatory mites. Because there were no consistent results based on leaf size, sampling efforts within orchards can accommodate a random selection of leaves of various sizes. For all evaluated orchards, except orchards 7 and 9A, phytoseiid levels were significantly higher on leaves infested with *O. perseae* than on uninfested leaves. This suggests that the presence of potential prey can influence the presence of naturally occurring phytoseiids.

The p-values of LRTs based on a comparison of spatial and non-spatial LMMs are listed in Table 4.4. Spatial LMMs based on a Gaussian spatial covariance structure significantly explained more of the variation in transformed phytoseiids counts for orchards 2 and 3A. Consequently, for orchards 2 and 3A, the practical range, r, of avocado trees that should be left in between sampled trees is 5 (i.e., 33.5 m) and 2 (i.e., 12 m), respectively (Table 4.1, 4.4). Based on LRTs, the evidence for spatial correlation at orchards 5, 7, 8, and 9A were not statistically significant and this outcome was also detected qualitatively in the similar AIC and BIC values between spatial and non-spatial LMMs for these sites (Table 4.4).

Additional spatial diagnostic tools used to analyze model residuals from non-spatial LMMs provided further insight into phytoseiid distributions. Formal testing of Moran's *I* coefficient revealed that spatial correlation was not statistically significant at any site (Table 4.5). These results conflicted with LRTs and thus, semivariograms were used to graphically describe the relationship between observations as a function of distance (Fig 4.4.) There were no strong trends detected in the semivariance values across orchards and this implied that there was no spatial correlation. However, for orchard 2, all estimated parameters (c_n, σ_0^2, and a_0) from a fitted Gaussian semivariogram model were significantly different than zero (results not shown) and the practical range, a_0, at which spatial correlation diminished between observations was estimated as a class distance of 4 trees (i.e., 27 m, Table 4.1, 4.5). For site 3, only the estimated nugget parameter, c_n, was statistically significant but a_0 was estimated as 1 tree (i.e., 6 m, Table 4.1, 4.5). Consequently, the majority of the spatial diagnostic methods suggest that spatial correlation for natural phytoseiid populations is only significant at orchard 2 and the practical sampling range is approximately a separation rule of 4-5 trees between sample collections.

Discussion

Fixed-Size Binomial Sampling. A binomial sampling strategy would be convenient for estimating densities of relatively small organism such as phytoseiids on avocado leaves because no counting is required and this approach is compatible with the binomial sampling structure already developed and validated for *O. perseae* based on a fixed sample size of 30 leaves (Lara and Hoddle 2013). Graphically, the empirical mean proportion relationship for phytoseiids looked promising across all orchards sampled during the summer (database A, Fig. 4.1) and the 10th orchard that was sampled throughout the year (database C, Fig. 4.2). The inclusion of database C was important because it was comprised of leaves of various ages whose trajectory was followed from spring flush (avocado trees have a spring and summer flush) and these are the types of leaves that would be available for initial sampling of *O. perseae* and phytoseiids. Therefore, the non-significant difference between parameters independently estimated from database A and C from equation 1 implied that the mean-proportion relationship was robust and could be used throughout the year to sample avocado leaves of various ages for phytoseiids. In a separate study using the same databases, a similar conclusion was reached for *O. perseae* (Lara, unpublished data).

However, practical convenience derived from reduced sampling efforts comes with a tradeoff in accuracy. A binomial sampling strategy is not recommended for estimating densities of natural phytoseiid populations in California avocado orchards because of high levels of over- and underestimation (validation database B, Table 4.2). Additional simulations were conducted by increasing the sample size (n=40, 60) but no substantial improvement in the accuracy of binomial sampling was observed (results not shown). Therefore, a binomial sampling strategy would be more appropriate for simply monitoring the presence of phytoseiids in avocado orchards without making an inference on density.

For *O. perseae* sampling, the tradeoff in accuracy was mitigated by developing a binomial sampling strategy that classified spider mite densities relative to a working economic threshold of 50 *O. perseae* per leaf (Maoz et al. 2011b) but the threshold concept for initiating control measures is only relevant for agricultural pests (Stern et al. 1959). It is possible to develop binomial sampling plans to classify ratios of pest to predator, including mite systems (Nyrop 1988, Nyrop and van der Werf 1994, Park et al. 2000), but the "critical ratio" below which effective natural biological control *O. perseae* populations would be expected to occur is not known for resident populations of phytoseiids in California and would require additional field research (see spatial analyses discussion section below).

Fixed-Size and Fixed-Precision Enumerative Sampling. Simulation results indicated that sequential fixed-precision sampling with D=0.30 performed better than both fixed-size enumerative sampling and fixed-size binomial sampling. The average sample size necessary to end sequential sampling for phytoseiids was 27 leaves (Fig. 4.6) for densities equal to or greater than 1 and across all densities, the average level of observed accuracy (PE) and precision (PR) was 6% and 0.25 (Fig. 4.5), respectively. This level of precision has been recommended for pest management programs (Southwood 1978). An average of 400 sequential leaf samples was required to maintain precision at

0.25 when observed mean predator densities were <1, but retaining this constant sensitivity at this lower density range is not practical for the purposes of pest management because this type of scenario would indicate that predator populations are not sufficient to control *O. perseae*.

Despite its statistical reliability, sequential enumeration of phytoseiids is not practical for use in avocado orchards because it requires the total number of phytoseiids to be counted on leaves and there is no upper limit on the leaves that need to be sampled. Alternatively, a fixed-size enumerative sampling strategy with a sample size of 20 leaves represents a compromise between optimal statistical reliability and convenience: this strategy does not control for precision but provides consistency and a good level of accuracy across leaves of various ages (database B and C). For densities greater than or equal to 0.10 (e.g., 1 phytoseiid found on 10 leaves) the average PE value was 6% and ranged between -3% and 25% while the average PR value was 0.56 and ranged between 0.08 and 0.84. (Table 4.3, Fig. 4.3) This alternative enumeration strategy still requires counting the total number of phytoseiids and would be difficult to combine with binomial sampling for *O. perseae* under field conditions. For the purposes of pest management, it would be best to employ enumeration of phytoseiids within specific areas of the orchard where damage on leaves (Aponte and McMurtry 1997) indicates *O. perseae* populations are elevated.

For research purposes aimed at evaluating the population dynamics of resident phytoseiids populations, maintaining an ideal fixed precision of D = 0.10 (average PR =0.08, Fig. 4.7) required an average of 333 leaves for detecting densities equal to or greater than 1 predator per leaf (Fig. 4.8). This is likely impractical for most research projects with limited resources available to cover the costs of labor. In contrast, maintaining an ideal fixed precision of D = 0.25 (average PR = 0.20, results not shown) required an average of 55 leaves and this is a realistic minimum sample size that can be accommodated in repeated measures studies. For example, during a two-year phenology study on avocado pests and beneficial predators, including phytoseiid populations, Yee et al. (2001) used a monthly random selection of 100 leaves within commercial avocado orchards. Similarly, database C is composed of periodic sampling (i.e., bimonthly and monthly) of 90 leaves during the period 2002-2012. A recommended sample size of 55 – 100 avocado leaves per sampling interval appears feasible based on previous research efforts and can provide constant precision that is compatible with pest management decision-making.

Spatial Analyses. The results from independent spatial analyses conducted for six orchards, determined that spatial dependence for phytoseiid populations was significant only at site 2 and diminished once pairs of selected trees were separated by a distance (range) of four to five trees (Tables 4.4, 4.5). Furthermore, counts of phytoseiids at this site were significantly lower on north-facing leaves relative to other cardinal directions. Interestingly, the spatial distribution of *O. perseae* at site 2 was previously analyzed and the range of spatial correlation was 3 trees (DePalma, unpublished data) and mean densities were lower on north-facing leaves relative to other cardinal directions (Li et al. 2012, Lara unpublished data). These parallel results, at one site, suggest that a biologically significant interaction between natural phytoseiid populations and *O. perseae* is at least possible in some orchards but does not appear to be consistent across multiple orchards. However, the underlying mechanisms enabling such high site fidelity are unknown. Furthermore, although significant spatial correlation for phytoseiids was only detected in orchard 2, a significantly higher number of phytoseiids were found on mite-infested leaves in four out of six sites (Table 4.4). This result is also biologically significant because it suggests that natural phytoseiid populations in California avocado orchards can potentially use *O. perseae* as a food source and that the distribution of these predators on leaves is not entirely a random process and could be influenced by the presence of potential prey.

The extent of the long-term impact that natural phytoseiid populations can have on *O. perseae* is currently unknown for the California avocado system. The natural enemy to spider mite ratio and overall mite densities (Table 4.1) suggested that, with the exception of orchard 9, populations of these predators did not provide sufficient natural control to suppress *O. perseae* densities at the time of evaluation. However, the spatial analyses from this study indicated that there was a nuanced biological relationship that would otherwise have gone undetected simply by looking at the densities listed in Table 4.1. Evidence for this relationship provides justification for considering the presence of natural phytoseiid populations in avocado orchards and possible benefits that could arise from conservation biological control efforts to enhance their efficacy. Time series analyses have been conducted for the data collected from orchard 10 using an extended version of database C (Lara unpublished) and will be used to study the temporal correlation between natural phytoseiid populations (*Euseius* sp.) and *O. perseae* over the approximate ten year sampling period.

These analyses using field-collected data may be suitable for determining the critical ratio between phytoseiids and *O. perseae* that is required to develop a binomial classification sampling plan that monitors biological control of *O. perseae* by resident phytoseiids. Investment in these types of long-term field studies is not always possible and an alternate mathematical approach for approximating an adequate pest: predator ratio for mite systems based on estimated predation rates, β, for the target pest, and the intrinsic rates of increase, r_m, of the candidate predator (these values can be obtained through simple lab experiments) was discussed by Jansenn and Sabelis (1992). Based on this theoretical work, Jansenn and Sabelis (1992) suggested that initial prey: predator ratios of 1:6 – 1:20 may be suitable for biological control of spider mites with phytoseiids. However, these ratio estimates apply only to local mite population dynamics (not large spatial scales such as an entire orchard) under certain conditions (see Jansenn and Sabelis 1992) and for this reason recommended pest: predator ratios at larger spatial scales should be determined empirically for each system. Obtaining a refined insight on the critical pest: predator ratio needed for effective *O. perseae* biological control with candidate natural enemies under field conditions from previous short-term studies has been limited.

Conservation biological control work from Spain reported an average of approximately 1-5 and 1-3 *E. stipulatus* predators per leaf on corn pollen treated and no-pollen treated avocado trees (control), respectively, during the period of June-August

2007 (González-Fernández et al. 2009). Necrotic leaf damage on trees supplemented with pollen was significantly lower than on control trees, thus providing evidence of the beneficial role of *Euseius* spp. for control of *O. perseae*. However, prey: predator ratios cannot be determined because *O. perseae* densities were not measured directly. In contrast, the results from pollen provisioning for field experiments in Israel using *E. scutalis* during 2007 (electrostatic corn pollen application) and 2008-2009 (pollen from rhode grass cover crop) did not provide evidence of a statistically significant difference in *O. perseae* densities between pollen treated and no-pollen treated avocado trees (Maoz et al. 2011a). Nevertheless, overall *O. perseae* densities were generally lower on pollen-treated trees. *E. scutalis* populations were generally higher on pollen-treated trees but predators were sampled using beat sampling and the units were reported as predators/branch rather than predators/leaf. Because the number of leaves per branch was not reported, it is difficult to convert these data into comparable densities needed to determine the prey: predator ratios observed on individual leaves.

Partial indication of suitable prey: predator ratios was derived from laboratory experimentation examining the effect of providing *E. scutalis* with pollen on avocado seedlings infested with *O. perseae* during a six week trial (Maoz et al. 2011a). After a two-week establishment period following inoculations of plants with phytoseiids and spider mites, observed prey: predator ratios on sampled avocado leaves from pollen-treated seedlings were approximately 1:1 and this led to a significant decrease in *O. perseae* densities (<1 per leaf) over the remaining four weeks compared to non-pollen treated seedlings (~10 *O. perseae* per leaf). The maximum pest: predator ratio observed on pollen treated seedlings was approximately 1:3 while predator populations on control seedlings crashed, thus allowing *O. perseae* populations to become established on these plants. These results may not reflect actual ratios needed under field conditions on mature avocado trees but they do provide supporting evidence that these types of ratios may be obtainable in California by providing pollen as a resource subsidy to boost densities of natural *E. hibisci* populations and consequently increase predation intensity of *O. perseae*.

For example, a 46-week greenhouse study conducted by McMurtry and Scriven 1966 with *E. hibisci* and *O. punicae* on *Persea indica* (in the same genus as avocado trees, *P. americana*) suggested that an initial pest: predator ratio of 8:1 in combination with weekly provisioning of *Mesembryanthemum* sp. pollen facilitated continuous suppression of *O. punicae* at densities below ~5 spider mites per leaf after an initial six weeks compared to densities of *O. punicae* on control trees with no predators and trees with *E. hibisci* but no pollen. *O. punicae* densities in these latter treatments with no pollen provisioning showed similar higher oscillations and peaked at 200 *O. punicae* per leaf. These results gave strong indication that pollen was crucial for maintaining *O. punicae* suppression and that *E. hibisci* alone without pollen could not perform as an effective biological control agent. These same studies however, have not been replicated with *O. perseae* and *E. hibisci* on avocado in either laboratory or field experiments. Consequently, the sampling plans presented here could be used in conservation biological control research to document phytoseiid densities and determine what the effective resident predator threshold for *O. perseae* control is and whether this threshold is similar to that seen for *Euseius* spp. in other countries.

Recommended Sampling Plan for Phytoseiids in California Avocado Orchards. The framework for a research-based enumerative sampling plan for natural phytoseiids populations was built using conclusions from sampling validations and spatial analyses in this study. This general sampling plan can be used by pest managers to reliably quantify levels of predatory mites in commercial avocado orchards. When there is a general interest in evaluating natural phytoseiid densities over mite-infested areas similar to the 5x6 blocks used in this study, a minimum of 20 leaves of various ages can be randomly collected throughout the block and the inference made on phytoseiid densities will only apply to this spatial scale. If the same area is selected for classification of *O. perseae* densities using the procedures outlined by Lara and Hoddle (2013) with a fixed sample size 30 leaves, the 20 leaves for phytoseiid monitoring can be chosen from this initial batch of leaves. Binomial sampling of *O. perseae* be can performed in the field with a hand lens but counting phytoseiid mites in the field may not be accurate so, when possible, leaves should be stored in a cooler and processed later using a microscope. During the summer, *O. perseae* will more likely be found on mature leaves that populations have been developing on since the spring season (Lara, personal observation) and given the significant interaction of phytoseiids with *O. perseae* infested leaves (Table 4.4) and favorable sampling validation results , these leaves can be targeted for systematic random sampling.

For larger spatial areas, avocado trees can be selected so that there is minimum separation distance of 4 trees between previously sampled trees (Table 4.5). This tree separation guideline accounts for potential autocorrelation so that a representative assessment of densities over larger spatial areas can be made. This same recommendation has been determined for *O. peseae* (see Depalma et al. 2012, Li et al. 2012). Furthermore, for sampling *O. perseae* over large spatial scales, trees are selected so as to maximize their separation distance (DePalma et al. 2012) and it is recommended that four leaves per tree for each of eight trees are collected and examined for pest presence. This sampling guideline for has been validated using field collected data over 200x200 blocks (Lara, unpublished data). This tree sampling pattern for these larger areas has not been validated for phytoseiids but given the primary interest in sampling for *O. perseae*, this sampling structure can be adopted for these predators as well and is expected to perform well because it optimizes the separation distance between trees.

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Table 4.1. Summary statistics for avocado leaves sampled from commercial orchards

Orchard	County[a]	Sample Date	Leaves sampled	Avg. tree distance (m)[b]	Spider mite density	Phytoseiid density	Phytoseiid: spider mite ratio
1	SD	July 2009	167	13.7	57	1	1:61
2	SB	Aug. 2009	240	6.7	42	3	1:16
3A	SB	Aug. 2009	240	6.0	342	1	1:292
3B	SB	Aug. 2009	240	6.0	307	2	1:155
4	SB	Aug. 2009	240	6.1	37	<1	1:104
5	SB	Sept. 2009	240	5.9	49	1	1:40
6	VC	June 2010	240	8.2	204	<1	1:562
7	VC	July 2010	240	6.8	208	1	1:247
8	OC	July 2010	256	8.5	519	<1	1:3689
9A	SB	Aug. 2010	120	6.1	17	1	1:15
9B	SB	Aug. 2010	120	6.1	19	1	1:15

[a] SD= San Diego County, SB= Santa Barbara County,VC= Ventura County, OC= Orange County.

[b] Distance was based on measurements taken at each site between pairs of neighboring trees.

Table 4.2. Precision (PR) and percent error (PE) results from simulations for density estimation based on binomial (BN) and enumerative (EN) sampling with a fixed sample size of 30 avocado leaves using database B

Orchard	No. of simulations	Sample size	BN mean	BN PE	EN mean	EN PR	EN PE
2	500	30	1	-81	3	0.19	0.5
3A	500	30	1	23	1	0.27	0.1
3B	500	30	1	-56	2	0.21	1
4	500	30	3	889	0	0.50	-1
5	500	30	1	29	1	0.29	-1
6	500	30	3	689	0	0.58	-2
7	500	30	2	117	1	0.31	0.3
8	485[a]	30	4	3483	0	0.63	8
9B	500	30	1	-41	1	0.20	0.2

[a] Simulation replicates with predicted densities equal to 0 from binomial sampling were not used for generating final PR and PE values to avoid calculation errors.

Table 4.3. Percent error (PE) and precision (PR) results for fixed-size enumerative sampling simulations based on 20 and 10 avocado leaves using database B

	20 leaves			10 leaves		
Orchard	Simulations[a]	PR	PE	Simulations[a]	PR	PE
2	500	0.23	1	500	0.31	-0.1
3A	500	0.33	-1	500	0.48	-2
3B	500	0.25	1	500	0.37	-0.3
4	491	0.60	2	441	0.77	17
5	500	0.34	1	500	0.47	1
6	487	0.66	-0.3	418	0.81	16
7	500	0.37	2	497	0.52	3
8	446	0.75	15	320	0.87	54
9B	500	0.24	0.1	500	0.35	1

[a] For each site, simulation replicates with predicted densities equal to 0 were not used for generating final PR and PE values to avoid calculation errors.

Table 4.4. Model fit statistics for spatial and non-spatial linear mixed models

Orchard	Lambda[a]	Fixed effects[b]	Spatial covariance	AIC	BIC	*r*	LRT p-value
2	0	length, cardinal, pm_pres	none	499.18	501.98	-	-
	0	length, cardinal, pm_pres	Gaussian	493.53	497.73	4.94	0.006
3	-0.75	pm_counts	none	162.49	165.3	-	-
	-0.75	pm_counts	Gaussian	155.83	160.04	2.28	0.003
5	-1.25	pm_pres	none	61.16	63.96	-	-
	-1.25	pm_pres	Gaussian	61.92	66.13	1.41	0.27
6	0	pm_pres	none	233.65	236.45	-	-
	0	pm_pres	Gaussian	235.65	239.85	0.43	1
7	0	ln_length	none	370.52	373.32	-	-
	0	ln_length	Gaussian	372.52	376.72	0.41	1
9A	-0.5	ln_length, cardinal	none	119.47	122.27	-	-
	-0.5	ln_length, cardinal	Gaussian	118.19	122.39	1.87	0.07

[a] Values used to perform box cox transformations to normalize counts of phytoseiids. Log transformations were used when lambda was equal to zero.

[b] Pm_pres refers to the scored presence (1, 0) of *Oligonychus perseae* on leaves. Pm_counts refers to full counts of *O. perseae* on leaves. Cardinal refers to cardinal direction of leaves on trees. Length refers the measured distance of leaves from the end of the leaf where it attaches to the petiole to the leaf tip. Fixed effects in each final model were significant with alpha=0.05

Table 4.5. Moran's *I* statistic and estimated parameters for fitted Gaussian semivariance models

	Moran's *I*				Semivariance parameters		
Site	Observed	Expected	Z	P-value	c_n	σ_0^2	a_0
2	-0.008	-0.004	-0.25	0.80	0.32	0.09	4.26
3A	-0.01	-0.004	-0.63	0.53	0.07	0.02	1.43
5	-0.02	-0.004	-0.75	0.45	0.05	0.02	3.76
6[a]	-0.02	-0.004	-1.37	0.17	-	-	-
7	-0.03	-0.004	-1.72	0.09	0.18	0.00	2.94
9A	0.002	-0.008	0.36	0.72	0.10	0.01	2.62

[a] None of the theoretical semivariance models fit the observed data at this site.

Figure Legends

Fig. 4.1. Observed and fitted mean-proportion relationship for the mean number of phytoseiids per leaf and the proportion of leaves infested with predators from database A.

Fig. 4.2. Comparison of observed mean-proportion values from database C and model predictions based on database B.

Fig. 4.3. Percent error (A) and precision (B) values based on an enumerative fixed sample size of 20 leaves using database C. Observed phytoseiid densities were scaled to a 100 leaf sample for clarity.

Fig. 4.4. Stoplines for Green's sequential sampling for three preferred levels of precision (D). Open circle, filled circle, and open triangle correspond to densities of 1, 2, and 3 phytoseiids per leaf, respectively.

Fig. 4.5. Simulation results for sequential fixed-precision sampling based on D = 0.30 and an initial sample size of 10 leaves using database B and C.

Fig. 4.6. Observed average number of leaves needed in simulations to maintain a fixed-precision of D = 0.30 using database B and C.

Fig. 4.7. Simulation results for sequential fixed-precision sampling based on D = 0.10 and an initial sample size of 10 leaves using database B and C.

Fig. 4.8. Observed average number of leaves needed in simulations to maintain a fixed-precision of D = 0.10 using database B and C.

Fig. 4.9. Empirical semivariance values for model residuals from six orchards: A) orchard 2, B) orchard 3a, C) orchard 5, D) orchard 6, E) orchard 7, and F) orchard 9b. Lag refers to the incremental separation distance between pairs of trees on an

approximate 5x6 grid and can be converted to equivalent physical measurements (m) based on the values reported in Table 4.1.

Fig. 4.1.

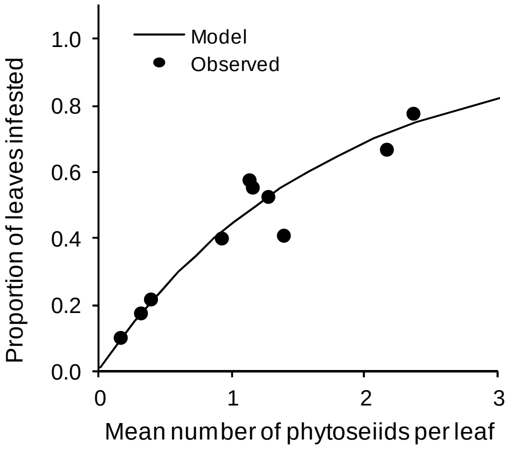

Model
Observed
Proportion of leaves infested
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
Mean number of phytoseiids per leaf

Fig. 4.2.

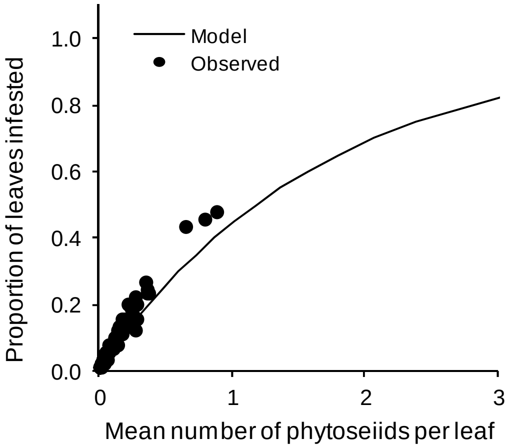
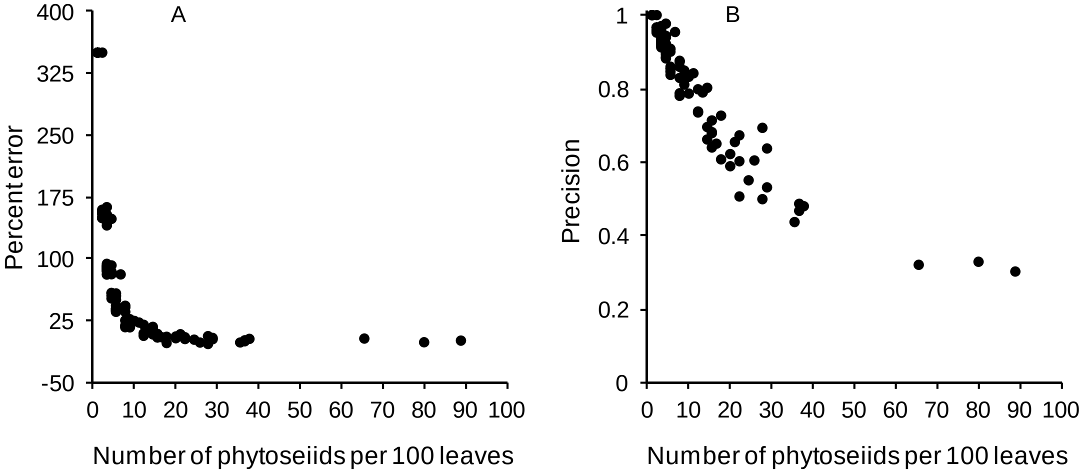
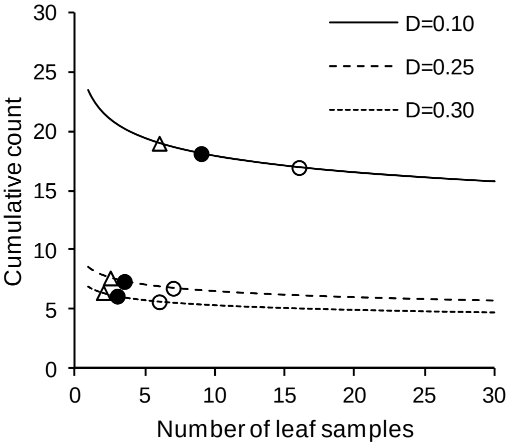

Model
Observed
Proportion of leaves infested
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
Mean number of phytoseiids per leaf

Fig. 4.3.



A
Percent error
400
325
250
175
100
25
-50
0 10 20 30 40 50 60 70 80 90 100
Number of phytoseiids per 100 leaves
B
Precision
1
0.8
0.6
0.4
0.2
0
0 10 20 30 40 50 60 70 80 90 100
Number of phytoseiids per 100 leaves

Fig. 4.4.



30
25
20
15
10
5
0
Cumulative count
0
5
10
15
20
25
30
Number of leaf samples
D=0.10
D=0.25
D=0.30

Fig. 4.5.

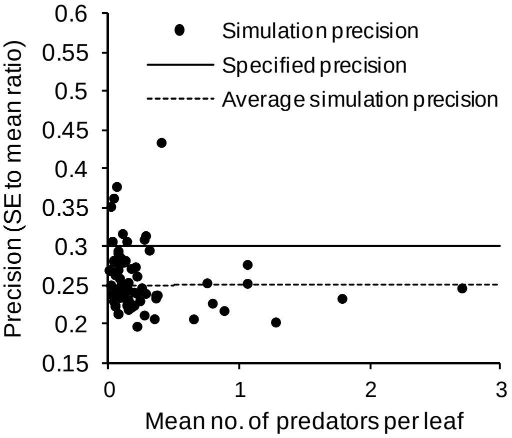
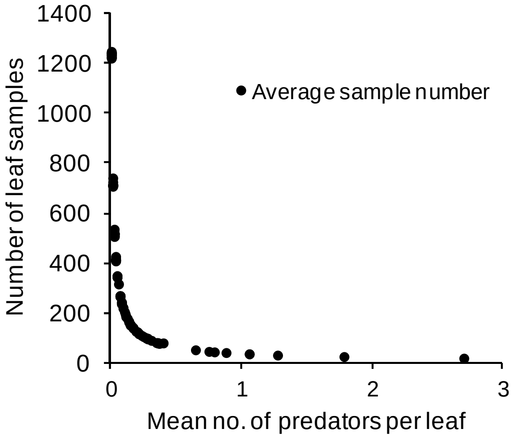

0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
Precision (SE to mean ratio)
0
1
2
3
Mean no. of predators per leaf
Simulation precision
Specified precision
Average simulation precision

Fig. 4.6.



1400
1200
1000
800
600
400
200
0
0
1
2
3
Number of leaf samples
Mean no. of predators per leaf
● Average sample number

Fig. 4.7.

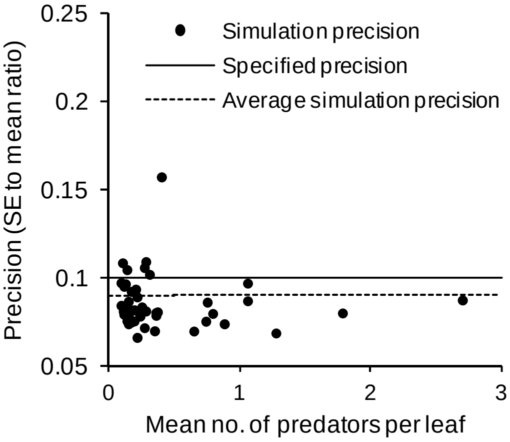

0.25
0.2
0.15
0.1
0.05
Precision (SE to mean ratio)
0
1
2
3
Mean no. of predators per leaf
Simulation precision
Specified precision
Average simulation precision

Fig. 4.8.

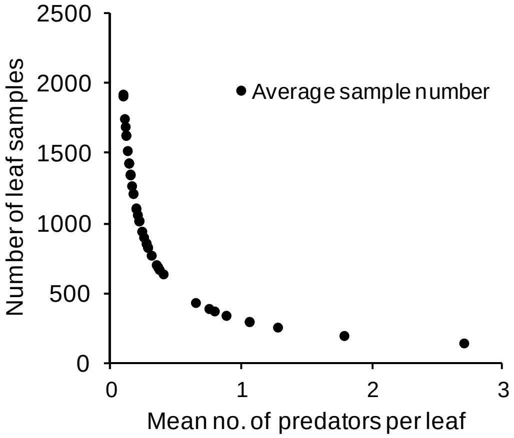
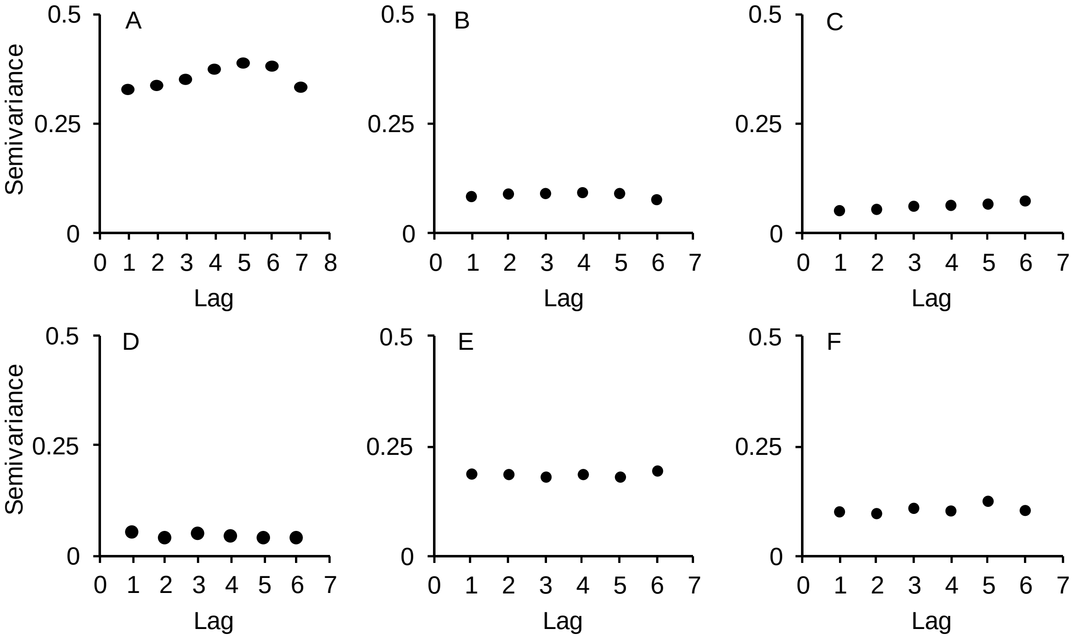

2500
2000
1500
1000
500
0
Number of leaf samples
● Average sample number
0
1
2
3
Mean no. of predators per leaf

Fig. 4.9.


A
B
C
D
E
F
Semivariance
Semivariance
Lag
Lag
Lag
Lag
Lag
Lag
0
0.25
0.5
0
1
2
3
4
5
6
7
8

Chapter 5

Examining the Synchrony Between *Oligonychus perseae* (Acari: Tetranychidae) and Resident Phytoseiid Populations in California Avocados

ABSTRACT *Oligonychus perseae* Tuttle, Baker, and Abatiello (Acari: Tetranychidae) is an economically important foliar pest of avocados. In California, candidate biological control agents, including indigenous populations of predatory mites, *Euseius* spp. (Acari: Phytoseiidae), have limited application against *O. perseae*. This problem has been documented in previous field studies but a statistical evaluation and conceptual understanding for the lack of effective biological control for *O. perseae* has not been provided. In this study, cross-correlation analyses based on Autoregressive Integrated Moving Average (ARIMA) models were used to examine the monthly synchrony between *O. perseae* and natural phytoseiid populations over an eleven year period (2002-2013) at an undisturbed Hass avocado orchard located in southern California. The cross-correlation results, after accounting for seasonality and autocorrelation in observed data, provided statistical evidence that resident phytoseiid populations did not provide control of *O. perseae* during the 11-year study period. This interpretation was corroborated with a gross examination of the original time series. Interestingly, a significant correlation was detected at time lag zero indicating a response of both resident phytoseiids and *O. perseae* populations to an environmental factor. The implications and possible mechanisms that generated these outcomes are discussed in the context of abiotic and biotic factors.

KEY WORDS *Oligonychus perseae*, *Persea americana*, cross correlation, ARIMA models

Oligonychus perseae is a widely distributed foliar spider mite pest of avocados (*Persea americana* Miller [Lauraceae]) in many areas of the world (i.e., California, Costa Rica, Israel, and Spain), including its native home range in Mexico. Invasive *O. perseae* populations were first detected in California sometime around 1990 (Bender 1994). Since then, *O. perseae* has established throughout approximately 90% of the avocado growing region and is most damaging to 'Hass' avocados (Aponte and McMurtry 1997, Kerguelen and Hoddle 2000). Annual California avocado production is valued at $381 million and the Hass variety accounts for 94% percent of the total production area (23,406 hectares) (CAC 2012).

O. perseae densities begin increasing during later spring months (April-May) when new avocado foliage is available to mites for colonization and feeding, followed by a peak in mite densities during summer (June-August) and eventual decline during fall (September – November) and winter months (December – February) (Yee et al. 2001, UC ANR 2008, Hoddle and Morse 2013). The working action threshold determined for chemical control, which is the principal management strategy for reducing *O. perseae* populations, is 50-100 mites per leaf (Moaz et al. 2011a).

For more than two decades the main problem in implementing a successful biological control program for reduction of *O. perseae* populations during the summer, when pest densities increase, has been dealing with the ecological limitations of natural enemies that could potentially respond to mite outbreaks. Initial monitoring data from 1992 (McMurtry 1993) showed that populations of *Euseius hibisci* (Chant) (Acari: Phytoseiidae), an abundant and widely distributed native species in California avocado orchards (McMurtry and Johnson 1966), failed to control populations of *O. perseae* which reached densities as high as 1,600 spider mites per leaf during that season. Furthermore, during 1998-2000 Yee et al. (2001) monitored populations of *O. perseae* and indigenous phytoseiid populations (mainly *E. hibisci*) at three Hass avocado orchards that were representative of coastal, intermediate, and warm-inland climates of the avocado growing region in southern California. Similarly, the results from that study indicated that there were no consistent trends between *O. perseae* and natural phytoseiid densities despite the fact that phytoseiids were the most abundant and continually present group of natural enemies at all three sites.

There are several potential reasons for this outcome. First, *Euseius* spp. belong to the economically important family Phytoseiidae which has long been a source of effective spider mite biological control agents in agricultural systems. However, not all phytoseiid species are effective predators of tetranychid spider mites (Gerson et al. 2003). Members of the genus *Euseius* are regarded as pollen specialists (McMurtry 1997). Laboratory studies demonstrated that *E. hibici* develops faster on a pollen diet than on spider mites alone (McMurtry and Scriven 1964, Zhimo and McMurtry 1990) and imbibing avocado leaf sap may provide nutrients required for optimal reproduction (McMurtry and Scriven 1965, Porres et al. 1975). Second, behavioral observations revealed that *Euseius* spp. could not penetrate the webbed nest of *O. perseae* and could only feed on exposed spider mites outside of the nest (McMurtry 1993). Together, these results suggest *E. hibisci* is a generalist facultative predator primarily dependent on obtaining plant-based nutrition rather than feeding exclusively on spider mites such as *O.*

perseae. Additionally, the leaf feeding habit might explain why populations of *E. hibisci* can subsist year round in the absence of pollen and when levels of *O. perseae* and other potential prey items are low in fall and winter.

The search for alternative biological control agents for *O. perseae* in California focused on screening commercially available phytoseiid species for deployment in commercial avocado orchards (Hoddle et al. 1999, 2000). The current recommended *O. perseae* biological control strategy depends on inoculative releases of *Neoseiulus californicus* (McGregor), which has characteristics of being a selective spider mite predator and also the capacity to reproduce on non-spider mite food sources (Croft et al. 1998). On avocados, *N. californicus* can penetrate the webbed nest of *O. perseae* and feed on all stages of this pest (Takano-Lee and Hoddle 2002). However, making the recommended seasonal releases of 2,000 *N. californicus* predators per tree during the growing season to obtain *O. perseae* control is not practical or cost-effective for commercial orchards when large areas need treatment.

Also, despite their generalist feeding habits, *N. californicus* populations cannot establish year-round in California avocado orchards and this is probably the result of at least two non-mutually exclusive mechanisms: competitive exclusion and intraguild predation. Mass reared *N. californicus*, unlike *E. hibisci*, are unable to subsist year-round on specific food resources available within the avocado system (e.g., avocado pollen, supplemental feeding on avocado leaf sap, *O. perseae*, other insect and mite species), and subsequent development and reproduction is insufficient to sustain year round populations and long-term biological control of *O. perseae*. Consequently, *N. californicus* populations may be competitively excluded by populations of *Euseius* spp. which may not only be better adapted for obtaining nutrition from the same available resources in avocado orchards, but they may also have a behavioral advantage in being more effective in detecting and finding these resources on avocados (see McMurtry and Johnson 1966). The reproductive potential of *N. californicus* and *Euseius* spp. reared on *O. perseae* has not been evaluated and results from this type of fundamental study might offer supporting evidence on the overall limited performance of both of these predators in the field when feeding exclusively on *O. perseae* (see Escudero and Ferragut 2005) or on a diet of this pest that is augmented with avocado pollen or some other readily obtainable resource.

Finally, a second potential mechanism for the lack of long-term establishment of *N. calfornicus* on avocados could be that asymmetric intraguild predation from native *Euseius* spp. prevent *N. californicus* from permanently establishing and effectively controlling *O. perseae* populations. This mechanism has been shown to occur between populations of *Euseius stipulatus* (Athias-Henriot) and *N. californicus* for control of *Tetranychus urticae* Koch on clementine plants (Abad-Moyano et al. 2010). Croft and Schausberger (2000) found that *E. hibisci* and *N. californicus* have a preference for heterospecific phytoseiids when given a choice. However, *N. californicus* may be at a disadvantage when released in orchards that have an abundant resident population of *E. hibisci*. Furthermore, the outcome of intraguild predation on *N. californicus* from resident populations of heterospecific phytoseiids in avocado may be compounded if *N. californicus* faces limited reproduction on avocado food resources compared to *E. hibisci* as discussed above.

Due to the practical limitations of making *N. californicus* releases, rescuing indigenous phytoseiid populations (*Euseius* spp.) which can subsist year-round in avocado orchards so that they can provide biological control services has been the on-going focus of several studies outside of California where *O. perseae* is also a pest. Research in Israel (Maoz et al. 2011b) and Spain (González-Fernández et al. 2009) demonstrated that supplemental pollen provisioning can boost levels of indigenous *Euseius* spp. populations. However, even with pollen provisioning, field releases of *E. stipulatus* and *N. californicus* were insufficient to control *O. perseae* (Monserrat et al. 2013). Monserrat et al. (2013) posit that this result was most likely due to a disruption of biological control when populations of phytoseiids experienced average temperatures above an estimated optimal value of 24.7°C. This potential influence of abiotic components points to a third mechanism that in some cases might explain why the biological control efficacy of these natural enemies in avocados is limited. However, Monserrat et al. (2013) did not rule out the possibility that intraguild predation between *E. stipulatus* and *N. californicus* may have also been a factor in explaining the lack of *O. perseae* suppression.

Previous studies on phytoseiid-*O. perseae* interactions have not examined the population dynamics of *O. perseae* and resident phytoseiids populations over an extended number of years (i.e., > 3 years, Yee et al. 2001). Consequently, the primary objective in this study was to examine the synchrony (i.e., cross-correlation) between *O. perseae* and natural phytoseiid population fluctuations over an eleven year period in an avocado orchard using quantitative methods. Cross-correlation analyses, a component of time series analyses, can be applied to biological systems to determine whether significant ecological relationships between two trophic groups occur over extended periods of time. This type of quantitative methodology has been used in previous studies to study predator-prey dynamics for both insect and mite systems in agricultural settings (Lingeman and van de Klashorst 1992, Scutareanu et al. 1999). However, these previous studies were based on spectral analyses whereby observations are processed in the frequency domain, in reference to the periodicity displayed by such data (Jenkins and Watts 1968).

In this study, cross-correlation analyses utilizing Autoregressive Integrated Moving Average (ARIMA) models were employed for further understanding of the role of natural phytoseiid populations in controlling *O. perseae* populations and to ascertain whether previous studies from the avocado system had failed to detect population regulation by these natural enemies because the time frame under consideration was too short. The ARIMA methodology for time series analyses was pioneered by Box and Jenkins (1970) and involves processing sequential observations in the time domain, in reference to the time scale under which data were collected (hourly, daily, weekly, monthly, etc.). The theory and application of time series analyses based on the ARIMA framework is provided by Yaffee and McGee (2000), Box et al. (2008), and Bisgaard and Kulahci (2011). The results of cross-correlation analyses for *O. perseae* and resident phytoseiid populations based on the ARIMA framework are presented herein.

Materials and Methods

Selected Study Site. South Coast Research and Extension Center (SCREC), Irvine, California is approximately 17 km from the Pacific Coast. During the period of study, hourly and daily weather information was collected by a CIMIS (California Irrigation Management Information System) weather station present on site. These data were averaged or summed (see below for distinction) for monthly intervals extending from the 15th day of the previous month to the 14th day of the current month. The resulting value was representative for the time period leading to the start of the 15th day of the current month and was paired with mite census data for the same interval length (see next subsection).

Monthly temperature and relative humidity values determined from hourly data fluctuated seasonally around stable mean values of 17°C (9-25°C) and 69% (49-85%), respectively (Fig. 5.1A, 5.1B). Total monthly rainfall was summed from daily values and showed two periods with relatively heavy rainfall (Fig 5.1C). Rainfall can sometimes disrupt mite colonization on leaves and has been previously implicated in influencing mite dynamics (Hanna et al. 2005). The cumulative number of days with maximum temperatures above 30°C for each month was monitored to determine whether relatively hot periods that might have otherwise been masked by simply taking the average of daily values over monthly intervals were important (Fig. 5.1C). This last calculation was repeated for other temperatures in the range of 24.7-35°C but the results are not reported here because no relevant patterns that might explain mite population dynamics at this site were found. Overall, the average temperature and relative humidity dynamics at SCREC were similar to those reported by Yee et al. (2001) for previous study sites in Ventura and Santa Barbara counties that are also close to the Pacific Coast.

Census of *O. perseae* and Phytoseiid Populations on Avocado. Hass avocado leaves were collected monthly during August 2002 - September 2013 (134 months) from a research plot within the SCREC grounds. In California, avocado tree shoot growth phenology is characterized by a spring flowering period and two distinct leaf flushes per year, a 'spring' (approx. March – May) and late 'summer' (approx. June-September) flush (Mickelbart et al. 2012). At each sampling occasion, ten spring and ten summer flush leaves were collected from each of the same nine untreated trees (i.e., 90 avocado leaves for each flush type). No attempt was made to stratify leaf age so that the age of leaves between months reflected the natural aging progression on avocado. Leaves of spring and summer flush types can be identified by examining the nodes on the branches. Sampled avocado leaves from each tree were stored in coolers in labeled paper bags and returned to the laboratory where the total number of motile *O. perseae* and phytoseiids on the undersurface of each leaf were counted under a stereomicroscope to estimate overall mean *O. perseae* and phytoseiid densities each month. No phytoseiids were intentionally introduced into the study site so it was assumed, based on previous studies (McMurtry and Johnson 1966, Yee et al. 2001), that the natural phytoseiid fauna at this site were mainly *Euseius* spp.

It is important to mention that while summer flush leaves were also sampled during the 11-year time frame from the same trees, data from these leaves are not included here for further time series analysis because *O. perseae* densities were always higher on spring flush leaves. This observation reflects the fact that *O. perseae* populations have a larger window of development (March-August) on aging spring flush leaves. Consequently, data collected from aging spring flush leaves provided better representation of the phenology and severity of *O. perseae* infestation and this is of practical importance for avocado pest managers who are sampling for this pest during summer months (Lara and Hoddle 2014).

Overview of ARIMA Methods for Conducting Cross Correlation Analyses. The underlying complication in performing cross-correlation analyses between two time series is that the serial correlation within each univariate time series may generate spurious correlations when both series are compared and this may lead to misleading interpretations. An approach for resolving this problem involves designating one time series as an input series and the second time series as a response series. Under this approach, autocorrelations for the input series are appropriately accounted for using ARIMA models.

The statistical framework of ARIMA models is equipped for handling serial correlation between current and previous observations of a univariate time series separated by time lag *k* (e.g., 1, 2, 3 months, etc.). The autoregressive (AR) component refers to observation values from time lag *k* multiplied by an estimated parameter ϕ for each lagged term. The integrated (I) component refers to the difference between successive observations so that the time series data can be regarded as a stable (stationary) process over the course of the evaluated time frame. The moving average (MA) component refers to error terms (random shocks) from time lag *k* multiplied by an estimated parameter θ for each lagged term. The designated number of lagged terms p and q (zero or a positive integer) for AR and MA components, respectively, and the level of differencing, d, for the integrated component define the order of the ARIMA model, which is denoted as (p, d, q). Not all ARIMA components may be required for fitting a final model to time series data (i.e., p, d, and q can be set to zero) but in some cases these components need to be expanded to account for seasonal patterns and the order of seasonal ARIMA components is denoted as (P, D, Q). Hence the representation for the order of a seasonal ARIMA model is (p, d, q) x (P, D, Q).

A suitable ARIMA model for characterizing the input time series is one that can significantly reduce the serial correlation between observations. Proper specification of ARIMA model components can be accomplished by identifying signature patterns of these components in plots of the autocorrelation (ACF) and partial auto correlation (PACF) function values (Yaffee 2000). ACF values, ranging from -1 to 1, refer to the measured correlation between observations of univariate time series at each time lag whereas PACF values, also ranging from -1 to 1, refer to the adjusted correlation between observations at each time lag having accounted for intermediate correlations (Bisgaard and Kulahci 2011). In general, fitting an ARIMA model to a time series is an iterative process controlled by the researcher. In cases where several models can describe the same time series data, preference should be given to a parsimonious model with the fewest parameters and with practical interpretation of its components (Yaffee and McGee 2000, Bisgaard and Kulahci 2011).

After fitting an ARIMA model to input series, the response time series is then filtered through the same ARIMA model, a combined process known as prewhitening (Box et al. 2008). The cross correlation values, ranging from -1 to 1, are then computed between the prewhitened input and prewhitened response time series at positive and negative time lags. Statistically significant correlation values at positive time lags imply that input series values are associated with future values of the response series at the indicated time lag. In the context of biological systems, this association might indicate there is a causal relationship between the input and response series. In contrast, significant correlation values at negative time lags imply that there is feedback from the response series to the input series.

Generally, when only positive time lags are significant, results from the cross-correlation analysis are used to specify a 'transfer function' for how the input series is related to the response variable and this information is then used to finalize the order of an ARIMA model for the response variable. This final ARIMA model can then be used to forecast future values of the response variable in the time domain. However, the ARIMA methodology cannot model feedback influences from the designated response series to the input series. When both potential feedback and causal relationships are detected, the pooled data from both time series can be treated as a bivariate response and modeled using an advanced state-space statistical framework. Depending on the research objectives, the state-space methodology may be more suitable for characterizing and forecasting the dynamics of predator-prey dynamics where significant feedback mechanisms are shown to occur between the different trophic groups. In this study, analyses were limited to calculating cross-correlations to find evidence for a significant causal and feedback relationship at positive and negative time lags, respectively, between populations of phytoseiid (input series) and *O. perseae* (output series) in the California avocado system.

Priming Biological Data for Cross-Correlation Analysis. The compilation of monthly mite population densities from spring flush leaves represent two discrete univariate time series, with general form X_{t_i}, for *O. perseae* (prey) and phytoseiids (generalist predator). Under this notation, X refers to the observed monthly organism density and t_i is the sequential numeric month identification starting with calendar month January 2002 as a reference point. Therefore, the time series ranges from t_8= August 2002 to t_{141}= September 2013.

Due to logistical difficulties it was not possible to collect spring flush leaves for estimating population densities of *O. perseae* and phytoseiids for monthly sample dates t_{53} (May 2006), t_{80} (August 2008), t_{122} (February 2012), and t_{130} - t_{135} (October 2012 – March 2013). These missing values represent less than 1% of the total sample dates but are required for complete time series analyses. Time series values for t_{53}, t_{80}, and t_{122} were estimated through linear interpolation as the midpoint between adjacent sample dates. Time series values for t_{130} - t_{135} were estimated as the average for each individual month based on monthly data from the full set of previous years (2002-2012). Also, zero values for both biological time series were replaced by 0.01 which represented cases where only 1 mite was found on a complete sample of 90 leaves. This data correction served two purposes: 1) to allow density zero values to be log transformed (see next subsection below) and 2) to reflect that fact the populations were more likely to have been estimated as zero at very low population densities because natural phytoseiid populations were detected throughout the course of the study (no phytoseiids were intentionally introduced into the study site). These initial modifications were incorporated into time series values for X_{t_i} (Fig. 5.2, 5.3).

Valid time series analyses assume that observations are stationary and equidistant. In basic terms, the property of stationarity requires that observations fluctuate around a constant mean (i.e., 0) with constant variance over time and that correlations between observations be only k- lag dependent so that the relationship between two observations can only be described in terms of their time separation and not based on when they occurred in the time series (Bisgaard and Kulahci 2011). Furthermore, seasonality, which would be expected to occur in biological populations monitored throughout the year, or strong non-seasonal deviations from a centered mean such as random walk behavior (i.e., the series values appears to wander haphazardly), can be a contributing factor for non-stationarity among observations and must be accounted for. Consequently, each time series of form X_{t_i} for phytoseiids and *O. perseae* was primed for analyses through a series of additional data filters.

To reasonably meet the constant variance assumption, a constant value 1 was added to all mean density values of *O. perseae* and phytoseiids and then log transformed so that all time series values were positive on the log scale. As a result of this transformation, the general biological time series form for *O. perseae* and phytoseiids could be expressed as:

$$Y_{t_i} = \ln [\, X_{t_i} + 1] \tag{1}$$

Next, to meet the equidistance assumption, log transformed values, Y_{t_i}, were calculated for the 15th of each month through linear interpolation (Fig. 5.4, 5.5). Equidistant Y_{t_i} values were also compared qualitatively with monthly weather data for signs of abiotic seasonal patterns. In general, non-stationarity due to trends or seasonality can be removed from the sequence of observations through differencing which creates a stationary time series variable, w_{t_i}, that is amenable for time series analysis. In other words, to reasonably obtain the stable characteristics of stationarity, the evaluation of the time series no longer focused on the raw data directly but on the population density changes that transpired from one period (e.g., year and/or month) to the next. For *O. perseae* and phytoseiid observations, 12-month seasonal differencing, $\nabla_{12}Y_{t_i}$, simple differencing, ∇Y_{t_i}, and a combination of seasonal and simple differencing, $\nabla\nabla_{12}Y_{t_i}$, were considered as potential candidates for w_{t_i}, and were calculated in the following manner,

$$\nabla_{12}Y_{t_i} = Y_{t_i} - Y_{t_i-12} \tag{2}$$

$$\nabla Y_{t_i} = Y_{t_i} - Y_{t_i-1} \tag{3}$$

$$\nabla\nabla_{12}Y_{t_i} = Y_{t_i} - Y_{t_i-1} - Y_{t_i-12} + Y_{t_i-13} \tag{4}$$

As previously indicated, the level of differencing refers to the integrated component of ARIMA models of order (p, d, q) x (P, D, Q). Therefore, a time series based on $\nabla_{12}Y_{t_i}$ implies d=0 and D=1. A time series based on ∇Y_{t_i} implies d=1 and D=0. Finally, a time series based on $\nabla\nabla_{12}Y_{t_i}$ implies d=1 and D=1. In practical terms, a time series based on D=1 refers to the seasonal (annual) population change for the same calendar period, d=1 refers to the successive monthly change, and the combination of d=1 and D=1 refers to the population change between both successive months and years. The type of differencing needed for each biological time series Y_{t_i} of phytoseiids and *O. perseae* was determined by visually assessing the stability of each potential differenced variable over the 11-year time frame. The selected stationary series w_{t_i} for the designated input series was then subjected to further time series analysis before fitting the final order of AR and/or MA components to both the input and response series (Fig. 5.6, 5.7) and finally calculating the cross-correlations between the set of prewhitened series.

Fitting an ARMA Model to the Designated Integrated Input Series. The time series Y_{t_i} for *O. perseae* and phytoseiids were seasonally differenced (integrated) to account for the seasonal influence of temperatures (1A) and reasonably meet the stationary assumption (Fig. 5.6, 5.7). The stationary time series w_{t_i} for phytoseiids (Fig. 5.7) was designated as the input series because the dynamics of the observations were easier to model using a simple ARMA model of order (1, 0, 0). A general mathematical representation of this model is given by

$$w_t = \hat{\phi}_1 w_{t-1} + \alpha_t \tag{7}$$

where ϕ_1 is an estimated AR parameter of order p=1 and α_t is an error term. This seasonal model implied that observations for phytoseiids Y_{t_i} could in part be explained by inspecting the changes that transpired from one year to the next, $w_{t_i} = \nabla_{12} Y_{t_i}$ (i.e., D=1), and further regressing these changes by those from the previous month at time lag *k* = 1. Therefore, Eq. (7) represents a seasonal ARIMA model for the phytoseiid time series Y_{t_i} with order (1, 0, 0) x (0, 1, 0).

The model represented by Eq. (7) accounted for the autocorrelations in $w_{t_i}$and proper fitting was determined iteratively using ACF and PACF plots for candidate models. The ACF values, $\hat{\rho}(k)$, for candidate models were calculated for each time lag k ranging from 1 – 48 months (4 years) using the following generalized equations adapted from Box et al. (2008):

$$\hat{\rho}(k) = \frac{\hat{\gamma}(k)}{\hat{\gamma}(0)} \tag{8}$$

$$\hat{\gamma}(k) = \frac{1}{T} \sum_{t=1}^{T-k} (w_{t+k} - \bar{w}) \cdot (w_t - \bar{w}) \tag{9}$$

where $\hat{\gamma}(k)$ refers to the estimated autocovariance between time series values w_t and w_{t+k} separated by time lag (interval) *k*, *T* is the total number of observation values in the time series, and $\bar{w}$ is the mean value for the time series. The term $\hat{\gamma}(0)$ in the denominator of Eq. (8) refers to the variance estimated from w_t. The mathematical representation for estimated PACF values, referred to as $\hat{\phi}_{kk}$, is more complex and a complete discussion is provided by Box et al. (2008). Simply, $\hat{\phi}_{kk}$ is a measure of the adjusted correlation between w_t and w_{t-k} that is not accounted by the influence of w_{t-1}, w_{t-2}, w_{t-3}, …, w_{t-k+1} (Box et al. 2008).

During model fitting iterations, parameter estimates based on maximum likelihood for AR and MA components of candidate models and diagnostic ACF and PACF values were calculated using PROC ARIMA in SAS 9.3 (SAS Institute 2011). ACF and PACF values were then plotted as a function of lag k to identify statistically significant correlation values. 95% statistical confidence boundaries for ACF and PACF values were defined by ± $2/\sqrt{T}$. For each candidate model, significant ACF and PACF values outside of the 95% confidence boundary implied that observations of time series w_t were not independent. Consequently, several ARIMA models (p, d, q) x (P, Q) were fit to w_t (the seasonal D term is already integrated into w_t) until there were no significant ACF and PACF values for $k > 0$.

To further confirm that a proper final model had been chosen, the significance of autocorrelations from each candidate ARIMA model was tested using a standard Ljung-Box test statistic (Ljung and Box 1978**)** for lags k=1-48. The null hypothesis for this test was that any remaining autocorrelations in the modeled data were not significantly different than zero (i.e., the observations are independent). Rejection of the null hypothesis meant that the candidate ARIMA model for w_t was not adequate and that an alternate ARIMA needed to be considered.

Synchrony between phytoseiid and *O. perseae* populations. The selected ARIMA model depicted in Eq. (7) was applied to the seasonally differenced time series w_t for phytoseiids (Fig. 5.7). The residuals from the prewhitened time series for phytoseiids were estimated as:

$$\alpha_t \approx \hat{\alpha}_t = w_t - \hat{\phi}_1 w_{t-1} \tag{10}$$

Similarly, Eq. 7 with the same value for $\hat{\phi}_1$ was applied to the seasonally differenced time series w_t of *O. perseae* (Fig. 5.6). The residuals for the prewhitened time series for *O. perseae* were estimated as:

$$\beta_t \approx \hat{\beta}_t = w_t - \hat{\phi}_1 w_{t-1} \tag{11}$$

Cross-correlation function values (CCF), $\hat{\rho}_{\alpha\beta}(k)$, between α_t and β_t at positive and negative time lags *k* were estimated using the generalized equations adapted from Box et al. (2008),

$$\hat{\rho}_{\alpha\beta}(k) = \frac{\hat{\gamma}_{\alpha\beta}(k)}{\hat{\sigma}_{\alpha}\hat{\sigma}_{\beta}} \qquad k = 0, \pm 1, \pm 2, \ldots \tag{12}$$

$$\hat{\gamma}_{\alpha\beta}(k) = \begin{cases} \frac{1}{n}\sum_{t=1}^{n-k}(\alpha_t - \bar{\alpha}\,) \cdot \left(\beta_{t+k} - \bar{\beta}\,\right) & k = 0, 1, 2, \ldots \\ \frac{1}{n}\sum_{t=1}^{n+k}(\beta_t - \bar{\beta}\,) \cdot (\alpha_{t-k} - \bar{\alpha}\,) & k = 0, -1, -2, \ldots \end{cases} \tag{13}$$

where $\hat{\sigma}_{\alpha}$ and $\hat{\sigma}_{\beta}$ are the estimated sample variances, $\hat{\gamma}_{\alpha\beta}(k)$ is the estimated covariance between α_t and b_t, $\bar{\alpha}$ and $\bar{\beta}$ are the sample means and n is the number of paired values (α_t, β_t). These calculations were conducted using PROC ARIMA in SAS 9.3 (SAS Institute 2011). 95% statistical confidence boundaries for CCF values were defined by ± $2/\sqrt{n}$.

Results and Discussion

The motivation of this study was to determine if resident populations of predatory mites contributed to biological control of *O. perseae* over extended periods of time. For this reason, the correlation between fluctuations of *O. perseae* and resident phytoseiid populations was examined. Data for this study were collected over a period of 134 months, a period sufficiently long to capture seasonal components that were therefore amenable to cross correlation analyses under the ARIMA framework (generally a minimum of 50 observations is recommended, Yaffee and McGee 2000). Conducting cross-correlation analyses under the ARIMA framework required the designation of phytoseiid and *O. perseae* time series as the input and response series, respectively, and dealing with the potential influence of seasonality and autocorrelation. This modeling specification operated on the assumptions that phytoseiid populations influenced *O. perseae* dynamics and that any deviations from this initial assumption would be revealed by a plot of cross-correlation values across time lags.

The ACF (Fig. 5.8) and PACF (Fig. 5.9) plots for the input phytoseiid time series w_t (Fig. 5.7) indicated there was significant autocorrelation in the observations for this group of organisms. This was also reflected in the significant Ljun-Box test statistics across all evaluated time lags, *k* = 1-48 (results not shown). After several iterations, the mathematical representation of the final AR model fit to the phytoseiid time series w_t of order (1, 0, 0) was

$$w_t = 0.44w_{t-1} + a_t \tag{14}$$

The ACF (Fig. 5.10) and PACF (Fig. 5.11) plots for the estimated residuals α_t of this model (Fig. 5.12) indicated that the autocorrelation for w_t were reduced to non-significant “white noise” Y_{t_i} after prewhitening. Eq. (14) was used to prewhiten the seasonally differenced time series w_t for *O. perseae* (Fig. 5.6) and estimate residuals β_t (Fig. 5.13). The computed cross-correlations between α_t (phytoseiids) and β_t (*O. perseae*) are shown in Fig. 5.14. For comparison, the correlations between the respective time series Y_t for *O. perseae* and phytoseiids without prewhitening or seasonal differencing are shown in Fig. 5.15. The contrast between Fig. 5.14 and Fig. 5.15 highlights the practical importance of accounting for the confounding influence of seasonality and autocorrelations which can generate misleading results. After accounting for these confounding effects, the lack of statistically significant correlations at non-zero time lags suggested that a strong causal and feedback relationship did not exist between natural *O. perseae* and phytoseiid populations at the evaluated site during the 11-year observation period. This result strongly implies that these generalist natural enemies did not act in a density dependent manner on *O. perseae* populations at any time scale during the course of this study.

The combination of statistically significant negative and positive time lags, had they occurred, would have implied, biologically, that increasing levels of *O. perseae* populations preceded increasing levels of responsive phytoseiid populations, which would have subsequently caused levels *O. perseae* to decrease. This type of predator-prey dynamic could then have modeled using a state-space model representation. However, the lack of evidence for the presence of this relationship in this study does not warrant these additional analyses.

The conclusions from statistical analyses regarding the absence of significant lagged interactions were also supported by a crude examination of the original biological time series shown in Fig. 5.2 and 5.3. Throughout the course of the study there were no significant increases in phytoseiids that were indicative of effective biological control for *O. perseae*. In 2003 ($t_{12} – t_{24}$) and 2005 ($t_{36} – t_{48}$), *O. perseae* levels exceeded the action threshold of 50 mites per leaf (Fig. 5.2) and peak ratios of *O. perseae*: phytoseiids were 760: 1 (t_{20} = August 2003) and 1,287: 1 (t_{42} = June 2005). During 2012-2013 there was an elevated rise in phytoseiids densities and peak ratios of *O. perseae*: phytoseiids were 23:

1 (t_{42} = June 2005) and 108: 1 (t_{139} = July 2013). However, the 2012-2013 increase in phytoseiids did not translate into effective *O. perseae* control because densities still exceeded the action threshold of 50 mites per leaf.

The significant synchrony between *O. perseae* and phytoseiids at time lag zero (Fig. 5.14) was initially perplexing. This result requires a comprehensive explanation as it could be interpreted as a spurious statistical result, the result of an inadequate sampling structure, or the result of an underlying biological mechanism that is unaccounted for. Based on the 95% confidence intervals, there is a 5% probability that at least two correlations (0.05 x 48) would be detected as significant but this outcome is unlikely (i.e., it has a probability of only 5%). In this system, this explanation would have more credibility if the significant correlation occurred at a greater time lag to which a logical biological interpretation would be difficult to assign (e.g., *k*=18, -9). Subsequently, the possibility that the monthly interval period in the study was too wide and masked any time-lagged responses of predators detectable only in the order of weeks was considered. To evaluate this second interpretation we refer to a previous study.

McMurtry and Johnson (1966) documented the phenology of *O. punicae* and phytoseiids (*Euseius* spp.) at six untreated avocado orchards during a variable 1-4 year time period with weekly, semimonthly, and or/monthly sampling intervals throughout the California avocado region. Quantitative time series analyses were not performed for the data collected in that study but the majority of the plots of the original data reveal a clear delayed response of phytoseiids populations to increasing levels of *O. punicae* with phytoseiid densities sometimes peaking at 10 or 15 predators per leaf. Although phytoseiid populations were for the most part considered ineffective in reducing *O. punicae* populations at the majority of the sites, the delayed (lagged) response in phytoseiid populations was still discernible over a period of one month. This pattern indicated that phytoseiids fed on *O. punicae* (this was confirmed visually by the authors) and this process translated into increased reproduction in subsequent weeks, including a period of one month. A statistical validation of this asymmetric feedback interpretation would have appeared on a CCF plot as significant positive spikes at negative time lags.

Similarly, in the present study, a lagged response of phytoseiid densities (*Euseius* spp.), if it existed, would have been apparent in graphs of the raw data (Fig. 5.2, 5.3) and in the CCF plot (Fig. 5.14). The absence of a significant lagged response does not exclude the possibility that individual phytoseiids were feeding and reproducing to some extent on *O. perseae*, as these predators have been observed feeding on this host in the laboratory. Instead, the absence of a lagged response reflects the low numerical response of phytoseiid populations during *O. perseae* infestations. At a fundamental level, this lack of numerical response from natural phytoseiid populations to *O. perseae* might be explained by differences in susceptibility between *O. punicae* and *O. perseae* to predation or differences in the reproductive potential of phytoseiids when feeding on these different hosts. These hypotheses have not been tested.

Therefore, the positive correlation at time lag zero, without the presence of other significant time lags, implies that populations of phytoseiids and *O. perseae* were most likely responding to the influence of an abiotic and/or biotic factor but the identity of these factors and the mechanisms by which they operate remain unresolved. It is important to clarify that for the modeling portion of this study, the precise identity of these factors and their mechanisms is not necessary when comparing *O. perseae* and phytoseiid fluctuations directly. This is because the sequences of observations (i.e., the time-series) for both groups are each assumed to be a realization of a stochastic process composed of all causal abiotic and biotic factors present at the study site (see Bisgaard and Kulahci 2011). Consequently, the ARIMA analyses were used appropriately to characterize the behavior (e.g., seasonality, autocorrelation) of each single realization based on its own past sequence of observed data. The cross correlation analyses were used to investigate whether there was lag-dependent "behavior" between both realizations that should be included in a more complex ARIMA model. If a significant correlation is detected, such as in this case where the correlation was significant at time lag zero, the environmental factor(s) responsible for this result are left for the researcher to determine based on available evidence.

However, the potential identity of this environmental factor(s) is discussed here briefly to underline the complexity of predator-prey mite dynamics and pest management research that is still needed on avocados. With regards to abiotic factors, fluctuations of weather variables (Fig. 5.1) were relatively stable over time and this, in part, explains the seasonal fluctuations in mite activity but not the dual rise in *O. perseae* and phytoseiid densities. Furthermore, with regards to periods with low *O. perseae densities*, it was initially suspected that extensive warm periods (e.g., heat waves with multiple days exceeding $30^{o}C$) may have been the source of natural spider mite control and/or reductions in phytoseiid populations (see Montserrat et al. 2013). However, no supporting evidence for this mechanism was found at this site as temperatures were relatively cool for the duration of this study. Rather, the mild weather patterns at this site would have been sufficiently permissive to allow continual elevated levels of *O. perseae* and would have been non-detrimental to phytoseiid densities throughout the study period. This again points to the inconsistency in *O. perseae* and phytoseiid fluctuations during the 11-year period where only some years (2003, 2005, 2012, and 2013) exhibited relatively higher spider mite and phytoseiid densities.

In addition to seasonal weather influences, it would seem likely that leaf nutrition might play a direct role in influencing population dynamics of phytophagous mite species. In theory, partial explanation for mite density fluctuations at SCREC may lie with physiological changes on Hass avocado trees that affected their suitability as host plants to herbivores during the period of study. It is known that feeding by herbivores can induce plant suitability changes that in turn are detrimental to the subsequent population growth of herbivores and this phenomenon is commonly referred to as induced resistance (Karban and Myers 1989). For agricultural systems, this understanding led to the practice of ‘vaccinating’ plants with less serious herbivores that would condition plants to become better protected against future damage by more economically important pests. An example of this was documented for spider mites on grapes in California (Karban et al. 1997). Induced resistance is typically thought of as affecting populations of plant-feeding pests but the underlying mechanisms could also affect co-occurring ‘non-target’ predators that feed or lay eggs on the same plant tissue (see Agrawal et al. 2000).

Mechanisms for induced resistance could be triggered changes in the expression of secondary metabolites for plant defense or nutrient composition after the plant is 'challenged' by a herbivore. Evidence of induced resistance has been documented for agricultural systems with regard to spider mites (Karban and English-Loeb 1988). However, induced resistance has not been well studied on avocado (*P. americana*) and the only readily available evidence comes from El-Hamalawi and Menge (1995a) who found that root inoculation of Topa-Topa avocado in California with the pathogen *Phytophthora citricola*, the causal agent of avocado stem cankers, conferred significant resistance to pre-treated plants against subsequent inoculations with the more serious causal agent of avocado root rot, *P. cinnamomi*. Also, McMurtry (1970) observed that *O. punicae,* a secondary foliar pest of avocados, had lower reproduction on *P. indica* leaves (an alternative host plant related to *P. americana*) that had received immediate conspecific mite damage than on leaves with a similar level of damage but with a lagged physiological recovery period of 2 months.

Based on the null hypothesis that induced resistance on Hass avocado can be a source of negative feedback to *O. perseae*, it would be expected that the observed period of heavy feeding in 2003 and 2005 induced physiological changes in host avocado trees. These host changes, either chemical or nutrient-based, subsequently caused a drop in *O. perseae* levels for several years and populations of *O. perseae* have only recently recovered in the last couple of years (2012-2013). By extension, one would also need to assume that resident phytoseiids (*Euseius* spp.) present in the system, which are capable of foliar feeding, were negatively affected by foliar changes induced by *O. perseae* feeding. This induced resistance phenomenon could offer a potential biological explanation for a statically significant correlation at time lag zero. It has been established, for example, that nutrients on Hass avocado leaves can vary naturally over time, as a function of season or with leaf age (Bingham 1961), but how *O. perseae* responds to those changes and whether it can secondarily modulate them via feeding is not known. On Hass avocado, El-Hamalawi and Menge (1995b) showed that seasonal nutrient changes on bark tissue explained the colonization pattern of *P. citricola*, a fungal pathogen, better than environmental temperature fluctuations. However, in the present study, data were not collected to test the hypothesis that foliar secondary metabolites and/or leaf nutrient concentration affected *O. perseae* and phytoseiids densities over time.

Conclusions and Future Directions. The results reported herein support the consensus from previous short-term studies conducted throughout the California avocado growing region that natural phytoseiid populations do not provide significant biological control *O. perseae* populations. However, this study differed in that quantitative methods were employed to study the parallel dynamics of both *O. perseae* and indigenous phytoseiid populations over 11 years, approximately half of the period since *O. perseae* was first detected in California in 1990.

Several mechanisms that could explain the limitations of these predators in the field were provided. Pollen-provisioning has been suggested as an alternative to rescue native phytoseiids and boost their populations in the avocado system but the effectiveness of this approach and practical wide-scale application in each avocado growing region (e.g., California, Mexico, Costa Rica) has not been demonstrated.

Inoculative releases of commercially available phytoseiids has also been unsuccessful in providing long-term biological control of *O. perseae* in California, as these predators don't establish permanent populations after release. Current studies are underway to determine the likely source of California *O. perseae* populations from Mexico and to identify candidate phytoseiid species that co-evolved with *O. perseae* in Mexico that may be established in California as part of a classical biological control program. This approach may be the best avenue for developing self-sustaining biologically-based control of *O. perseae* in California grown avocados.

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Figure Legends

Fig. 5.1. Monthly data for selected weather variables at study site from August 2002 – September 2013: A) temperature, B) relative humidity, C) rainfall, and D) number of days above 30°C.

Fig. 5.2. Time series for untransformed monthly densities of *Oligonychus perseae* from August 2002 to September 2013.

Fig. 5.3. Time series for untransformed monthly densities of *Oligonychus perseae* from August 2002 to September 2013.

Fig. 5.4. Time series for log transformed monthly densities of *Oligonychus perseae* (X_t +1) from August 2002 to September 2013.

Fig. 5.5. Time series for log transformed monthly phytoseiid densities (X_t +1) from August 2002 to September 2013.

Fig. 5.6. Seasonally differenced time series w_t for log transformed monthly densities of *Oligonychus perseae* from August 2003 to September 2013. Due to seasonal differencing (12 month period) observations from August 2002 to July 2003 were eliminated.

Fig. 5.7. Seasonally differenced time series w_t for log transformed monthly densities of phytoseiids from August 2003 to September 2013. Due to seasonal differencing (12 month period) observations from August 2002 to July 2003 were eliminated.

Fig. 5.8. Autocorrelation values for time series w_t for phytoseiids for 48 time lags prior to prewhitening. Correlation values above 2 standard errors for time lags $k > 0$ indicated there were significant serial correlations in the time series.

Fig. 5.9. Partial autocorrelation values for time series w_t for phytoseiids for 48 time lags prior to prewhitening. Correlation values above 2 standard errors for time lags $k > 0$ indicated there were significant serial correlations in the time series.

Fig. 5.10. Autocorrelation values for time series w_t for phytoseiids for 48 time lags k after prewhitening. Correlation values below 2 standard errors for time lags $k > 0$ indicated there were no significant serial correlations in the time series.

Fig. 5.11. Partial autocorrelation values for time series w_t for phytoseiids for 48 time lags k after prewhitening. Correlation values below 2 standard errors for time lags $k > 0$ indicated there were no significant serial correlations in the time series.

Fig. 5.12. Residual time series, α_t, for phytoseiids from August 2003 to September 2013after prewhitening series w_t with seasonally differenced autoregressive model. Due to seasonal differencing, 12 observations from August 2002 to July 2003 were eliminated.

Fig. 5.13. Residual time series, β_t, for *Oligonychus perseae* from August 2003 to September 2013after prewhitening series w_t with the same seasonally differenced autoregressive model for phytoseiids. Due to seasonal differencing (12 month period) observations from August 2002 to July 2003 were eliminated.

Fig. 5.14. Cross correlation values between prewhitened time series α_t and β_t for 24 positive and negative time lags k. Correlation values above 2 standard errors were significant.

Fig. 5.15. Cross correlation values between time series Y_t for *O. perseae* and phytoseiids for 24 positive and negative time lags *k* with no prewhitening and no seasonal differencing. Correlation values above 2 standard errors were significant.

Fig. 5.1.

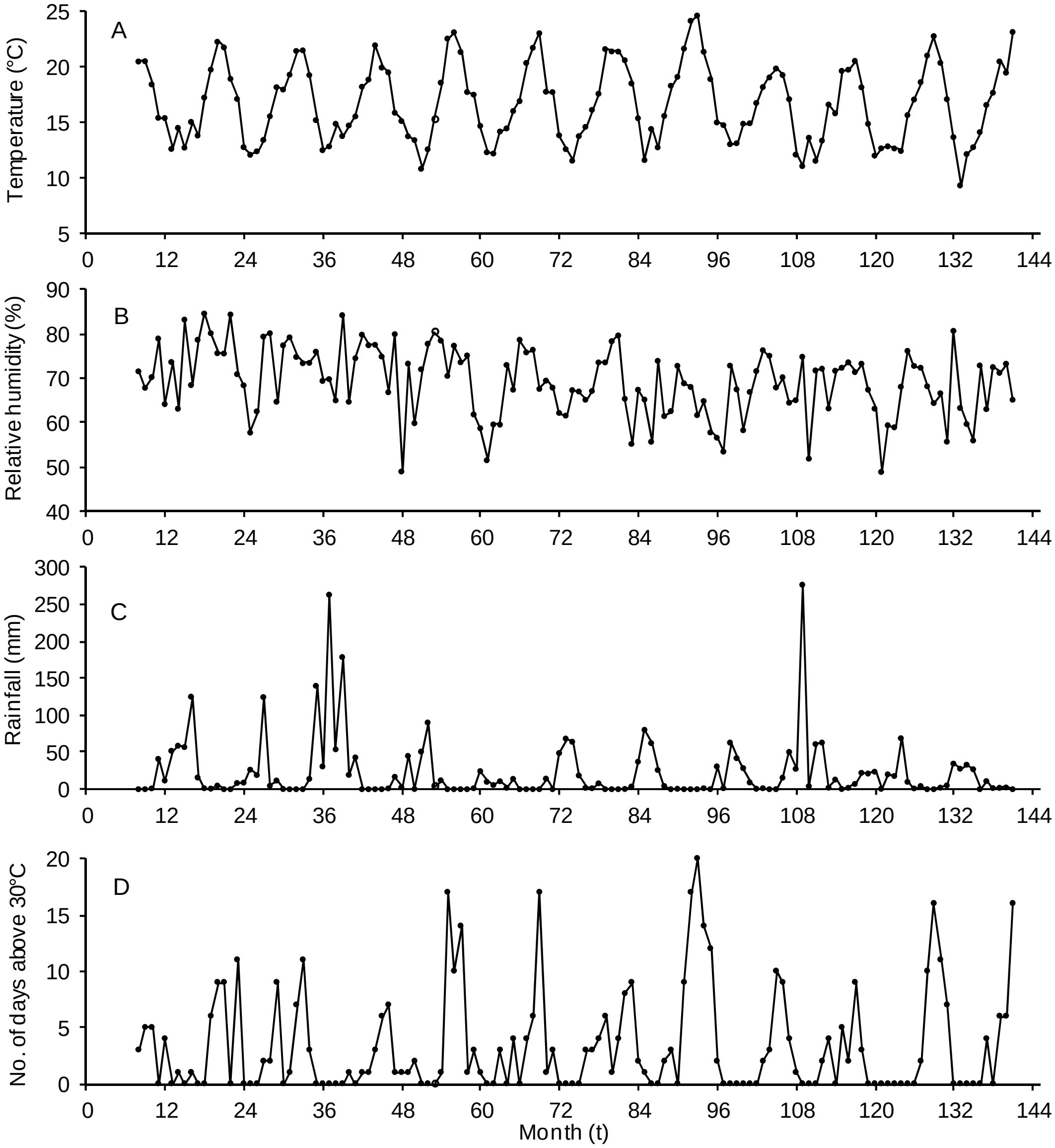

A
Temperature (°C)
25
20
15
10
5
B
Relative humidity (%)
90
80
70
60
50
40
C
Rainfall (mm)
300
250
200
150
100
50
0
D
No. of days above 30°C
20
15
10
5
0
0 12 24 36 48 60 72 84 96 108 120 132 144
Month (t)

Fig. 5.2.

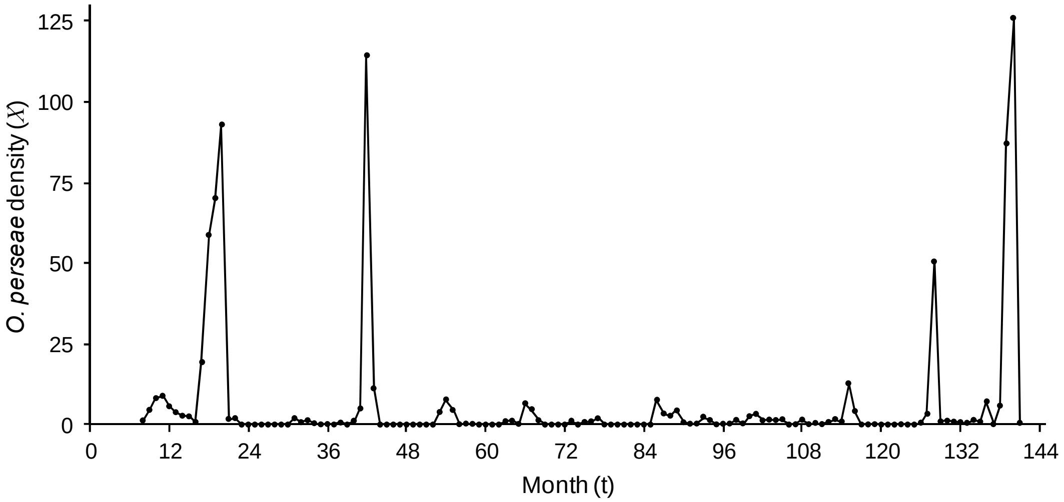
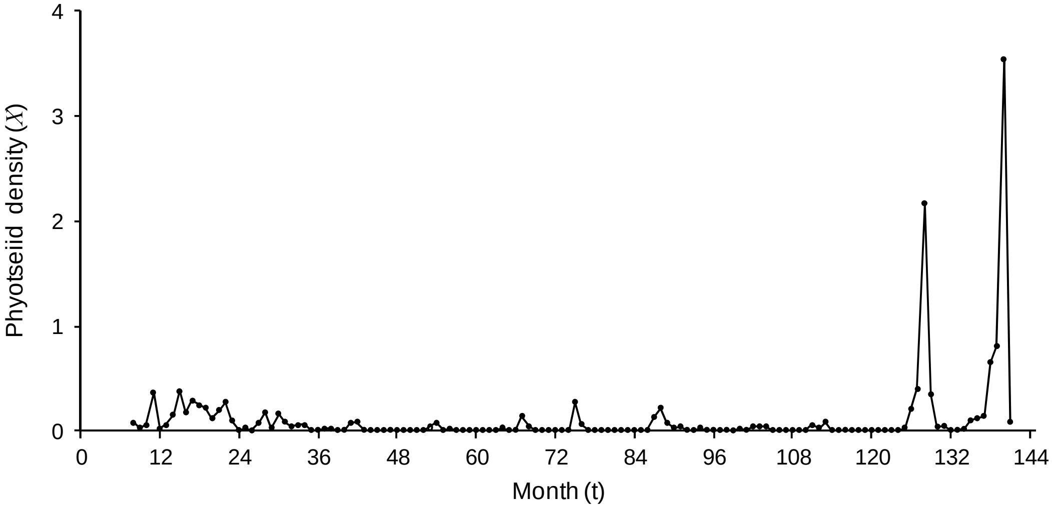

125
100
75
50
25
0
O. perseae density (X)
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.3.



4
3
2
1
0
Phyotseiid density (X)
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.4.

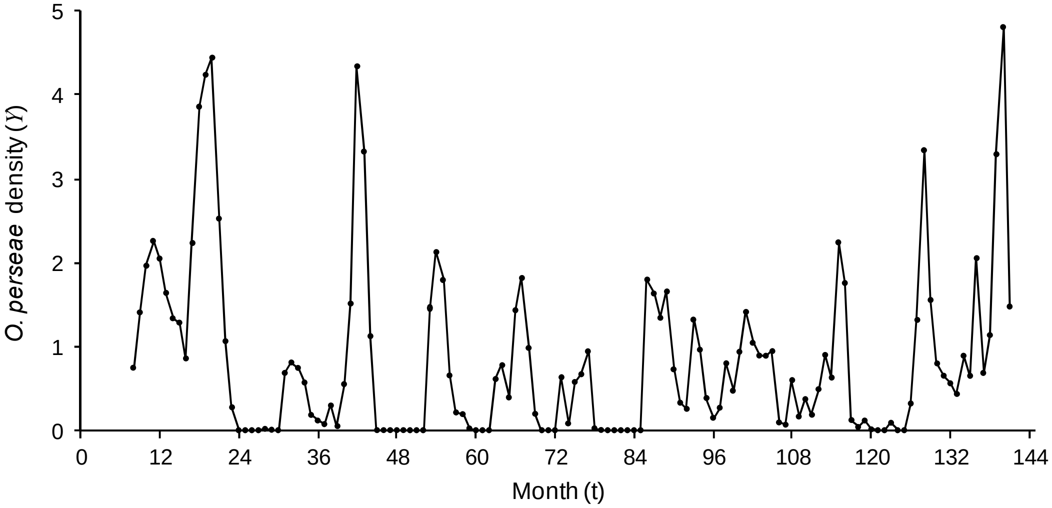
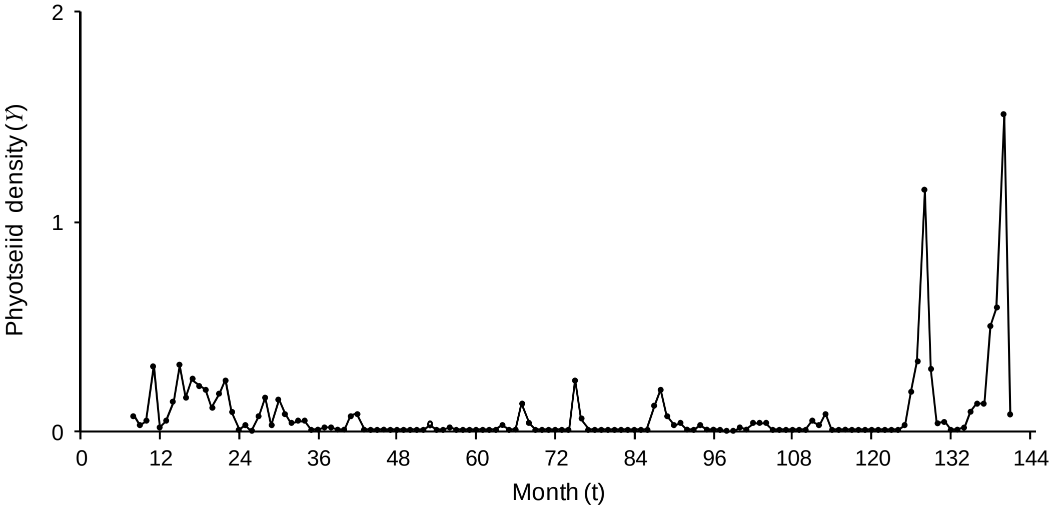

O. perseae density (Y)
0
1
2
3
4
5
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.5.



Phyotseiid density (*Y*)
2
1
0
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.6.

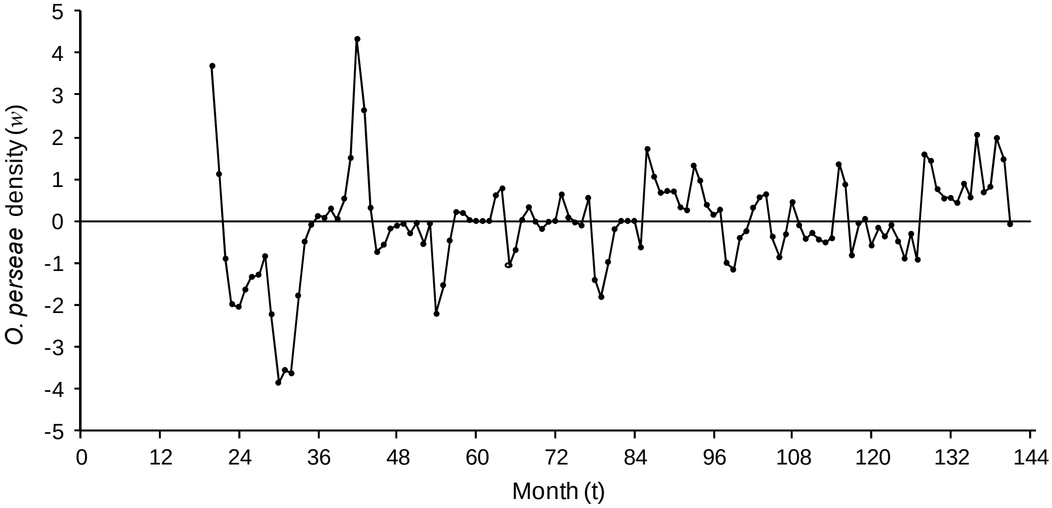
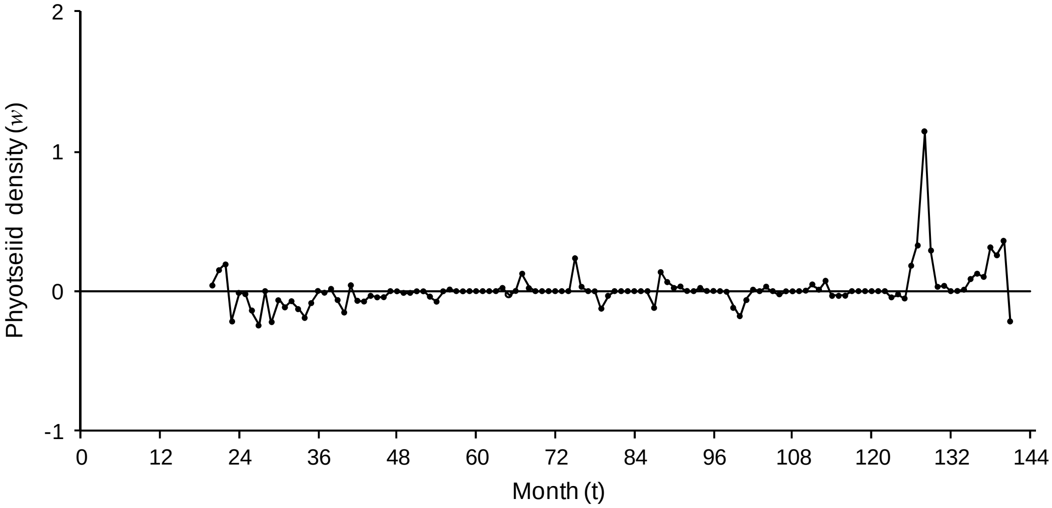

5
4
3
2
1
0
-1
-2
-3
-4
-5
O. perseae density (w)
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.7.



2
1
0
-1
Phyotseiid density (*w*)
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.8.

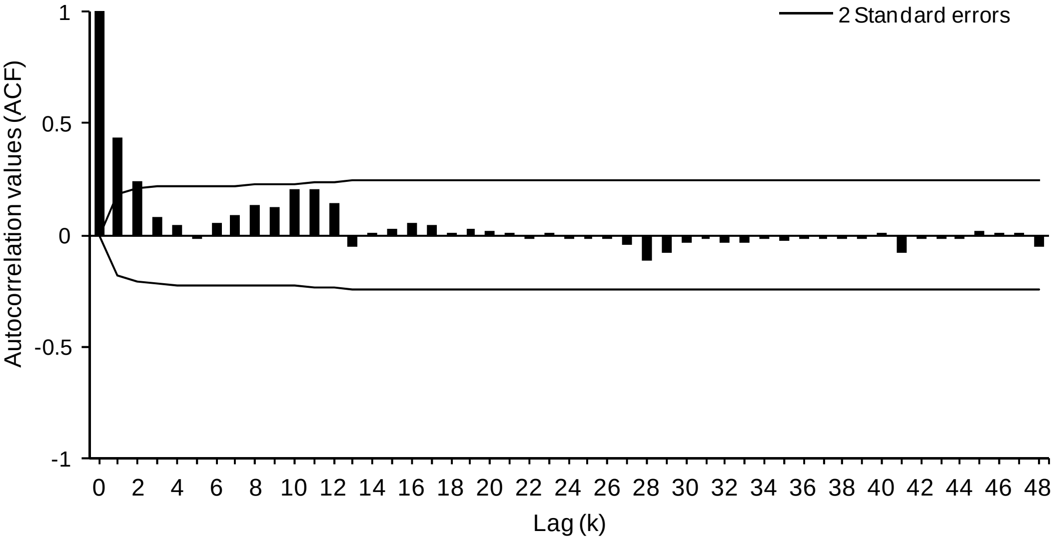
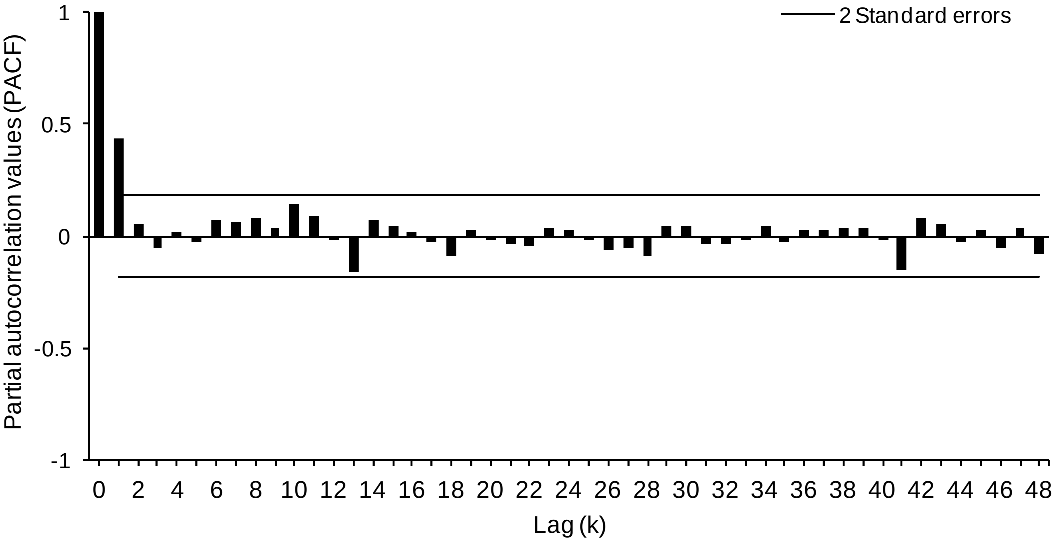

2 Standard errors
Autocorrelation values (ACF)
1
0.5
0
-0.5
-1
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
Lag (k)

Fig. 5.9.



2 Standard errors
Partial autocorrelation values (PACF)
1
0.5
0
-0.5
-1
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
48
Lag (k)

Fig. 5.10.

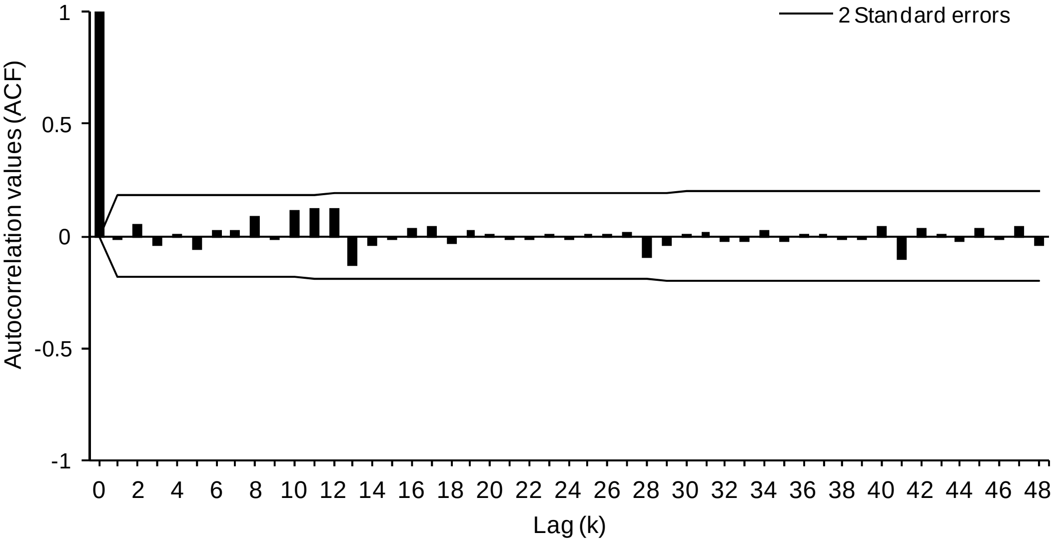
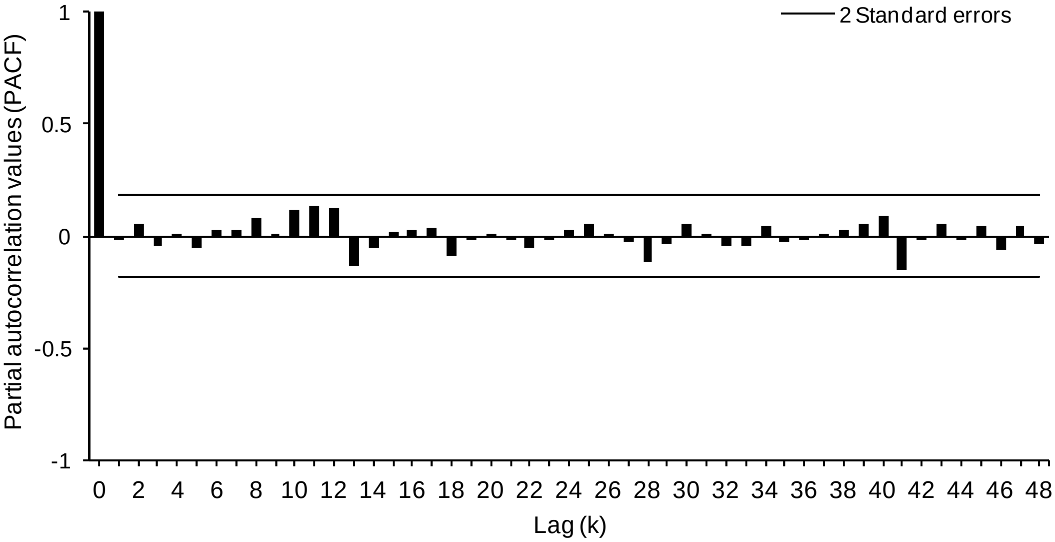
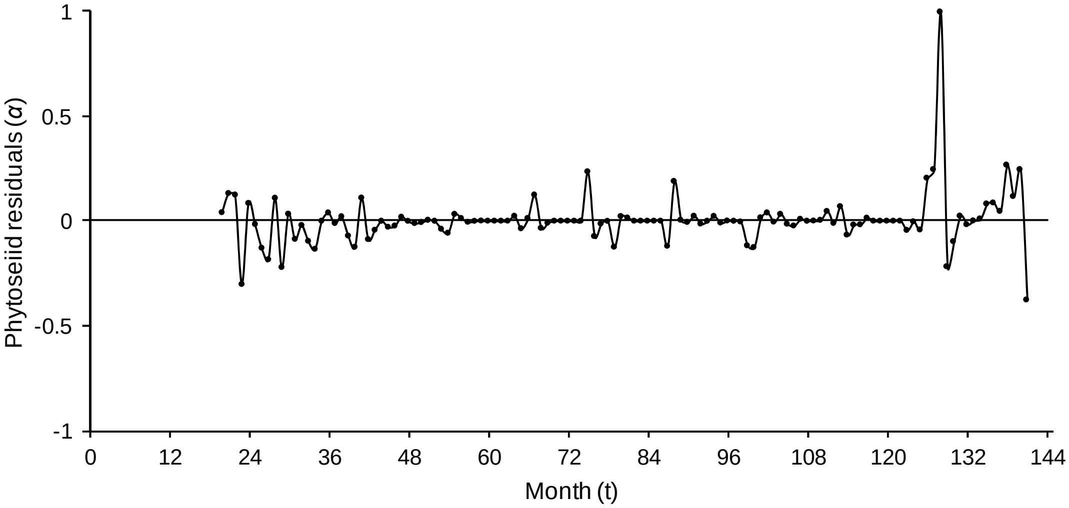

2 Standard errors
Autocorrelation values (ACF)
1
0.5
0
-0.5
-1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Lag (k)

Fig. 5.11.



2 Standard errors
Partial autocorrelation values (PACF)
1
0.5
0
-0.5
-1
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38 40 42 44 46 48
Lag (k)

Fig. 5.12.



Phytoseiid residuals (α)
1
0.5
0
-0.5
-1
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.13.

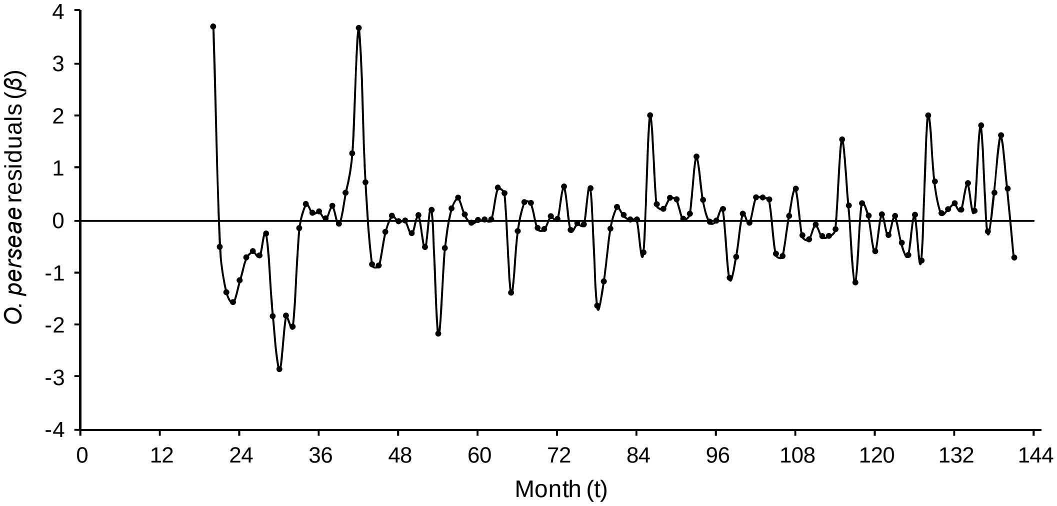

O. perseae residuals (β)
4
3
2
1
0
-1
-2
-3
-4
0
12
24
36
48
60
72
84
96
108
120
132
144
Month (t)

Fig. 5.14.

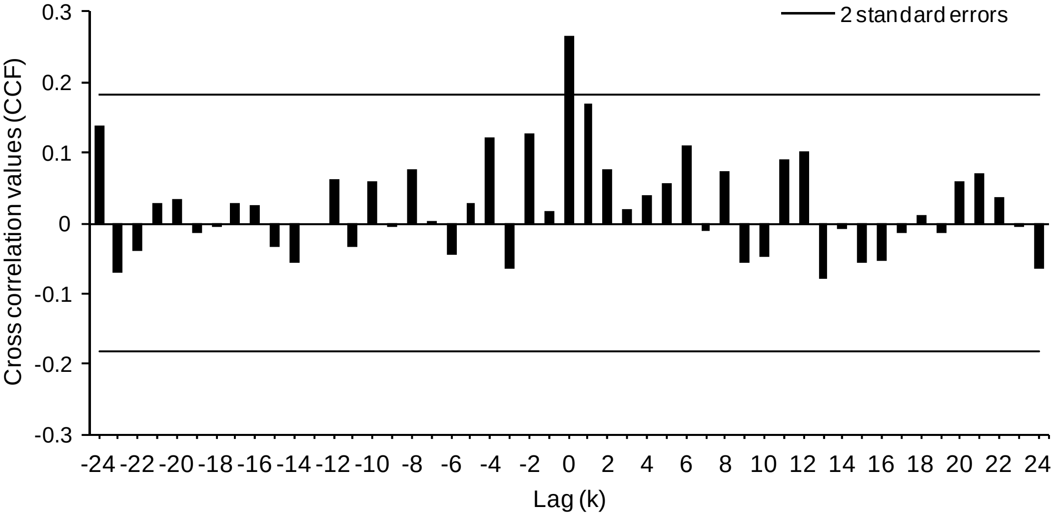
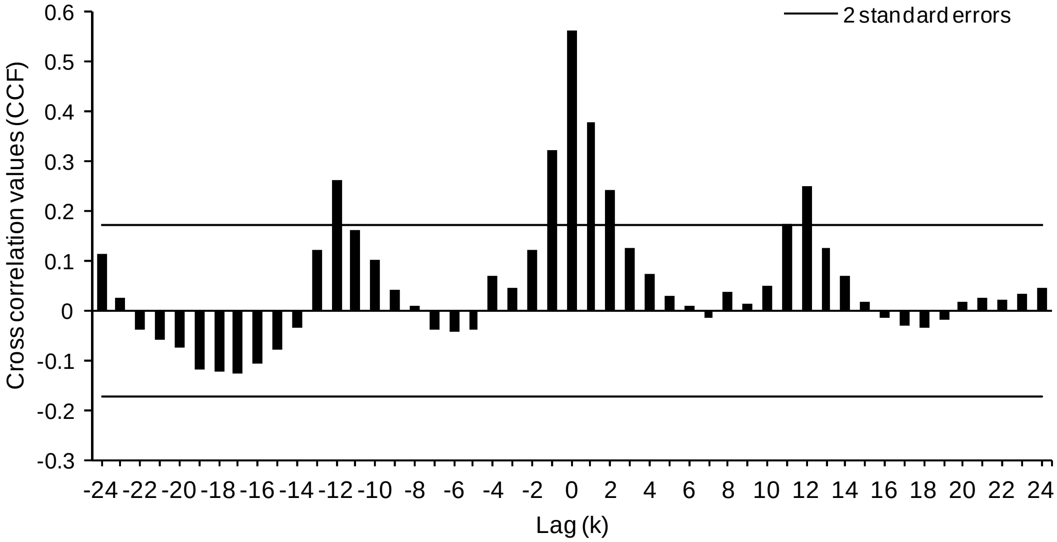

2 standard errors
Cross correlation values (CCF)
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-24 -22 -20 -18 -16 -14 -12 -10 -8 -6 -4 -2 0 2 4 6 8 10 12 14 16 18 20 22 24
Lag (k)

Fig. 5.15.


2 standard errors
Cross correlation values (CCF)
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-24
-22
-20
-18
-16
-14
-12
-10
-8
-6
-4
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
Lag (k)