Molecular ecological study on spider mite control using phytoseiid mites with emphasis on the influence of insectary plants in Japanese peach orchards

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DEDICATION

To the woman that taught me the essentials and fundamentals of life,
Grandma and Meemaw; Tania Tau

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Abstract

Spider mites, a major pests of fruits have developed resistance to pesticides of various types. Phytoseiid mites on the other hand, have been proven to be potential biological control agents in suppressing spider mites. The importance of some groundcover plants have been suggested to promote the occurrence of phytoseiid mites as insectary plants providing refuges, alternate foods, plant resources such as nectar and pollen, and places to diapause, develop, and reproduce. However, there is little information on the current status of phytoseiid mites as biological control agents in peach orchards and the functions of insectary plants in controlling spider mite in peach orchards. Six different orchards with different pest management practices were used in this study. Site I, an organic orchard with ground cover. Site II, IV, V and VI are conventional orchards with groundcover while Site III, a conventional orchard without groundcover. A consecutive annual population survey were conducted between 2012 and 2015 on phytoseiid mites and spider mites at Japanese peach orchards. Population survey revealed that the organic orchard with wild groundcover and no synthetic chemicals used for pest control and a conventionally managed orchard with bare ground had no trees on which spider mite density was beyond the control threshold density (one mite per leaf). Furthermore, organic orchard had an appearance in which the occurrence of phytoseiid mite preceded that of spider mites henceforth inferring the decrease in the population of spider mites below control threshold density. While in a conventional orchard without groundcover, though phytoseiid mites occurred after spider mites, the populations of spider mites were well suppressed. On the other hand, spider mite densities in some trees at conventionally managed orchards with wild groundcover were temporarily beyond the control threshold level. To justify the dynamics and occurrence pattern of phytoseiid mites and spider mites in orchards with different pest management practices, phytoseiid mite species composition on peach leaves was conducted using a quantitative sequencing method, a molecular technique for differentiating phytoseiid mite species. The results revealed that phytoseiid mite species composition changed during the survey period and varied among study sites. *Euseius sojaensis*, pollen feeders/generalist predators, was dominant in organic orchard and might have the ability to occur ahead of spider mites and maintain spider mites at lower levels. *Neoseiulus californicus*, selective predators of tetranychid mites, was detected to be dominating in conventional orchards without groundcover, thus might effectively suppress spider mite populations below control threshold densities. In the other orchards with groundcover, *Amblyseius eharai*, generalist predators, dominated sparsely in the conventional orchards that had ground cover vegetation. Wild plants species inhabiting the undergrowth vegetation of peach orchards were sampled, identified and evaluated for their potential as insectary plants. The survey revealed that *Paederia foetida* harbored large quantities of phytoseiid mites from May to October. The largest quantity of phytoseiid mites was detected on *Persicaria longisetum* in October. *Oxalis corniculata* had large quantities of phytoseiid mites in June and September. Thus, *P. foetida*, *O. corniculata*, and *P. longisetum* were selected as insectary plants. Generally, the phytoseiid mite species compositions were similar between peach leaves and *P. foetida, P.*

longisetum, and *O. corniculata*, especially after mid-summer. To determine the movement of phytoseiid mites from groundcover vegetation to peach leaves, *Petrobia harti* which occurs exclusively on *Oxalis* spp. was used. *P. harti* ribosomal Internal Transcribed Spacer sequences were detected from *E. sojaensis, A. eharai,* and *N. californicus* collected on peach leaves by PCR. These results suggest that insectary plants selected might contribute towards spider mite control through provision of phytoseiid mites on peach leaves. The functional significate of *P. foetida* as an insectary plant in regulating spider mite populations in peach orchards was examined. Field results showed that an exclusive occurrence of *P. foetida* generated a decreased spider mites population on peach leaves while presence of other wild plants diminished the effectiveness of *P. foetida* in keeping the spider mite below control threshold density. In conjunction with field studies, the decreased survival and fecundity of *Tetranychus kanzawai* on *P. foetida* at least implies that *P. foetida* was not a suitable host for spider mites. Increased survival of *N. californicus* on *P. foetida* in the laboratory studies and detection of *P. foetida* chloroplast sequences in phytoseiid mites suggest that phytoseiid mites can be promoted by *P. foetida* and can also utilize *P. foetida* when food resources are scares. In general, utilization of insectary plants like *P. foetida* can increase the efficiency of phytoseiid mites in managing the spider mite pests below control threshold densities.

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Chapter One

Spider mites are one of the most important plant-feeding mites worldwide and cause significant yield losses in many economically important fruit crops (van Leeuwen et al. 2013). They have the ability to develop faster with high fecundity rates in a conducive environment (Carrière 2003). Furthermore, as a specie regarded as haplodiploids, they have high potential of developing resistance to acaricides and can become troublesome to control (Croft and van de Baan 1988). Phytoseiid mites on the other hand are proven to be of great potential in managing outbreaks of spider mites (Helle and Sabelis 1985). However, mechanisms to which the efficiency of phytoseiid mites can be improved to its maximum potency are still a study in progress. This dissertation explores the mechanisms involved in improving the potential of phytoseiid mites through utilization of environmental factors such as groundcover plants in effectively managing spider mites at peach orchards.

1.1 Problems of spider mites in Japanese fruit orchards

Spider mites are one of the most serious pests on fruit orchards in Japan, as well as in other horticultural crops and to date, 78 spider mite species have been recorded in Japan (Takafuji et al. 2000). Six spider mite species: *Amphitetranychus viennensis*, *Panonychus citri*, *P. mori*, *P. ulmi*, *Tetranychus urticae*, and *T. kanzaw*ai are considered to be important pests for fruits including apple, Japanese pear, citrus, and clementine mandarin (Osakabe 1967; Ehara and Shinkaji 1975; Nakagaki 1980; Uchida 1982; Gomi and Gotoh 1996; Ehara 1999; Funayama 2010). Among them, *T. urticae, T. kanzawai, P. citri*, and *P. ulmi* are serious cosmopolitan pests and are now listed as pests of agricultural importance in the Plant Protection Law of Japan (Osakabe et al. 2009). In Japanese peach orchards, *P. mori* and *T. kanzawai* are dominant polyphagous pest spider mites (Ehara and Shinkaji 1996; Fujimto et al. 1996; Gotoh and Higo 1997; Sonoda et al. 2012).

1.1.1 Life cycle of spider mites

The life cycle of *T. kanzawai* consists of eggs, larva, quiescent nymph (protochryssalis) protonymph, quiescent protonymph (deutochrysalis), deutonymph, quiescent deutonymph (teleiochrysalis) and adult stages (Oku 2014). The development from eggs to adult stage takes as long as 10 days at an optimum conditions (25℃, 16L:8D) (Osakabe 1967). Female adults of *T. kanzawai* lay about 1 to 12 eggs per day for more than 20 days at the optimum conditions after ca. 1 day of pre-oviposition period (Kondo and Takafuji 1985). The morphology of *T. kanzawai* differs between sexes in that, females are deemed larger than the males (Macke et al. 2011). Females normally accepts only the first copulation for fertilization (Helle 1967; Oku 2008; Potter and Wrensch 1987; Satoh et al. 2001). Fertilized females disperse from their natal colonies and establish new colonies (Hussey and Parr 1963) while males can repeatedly mate (Krainacker and Carey 1989) hence remaining at the natal colony they originally settled in (Enders 1993; Kondo and Takafuji 1982).

It is known that *T. kanzawai* in moderate-temperature regions usually produce bimodal population peaks in early summer (June–July) and autumn (September–November) and remain at low density in mid-summer in tea (Osakabe 1959, 1967), Japanese pear (Uchida 1982) and hydrangea (Gotoh and Gomi 2000). Gotoh and Gomi (2000) suggested that host plant quality and predation are considered as factors responsible for the summer decline in population density of *T. kanzawai*. *T. kanzawai* passed the winter as diapausing females at Shizuoka Prefecture, central Japan, while in southern Japan, all stages could be found in winter, including both diapausing and nondiapausing females (Takafuji et al. 2003). Dispersion of *T. kanzawai* is affected by crawling, air currents, and plantation laborers. Fertilization of the tea bushes was also said to have been influencing the population development (Osakabe 1959). A positive correlation was found between the levels of nitrogen and phosphorus in the soil and mite density, but a negative correlation was found with potassium and manganese (Osakabe 1959, 1967). *T. kanzawai* feeds on a broad spectrum of naturally occurring plant species (Ho et al. 1989; Ho 1993; Ehara and Shinkaji 1996; Zhang et al. 1996; Gotoh and Gomi 2000).

P. mori distributes widely on peach trees in Okayama Prefecture, western Japan. Their occurrence is quite consistent and the densities of diapuasing eggs tend to be lower in orchards where densities of adult *P. citri* are high (Fujimoto and Hiramatsu 1995).

1.1.2 Haplodiploidy in spider mites

Evolution of insecticide/acaricide resistance is a global problem that provides some of the most compelling examples of adaptation by natural selection (Denholm and Rowland 1992; Onstad 2007; Tabashnik et al. 2008). Although nearly all techniques for analyzing inheritance of insecticide/acaricide resistance are focused on diploid insects, haplodiploidy is a key factor in influencing evolution of resistance in some of the important arthropod pests, including spider mites (Denholm et al. 1998; Carrière 2003; Crowder et al. 2006). Rapid development of insecticide/acaricide resistance is usually observed more in haplodiploid arthropods than in diploids (Croft and van de Baan 1988).

T. kanzawai has been observed as haplodiploids (Oku 2014) (Croft and van de Baan, 1988). The reproduction is parthenogenetic arrhenokous in *T. kanzawai*, to which unmated females produce only haploid male offspring while mated females produce both haploid male and diploid female offspring (Bull 1983; Byrne and Devonshire 1996; Heimpel and de Boer 2008). The sex ratios of offspring produced by mated females usually are female-biased, female: male ratio ranging from 2:1 to 3:1 (Kondo and Takafuji 1982; Laing 1969; Overmeer 1972). Carrière (2003) reviewed the haplodiploidy, sex and their evolution in insecticide/ acaricide resistance and indicated that haploid males are more susceptible against insecticides and acaricides compared to diploid females. Carrière (2003) further pointed out that males are generally less tolerant than females in both haplodiploid and diploid arthropods. Hence, perceiving the higher frequencies of female emergence compared to low males (Kondo and Takafuji 1982; Laing 1969; Overmeer 1972) alone could be the reason to the emergence of acaricide resistance in spider mites particular in that of *T. kanzawai*.

There are no reports of *P. mori* being observed to be haplodiploids in respect to acaricides, although there are reports on interaction of *P. mori* with wolbachia manipulating its reproductive incompatibities (Breeuwer and Jacobs 1996; Gotoh et al. 2005). Populations of *P. mori* are deemed to be sensitive to acaricides (Fujimoto and Hiramatsu 1995).

Haplodiplody has been documented to be a phenotypic trait that can be affected by abiotic factors such as temperature, humidity, photoperiod, and light conditions (Pianka 1988; Wrensch 1993). Female age was also reported to be influencing sex allocation in tetranychid mites (Shih 1979; Hamilton et al. 1986; de Moraes and McMurtry 1987; Krainacker and Carey 1988) and sexual size dimorphism and sex-dependent selection (Carrière 2003).

1.1.3 Pesticide resistance in spider mites

Insecticide/acaricide resistance is a genetic change in response to selection by toxicants that may impair control in the field and is termed as “development of an ability in a strain of pests to tolerate doses of toxicant which would prove lethal to the majority of individuals in a normal population of the same species" (Sawicki 1987).

In the mid-2010, Arthropod Pesticide Resistance Database (APRD); managed by scientists from Michigan State University and supported by Insecticide Resistance Action Committee (IRAC) compiled 9394 reports on resistance developed in 572 species of arthropods, of which 1130 reports refer to 82 species from the Acari subclass. Out of this number, 745 reports concern 39 species belonging to four families of phytophagous mites: Tetranychidae, Acaridae, Eriophyidae and Tenuipalpidae. Approximately 93% of reports deal with the resistance of spider mites, with *T. urticae* (53% of spider mite reports) and *P. ulmi* (26% of spider mite reports). The authors of the APRD created the list of the "top 20" resistant arthropod pests in the world, ranked by number of compounds with reported resistance. On this list, *T. urticae* and *P. ulmi* rank first and ninth, respectively, by data for 92 and 42 compounds for which the information about resistant populations exist (Whalon et al. 2008, 2010; Marčić et al. 2011). Resistance in mites to acaricides was first observed in *T. urticae* against ammonium potassium selenosulfide in 1937 (Singh 2010). Organophosphate resistance in *P. citri* was found in 1958 and it was the first case of resistance in mites reported in Japan (Seki 1958). Methyl demeton resistance in camine spider mite; *Tetranychus cinnabarinus* on ornamental plants was reported in Japan (Nomura and Nakagaki 1959). Yamada et al. (1986) reported *P. citri* showing resistance against benzomate (benzoxamate) in New York, USA. *T. kanzawai* is one of the major pests of agricultural crops in Japan developing resistance to various acaricides (Kuwahara 1984; Hamamura 1985; Tatara 1991; Mochizuki 1991). Osakabe (1971) reported resistance of *T. kanzawai* on tea, against phenocapton in Japan. *T. kanzawai* also has developed resistance against three different acaricides such as fenpyroximate, pyridaben, and tebufenpyrad released in 1991-1992 (Goka 1999). *P. mori* on the other hand, has not shown any resistance traits in the peach orchards of Okayama Prefecture (Fujimoto and Hiramatsu 1995).

1.2 Phytoseiid mites

Phytoseiid mites belonging to the family Phytoseiidae can be roughly estimated up to 1,700 species mostly of predatory function, free-living, terrestrial, and are quite common around the world, except for the Antarctica (Muma et al. 1970; McMurtry and Croft 1997). The life cycle consists of the egg, larva, protonymph, deutonymph and adult (Hoy 2011). The sources of food for phytoseiid mites covers pollen, fungi, scale insects, white flies, mites and other small arthropods (McMurtry et al. 2013). Phytoseiid mites have so far gained more attention because of their biological potential to manage important agricultural arthropods including spider mites. They have been included in some integrated pest management (IPM) programs. Some of the phytoseiid mite species reported so far for their biological potential and sold for use in biological control programs are: *Phytoseiulus persimilis*, a predacious mite to many spider mite species worldwide (Dosse 1958; McMurtry et al. 1978; Khalequzzaman et al. 2007); *Metaseilus* *occidentalis*, a predator on spider mites on apples, grapes, peaches and almonds, except the genera *Panonychus*, *Bryobia*, and *Eotetranychus* (Flaherty et al. 1981); *Typhlodromus pyri,* a predator on *T. urticae* and *P. ulmi* in Europe and apples in New Zealand (Wysoki 1985; Benuzzi et al. 1992; Easterbrook 1992; Decou 1994; Zacharda and Hluchy 1996); *Neoseiulus alpinus*, a predator of *Steneotarsonemus pallidus* on strawberries in Florida (Muma et al. 1970); *Euseius hibisci*, a predator of the six spotted mite, *Eotetranychus sexmaculatus* in California, USA (Muma and Denmark 1971). Phytoseiid mites including *Neoseiulus reticulatus*, *Amblyseius barkeri*, *Amblyseius cucumeris, Iphiseius degenerans, Amblyseius swirskii*, *Neoseiulus fallacis*, and *N. californicus* are also commonly used in managing agriculturally important pests such as spider mites, thrips, and other arthropod pests (Greco et al. 2005; Janssen and Sabelis 2015). The cost of pesticides, the development of pesticide resistance and sustaining competitive food exports to foreign markets dictate the need for new tactics of control. IPM programs can always use additional predators to help reduce pest problems (Capinera 2008).

1.2.1 Biological control potency of phytoseiid mites

In a broad sense, the concept of biological control is the regulation of another organism's population density by natural enemies at a lower average than would otherwise occur (Debach 1974). More specifically, biological control has been defined as the study, importation, augmentation and conservation of beneficial organisms for the regulation of population densities of other organisms rendered as pests (Debach 1964). When implementing a biological control program, there are certain desirable characteristics to look for in a control agent. Essentially, the natural enemy needs to be adapted biologically, physiologically and ecologically to the host (Doutt and Debach 1964). What constitutes a successful biological control agent varies depending on the situation, however, common desirable attributes include: high powers of dispersal and searching capacity, prey specificity, the ability to survive at low prey densities, reproductive potential, a potency of increase in population greater than the prey, voracity, synchronous distribution with prey, and the ability to tolerate pesticides (Messenger et al. 1976; Gerson and Smiley 1990; McMurtry 1982). McMurtry and Croft (1997) have suggested that the efficacy of phytoseiid mites as control agents depends on the rapid rate of population increase, the ability as predators to regulate spider mites at low equilibrium densities. Phytoseiid mites that regulate spider mites at these low densities generally have the ability to survive when starved, and can use alternative food, cannibalism, or predation on other phytoseiid species as means of survival during times when the favored prey are scarce (McMurtry and Croft 1997).

1.2.2 Susceptibilities of phytoseiid mites against insecticides and acaricides

The extensive use of acaricides has provoked an exceptionally intrinsic potential of mites in rapid development of acaricide resistance (Cranham and Helle 1985; Croft and van de Baan 1988; van Leeuwen et al. 2009), particularly in that of species from

Tetranychidae family, which has become a global phenomenon according to APRD and IRAC (Marčić et al. 2011). Biological control of spider mites by phytoseiid mites and other predators continually prove to be a successful alternative to conventional chemical control (Gerson and Weintraub 2007). Actually, the significance of phytoseiid mites for the management of spider mites has been increasingly realized during the last three decades; however use of phytoseiid mites is not a panacea for all spider mite pest problems (Talebi et al. 2008).

Acaricide resistance of phytoseiid mites both in field-developed resistance and in artificial selection has been observed in species including *A. fallacis* (Croft et al. 1976; Croft and Whalon 1982), *P. persimilis* (Fournier et al. 1985), *M. occidentalis* (Roush and Hoy 1981; Roush and Plapp 1982; Hoy 1985), and *T. pyri* (Overmeer and van Zon 1983). Most field-developed resistances have been of a monogenic, dominant type aroused from highly susceptible populations (Croft and van de Baan 1988).

1.3 Groundcover vegetation

Groundcover vegetation management may prove useful in the control of certain agricultural pests (Bugg and Ellis 1990). Groundcover vegetation management has been suggested as useful in attracting and providing food sources for certain insects. Bugg (1992) evaluated the dispersal of arthropods from groundcover vegetation to vegetable crops and suggested that groundcover vegetation manipulation may prove useful in management of natural enemies in pest control. A number of studies have evaluated the potential of phytoseiid mites as useful predators of spider mites as discussed in the previous sub-header. Groundcover vegetation provides important habitats for phytoseiid mites (McMurtry 1982). Muma (1961) found a greater number of phytoseiid mites in citrus trees with established groundcovers around them. Populations of *Tetranychus pacificus* were reduced when grasses were maintained in vineyards (Flaherty et al. 1972). Flaherty (1969) found that *Eotetranychus willamettei* was effectively kept at low levels by the predator *M. occidentalis* because the predator was feeding on alternative prey dwelling in the grasses.

M. occidentalis and *N. fallacis* overwintered in groundcover and dispersed to tree trunks and the canopy of apple trees (Lee and Davis 1968; McGroarty and Croft 1978; Johnson and Croft 1981; Tanigoshi et al. 1983). Tanigoshi et al. (1983) found that groundcover vegetation at the base of the apple trees aided in the survival of *N. fallacis* during the winter. However, Nyrop et al. (1994) found that at apple orchards in Massachusetts, USA, significant numbers of *N. fallacis* overwinter on the trees. *N. fallacis* also shows a strong tendency to disperse aerially when its prey are in short supply in the groundcover hence their movement up the trees (Johnson and Croft 1981; McMurtry and Croft 1997; Tixier et al. 1998).

The availability of pollen in early spring was also thought to have a greater influence in the population increases of the phytoseiid mites, *T. pyri* and *Zetzellia mali* than the abundance of the prey mite *Aculus schlechtendali* (Addison et al. 2000). On natural hedgerows consisting of elderberry and hornbeam located adjacent to vineyards in Italy, Duso et al. (2004) demonstrated a potential relationship between pollen availability and phytoseiid abundance. Non-crop vegetation may provide resources for natural enemies not found in crops such as shelter, overwintering sites and food sources particularly for a wide range of arthropods with primarily carnivorous feeding habits that need plants for pollen or nectar to complement prey (Gurr et al. 2004).

By understanding characteristics of vegetation that promote natural enemies, potential costs and losses in pest control could be decreased (Gurr et al. 2004). For instance, vegetation that provides nectar resources can increase activity of predators and parasitoids (Landis et al. 2000; Hooks et al. 2006; Winkler et al. 2006). This increased activity at field edges can translate into decreased crop damage in adjacent crops and therefore could provide direct benefits to offset costs and losses (Landis et al. 2000; Tscharntke et al. 2002; Bianchi et al. 2006; Tsitsilas et al. 2006). However the connection between natural enemy activity and pest control is not always clear (Gurr et al. 2000). Sustainable pest control usually involves many enemy species that have an impact on a particular prey and whose importance may change over time (Rosenheim 1998; Memmott et al. 2000; Symondson et al. 2002; Cardinale et al. 2003). Therefore, when assessing the overall benefits of adjacent vegetation on decreasing crop damage, the impact of a range of host/predator/prey interactions therefore needs to be assessed (Schmidt et al. 2003).

1.4 Objectives

While this study explores the mechanisms involved in improving the potential of phytoseiid mites through utilization of environmental factors such as groundcover plants in effectively managing spider mites at peach orchards, the supporting objective involves in evaluating the predator-prey interactions from community, behavioral, and population perspectives between the natural habitats and hosts. First, I examined the population dynamics of phytoseiid mites and spider mites at both aerial (peach leaves) and ground vegetation. Second, I determined the species composition of phytoseiid mites and their relevance to spider mite control. Third, I selected potential insectary plants harboring large amount of phytoseiid mites. Finally, I examined the effectiveness of insectary plants selected in spider mite control through both laboratory and field studies.

Chapter Two

Population dynamics and species composition of phytoseiid mites and spider mites on peach leaves

2.1 Introduction

Excessive use of pesticides for agricultural pest control has engendered the worldwide emergence of resistant pests, including spider mites. Spider mites have developed resistance to acaricides of various types. In spider mites, more than 700 cases of acaricide resistance have been reported (Mačić et al. 2011). Therefore, the importance of biological control of spider mites using natural enemies, including phytoseiid mites, has become increasingly attractive as an alternative to conventional chemical control (Gerson and Weintraub 2007). Phytoseiid mites have been successfully used to suppress densities of pests, such as spider mites, thrips, and whiteflies (Helle and Sabelis 1985; McMurtry and Croft 1997; van Lenteren 2001; Nomikou et al. 2002). Even though phytoseiid mites are not particularly voracious when compared with insect predators, the fact that some species prefer to feed on tetranychid mites, have short life cycles, high survivorship, better ability to thrive at low prey levels than most insects, and mass-rearing, make them the most important biological control agents of spider mites (van Lenteren 2001).

In all, 90 phytoseiid mite species have been reported in Japan (Ehara and Gotoh 2009). Phytoseiid mite species identification based on morphological characteristics alone is sometimes difficult because interspecific differences are often subtle and the collected individuals can lack distinguishing morphological features (Navajas et al. 1999; Jeyaprakash and Hoy 2002). Furthermore, the morphological identification of a large quantity of phytoseiid mites at the same time is almost impossible. Earlier, Sonoda et al. (2012) developed a high-throughput population survey method to examine numerous phytoseiid mite individuals collected on peach leaves and estimated phytoseiid mite species composition to evaluate the contribution of each phytoseiid mite species in spider mite control. However, the method was applicable for five phytoseiid mite species, *Neoseiulus califirnicus* (McGregor), *Amblyseius eharai* Amitai and Swirski, *Neoseiulus womersleyi* (Schicha), *Amblyseius tsugawai* Ehara, and *Euseius sojaensis* (Ehara) (Sonoda et al. 2012). Morphological characterization of phytoseiid mites collected on peach leaves and wild plants in peach orchards in 2011 showed the presence of additional phytoseiid mite species.

In this study, first I examined population dynamics of phytoseiid mites and spider mites at peach orchards with different pesticide practices. Second, I improved the method developed by Sonoda et al. (2012) to be applicable as a monitoring tool for the additional phytoseiid mite species and finally, using this improved method, I estimated the species composition of phytoseiid mites sampled from peach leaves.

2.2 Materials and methods

2.2.1 Study sites

This study was conducted at six and five commercial peach orchards in Kurashiki City in 2012 and 2013 respectively. The detailed information of the peach orchards (Sites I, Site II, Site III, Site IV, Site V and Site VI) is presented in Table 2.1. Site I was managed according to the Japanese Agricultural Standard for organic agricultural products, which does not allow the use of any synthetic chemicals for pest control. The wild ground-cover vegetation for Site I, Site II, Site IV, Site V, and Site VI was managed using a mowing machine. Non-selective herbicides, glyphosate and paraquat dichloride, were applied at Site III. Samplings were conducted every week in 2012 (from April 23 to November 12) and 2013 (from May 2 to October 22).

2.2.2 Sampling procedure

In 2012, a total of 160 leaves from 8 trees (20 leaves of each tree) were sampled for each orchard in that, Site I, Site II, Site III, Site IV, Site V, and Site VI. In 2013, survey was conducted at tree level at five orchards. Five, seven, four, nine, and five trees were selected, respectively, at Site I, Site II, Site III, Site IV and Site VI. Thirty leaves were sampled from each tree. Leaves were brushed using a brushing machine (Daiki Co. Ltd., Konosu, Japan) to collect mites. Mites collected in each sampling were mixed in a petri dish filled with 70% ethanol. After counting of mites, the phytoseiid mites in each sampling were collected and pooled irrespective of species, sex, and developmental stage in glass containers and were stored in 99.5% ethanol until DNA extraction.

2.2.3 DNA extraction

DNA extraction was performed according to the method described in Sonoda et al. (2012). Briefly, pooled samples of phytoseiid mites with more than five individuals were homogenized in 400 µl of extraction buffer (50 mM Tris–HCl pH 8.5, 10 mM EDTA, 100 mM NaCl, 2% SDS). The DNA was extracted twice with phenol: chloroform: isoamyl alcohol (25: 24: 1) and precipitated using 99.5% ethanol in the presence of 3 M sodium acetate (pH 5.2). The DNA pellet was washed with 70% ethanol and dissolved in 100 µl of TE (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). After treatment with RNase A, the DNA was purified once with phenol: chloroform: isoamyl alcohol (25: 24: 1), once with chloroform: isoamyl alcohol (24: 1) and was precipitated using 99.5% ethanol in the presence of 3 M sodium acetate (pH 5.2). The DNA pellet was washed with 70% ethanol and was dissolved in H_2O.

2.2.4 Identification of species-specific polymorphic sites on the 28S ribosomal gene

Morphological characterization of phytoseiid mites collected on peach leaves and wild plants in peach orchards in 2011 showed the presence of six (*N. californicus, N. womersleyi, A. eharai, A. tsugawai, E. sojaensis,* and *Typhlodromus vulgaris* (Ehara) and eight (*N. californicus, N. womersleyi, A. eharai, A. tsugawai, E. sojaensis, T. vulgaris, Scapulaseius okinawanus* (Ehara), and *Neoseiulus makuwa* (Ehara)) phytoseiid mite species, respectively. The nucleotide sequences of the 28S ribosomal gene (728 bp) from *N. californicus* (GenBank/EMBL/DDBJ accession nos. AB618055, AB618056, and AB618057), *N.*

womersleyi (accession nos. AB618060, AB618061, and AB618062), *A. eharai* (accession nos. AB618058 and AB618059), *A. tsugawai* (accession no. AB618063), *E. sojaensis* (accession nos. AB618064 and AB618065), *T. vulgaris* (accession no. AB862881), *S. okinawanus* (accession no. AB862880), and *N. makuwa* (accession no. AB862882) were compared (Fig. 2.1). Species-specific polymorphic sites for *N. californicus*, *N. womersleyi*, *A. eharai*, *A. tsugawai*, *E. sojaensis, T. vulgaris*, *S. okinawanus*, and *N. makuwa* were chosen, respectively at 492, 705, 624, 652, 465, 656, 475 and 479 to optimize the measurements of the peak signal intensities in the chromatogram in our sequence system (Fig. 2.1). The nucleotide sequence identity of the eight species was 93—98%.

2.2.5 Cloning of the 28S ribosomal gene sequences of phytoseiid mite species

The genomic DNA fragments corresponding to the 28S ribosomal gene were amplified from each DNA sample using PCR with primer set rD43 (5′-gacccgctgaacttaagcat-3′) and rD13dp (5′-cgtgtttcaagacgggtcaaataact-3′) (Sonoda et al. 2012). The PCR conditions were 30 cycles of 15 s at 94°C, 30 s at 60°C and 1 min at 72°C with subsequent final extension of 72°C for 7 min. Direct sequencing of amplified PCR products was conducted using a dye terminator cycle sequencing kit (Applied Biosystems, Carlsbad, CA, USA) and a DNA sequencer (3130xl; Applied Biosystems) with the internal primer rD25 (5´-gggaaagttgaaaagaactc-3´) (Sonoda et al. 2012) to confirm the presence or absence of each phytoseiid mite species in the products. A series of PCR products covering the eight phytoseiid mite species were cloned into pGEM-T East vector (Promega Corp., Madison, WI, USA). The nucleotide sequences inserted into the vector were confirmed by nucleotide sequencing. The nucleotide sequences were analyzed using Genetyx ver. 11 (Genetyx Corp., Tokyo, Japan). Thus, plasmids containing the 28S ribosomal gene sequences of the eight phytoseiid mite species were obtained.

2.2.6 Quantitative sequencing (QS) for the prediction of phytoseiid mite proportions

For the prediction of phytoseiid mite proportions, QS was conducted according to the method described by Sonoda et al. (2012). PCR was conducted using a plasmid containing the 28S ribosomal gene sequence of each species as templates under the same conditions as those described above to generate standard templates for QS. The PCR products derived from each species were purified using a PCR purification kit (QIAquick; Qiagen Inc., Hilden, Germany) and quantified. Purified PCR products of one species were mixed with those of the other species to produce the following molar ratios for the standard DNA template mixtures: 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, and 4:1. The standard DNA template mixtures were sequenced directly with the internal primer rD25 as described above. The peak nucleotide sequence signal intensities of both nucleotides at each species-specific polymorphic site were measured from the sequence chromatogram using software (Photoshop CS3 ver. 10.0.1; Adobe Systems Inc., San Jose, CA, USA) (Fig. 2.2). Then the signal ratio was calculated. The signal ratios were assessed against the corresponding molar ratio. Standard regression equations were generated using software (SIGMA plot ver. 11.2; Systat Software, Inc., San Jose, USA) (Fig. 2.3). Regression equations to estimate the phytoseiid mite species composition in unknown samples collected on wild plants were developed using the all eight phytoseiid mite species. For unknown samples collected on peach leaves, regression equations were developed using *N. californicus*, *N. womersleyi*, *A. eharai*, *A. tsugawai*, *E. sojaensis,* and *T. vulgaris*. Quadratic equations showed higher regression coefficients (r^2) than linear equations for both cases (data not shown). Therefore, the quadratic equations shown in Table 2.2 were used in this study. In the equations, x-axis and y-axis were nucleotide signal ratio [species-specific nucleotide signal/ (species-specific nucleotide signal + the other nucleotide signal)] and expected proportion at species-specific polymorphic site, respectively. For signal ratios of 0 and 1.0, the resulting frequencies were regarded as 0% and 100%, respectively, without incorporating it into the prediction equations.

PCR with primer set rD43 and rD13dp was conducted for unknown samples collected from peach leaves and wild plants. Amplified fragments were sequenced directly using the primer rD25. The presence or absence of phytoseiid mite species in unknown samples was confirmed at their respective species-specific polymorphic sites. Then, the prediction of proportions for phytoseiid mite species was conducted using the quadratic equations described above (Table 2.2). It is noteworthy that the proportions of phytoseiid mite species estimated using the QS-based method cannot be a prediction of their number, but their biomass (Sonoda et al. 2012)

2.3 Results

2.3.1 Population dynamics of phytoseiid mites and spider mites on peach leaves

Seasonal fluctuations of phytoseiid mites and spider mites at orchard level in 2012 were shown in Fig. 2.4. At Site I, the spider mite appearance was preceded by that of phytoseiid mites. At Site II, Site IV, Site V, and Site VI, phytoseiid mites populations generally appeared in response to the occurrence of spider mites. However, no such peak of phytoseiid mites was observed at Site III. During the survey period, no significant damage caused by spider mites was observed at any orchards including Site IV, where the spider mite density was temporary beyond the control threshold density for spider mites in Japanese peach orchards (one mite/ leaf) (Sonoda, S., personal communication).

The population dynamics examined at tree level in 2013 were shown in Fig. 2.5. Preceded appearance of phytoseiid mites was observed at all trees at Site I. Site II was the only site where the phytoseiid mite appearance responded to that of spider mites in all trees surveyed. Such phytoseiid mite appearance in response to spider mites was observed in some trees at Site IV and Site VI. At Site III, no such clear response of phytoseiid mites against spider mites was observed in any trees surveyed in 2013. Site II, Site IV, and Site VI had trees for which the spider mite densities were temporarily beyond the control threshold density for spider mites in Japanese peach orchards. In contrast, at Site I and Site III, spider mite densities were below the control threshold in all trees during the survey period.

2.3.2 Species composition of phytoseiid mites on peach leaves

The phytoseiid mite species composition on peach leaves at orchard level in 2012 is depicted in Fig. 2.6. The phytoseiid mite species composition changed during the survey period, as reported by Sonoda et al. (2012). Nevertheless, at orchard level in general, *N. californicus* was dominant at Site III. At Site II, Site V, and Site VI, *A. eharai* was more dominant than *N. californicus*. Further dominant existence of *A. eharai* was observed at Site IV. At Site I, *E. sojaensis* constituted a large share (Fig. 2.6).

Survey on the phytoseiid mite species composition was conducted at tree level in 2013 (Fig. 2.7). *E. sojaensis*, *N. californicus*, and *A. eharai* were dominating respectively at Site I, Site III, and Site II. These observations as they previously appeared at the survey conducted at orchard level was confirmed at tree level with some exceptions at Site IV. At Site II, in general, *N. californicus* constituted a large share from June to early July and then *A. eharai* became dominant after late July. Phytoseiid mite species composition varied among trees at Site VI. In addition to *N. californicus* and *A. eharai*, *T. vulgaris* showed a large share in some trees at Site VI.

2.4 Discussion

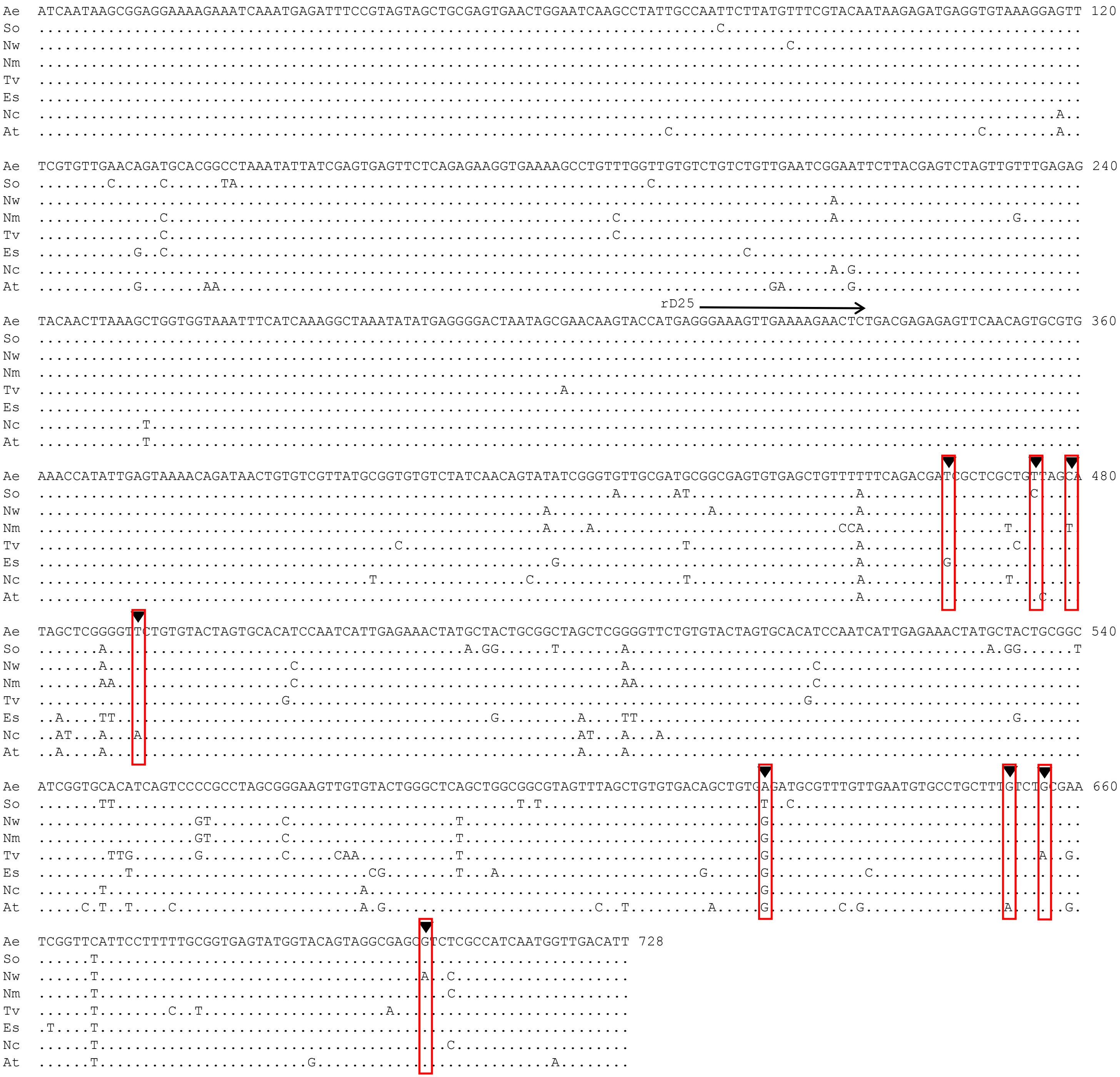
Population survey of spider mites and phytoseiid mites were conducted for two consecutive years at orchard level in 2012 and at tree level in 2013. In 2012 study, trees were collectively sampled in an orchard. The spider mite density was temporary beyond the control threshold density for spider mites in Japanese peach orchards only at Site IV. However, spider mite densities might be varied among peach trees even in the same peach orchard. Therefore, in 2013, trees were separately sampled in an orchard. Results revealed that no outbreak of spider mites beyond the control threshold density was only observed at Site I and Site III. In general, phytoseiid mites appeared in response to the occurrence of spider mites (Gotoh 1987), as observed at Site II, Site IV, Site V, and Site VI (Fig. 2.4). By contrast, at Site I, the appearance of spider mites was preceded by that of phytoseiid mites. The preceded appearance of phytoseiid mites, possibly significant for the successful spider mite control at Site I, might be attributable to the distinct phytoseiid mite species composition.

To examine the possibility, phytoseiid mite species composition were examined at orchard and tree levels. Results showed that *E. sojaensis* was dominant at Site I. Dominant existence of *E. sojaensis* was not observed at any other study sites. *E. sojaensis* is a generalist phytoseiid mite (type IV: specialized pollen feeders/ generalist predators) (McMurtry and Croft 1997). McMurtry (1992) reported that generalist phytoseiid mites play a vital role when spider mite densities are low. Ozawa and Yano (2009) showed that *E. sojaensis* can prey on adults of *T. kanzawai* as long as the predator settled on a plant before the prey in the laboratory. The successful suppression of spider mites by the generalist phytoseiid mite at Site I might be attributable to the earlier settlement of *E. sojaensis*. No temporal local outbreak of spider mites was also observed at Site III where *N. californicus* was dominant during the survey period (Figs. 2.5 and 2.7). On the other hand, at Site II, Site IV, and Site VI where *A. eharai* showed a large share, the temporal local outbreaks were observed in some trees (Fig. 2.5). Type II phytoseiid mites including

N. californicus have higher impact on tetranychid infestations than type III phytoseiid mites including *A. eharai* (McMurtry and Croft 1997). Total numbers of pesticide application up to harvest at Site II and Site IV were equal to that at Site III (Table 2.1). No temporary local outbreak observed at Site III might be attributable to differences in phytoseiid mite species composition (Figs. 2.6 and 2.7).

Reportedly, *Tetranychus urticae* Koch populations developed more quickly and with higher densities in trees over wild groundcover than bare ground in peach orchards (Meagher and Meyer 1990). Wild plants belonging to *Vicia*, *Geranium*, *Lamium*, and *Lepidium* hosted high densities of spider mites during early spring and might have become the source for later peach-tree infestation (Meagher and Meyer 1990). Therefore, the temporary local outbreaks of spider mites might also be attributable to quantitative differences in the ground cover harboring specific wild plants with larger quantities of spider mites.

Ground cover is reported to have potentials in promoting and or relegating phytoseiid mites (predators) (Muma 1961; Smith and Papacek 1991; Gravena et al. 1993; Liang and Huang 1994; Kasai et al. 2005; Aguilar-Fenollosa et al. 2011) as well as spider mites (pests) (Brandenburg and Kennedy 1981; Tedders et al. 1984, Meager and Meyer 1990) in a given orchard. Ground cover might contain wild plants harboring large quantities of spider mites and causing the temporal local outbreak of spider mites as observed at Site II, Site IV, and Site VI. On the other hand, possible insectary plants that enhances the functional significance of phytoseiid mites in spider mite control might be included in the ground cover. In the next chapter, groundcover plants inhabiting the undergrowth vegetation of peach orchards were sampled to select possible insectary plants that promotes phytoseiid mites in spider mite control.



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Ae  ATCAATAAGCGGAGGAAAAGAAATCAAATGAGATTTCCGTAGTAGCTGCGAGTGAACTGGAATCAAGCCTATTGCCAATTCTTATGTTTCGTACAATAAGAGATGAGGTGTAAAGGAGTT 120
So  ..............................................................................C.........................................
Nw  ......................................................................................C.................................
Nm  ........................................................................................................................
Tv  ........................................................................................................................
Es  ........................................................................................................................
Nc  .....................................................................................................................A..
At  ........................................................................C...................................C........A..

Ae  TCGTGTTGAACAGATGCACGGCCTAAATATTATCGAGTGAGTTCTCAGAGAAGGTGAAAAGCCTGTTTGGTTGTGTCTGTCTGTTGAATCGGAATTCTTACGAGTCTAGTTGTTTGAGAG 240
So  ........C.....C......TA...............................................C.................................................
Nw  ...........................................................................................A............................
Nm  ..............C...................................................C........................A....................G.......
Tv  ..............C...................................................C.....................................................
Es  ...........G..C..................................................................C......................................
Nc  ...........................................................................................A.G..........................
At  ...........G.......AA...............................................................GA.......G..........................
                                                                           rD25 --------------------->
Ae  TACAACTTAAAGCTGGTGGTAAATTTCATCAAAGGCTAAATATATGAGGGGACTAATAGCGAACAAGTACCATGAGGGAAAGTTGAAAAGAACTCTGACGAGAGAGTTCAACAGTGCGTG 360
So  ........................................................................................................................
Nw  ........................................................................................................................
Nm  ........................................................................................................................
Tv  ............................................................A...........................................................
Es  ........................................................................................................................
Nc  ............T...........................................................................................................
At  ............T...........................................................................................................

Ae  AAACCATATTGAGTAAAACAGATAACTGTGTCGTTATGCGGTGTGTCTATCAACAGTATATCGGGTGTTGCGATGCGGCGAGTGTGAGCTGTTTTTTCAGACGATCGCTCGCTGTTAGCA 480
So  ..................................................................A......AT...................A...................C.....
Nw  ..........................................................A..................A................A.........................
Nm  ..........................................................A....A............................CCA................T......T.
Tv  .........................................C................................T...................A.................C.......
Es  ...........................................................G..................................A.........G...............
Nc  ......................................T.................C.................T...................A................T........
At  ..............................................................................................A....................C....

Ae  TAGCTCGGGGTTCTGTGTACTAGTGCACATCCAATCATTGAGAAACTATGCTACTGCGGCTAGCTCGGGGTTCTGTGTACTAGTGCACATCCAATCATTGAGAAACTATGCTACTGCGGC 540
So  .......A.........................................A.GG......T.......A.........................................A.GG......T
Nw  .......A.....................C.....................................A.....................C..............................
Nm  .......AA....................C.....................................AA....................C..............................
Tv  ............................G...........................................................G...............................
Es  ..A....TT...........................................G.........A....TT...........................................G.......
Nc  ..AT...A...A..................................................AT...A...A................................................
At  ..A....A......................................................A....A....................................................

Ae  ATCGGTGCACATCAGTCCCCGCCTAGCGGGAAGTTGTGTACTGGGCTCAGCTGGCGGCGTAGTTTAGCTGTGTGACAGCTGTGAGATGCGTTTGTTGAATGTGCCTGCTTTGTCTGCGAA 660
So  .......TT..............................................T.T.........................T..C.................................
Nw  ..................GT........C...................T..................................G....................................
Nm  ..................GT........C...................T..................................G....................................
Tv  ........TTG.......G.........C.....CAA...........T..................................G...............................A..G.
Es  ..........T...........................CG........T...A.......................G......G...........C........................
Nc  .......T.............................A.............................................G....................................
At  .....C.T..T....C.....................A.G........................C..T.........A.....G........C.G................A......G.

Ae  TCGGTTCATTCCTTTTTGCGGTGAGTATGGTACAGTAGGCGAGCGTCTCGCCATCAATGGTTGACATT 728
So  ......T.............................................................
Nw  ......T.....................................A..C....................
Nm  ......T........................................C....................
Tv  ......T........C..T.....................A...........................
Es  .T....T.............................................................
Nc  ......T........................................C....................
At  ......T........................G...........................A........
```

Fig. 2.1. Alignment of the 28S ribosomal gene sequences in eight phytoseiid mite species. Identical nucleotides are represented by a dot. Ae, *Amblyseius eharai* (DDBJ/EMBL/GenBank accession no. AB621632); So, *Scapulaseius okinawanus* (accession no. AB862880); Nw, *Neoseiulus womersleyi* (accession no. AB621633); Nm, *Neoseiulus makuwa* (accession no. AB862882); Tv, *Typhlodromus vulgaris* (accession no. AB862881); Es, *Euseius sojaensis* (accession no. AB621635); Nc, *Neoseiulus californicus*, (accession no. AB621631); At, *Amblyseius tsugawai* (accession no. AB621634). Species-specific polymorphic sites are boxed.

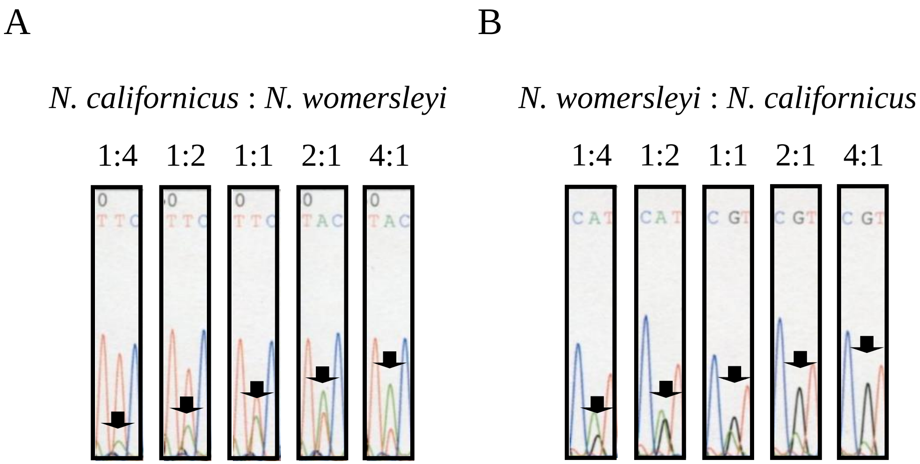


Fig. 2.2. Chromatograms for validation of quantitative sequencing for *N. californicus* (A) and *N. womersleyi* (B). Mixtures of genomic DNA of *N. californicus* and *N. womersleyi* with different ratios were subjected to PCR using the primer sets rD43 and rD13dp. Amplified PCR products were sequenced directly using the primer rD25. The numbers on the top of each column indicate the molar ratios of genomic DNA for each species. The relative intensities of the signals are indicated with arrows.

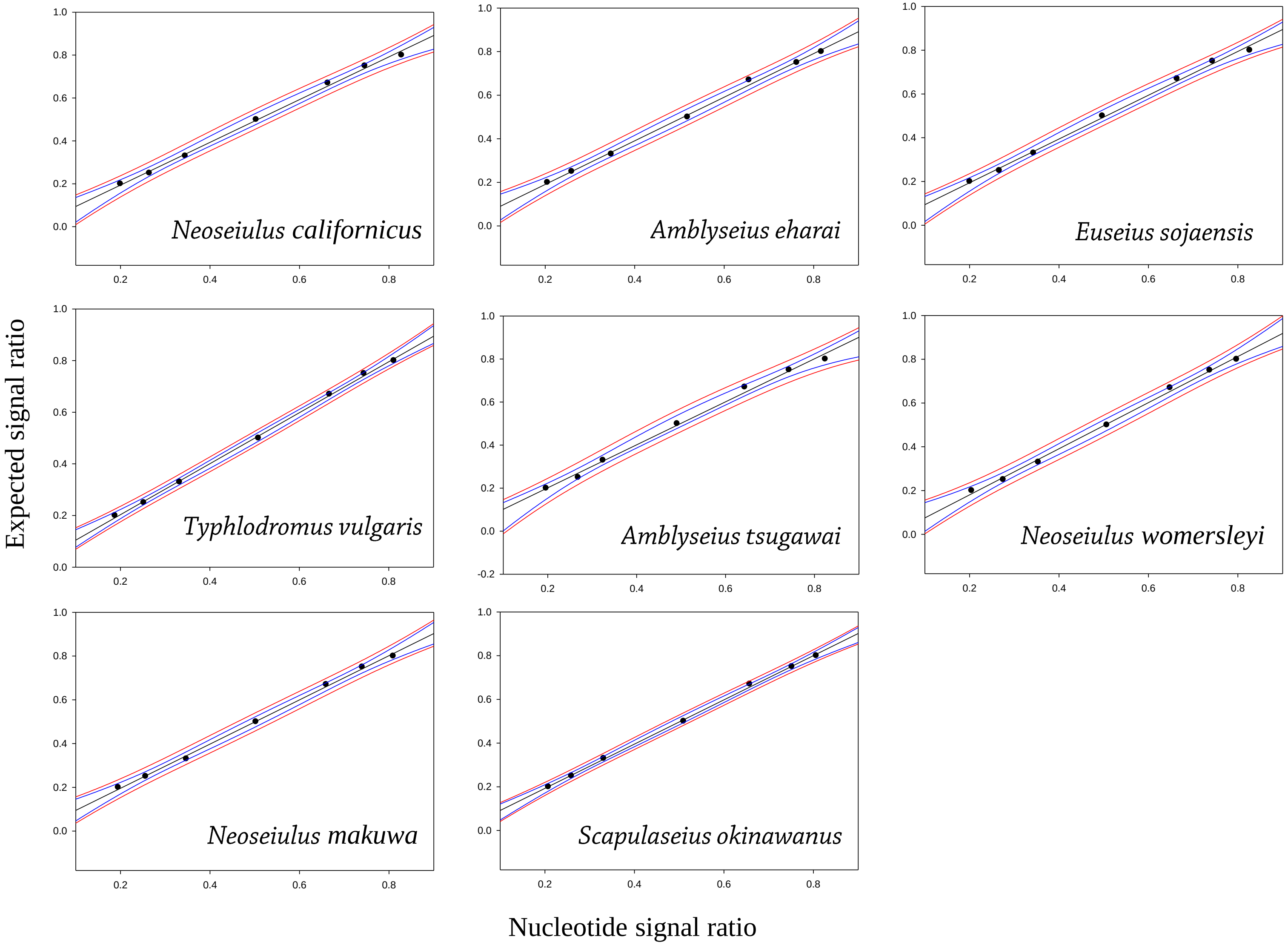


Fig. 2.3. Nucleotide signal ratios at species-specific sites for eight phytoseiid mite species obtained from direct sequencing were shown with corresponding expected signal ratios. Quadratic regression lines are shown as *solid black lines* with 95 % confidence lines (*blue*) and 95 % prediction lines (*red*). The nucleotide signal ratio (*x*-axis) was calculated as [species-specific nucleotide signal/(species-specific nucleotide signal + the other nucleotide signal)].

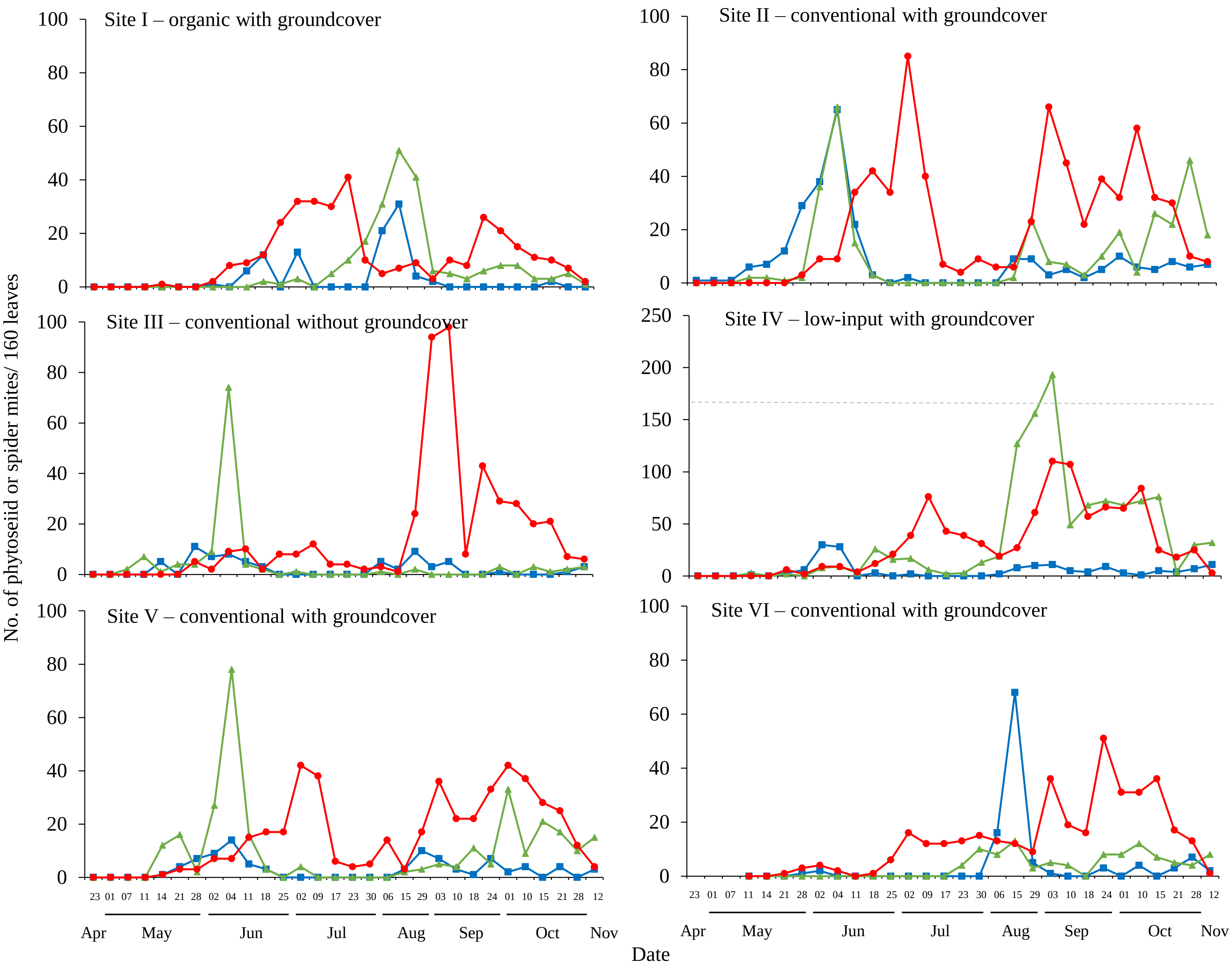


Fig. 2.4. Seasonal fluctuation in the number of phytoseiid mites and spider mites collected on peach leaves. Population survey was conducted at orchard level in 2012. Red, green and blue lines, respectively, present the number of phytoseiid mites, *Panonychus mori* and *Tetranychus kanzawai*. Broken line shows the control threshold density for spider mites in Japanese peach orchards (one mite per leaf).

Site I – organic with groundcover

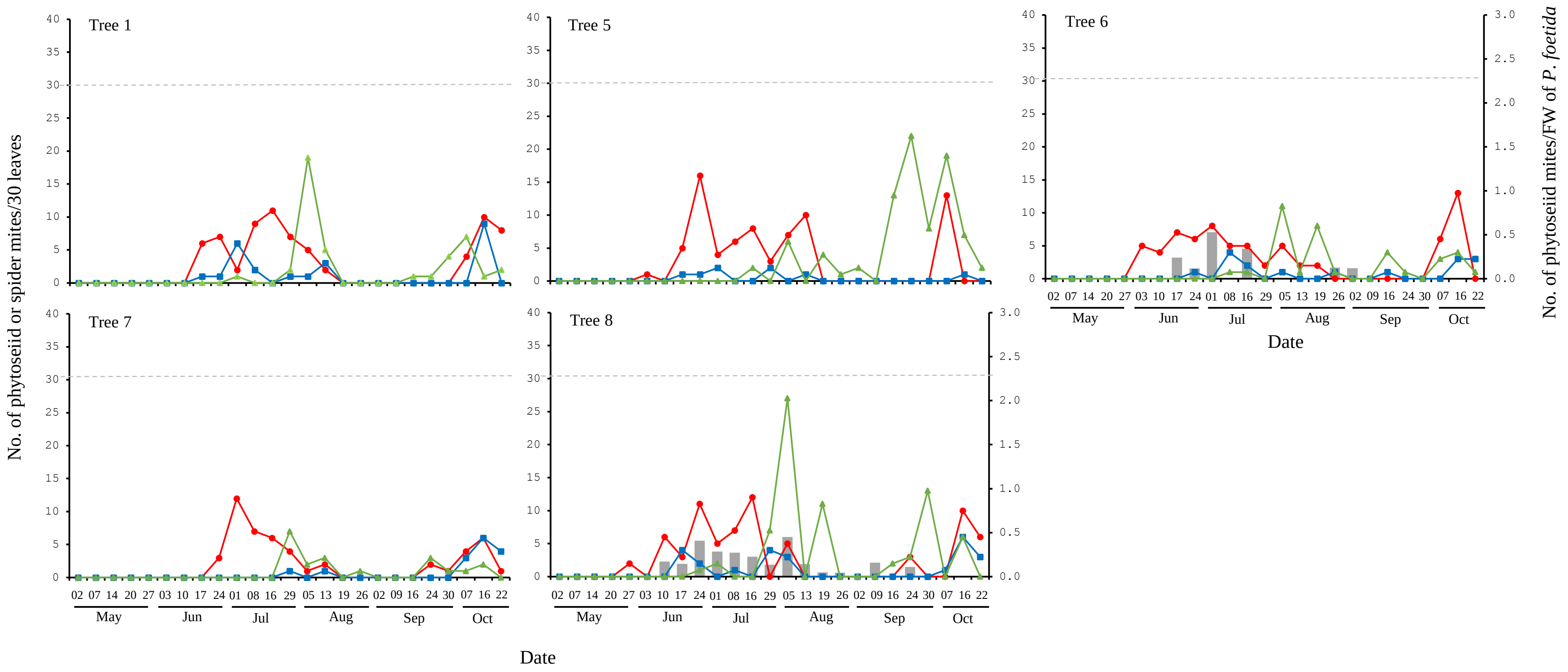


Fig. 2.5. Seasonal fluctuation in the number of phytoseiid mites and spider mites collected on peach leaves.Population survey was conducted at tree level in 2013. Red, green and blue respectively present the number of phytoseiid mites, *Panonychus mori* and *Tetranychus kanzawai*. Broken line shows the control threshold density for spider mites in Japanese peach orchards (one mite per leaf). Bars at Site I, Site II, and Site IV show seasonal fluctuation in the number of phytoseiid mites on *Paederia foetida* (see Chapter 3).

Site II – conventional with groundcover

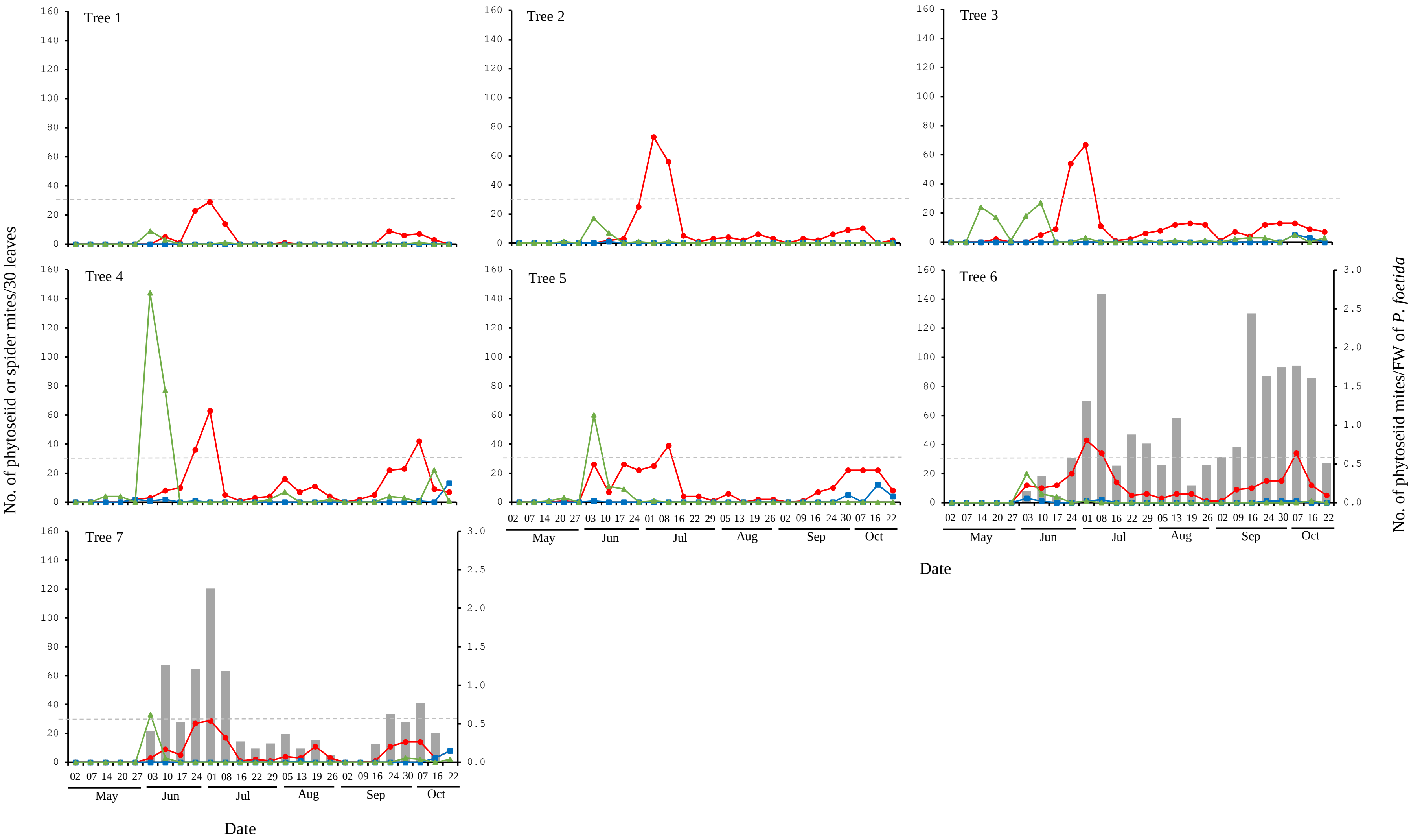


Fig. 2.5 Continued

Site III – conventional without groundcover

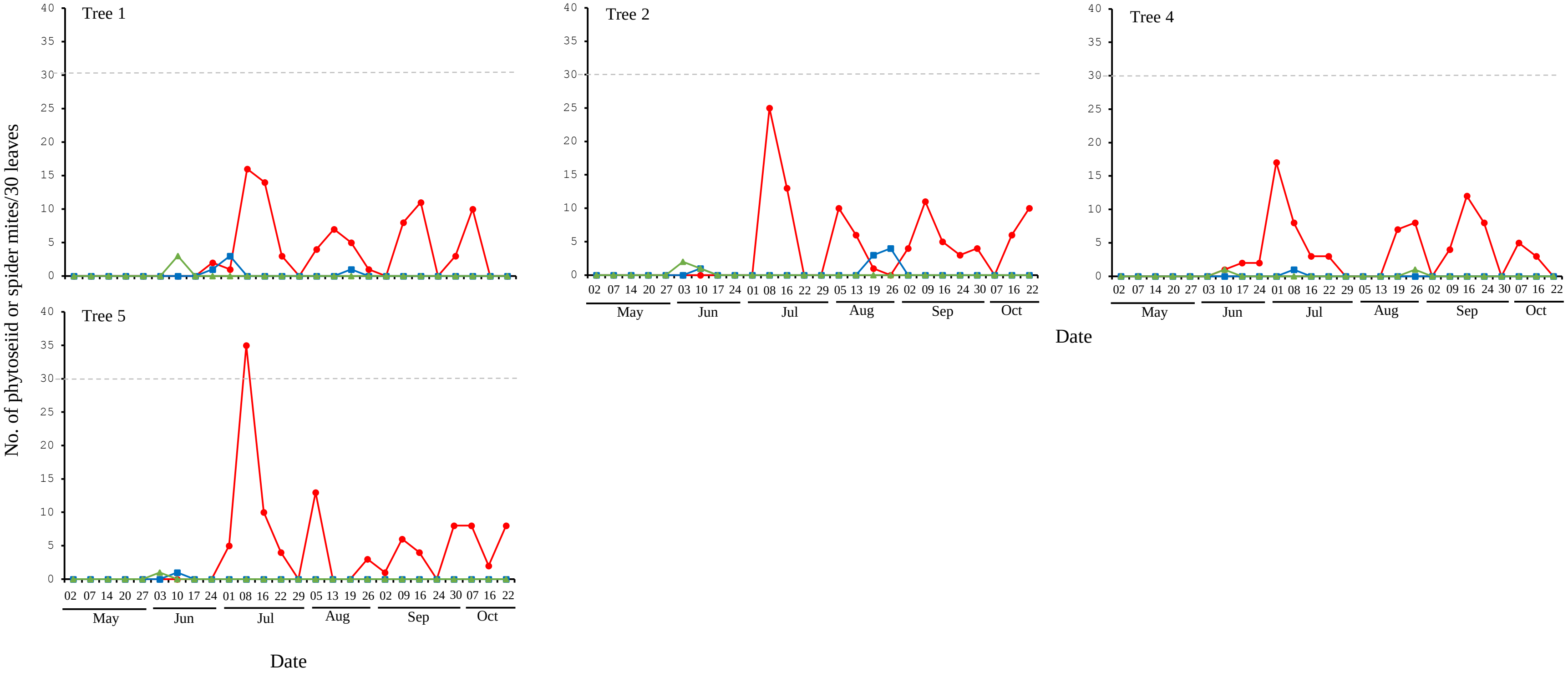


Fig. 2.5 Continued

Site IV – Conventional with groundcover

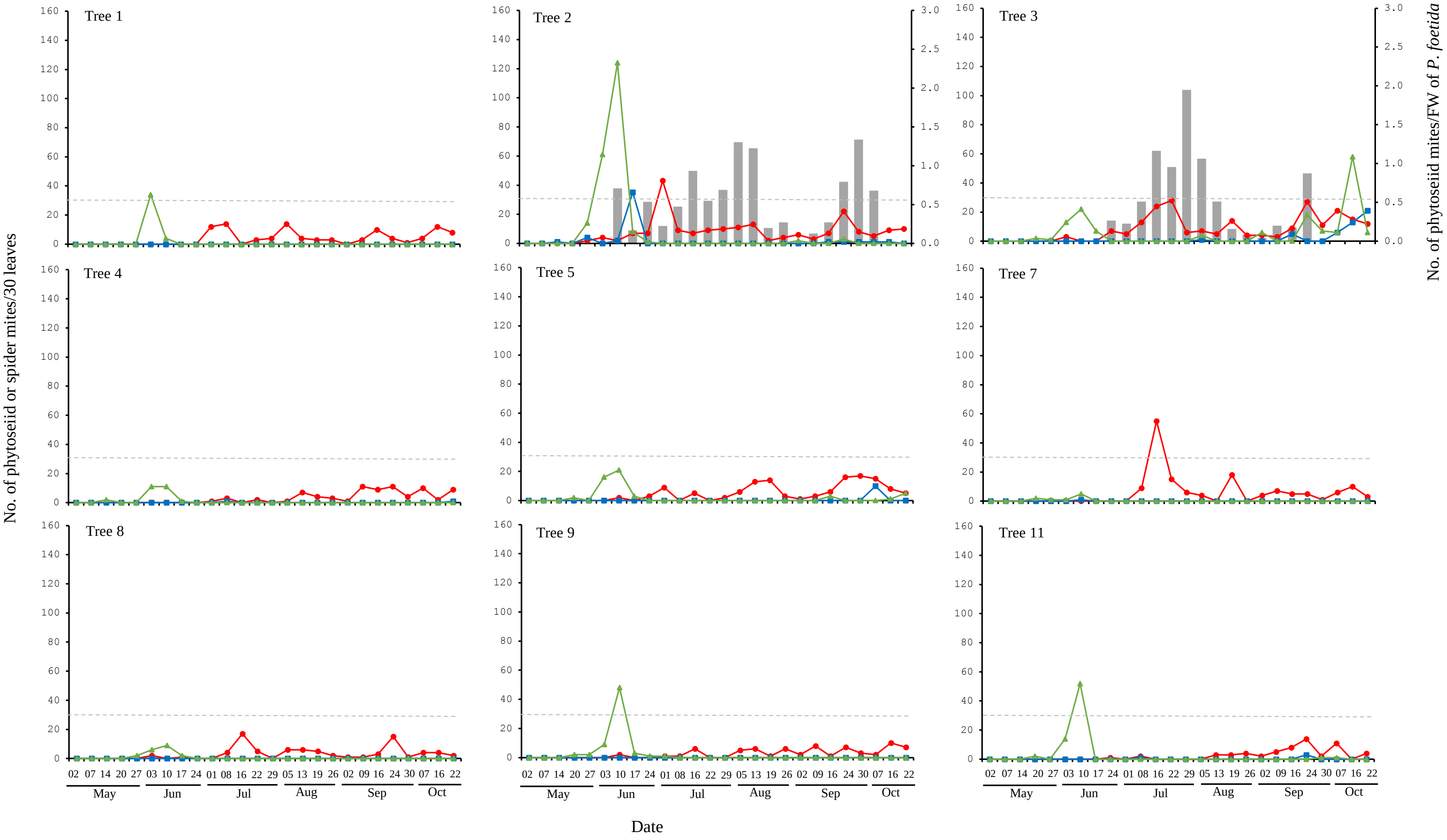
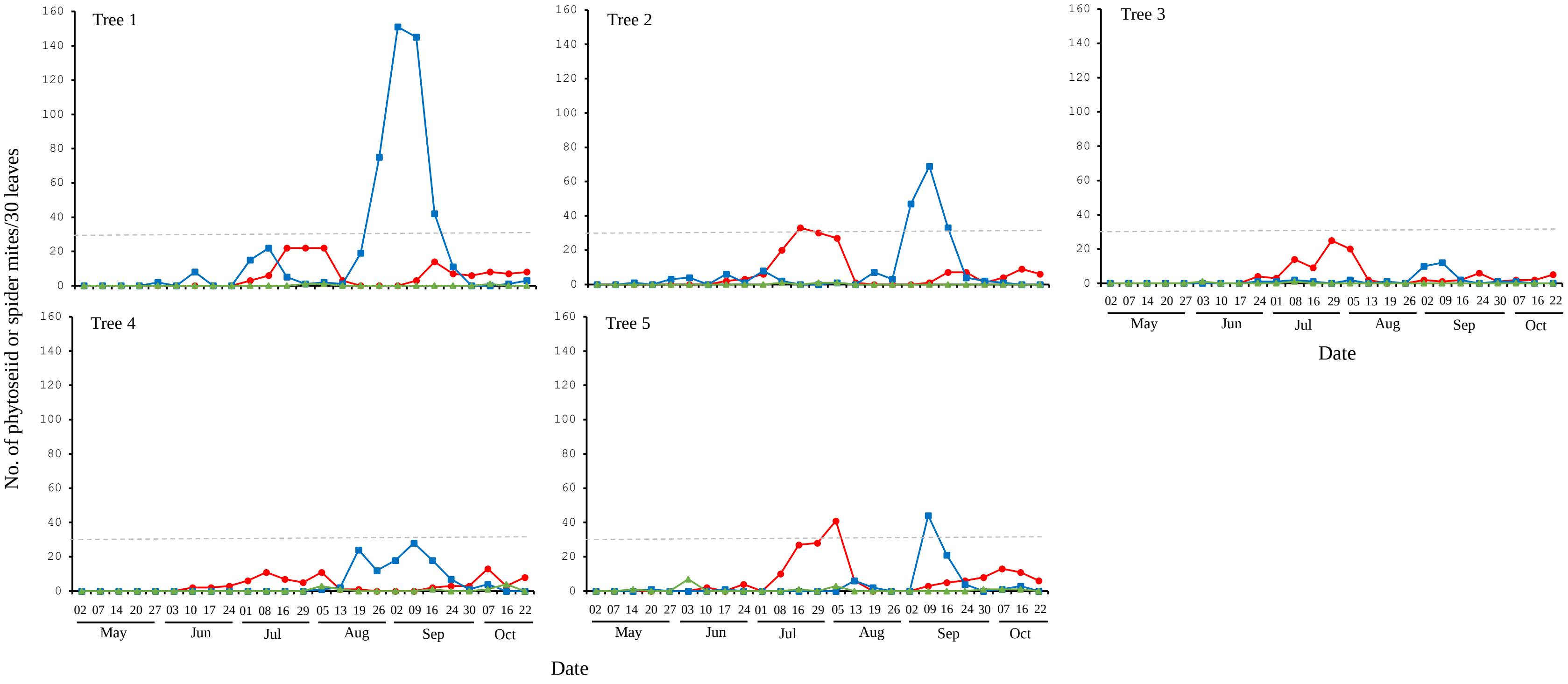


Fig. 2.5 Continued

Site VI – conventional with groundcover



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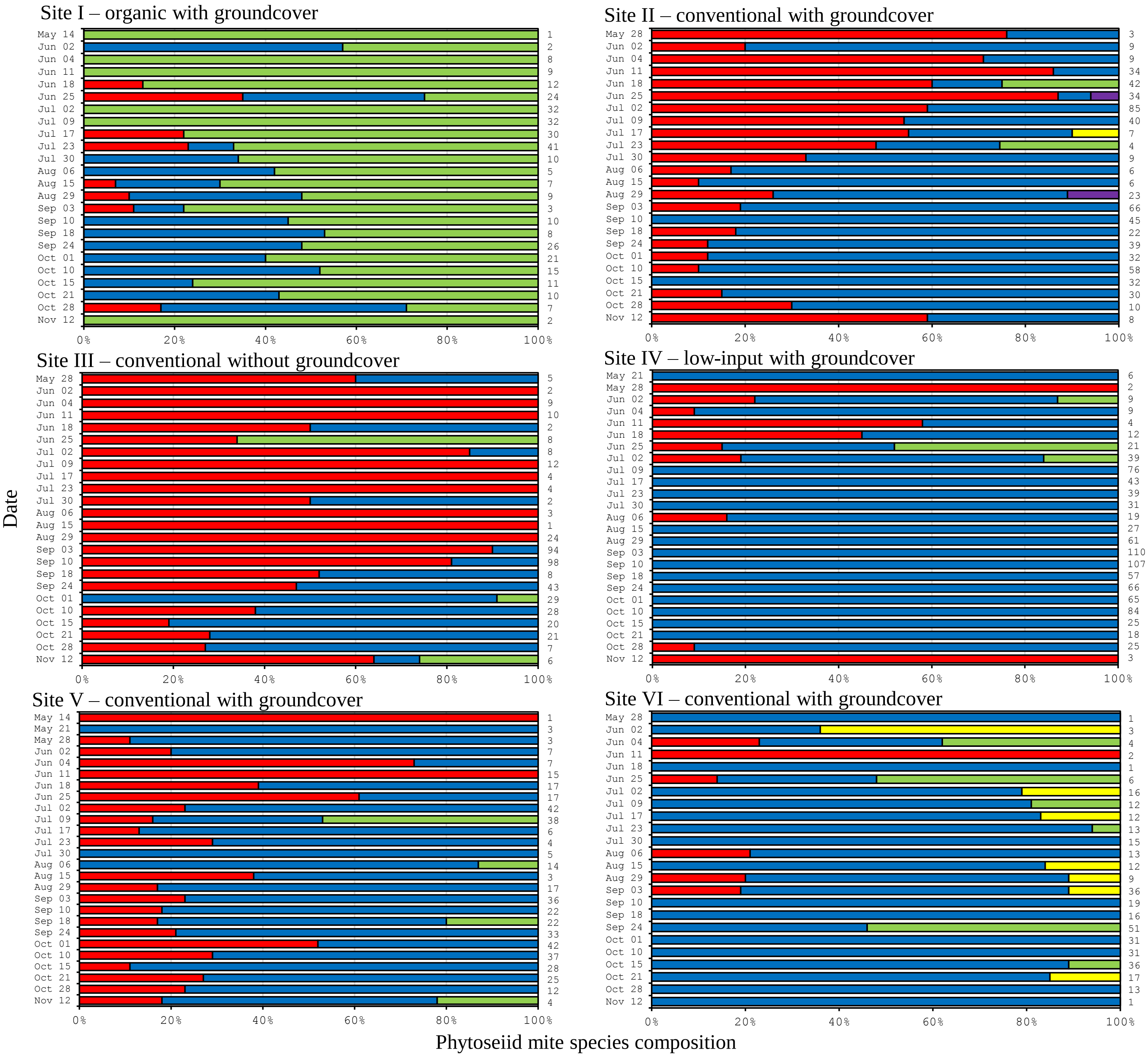

Site I – organic with groundcover

Site II – conventional with groundcover

Site III – conventional without groundcover

Site IV – low-input with groundcover

Site V – conventional with groundcover

Site VI – conventional with groundcover

Fig. 2.6. Seasonal fluctuation of phytoseiid mite species composition on peach leaves at orchard level. The frequency of each species was normalized as the sum of the frequencies 100%. Green, red, blue, yellow and purple bars respectively represent proportions of *Euseius sojaensis*, *Neoseiulus californicus*, *Amblyseius eharai*, *Typhlodromus vulgaris* and *Amblyseius tsugawai*. Numbers on the right column represent the number of individuals examined per survey.

Site I – Organic with groundcover

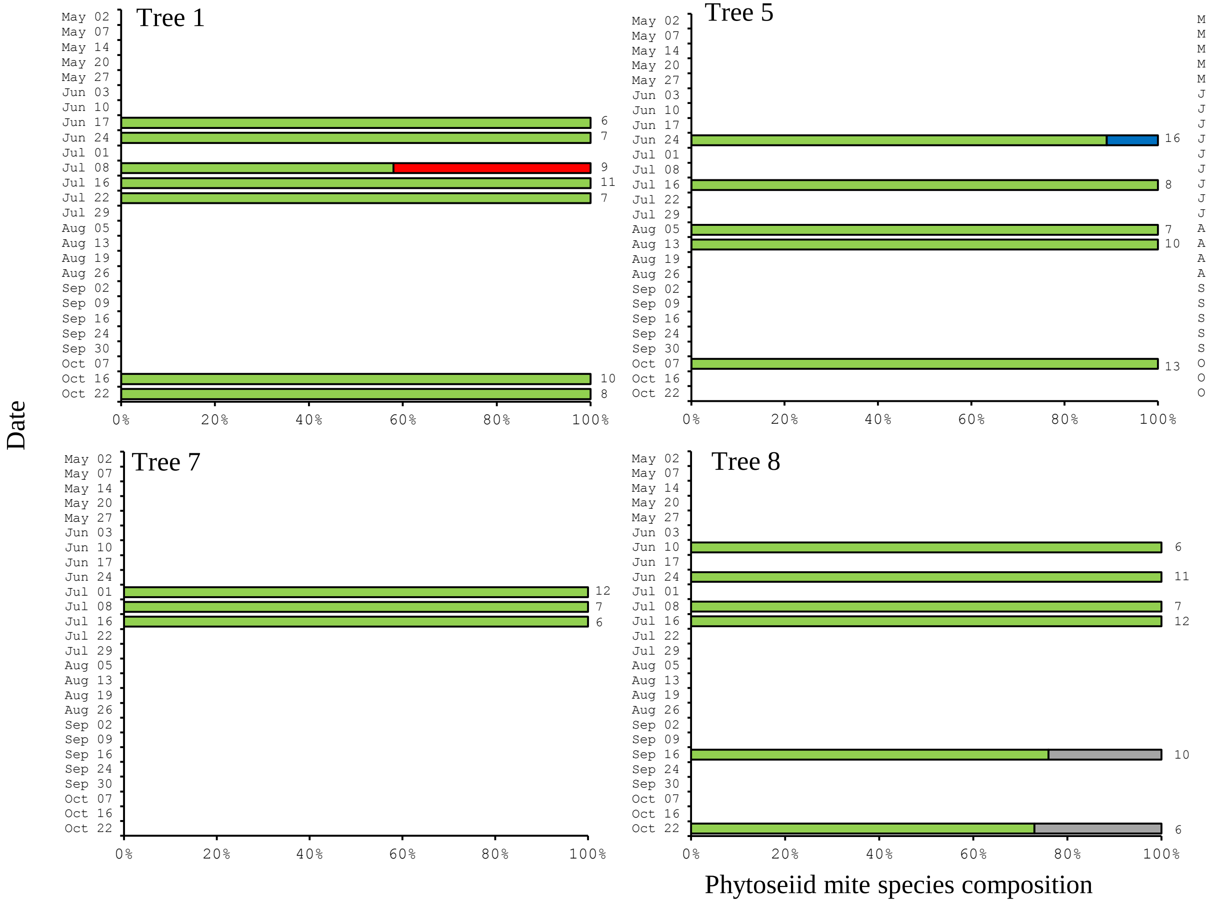

Fig. 2.7. Seasonal fluctuation of phytoseiid mite species composition on peach leaves at tree level. The frequency of each species was normalized as the sum of the frequencies 100%. Green, red, blue, yellow, purple and grey bars respectively represent proportions of *Euseius sojaensis, Neoseiulus californicus*, *Amblyseius eharai*, *Typhlodromus vulgaris*, *Amblyseius tsugawai* and *Neoseiulus* makuwa. Numbers on the right column represent the number of individuals examined per survey.

Site II – conventional with groundcover

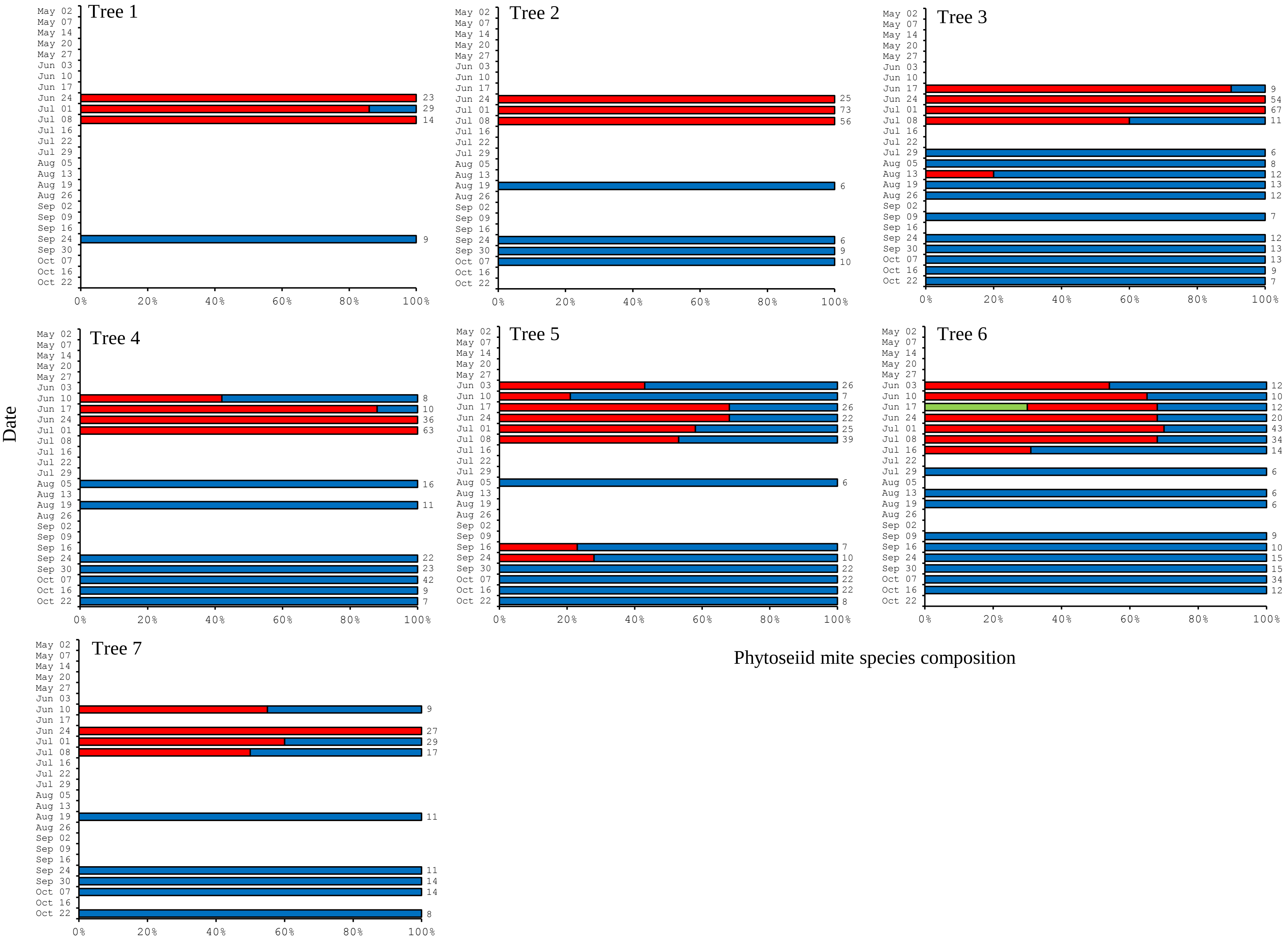

Fig. 2.7 Continued

Site III – conventional without groundcover

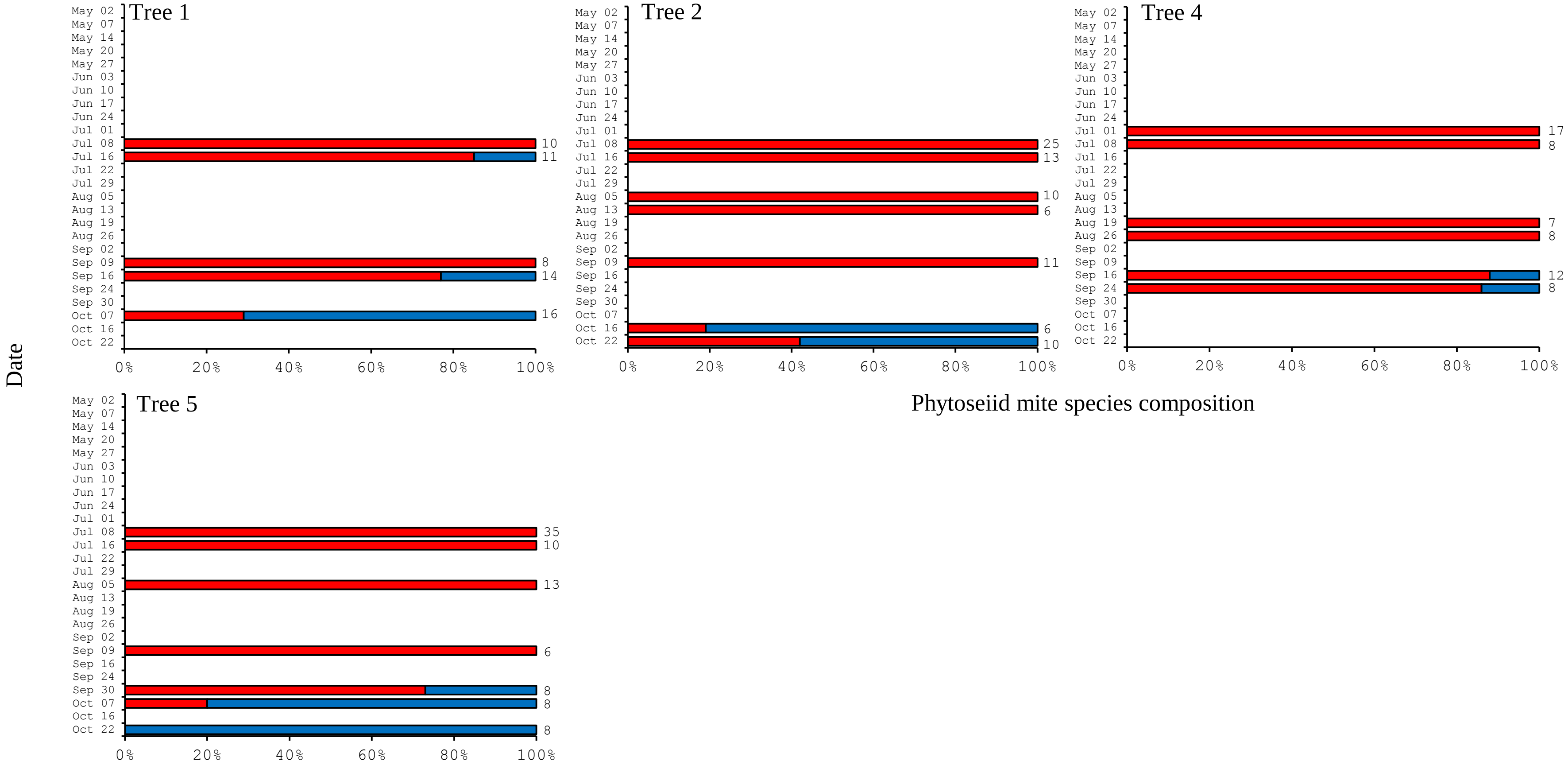

Fig. 2.7 Continued

Site IV – Conventional with groundcover

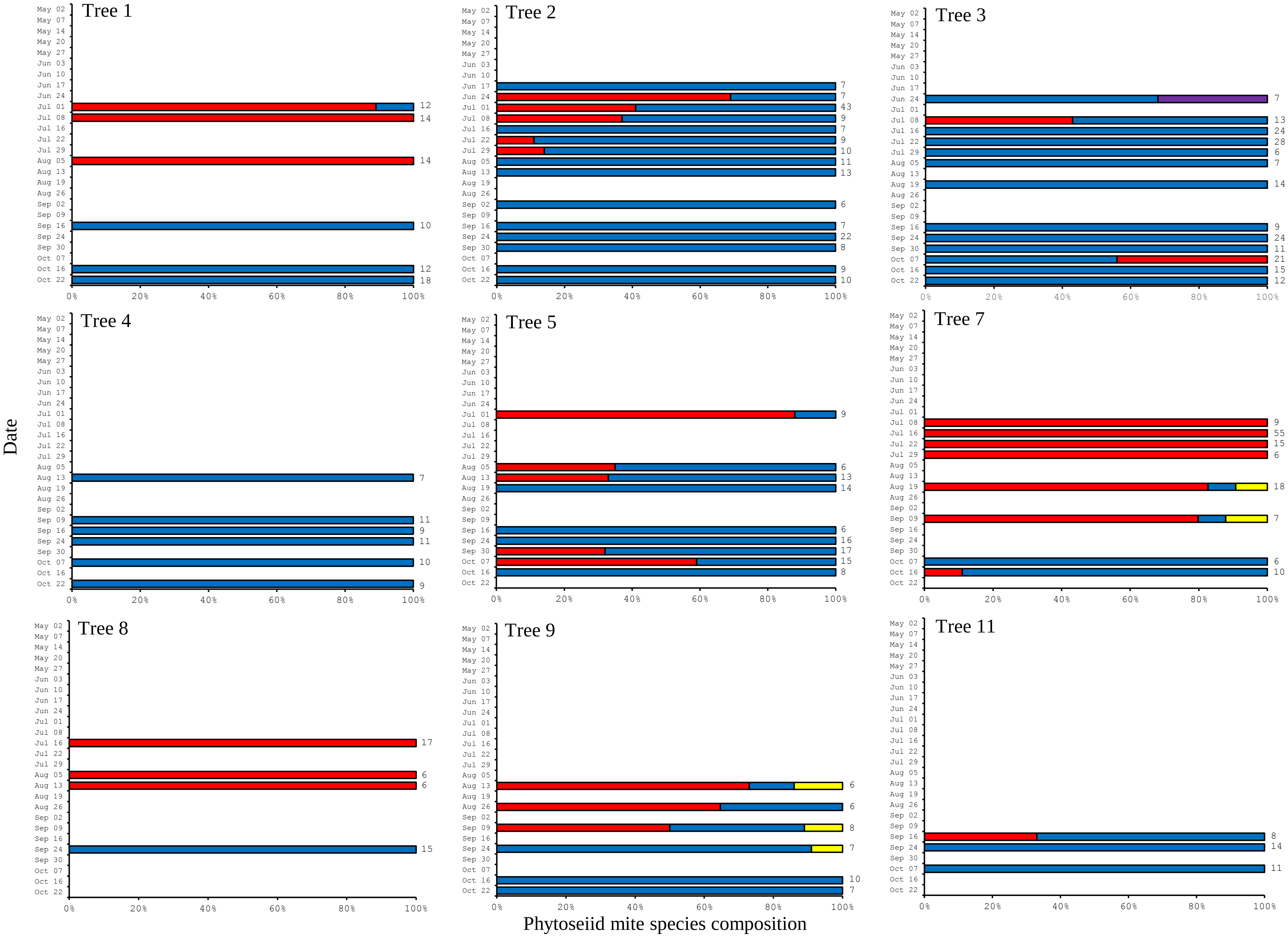

Fig. 2.7 Continued

Site VI – Conventional with groundcover

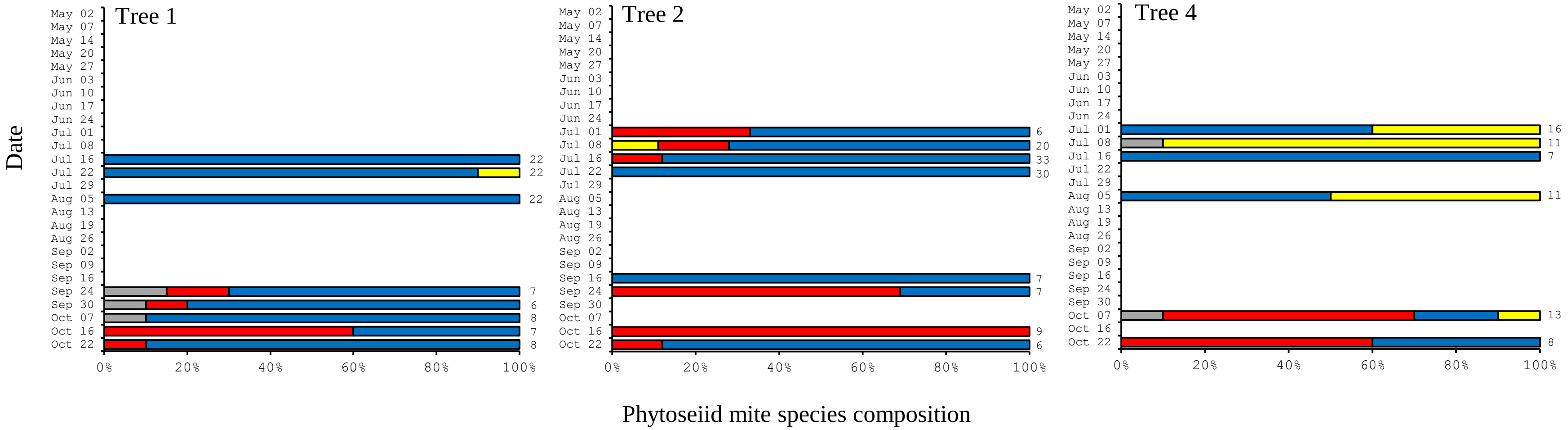

Fig. 2.7 Continued

Table 2.1 Location, area, and pest control of each study site in 2012 and 2013

Plot	Latitude/ longitude	Area (m^2)	2012		2013	
			Product applied	Farm type and weed management	Product applied	Farm type and weed management
Site I	N34° 35'05.9" E133° 39'36.7	2440	No synthetic chemicals	Organic/ groundcover	BT (Apr 17)	Organic/ groundcover
Site II	N34° 35'02.7" E133° 39'41.1"	1500	permethrin (Apr 24) chlorantraniliprole, etoxazole (Jun 3) thiacloprid (Jun 14) dinotefuran (Jun 30) tolfenpyrad (Jul 10) DMTP (Sep 3) MEP (Oct 12)	Conventional/groundcover	permethrin (Apr 22) buprofezin (May 14) chlorantraniliprole, etoxazole (Jun 3) thiacloprid (Jun 14) dinotefuran (Jun 30) tolfenpyrad (Jul 10) DMTP (Sep 6) MEP (Oct 12)	Conventional/groundcover
Site III	N34° 35'04.0" E133° 39'40.2"	400	permethrin (Apr 23) chlorantraniliprole, etoxazole (Jun 9) thiacloprid (Jun 17) dinotefuran (Jun 27) tolfenpyrad (Jul 7) acetamiprid (Jul 19) flubendiamide (Aug 18)	Conventional/No groundcover	permethrin (Apr 28) chlorantraniliprole, etoxazole (Jun 6) thiacloprid (Jun 17) dinotefuran (Jun 30) tolfenpyrad (Jul 7) acetamiprid (Jul 19) flubendiamide (Aug 15)	Conventional/No groundcover
Site IV	N34° 35'02.4" E133° 39'37.8"	2900	dinotefuran (May 13) dinotefuran, bifenazate (Jun 2) thiacloprid (Jun 23) acetamiprid (Jul 10)	Low-input/groundcover	tolfenpyrad (Apr 16) thiacloprid (May 5) alanycarb, bufezin (Jun 1) etoxazole (Jun 10) acetamiprid (Jun 23) thicloprid, cyenopyrafen (Jul 12)	Conventional/groundcover
Site V	N34° 35'02.3" E133° 39'39.9	3000	permethrin (Apr 24) chlorantraniliprole, etoxazole (Jun 3) thiacloprid (Jun 14) dinotefuran (Jun 30) tolfenpyrad (Jul 10) DMTP (Sep 3) MEP (Oct 12)	Conventional/groundcover	permethrin (Apr 22) buprofezin (May 14) chlorantraniliprole, etoxazole (Jun 3) thiacloprid (Jun 14) dinotefuran (Jun 30) tolfenpyrad (Jul 10) DMTP (Sep 6) MEP (Oct 12)	Conventional/groundcover
Site VI	N34° 35'06.7" E133° 39'38.8"	1400	alanycarb (Apr 19) permethrin (Apr 25) diazinon (Apr 30) buprofezin (May 8) acrinathrin (May 19) teflubenzuron (May 27) acetamiprid, cyenopyrafen (Jun 4) alanycarb (Jun 7) fenpropathrin (Jun 11) flubendiamide (Jun 12) No information after Jun 12	Conventional/groundcover	permethrin (Apr 17) alanycarb (Apr 29) buprofezin (May 9) alanycarb (May 22) acetamiprid, cyenopyrafen (Jun 4) thiacloprid (Jun 4) dinotefuran (Jun 28) tolfenpirad (early Jun) acetamiprid (mid Jun) flubendiamide (early Aug)	Conventional/groundcover

Table 2.2 Quadratic regression equations of phytoseiid mite proportions on peach leaves and wild plants

Plants	Species	Regression equation	Regression coefficient (r^2)	95% prediction equation	
				Upper	Lower
Peach	*Euseius sojaensis*	$y = 15.7 + 46.4x + 50.2x^2$	0.9967	$y = 22.9 + 27.6x + 68.9x^2$	$y = 8.6 + 65.2x + 31.4x^2$
	Amblyseius eharai	$y = 4.4 + 100.0x - 6.1x^2$	0.9912	$y = 15.6 + 69.9x + 24.0x^2$	$y = -7.1 + 130.1x - 36.2x^2$
	Neoseiulus californicus	$y = 3.1 + 89.2x + 3.5x^2$	0.9912	$y = 14.4 + 59.5x + 33.2x^2$	$y = -8.5 + 118.9x - 26.2x^2$
	Neoseiulus womersleyi	$y = 3.1 + 105.3x - 10.1x^2$	0.9982	$y = 8.4 + 91.5x + 3.7x^2$	$y = -2.1 + 119.1x - 23.9x^2$
	Amblyseius tsugawai	$y = 10.7 + 56.2x + 38.7x^2$	0.9973	$y = 17.1 + 39.5x + 55.4x^2$	$y = 4.3 + 72.9x + 22.0x^2$
	Typhlodromus vulgaris	$y = 0.5 + 119.0x - 24.9x^2$	0.9982	$y = 5.65 + 105.4x - 11.3x^2$	$y = -4.7 + 132.5x - 38.4x^2$
Wild plant	*Euseius sojaensis*	$y = -4.2 + 117.4x - 15.0x^2$	0.9978	$y = 1.8 + 102.0x - 2.1x^2$	$y = -10.1 + 132.8x - 31.9x^2$
	Amblyseius eharai	$y = -1.6 + 103.3x - 3.2x^2$	0.9978	$y = 4.5 + 87.5x + 12.2x^2$	$y = -7.7 + 119.2x - 18.5x^2$
	Neoseiulus californicus	$y = -3.4 + 113.4x - 13.5x^2$	0.9978	$y = 2.6 + 98.2x + 1.3x^2$	$y = -9.3 + 128.6x - 28.2x^2$
	Neoseiulus womersleyi	$y = -2.3 + 101.6x + 3.8x^2$	0.9976	$y = 4.9 + 80.9x + 243x^2$	$y = -9.5 + 122.3x - 16.7x^2$
	Amblyseius tsugawai	$y = -6.0 + 130.4x - 29.9x^2$	0.9971	$y = 0.8 + 112.8x - 12.7x^2$	$y = -12.8 + 148.04x - 47.3x^2$
	Typhlodromus vulgaris	$y = 1.8 + 92.8x + 5.9x^2$	0.9992	$y = 5.2 + 83.9x + 14.8x^2$	$y = -1.7 + 101.6x - 2.9x^2$
	Neoseiulus makuwa	$y = -0.3 + 99.4x + 1.6x^2$	0.9983	$y = 4.9 + 85.5x + 15.5x^2$	$y = -5.6 + 113.4x - 12.3x^2$
	Scapulaseius okinawanus	$y = -2.3 + 107.8x - 6.5x^2$	0.9992	$y = 1.5 + 97.7x + 3.4x^2$	$y = -6.1 + 117.9x - 16.5x^2$

Chapter Three

Selection of insectary plants and their possible role in spider mite control

3.1 Introduction

Insectary plants providing refugia, alternate food, and places to breed has been suggested for promotion of the occurrence of phytoseiid mites (Gravena et al. 1993). Gravena et al. (1993) showed that the presence of *Ageratum conyzoides* L. (Asteraceae) and *Eupatorium pauciflorum* Humboldt, Bonpland and Kunth (Asteraceae) in citrus orchards in Brazil led to an increase in phytoseiid mite densities, compared to a bare ground management. *A. conyzoides* planted or conserved for citrus production in China is also recognized as a beneficial weed to natural enemies including phytoseiid mites, *Amblyseius* spp., for control of the citrus red mite, *P. citri* McGregor, through provision of the pollen as alternative food (Liang and Huang 1994). In citrus production in Australia, the Rhodes grass *Chloris gayana* Kunth is sown to provide pollen as alternative food for a phytoseiid mite, *Amblyseius victoriensis* (Womersley) (Smith and Papacek 1991). Leguminous cover crops were shown to enhance population densities of a phytoseiid mite *Euseius tularensis* (Congdom) in citrus production in California (Grafton-Cardwell et al. 1999). Leguminous cover crops were also shown to act as a reservoir for phytoseiid mites in citrus orchards in Guadeloupe (Mailloux et al. 2010). Aguilar-Fenollosa et al. (2011) showed that a mono-specific grass ground cover, e.g. *Festuca arundinacea,* exhibits better regulation of *T. urticae* than wild cover, because of increase of phytoseiid mites represented by *Galendromus* and some *Neoseiulus* species in clementine mandarin orchards.

In this study, wild plants species inhabiting the undergrowth vegetation of peach orchards were sampled, identified and evaluated for their potential as insectary plants. Wild plants species harboring larger quantities of phytoseiid mites were selected. Population dynamics of phytoseiid mites on *Paederia foetida* (L.) harboring large quantities of phytoseiid mites during survey period was examined. Phytoseiid mite species composition was also examined on *P. foetida* using the QS-based method described in Chapter two. Subsequently I examined feeding preferences of phytoseiid mites for spider mites in the field using PCR. Finally, I used PCR to examine the dispersal of phytoseiid mites from groundcover plants to peach leaves to evaluate the usefulness of groundcover plants as a source for phytoseiid mites on peach trees.

3.2 Materials and methods

3.2.1 Study site and sampling

The study sites are described in Table 2.1. The survey was conducted from April 23 to November 12 in 2012 and from May 2 to October 22 in 2013.

A total of 219 wild plant species inhabiting the five orchards (Site I, Site II, Site IV, Site V, and Site VI) with enough quantities for sampling were randomly collected at each orchard. Wild plants were not collected at Site III where the ground-cover vegetation was managed with herbicides. Wild plant species collected in each sampling were weighed for their fresh weights before collecting mites. Mites were collected from each wild plant species through washing and dipping in 70% ethanol. After counting of mites, phytoseiid mites in each sampling were selected irrespective of species, sex, and developmental stage and were stored in 99.5% ethanol until DNA extraction.

3.2.2 DNA extraction and species composition analysis for mites sampled from wild plants

Methods for DNA extraction and estimation of phytoseiid mite species composition are described in Chapter two.

3.2.3 Detection of spider mite DNA from phytoseiid mites

In total, 17, 78 and 14 DNA samples of phytoseiid mites were predicted to contain *E. sojaensis*, *A. eharai* and *N. californicus* through the QS-based method described in Chapter two. These samples were used to amplify the internal transcribed spacer (ITS) region of ribosomal genes of spider mites. First, PCR was conducted using primer set T/P-ITS-5′-2 (5′-cctgcggaaggatcattaac-3′) and T/P-ITS-3′-2 (5′-ggtaattcgagtgatccacc-3′) at PCR conditions of 40 cycles of 15 s at 94°C, 30 s at 60°C and 1 min at 72°C with subsequent final extension of 72°C for 7 min. The primer set was designed to amplify the ITS sequences of both spider mite species. The PCR products were used for re-amplification using primer sets PM-ITS-5´ (5´-atgcaggcacacataccgt-3´) and PM-ITS-3´ (5´-ccgtgggacttttattctc-3´) and TK-ITS-5´ (5´-caacatgattctatttgtg-3´) and TK-ITS-3´ (5´-

gccaccgtgggacttttaa-3´) at PCR conditions of 40 cycles of 15 s at 94°C, 30 s at 50°C, and 1 min at 72°C with subsequent final extension of 72°C for 7 min. The former and latter primer sets exclusively amplify PCR products from *P. mori* (415 bp) and *T. kanzawai* (323 bp), respectively. The PCR conditions amplified detectable amounts of spider mite DNA from 1.0^{-10} ng of genomic DNA of both spider mite species (data not shown).

In addition, PCR amplification for the ITS sequences of *Petrobia harti* (Ewing) (Acari: Tetranychidae) from the phytoseiid mite DNA samples was also conducted. First PCR conducted using primer set Ph-ITS-5′-1 (5′-gcataaattctgcgggtagc-3′) and Ph-ITS-3′-1 (5′-ctgtggcatactctcccttg-3′) was followed by second PCR using primer set Ph-ITS-5´-2 (5´-taccatccattagtgcggtg-3´) and Ph-ITS-3´-2 (5´- caccgcttgtaggtgtatct -3´). These primer sets for *P. harti* were designed based on the nucleotide sequences deposited in the DNA data bank (GenBank/EMBL/DDBJ accession no. GQ141935). The primer sets exclusively amplify PCR products from *P. harti* (416 bp) (data not shown). The PCR conditions were 30 cycles of 15 s at 94°C, 30 s at 60°C and 1 min at 72°C, with subsequent final extension of 72°C for 7 min. PCR products were size-fractionated on 1.0% agarose gel and were observed under ultraviolet light after staining with ethidium bromide.

3.3 Results

3.3.1 Selection of wild plants harboring large quantities of phytoseiid mites

The number of wild plant species harboring phytoseiid mites was limited in April (Table 3.1). However, in July 80% of the wild plant species had phytoseiid mites (Table 3.1).

The top ten wild plant species harboring larger quantities of phytoseiid mites are shown for each month from April (one species) to October in Table 3.2. Phytoseiid mites were the most abundant on *Veronica persica* Poir. in May and June. *P. foetida* harbored the largest quantities of phytoseiid mites from July to September. In October, a larger quantity of phytoseiid mites was detected on *Persicaria longiseta* (Bruijin) Kitag. than on *P. foetida*. *Oxalis corniculata* L. also had large quantities of phytoseiid mites in June and September. Thus, wild plant species harboring large quantities of phytoseiid mites change seasonally (Table 3.2).

3.3.2 Species composition of phytoseiid mites on *V. persica, O corniculata, P. foetida* and *P. longiseta*.

The phytoseiid mite species composition on four wild plant species (*V. persica*, *O. corniculata*, *P. foetida,* and *P. longiseta*), harboring larger quantities of phytoseiid mites (Table 3.2), at Site I, Site II, and Site IV was examined to compare with that on peach leaves (Figs. 2.6, 2.7, 3.1 and 3.2). *N. makuwa*, *S. okinawanus,* and *N. womersleyi*, which did not appear on peach leaves during the survey period, were detected on the wild plant species. The phytoseiid mite species composition on the four wild plant species was varied among peach orchards. Several phytoseiid mite species were generally detected on the wild plant species through late July. However, the phytoseiid mite species composition became simpler, resembling the composition on the peach leaves after mid-summer (Figs. 2.6, 2.7, 3.1 and 3.2). Typically, on *P. foetida*, proportions of *N. californicus*, *A. eharai*, and *E. sojaensis* were, respectively, higher at Site II, Site IV, and Site I (Fig. 3.1). Phytoseiid mite species composition on the four wild plants (Figs. 3.1 and 3.2) seemed to be resembling with the phytoseiid mite species composition on peach leaves (Figs. 2.6 and 2.7), suggesting that the dominance of phytoseiid mite species on wild plants is also affected at least in part by pesticide application (Table 2.1) as observed on peach leaves. For wild plants other than the four species mentioned above, enough quantities of phytoseiid mites were not obtained for continuous survey on the species composition.

3.3.3 Detection of spider mite DNA from phytoseiid mites collected on tree leaves

A total of 109 DNA samples from a pool of phytoseiid mites with more than five individuals collected on peach leaves and predicted to contain only one species (Figs. 2.6 and 2.7) were used for amplification of the ITS sequences of *P. mori* and *T. kanzawai*. All *E. sojaensis* samples were derived from Site I. Samples for *A. eharai* and *N. californicus* were derived respectively from four sites (Site II, Site III, Site IV, and Site VI) and two sites (Site III and Site IV). Total number of *T. kanzawai* and *P. mori* collected at Site I were, respectively, 87 individuals and 257 individuals (data not shown). Total number of both spider mites collected at Site II, Site III, Site IV and Site VI were similar (*T. kanzawai*, 1132 individuals; *P. mori*, 1374 individuals) (data not shown). Actually, *P. mori* was collected more (total 758 individuals) than *T. kanzawai* (total 132 individuals) at Site III and Site IV (data not shown). The ITS sequences of *T. kanzawai* were amplified more frequently than those of *P. mori* for *E. sojaensis* (Table 3.3). More frequent amplification of the ITS sequences of *T. kanzawai* was also observed for *A. eharai* (Table 3.3). The ITS sequences of both spider mite species were amplified with similar frequencies for *N. californicus* (Table 3.3). However, this was observed in the condition at which *P. mori* shared a higher proportion, as described above. Therefore, *N. californicus* might also show prey preference on *T. kanzawai*.

3.3.4 Detection of *P. harti* DNA from phytoseiid mites collected on peach leaves

Amplification of the ITS sequences of *P. harti* was conducted from 109 DNA samples as described above. Results showed that the ITS sequences of *P. harti* were amplified from two *E. sojaensis* samples out of 17, three *A. eharai* samples out of 78, and three *N. californicus* samples out of 14 (Table 3.3).

3.4 Discussion

V. persica, O. corniculata, P. foetida and *P. longiseta* harbored large quantities of phytoseiid mites (Table 3.2). It is noteworthy that densities of phytoseiid mites on *V. persica, O. corniculata, P. foetida,* and *P. longiseta* are not necessarily the highest (Table 3.2). Nevertheless, these wild plant species appeared in peach orchards in order with large biomass at their growing seasons. The occurrence of phytoseiid mites on *V. persica* (Ferla et al. 2007) and *O. corniculata* (Abbas et al. 2012) was reported previously. However, *V. persica* contained a large quantity of *T. kanzawai* (Table 3.2) and might become the origin of spider mites. Therefore, *V. persica* was excluded from candidates of insectary plants. *Taraxacum* spp. and *Solidago* spp. were also reported to harbor phytoseiid mites in commercial apple orchards (Coli et al. 1994). These plants were also shown to have considerable quantities of phytoseiid mites in peach orchards (Table 3.2). However, the occurrence of these wild plants is less consistent. Biomass is also limited, at least in peach orchards. The functional significance of *O. corniculata, P. foetida,* and *P. longiseta* as insectary plants in spider mite control remains to be examined for the next stage.

Croft et al. (1998) showed that fecundity of *N. californicus* fed with *P. ulmi* was lower than that fed with *T. urticae*. Miya et al. (2004) reported that the fecundity of *N. californicus* was slightly higher on *T. urticae* eggs than on *P. mori* eggs. Feeding on *P. ulmi* eggs caused slightly reduced consumption and developmental rates and shorter post oviposition period of *N. californicus* than those of the eggs of the other spider mites including *T. urticae, T. kanzawai*, and *P. citri* (Gotoh et al. 2006). The survival rate in immature stages of *Typhlodromus annectens* De Leon is significantly lower when fed on *P. citri* than when fed on *Tetranychus pacificus* McGregor (Badii et al. 1990). The survival, reproduction, and development rates of *Neoseiulus fallacis* (Garman) are higher when held with *Tetranychus* species than when held with other genera of tetranychid mites including *P. ulmi* and *P. citri* (Pratt et al. 1999). *P. citri* eggs were not suitable for generalist phytoseiid mite species, *Typhlodromus laurentii* Ragusa et Swirski and *Typhlodromus* *rhenanoides* Athias-Henrio compared to *T. urticae* eggs (Tsolakis et al. 2013). Results in this study showed that *N. californicus*, *A. eharai,* and *E. sojaensis* prefer to feed *T. kanzawai* rather than *P. mori* in the field (Table 3.3). Although, reportedly, *N. californicus* can grow and oviposit by preying on *P. citri* eggs no less than *T. urticae* (Katayama et al. 2006), *Panonychus* species might be less preferred by phytoseiid mites than *Tetranychus* species, irrespective of their feeding types. No data are available to explain the growth stages of spider mites at which phytoseiid mite species mostly prey in the peach orchards. Predatory performance of the phytoseiid mite species on various growth stages of *T. kanzawai* and *P. mori* remains to be examined to ascertain whether the preference depends on the food quality or on the defense strategy of the prey stage.

Kawashima and Jung (2010) investigated the overwintering sites of *N. californicus* in mandarin orchards in Korea. They reported that most *N. californicus* individuals were collected on herbaceous plants on the ground rather than on mandarin trees or woody plants surrounding the orchards. That observation seems reasonable considering that *N. californicus* on the tree leaves was dispersed from the groundcover in this study. However, in persimmon orchards, a considerable number of overwintering *A. eharai* were collected on the trees (Kawashima, unpublished data). Overwintering sites for *E. sojaensis* have not been reported. Reportedly, in peach orchards of northern Greece, *Euseius finlandicus* Oudemans overwinters in various sites on the trees (Broufas et al. 2002). Therefore, it remained unclear if *A. eharai* and *E. sojaensis* on the tree leaves were supplied by the groundcover. In the present study, I detected *P. harti* DNA from phytoseiid mites including *E. sojaensis* and *A. eharai* collected on tree leaves at Site I, Site II, Site IV, and Site VI with wild groundcover. Also, *P. harti* DNA was detected from *N. californicus* collected on tree leaves at Site III with bare ground. Actually, distribution of *P. harti* is worldwide, where they are usually found on *Oxalis* spp. (Dubitzki and Gerson 1987), but they have never been observed on peach leaves (Sonoda, unpublished data). Reportedly, the walking speed of phytoseiid mites differs among species depending on their feeding types (Jung and Croft 2001). The specialist predator *Neoseiulus fallacis* (Garman), an 'active' mite, requires ca. 10 h of continuous walking to go 1 m linear distance (Croft and Jung 2001). That rate seems reasonable considering that the ambulatory and aerial dispersal of the phytoseiid mites occurred from *O. corniculata* to peach trees as reported by Jung and Croft (2001). These results suggest that some groundcover plants such as *O. corniculata*, a host of *P. harti*, promote the occurrence of phytoseiid mites by providing food resources.

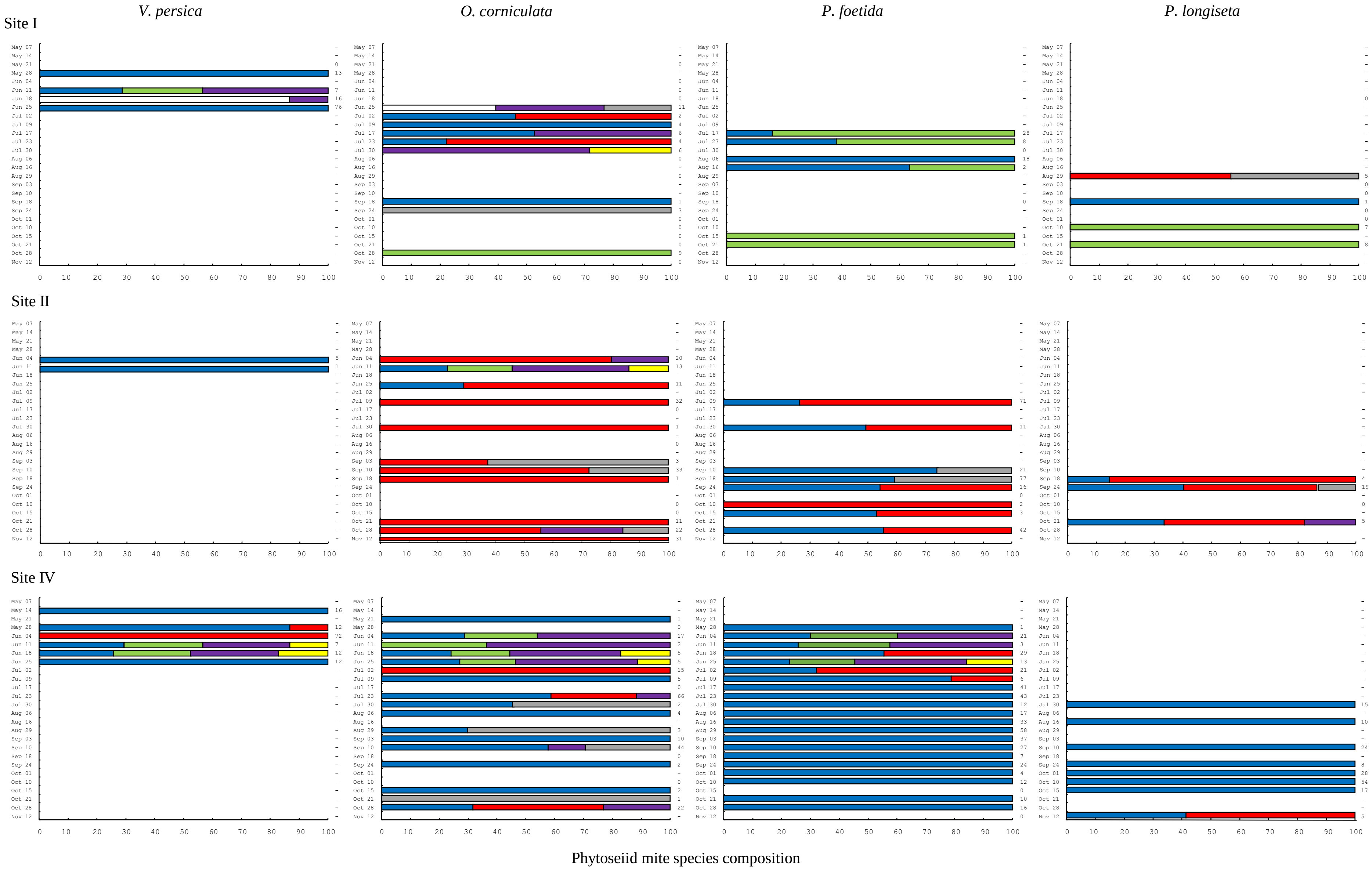

Fig. 3.1. Seasonal fluctuation of phytoseiid mite species composition on four wild plant species (*Veronica persica, Oxalis corniculata, Paederia foetida,* and *Persicaria longiseta*) at Site I, Site II, and Site IV. The frequency of each species was normalized as the sum of the frequencies 100%. Green, red, blue, yellow, purple, grey, white and black bars respectively represent proportions of *Euseius sojaensis, Neoseiulus californicus*, *Amblyseius eharai*, *Typhlodromus vulgaris*, *Amblyseius tsugawai, Neoseiulus makuwa, Scapulaseius okinawanus,* and *Neoseiulus womersleyi*. Numbers on the right column represent the number of individuals examined per survey.

Fig. 3.2 Seasonal fluctuation of phytoseiid mite species composition on *Paederia foetida* at Site I, Site II, and Site IV. The frequency of each species was normalized as the sum of the frequencies 100%. Green, red, blue, yellow, purple, grey, white and black bars respectively represent proportions of *Euseius sojaensis*, *Neoseiulus californicus*, *Amblyseius eharai*, *Typhlodromus vulgaris*, *Amblyseius tsugawai*, *Neoseiulus makuwa*, *Scapulaseius okinawanus*, and *Neoseiulus womersleyi*. Numbers on the right column represent the number of phytoseiid mite individuals sampled.

Table 3.1 Number of wild plant species harboring phytoseiid mite

	Apr	May	Jun	Jul	Aug	Sep	Oct
No. of wild plant species	32	41	35	41	34	40	44
No. of wild plant species harboring phytoseiid mites	1	14	28	34	21	32	33
Detection efficiency (%)	3.1	34.1	80.0	82.9	61.8	80.0	75.0

Table 3.2 The number of phytoseiid mites and spider mites sampled from wild plant species

Month	Wild plant species	Phytoseiid mites		*T. kanzawai*		*P. mori*		*P. citri*		*P. harti*	
		No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No.of mites	No. of mites/ FW (g)
April	*Lamium amplexicaule*	2	0.01	-	-	-	-	-	-	-	-
May	*Veronica persica*	34	0.08	41	0.10	-	-	-	-	-	-
	Solidago altissima	15	0.04	-	-	-	-	-	-	-	-
	Paederia foetida	8	0.05	-	-	-	-	-	-	-	-
	Prunus salicina	7	0.06	-	-	-	-	-	-	-	-
	Taraxacum sp.	5	0.03	2	0.01	-	-	-	-	-	-
	Youngia japonica	5	0.03	2	0.01	-	-	-	-	-	-
	Vicia sativa subsp. *nigra* var. *segetalis*	4	0.01	106	0.36	-	-	-	-	-	-
	Rosa multiflora var. *multiflora*	4	0.01	1	0.01	2	0.01	-	-	-	-
	Lamium purpureum	3	0.02	20	0.13	-	-	-	-	-	-
	Quercus serrata subsp. *serrata* var. *serrata*	2	0.02	-	-	-	-	-	-	-	-
June	*Veronica persica*	218	0.45	24	0.05	1	0.00	-	-	-	-
	Oxalis corniculata	92	0.11	1	0.00	8	0.01	-	-	137	0.17
	Paederia foetida	67	0.33	-	-	-	-	-	-	-	-
	Achyranthes bidentata var. *japonica*	52	0.25	-	-	-	-	-	-	-	-
	Prunus salicina	42	0.25	-	-	-	-	-	-	-	-
	Trifolium repens	30	0.06	1	0.00	3	0.01	-	-	-	-
	Commelina communis var. *communis*	27	0.05	1	0.00	-	-	-	-	-	-
	Solidago altissima	26	0.06	3	0.01	-	-	-	-	-	-
	Taraxacum	21	0.07	1	0.00	-	-	-	-	3	0.01
	Citrus sp.	19	0.04	2	0.00	-	-	106	0.22	-	-
July	*Paederia foetida*	310	0.59	-	-	-	-	-	-	-	-
	Prunus salicina	235	1.15	-	-	-	-	-	-	-	-
	Oxalis corniculata	207	0.27	1	0.01	-	-	-	-	-	-
	Commelina communis var. *communis*	124	0.24	-	-	-	-	-	-	-	-
	Trifolium repens	79	0.32	-	-	-	-	-	-	-	-
	Citrus sp.	59	0.15	-	-	-	-	22	0.06	-	-
	Phytolocca americana	56	0.25	9	0.04	-	-	-	-	-	-
	Ipomoea purpurea	44	1.32	-	-	-	-	-	-	-	-
	Achyranthes bidentata var. *japonica*	40	0.38	-	-	-	-	-	-	-	-
	Bidens frondosa	37	0.41	-	-	-	-	-	-	-	-

Table 3.2 Continued

Month	Wild plant species	Phytoseiid mites		*T. kanzawai*		*P. mori*		*P. citri*		*P. harti*	
		No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)	No. of mites	No. of mites/ FW (g)
August	*Paederia foetida*	154	0.67	4	0.02	-	-	-	-	-	-
	Taraxacum sp.	66	0.50	-	-	-	-	-	-	-	-
	Youngia japonica	36	0.96	2	0.05	-	-	-	-	-	-
	Prunus salicina	30	0.33	-	-	-	-	-	-	-	-
	Cocculus trilobus	27	0.70	2	0.05	-	-	-	-	-	-
	Solanum ptychanthum	26	0.66	-	-	-	-	-	-	-	-
	Ipomoea purpurea	26	0.59	34	0.77	-	-	-	-	-	-
	Achyranthes bidentata var. *japonica*	24	0.26	-	-	-	-	-	-	-	-
	Oxalis corniculata	24	0.09	-	-	-	-	-	-	674	2.43
	Fallopia japonica var. *japonica*	18	0.14	-	-	-	-	-	-	-	-
September	*Paederia foetida*	219	0.65	2	0.01	3	0.01	-	-	-	-
	Oxalis corniculata	107	0.25	-	-	-	-	-	-	328	0.76
	Acalypha australis	79	0.55	-	-	-	-	-	-	-	-
	Ipomoea hederacea var. *integriuscula*	77	0.64	-	-	-	-	-	-	-	-
	Solanum ptychanthum	68	0.19	-	-	-	-	-	-	-	-
	Persicaria longiseta	56	0.19	-	-	1	0.00	-	-	-	-
	Youngia japonica	48	0.45	-	-	-	-	-	-	-	-
	Taraxacum sp.	48	0.35	-	-	2	0.01	-	-	-	-
	Commelina communis var. *communis*	46	0.08	-	-	10	0.02	-	-	-	-
	Citrus sp.	34	0.10	-	-	-	-	42	0.12	-	-
October	*Persicaria longiseta*	119	0.37	-	-	-	-	-	-	-	-
	Paederia foetida	91	0.24	-	-	-	-	-	-	-	-
	Solanum ptychanthum	69	0.18	-	-	-	-	-	-	-	-
	Youngia japonica	64	0.45	-	-	-	-	-	-	-	-
	Commelina communis var. *communis*	60	0.12	-	-	-	-	-	-	-	-
	Lamiaceae sp.	47	0.28	2	0.01	3	0.02	-	-	-	-
	Ipomoea hederacea var. *integriuscula*	37	0.21	-	-	3	0.02	-	-	-	-
	Ampelopsis glandulosa var. *heterophylla*	36	1.36	-	-	-	-	-	-	-	-
	Prunus salicina	26	0.17	-	-	-	-	-	-	-	-
	Ipomoea triloba	26	0.14	-	-	-	-	-	-	-	-

Table 3.3 Detection of ribosomal ITS sequences of spider mites from phytoseiid mites

Phytoseiid mite species	n[a]	*P. harti*	*T. kanzawai*	*P. mori*	Both[b]	None[c]
E. sojaensis	17	2	5	1	11	0
A. eharai	78	3	24	3	49	2
N. californicus	14	2	0	3	11	0

[a] number of phytoseiid mite samples (*E. sojaensis*, *A. eharai* and *N. californicus*) examined

[b] detection of both *T. kanzawai* and *P. mori* DNA

[c] no detection of both *T. kanzawai* and *P. mori* DNA

Chapter Four

Evaluation of functional significance of *Paederia foetida* L. as an insectary plant in regulating spider mite populations in peach orchards; a bottom-up regulation mechanism

4.1 Introduction

Phytoseiid mites (Phytoseiidae) have received considerable attention for the last four decades because of their potential as biological control agents of spider mites (Tetranychidae) and, more recently, of thrips on various crops (McMurtry et al. 2013). The early part of the period saw considerable controversy about the effectiveness of these predators in suppressing and regulating populations of spider mites (Wilson et al 1991). As more studies were undertaken, there was increasing evidence to support the contention that phytoseiid mites can be important biological control agents and essential elements in spider mite control programs (McMurtry et al. 2013).

Utilization of resources through habitat management in the form of insectary plants has been noted to be of favorable mechanism towards enhancing the efficacy of natural enemies including phytoseiid mites (Jonsson et al. 2008; Landis et al. 2000). In particular, the provision of floral resources for natural enemies (Gurr et al. 2005; Tylianakis et al. 2004) that enhances their effectiveness through aggregation, sex-ratio changes, increases in longevity, fecundity, and predation rates might ultimately reduce pest populations (Wratten et al. 2002). However, insectary plant species can differ between each other on how they improve the performances of natural enemies in controlling arthropod pests (Ambrosino et al. 2006; Colley and Luna 2000; Cowgill et al. 1993). Therefore, screening insectary plants for traits that are appropriate for the targeted natural enemies in the purpose of controlling agricultural pests should be of paramount importance (Baggen and Gurr 1998; Pontin et al. 2006). There have been relatively little research into the selection of insectary plants from native plant species inhabiting fruit orchards to date (Gurr et al. 2004; Landis et al. 2000).

In the previous chapter, three wild plant species, *P. foetida, O. Corniculata*, and *P. longiseta* were selected as insectary plants in peach orchards. Among them, *P. foetida* harbored considerable number of phytoseiid mites for longer period as compared to any other wild plants including *O. corniculata* and *P. longiseta* (Table 3.2). As such, *P. foetida* could be a possible insectary plant with a potent of reducing spider mite populations on peach trees/leaves. In this study, a field survey on *P. foetida* was further tested for its potency in spider mite control through comparing the population dynamics of spider mites and phytoseiid mites on peach leaves. Bioassays on the biological performances of *T. kanzawai* and *N. californicus* on *P. foetida* leaves were also conducted at laboratory conditions to substantiate the data gathered from the field. Finally, we examined the utilization of *P. foetida* as a food source using PCR.

4.2. Materials and Methods

4.2.1 Study sites and sampling

This study was conducted at Site II and Site V in 2014, and at Site V in 2015. Pesticide practices at the study sites in 2014 and 2015 were described in Table 4.1. Sampling was conducted every week in 2014 (from April 28 to October 27) and 2015 (from May 11 to Aug 24). Sampling procedures were described in Chapter two however with some modifications. Five trees were selected from Site II and Site V. In 2014, Site II and Site V have one tree and three trees respectively having *P. foetida* exclusively occurring around the stumps of the trees (tree 2, tree 3 and tree 4 at Site V shown in Fig. 4.1a). The other trees apart from the aforementioned trees were observed to have no *P. foetida* (tree 1 and tree 5 at Site V shown in Fig. 4.1). In 2015, Site V was again selected to further study the role of *P. foetida* and its relationship with densities of spider mites and phytoseiid mites on peach leaves. The occurrence of *P. foetida* however, was observed to be in the mixture with other wild ground cover unlike the previous year (Fig. 4.1b). It is noteworthy that herbicide, glyphosate, was sprayed around the stamps of the trees at both sites in early spring, 2014. By contrast, no herbicide was used at Site V in 2015.

Thirty leaves were sampled every week from each tree. The number of spider mites and phytoseiid mites collected in each sampling of 30 leaves was counted in a petri dish filled with 70% ethanol. Then phytoseiid mites were separated from spider mites. Separated phytoseiid mites in each sampling were used for DNA extraction or stored in a glass container filled with 99.9% ethanol until DNA extraction.

P. foetida collected from each sampling were weighed for their fresh weights before collecting mites. Mites were collected from *P. foetida* through washing and dipping in 70%

ethanol. After counting of mites, phytoseiid mites in each sampling were selected irrespective of species, sex, and developmental stage and were stored in 99.9% ethanol until DNA extraction.

4.2.2 DNA extraction

DNA extraction and analysis of phytoseiid mite species composition were conducted according to the methods descried in Chapter two.

4.2.3 Stock colonies

T. kanzawai and *N. californicus* were collected at peach orchards in Kurashiki City, Okayama Prefecture, western Japan in 2014. *T. urticae* was obtained from H. Amano (Kyoto University, Japan) in 2015. Mites have been maintained at 25°C under a long photoperiod (16L8D). *T. kanzawai* and *T. urticae* were maintained on fava bean (*Vicia faba*) leaves. *N. californicus* colonies were maintained on *V. faba* leaves using *T. urticae* as the food source.

4.2.4 Survival of *N. californicus* on *P. foetida*

Each leaf disc of *Ipomoea nil*, *V. faba* and *P. foetida* was placed on a cotton in a plastic petri dish (9 cm in diameter) soaked with water to keep *N. californicus* protonymps from crawling off the leaves (Fig. 4.2a). One two-day-old female protonymph was placed on the leaf disks of *I. nil*, *V. faba*, and *P. foetida*. The survival of the protonymphs were observed and dead or alive was recoded every 24 h. One assay with three replicates was repeated three times. Survival means of *N. carlifornicus* on *P. foetida*, *V. faba* and *I. nil* was analyzed using R, version 3.2.1 (2015 The R Foundation for Statistical Computing Platform). When significant differences were found between the means ($P<0.001$), the means were separated using Tukey HSD test.

4.2.5 Survival and fecundity of *T. kanzawai* on *P. foetida*

Ten two-day-old *T. kanzawai* protonymphs were placed on the leaf discs of *I. nil*, *V. faba* and *P. foetida* as described above (Fig. 4.2b). The survival of the protonymphs were observed and dead or alive was recoded after every 24 h. Survival means of *T. kanzawai* on *P. foetida V. faba* and *I. nil* was analyzed using R, version 3.2.1. When significant differences were found between the means ($P<0.001$), the means were separated using Tukey HSD test.

Ten newly emerged *T. kanzawai* adult females were placed on the leaf discs of *I. nil*, *V. faba* and *P. foetida.* Number of eggs laid was counted every 24 h for five days (Fig. 4.2c). The eggs laid were examined for hatchability (Fig. 4.2d). Number of eggs hatched were counted every 24 h. The assays for survival and fecundity were replicated three times. Fecundity and hatchability means of *T. kanzawai* on *P. foetida V. faba* and *I. nil* was analyzed using R, version 3.2.1. When significant differences were found between the means of T. kanzawai fecundity ($P<0.01$) and hatchability ($P<0.05$), the means were separated using Tukey HSD test.

4.2.6 Detection of *P. foetida* DNA in *N. californicus*

Genomic DNA was extracted from ten *N. californicus* individuals which were kept on *P. foetida* and *V. faba* leaves without spider mites as food source for five days as described in Chapter two. DNA from the ten *N. californicus* individuals were used for PCR amplification of the chloroplast sequences of *P. foetida*. First PCR was conducted using the universal primers C (5′-cgaaatcggtagacgctacg-3′) and F (5′-atttgaactggtgacacgag-3′) (Taberletet et al. 1991). The PCR products were used for second PCR using *P. foetida* specific primer PF-Chloroplast 5’ (5′-atggaaacccactgagtgag-3′) and PF-Chloroplast 3’ (5′-gagtgaatagaaagggatttta-3′). The primer set exclusively amplify chloroplast DNA of *P. foetida*. The PCR conditions were 40 cycles of 15 s at 94°C, 30 s at 60°C and 1 min at 72°C for both first and second PCR. The PCR products were size-fractionated on 1.0% agarose gel and were observed under ultraviolet light after staining with ethidium bromide.

4.2.7 Detection of *P. foetida* chloroplast sequences from phytoseiid mites collected on *P. foetida* from the field

Phytoseiid mites collected on *P. foetida* from the field in 2013 and 2014 population surveys were analyzed to determine the feeding frequencies of phytoseiid mites on *P. foetida*. Species compositions of the phytoseiid mite samples collected from field were first conducted using QS-based method described in Chapter two. Then, samples having more than two or more phytoseiid mite species in one sample were separated from samples that had only one species. A total of 19 and 57 DNA samples of phytoseiid mites predicted to contain only *N. californicus* and *A. eharai* respectively collected from *P. foetida* were used to examine the feeding frequencies of phytoseiid mites on *P. foetida*. PCR amplification in detection of *P. foetida* chloroplast sequences as described above with universal primers C and F (Taberletet et al. 1991) and *P. foetida* specific primer PF-Chloroplast 5' and PF-Chloroplast 3' were used.

4.3. Results

4.3.1 Population dynamics of spider mites and phytoseiid mites on peach trees with and without *P. foetida*

Seasonal population dynamics of phytoseiid mites and spider mites on peach leaves in 2014 and 2015 are shown in Fig. 4.3. In 2014, Site II had four trees for which the spider mite densities were temporally beyond the control threshold level in early June. Tree 7 with *P. foetida* was included among them. Spider mite densities were below the control threshold level in all trees at Site V. However, tree 1 and tree 5 without *P. foetida* showed small but distinct peaks of spider mite occurrence at Site V in 2014. In 2015, tree 1 at Site VI showed spider mite density beyond the control threshold. Small but distinct peaks of spider mite occurrence were observed in tree 2.

4.3.2 Survival and fecundity of *N. californicus* on *P. foetida*

In Chapter three, larger number of phytoseiid mites were observed on *P. foetida* which occurred year round (Table 3.2). In the laboratory experiments, the survival of *N. californicus* was examined on *P. foetida*, *V. faba* and *I. nil*. Comparatively, the survival of *N. californicus* on *P. foetida* was significantly longer than on *V. faba* and *I. nil* (Fig. 4.4). However, *N. californicus* adults were never observed to have being laying eggs on *P. foetida* (data not shown).

4.3.3 Survival of *T. kanzawai* on *P. foetida*

P. foetida was observed to have few spider mites in field conditions in the previous study (Table 3.2). Little mortality was observed for *T. kanzawai* protonymphs on *V. faba* (Fig. 4.5). By contrast, only 10% and 40% of *T. kanzawai* protonymphs survived to up to six days on *P. foetida* and *I. nil* respectively (Fig. 4.5). *T. kanzawai* laid lower number of eggs on *P. foetida* and *I. nil* compared to *V. faba* (Fig. 4.6a). Hatchability of *T. kanzawai* eggs laid on *P. foetida* and *I. nil* was lower than that on *V. faba* (Fig. 4.6b).

4.3.4 *P. foetida* DNA detection in *N. californicus* and phytoseiid mites collected on *P. foetida* from the field.

Chloroplast sequence of *P. foetida* was amplified from *N. californicus* which were kept on *P. foetida* leaves for five days (data not shown). A total of 76 DNA samples of phytoseiid mites collected from *P. foetida* and predicted to contain only one species were used for amplification of the chloroplast sequence of *P. foetida*. Both *N. californicus* and *A.*

eharai showed amplification of *P. foetida* chloroplast sequences though, the frequencies were observed to be abridged.

4.4. Discussion

Trees with and without exclusive occurrence of *P. foetida* around the stumps were identified at Site II and Site V and were used to compare the population dynamics of spider mites on peach leaves in 2014. Results showed that trees with exclusive occurrence of *P. foetida* (tree 2, tree 3, and tree 4) showed diminished populations of spider mites in June at Site V. On the other hand, no such successful suppression of spider mite appearance was observed in tree VII with exclusive occurrence of *P. foetida* at Site II. One possible reason for this unsuccessful spider mite control could be the age of the tree. Lehman (2002) reported that spider mites generally prefer older leaves. Sadof and Gibb (2010) also reported that spider mites had extensive feeding on mature leaves of older trees. These observations are further confirmed in Puchalska (2014) in which very numerous populations of spider mites were found on older *Picea abies* trees indicating that a possible role of those trees as a source of the spider mites (Puchalska 2014). Moreover, Leetham and Jorgensen (1969) reported that in a study of overwintering sites at peach orchards in Ontario, Canada, larger number of mites were found in the bark crevices and deep protected cracks of cankers of older trees. Thus, it could be proposed that older trees are less tolerant to pest invasion (Jennings et al. 2015). In fact, tree 7 at Site II was terminated because of its old age in 2015.

The exclusive occurrence of *P. foetida* however was not observed in 2015 rather a mixture in the occurrence with other wild groundcover. The resulting occurrence of spider mite population dynamics showed a small peak in tree 2 at Site V (Fig. 4.3) which was previously not observed in 2014. The occurrence in the random mixture of wild groundcover could be influencing the increase in spider mite populations on peach leaves as per the studies in von Klinger (1987); Welling et al. (1988); Boatman and Wilson (1988); Chiverton and Sotherton (1991); Hassal et al. (1992); Lagerlöf and Wallin (1993); Boatman (1994) and Frank (1997). Hence, it can be anticipated that occurrence of *P. foetida* with other wild groundcover diminished at least in part the functional significance in spider mite control.

Laboratory assays showed decreased survival of *T. kanzawai* when cultured on *P. foetida* (Fig. 4.5). The number of eggs laid per ten female adults and the hatchability of the eggs were also lower compared to *V. faba* (Fig. 4.6). In the previous study, *P. foetida* was shown to harbor little spider mites during survey period (Table 3.2). Zheng et al. (2004) reported one peculiar characteristics of *P. foetida*, in that, it releases a strong sulfur odor when its leaves or stems are crushed or bruised. Sulfur is a good acaricide and fungicide and is probably the oldest known acaricide (Goodwin and Martin 2008). Indeed, effectiveness of multiple sulfur application in spider mite control has been reported in vineyards and hop yards (James et al. 2001; James and Prischmann 2006; Costello 2007; Gent et al. 2009). Decreased survival and fecundity of *T. kanzawai* observed in this study might be correlated with sulfur in *P. foetida*. *I. nil* might also contain chemicals or other structural features which might be affecting adversely the survival and fecundity of *T. kanzawai*. It is still unclear why spider mite populations were decreased in *P. foetida*. Nevertheless, these results suggest that *P. foetida* is not a suitable host for spider mites in particular, *T. kanzawai*. This is a vital progress taking into account the role of *P. foetida* as an insectary plant for phytoseiid mites.

McMurtry et al. (1970) reported that some acaricides in the group of sulfonates, sulfides, sulfonamides and sulfite derivatives have been observed to be relatively non-toxic to predacious mites. A decade later, Hoy et al. (1982) reported that phytoseiid mites are rendered to have a higher tendency to tolerate all formulations of sulfur particularly in that of *Metaseiulus occidentalis*. They also reported that the tolerance to sulfur in phytoseiid mites is based on a single major dominant gene (Hoy et al. 1982). As reported in the third chapter, the higher number of phytoseiid mites collected from *P. foetida* could entail the fact that perhaps, phytoseiid mites could tolerate *P. foetida* which contains sulfur (Zheng et al. 2004) while the spider mites could not. The laboratory bioassay showed *N. californicus* nymphs could live longer on *P. foetida* (Fig. 4.4) whereby, their survival increased four days more than spider mites. The adult females of *N. californicus* however did not lay eggs on *P. foetida* (data not shown). Thus, *P. foetida* itself does have the nutrients enough for nymphal development and fecundity of *N. californicus* which at least can sustain larger numbers of phytoseiid mites.

While it could be said that spider mites populations can be reduced with an exclusive occurrence of *P. foetida*, it is still unclear whether the larger populations of phytoseiid mites are the direct cause to the decreased populations of spider mite or the effect of sulfur from the *P. foetida* that drives them away, making *P. foetida* a possible spider mite non-host plant. Moreover, it is still unknown why larger numbers of phytoseiid mites are observed when there is no sources of food available in the likes of spider mites present on *P. foetida*. The numerous number of phytoseiid mites with sustained survival trait while lacking food sources in the form of spider mites remains for future investigations.

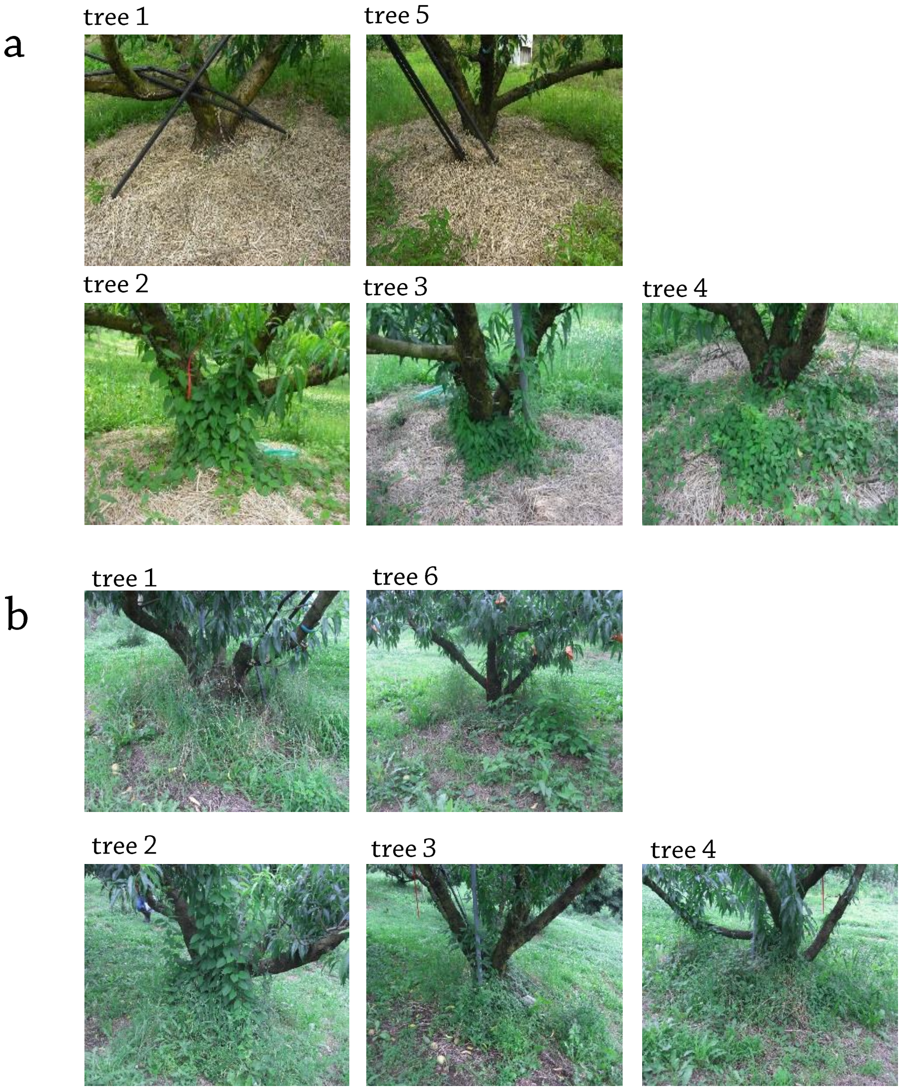

Fig. 4.1 Trees with *Paederia foetida*; tree 2, tree 3 and tree 4; tree 1, tree 5 (2014) and tree 6 (in 2015) with no *P. foetida* for surveys conducted in 2014 (a) and 2015 (b).

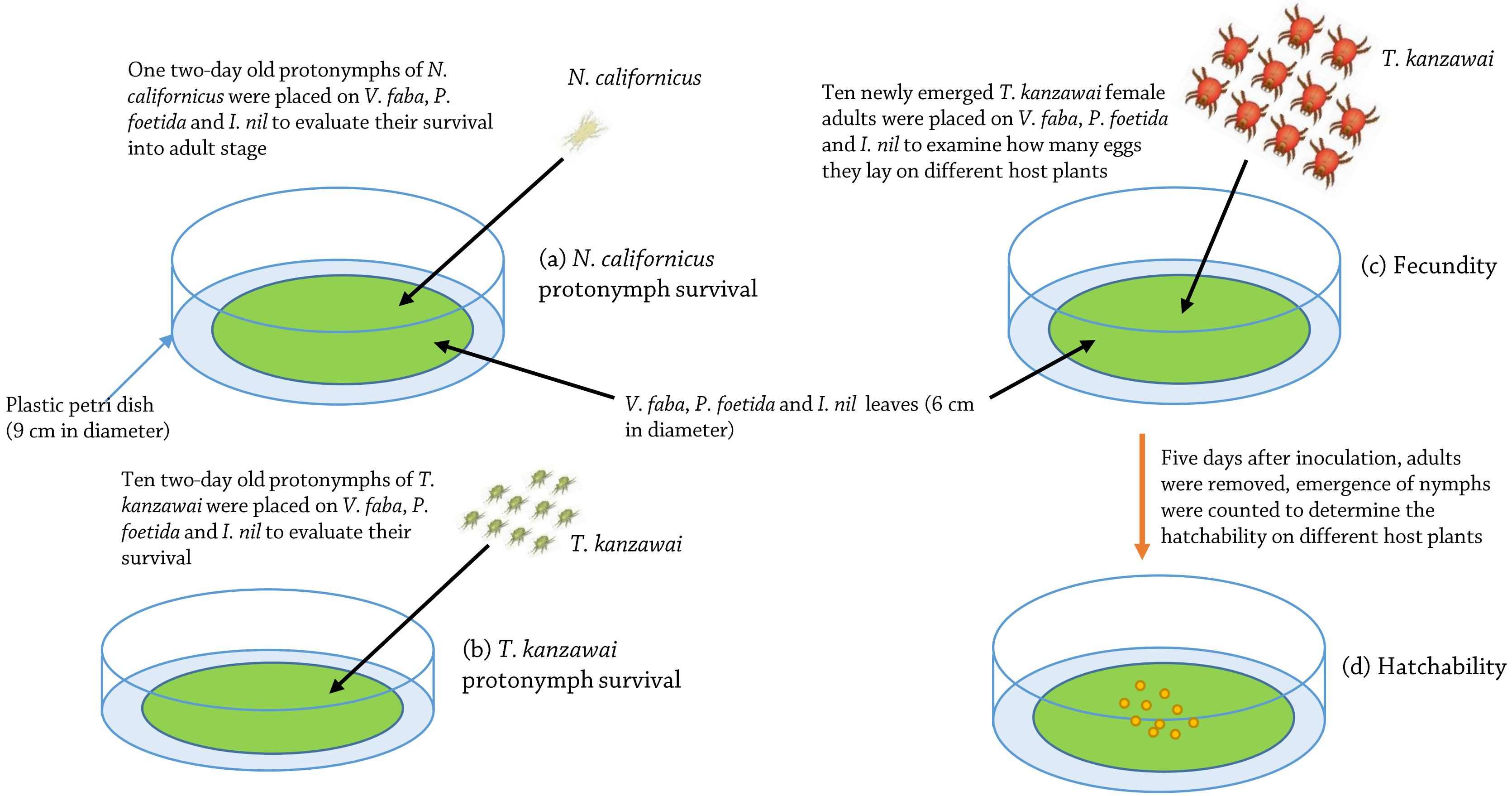

Fig. 4.2. Schematic representation of the bioassay conducted in laboratory condition for survival of *Neoseiulus californicus* on *V. faba*, *P. foetida* and *I. nil* (A), survival (B), fecundity (C) and hatchability (D) of *Tetranychus kanzawai* on *Vicia faba*, *Paederia foetida* and *Ipomoea nil*.

Site II Conventional orchard with groundcover in 2014

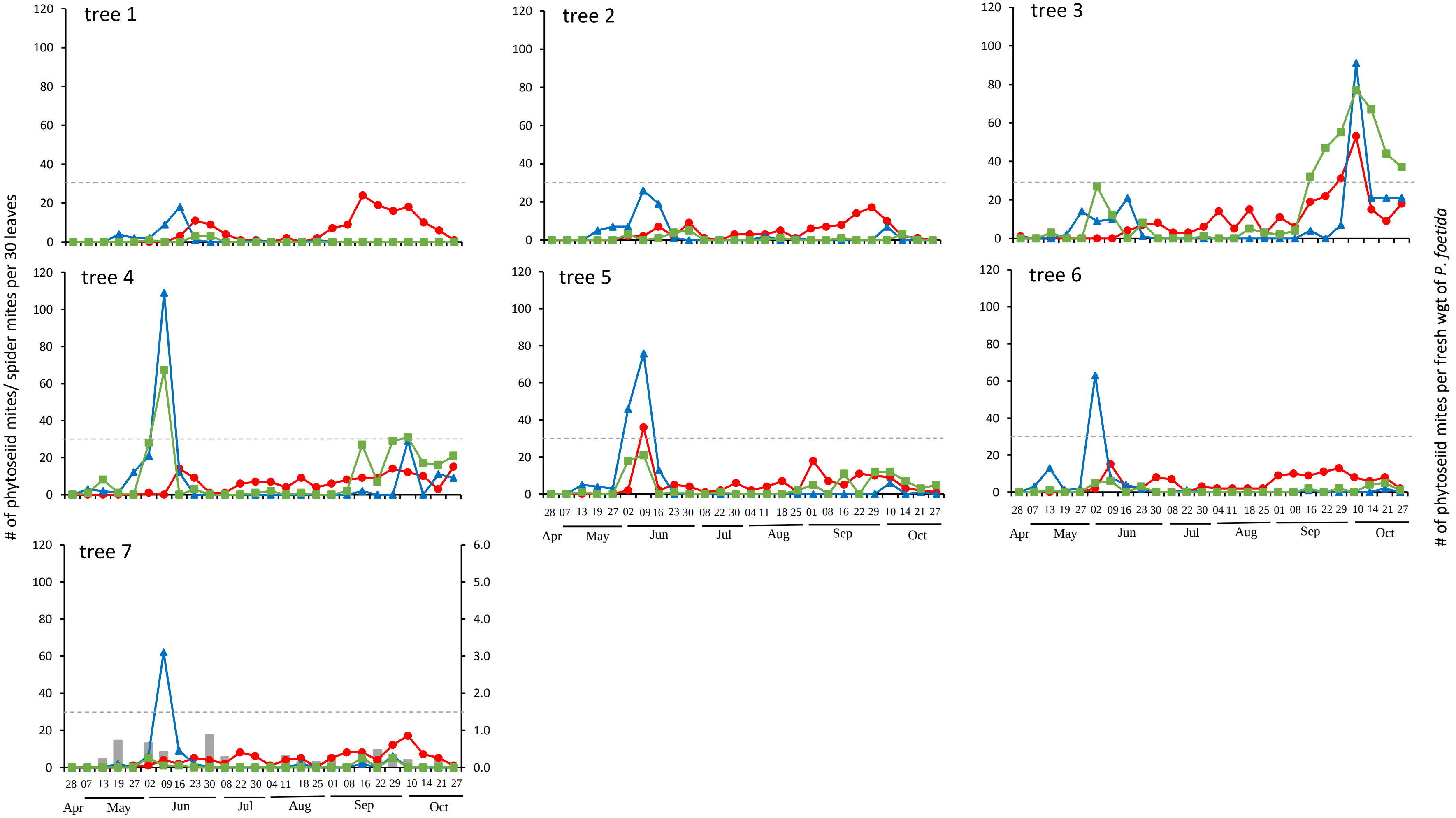

Fig. 4.3 Seasonal fluctuation in the number of phytoseiid mites and spider mites collected on peach leaves at Site II and Site V in 2014 and 2015. Red, green and blue lines, respectively, present the number of phytoseiid mites, *Panonychus mori*, and *Tetranychus kanzawai*. Broken line shows the control threshold density for spider mites in Japanese peach orchards (one mite per leaf). Bars in tree VII at Site II and tree II, tree III and tree IV at Site V shows seasonal fluctuation in the number of phytoseiid mites on *Paederia foetida* (see Chapter 3).

Site V Conventional orchard with groundcover in 2014

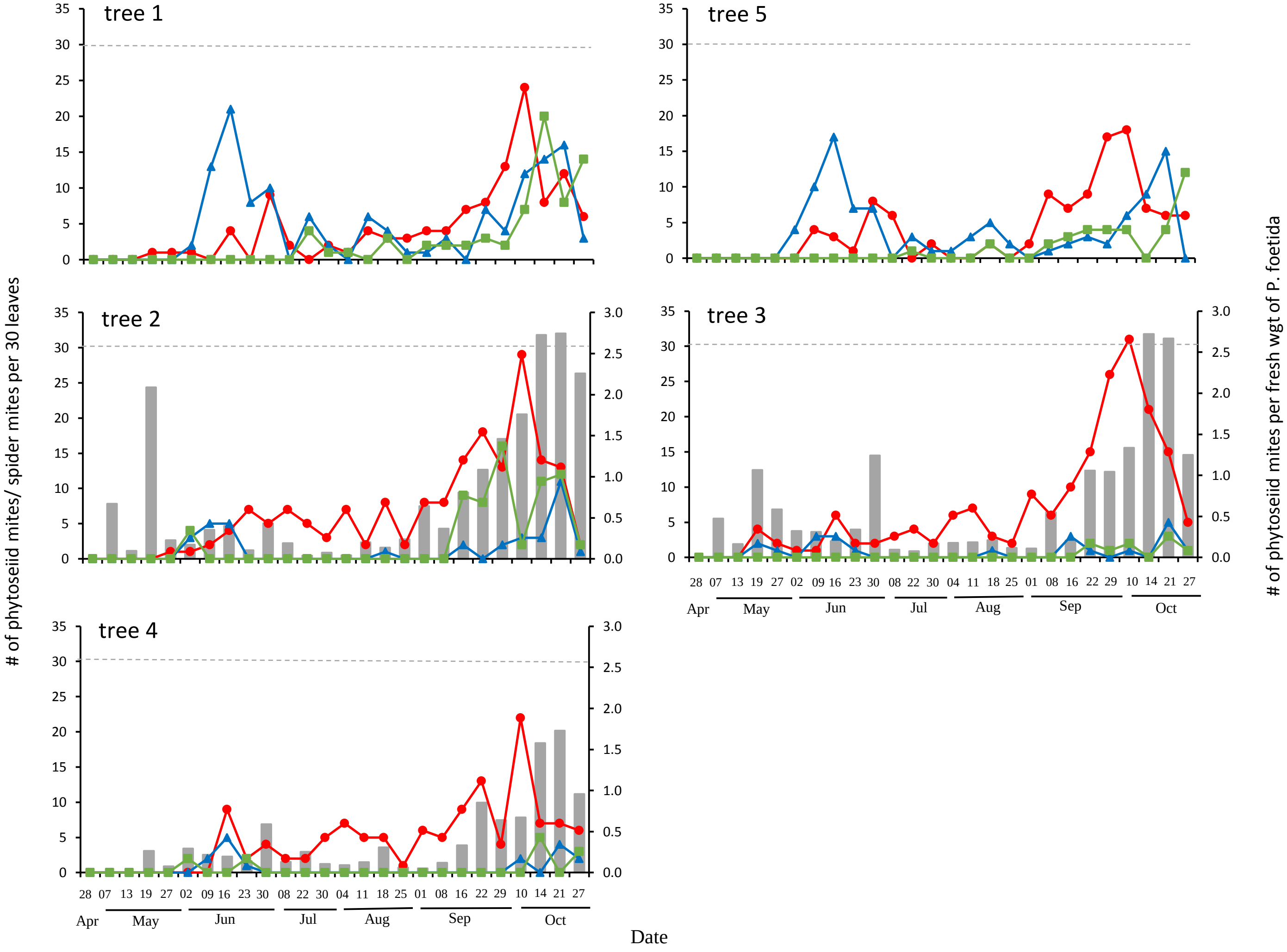

Fig. 4.3 (Continued)

Site V Conventional orchard with groundcover in 2015

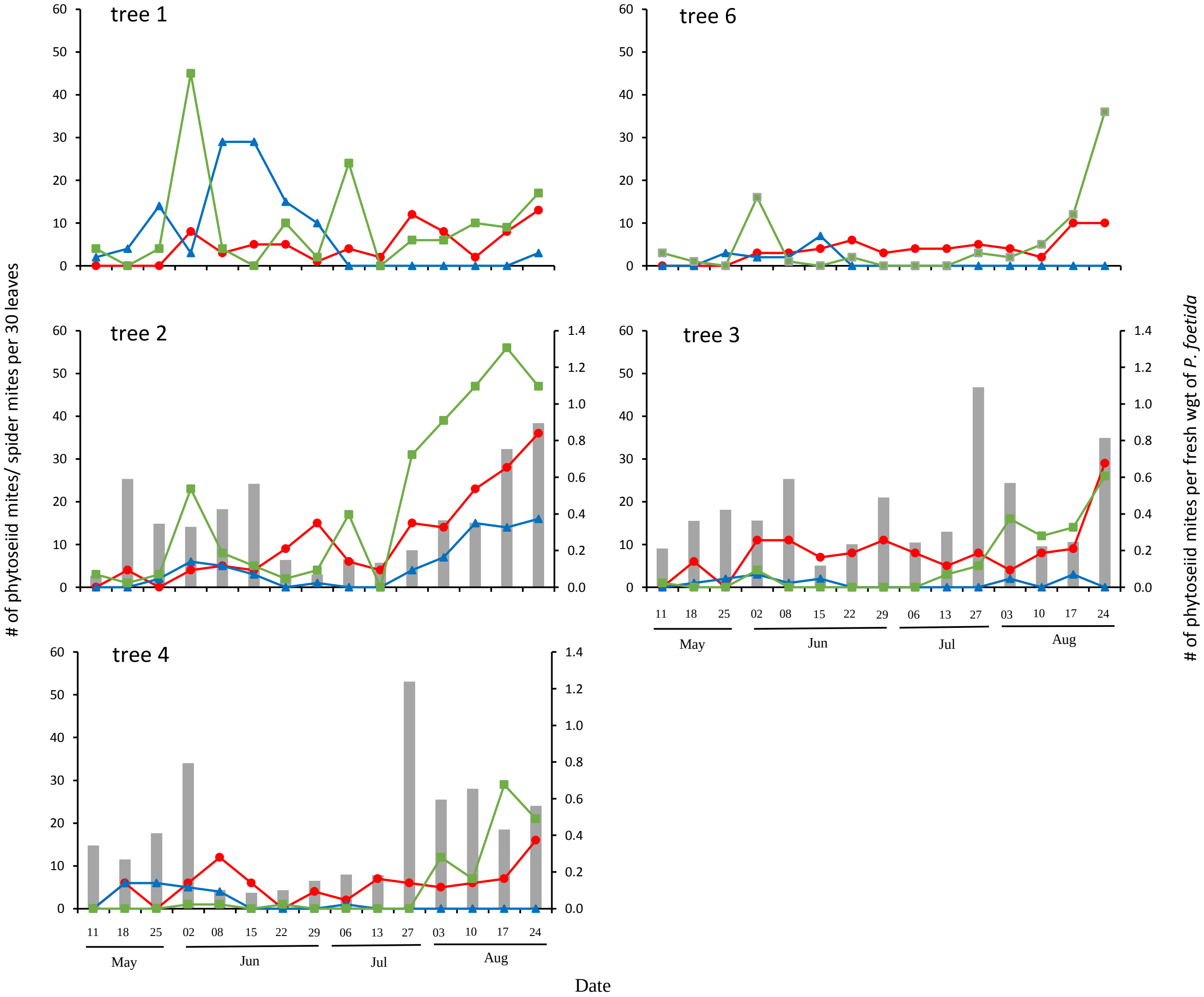

Fig. 4.3 Continue

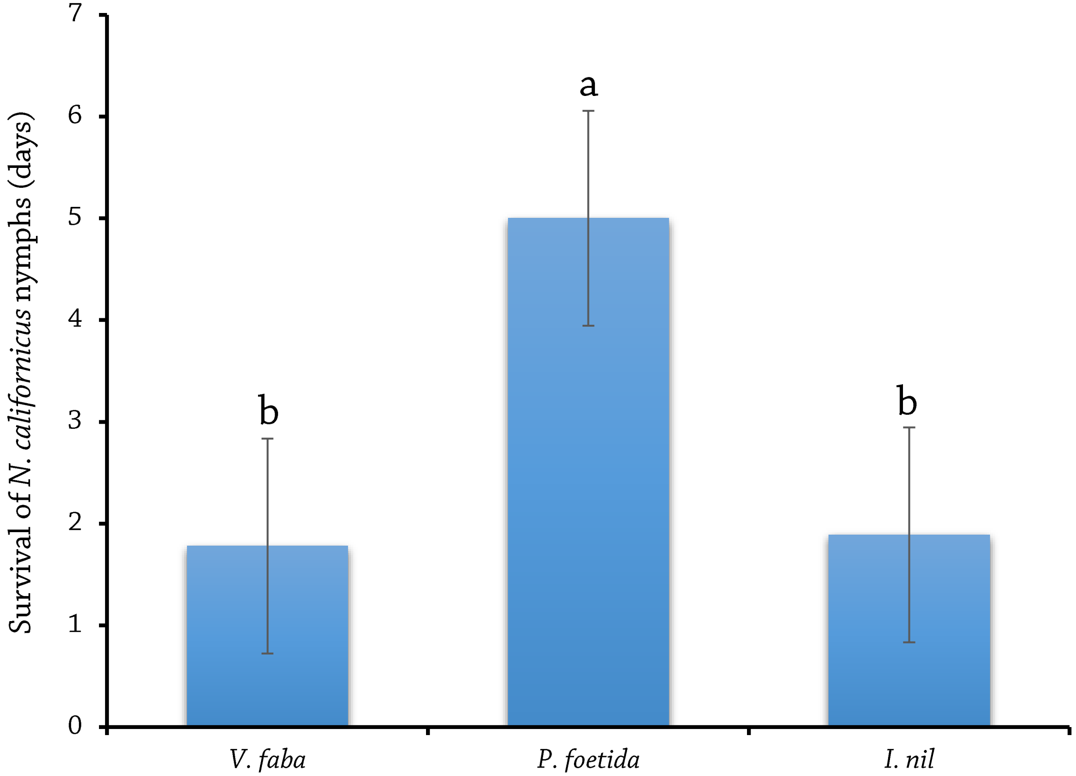

Fig. 4.4 Survival of two-day old nymphs of *Neoseiulus californicus* on *Vicia faba*, *Paederia foetida* and *Ipomoea nil* (mean ±SE). Different letters above the bars indicate significance difference ($P < 0.001$; Tukey HSD test) between the treatments.

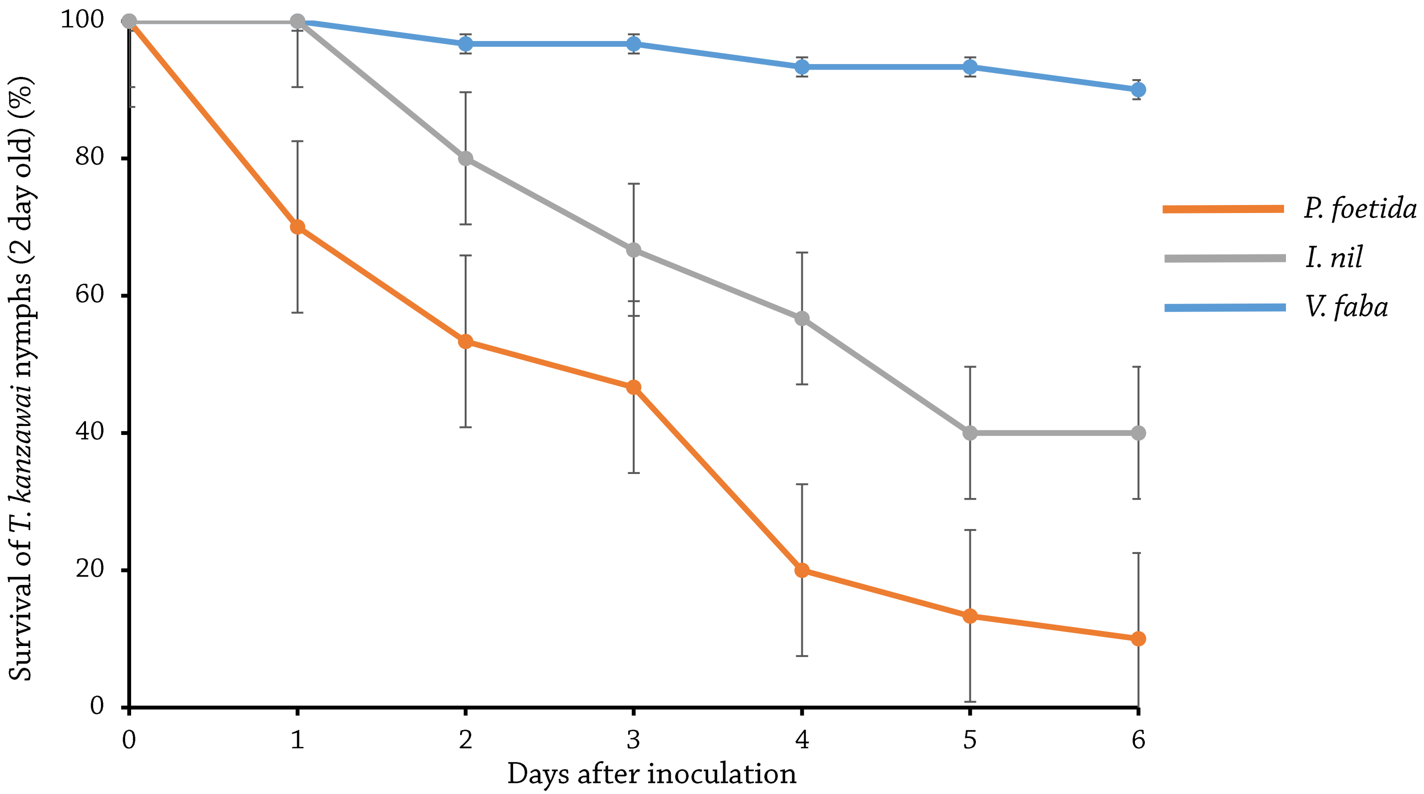

Fig. 4.5 Survival of *Tetranychus kanzawai* two-day old nymphs (mean number of mites ±SE) on *Paederia foetida*, *Ipomoea nil* and *Vicia faba*.

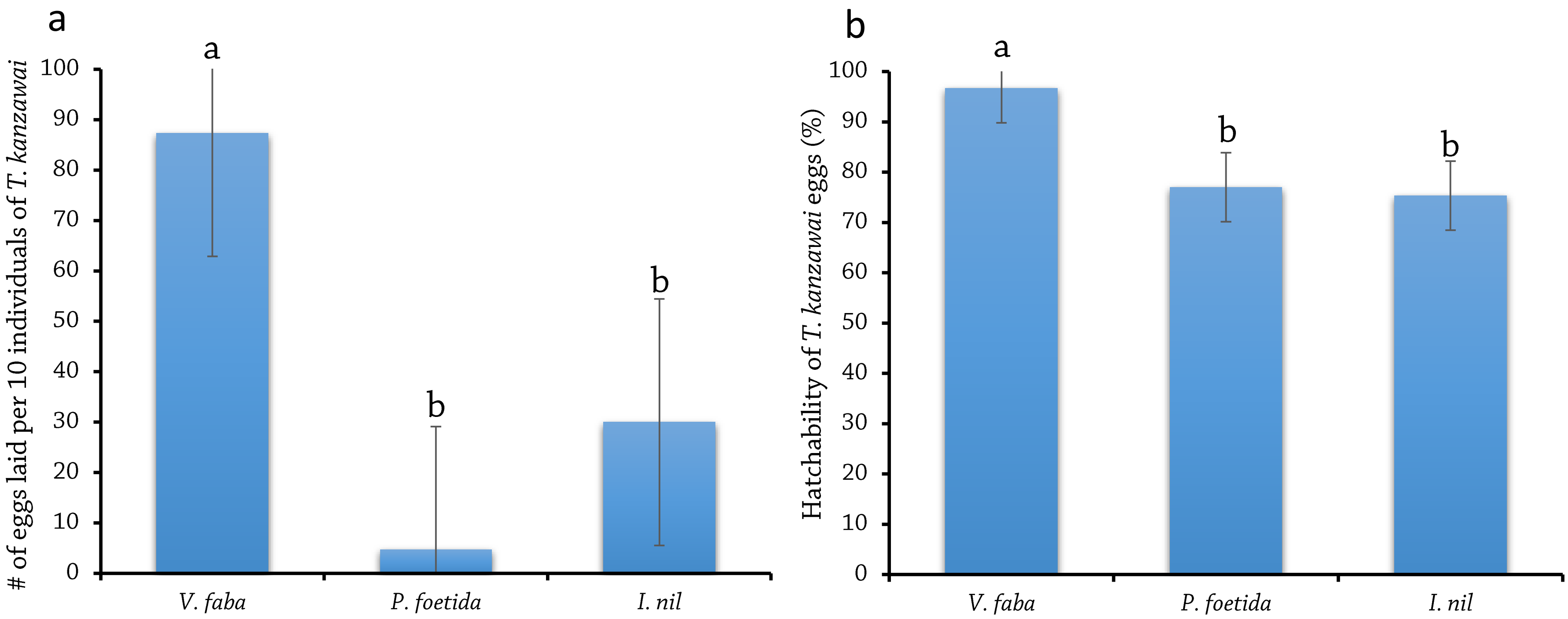

Fig. 4.6 Effect of host plants *Vicia faba*, *Paederia foetida* and *Ipomoea nil* on the fecundity of *Tetranychus kanzawai*. (a) shows the number of eggs laid per 10 newly emerged adult females of *T. kanzawai* and (b) shows the proportions of *T. kanzawai* eggs (%) hatched on each host plants. Different letters above the bars indicate significance difference (*P*< 0.01; Tukey HSD test for (a) and *P*< 0.05; Tukey HSD test for (b) between the treatments.

Table 4.1 Location, area, and pest control of each study site in 2014 and 2015

Plot	Latitude/ longitude	Area (m^2)	2014		2015	
			Product applied	Farm type and weed management	Product applied	Farm type and weed management
Site II	N34° 35’02.7” E133° 39’41.1”	1500	permethrin (Apr 18) chlothianidine (May 14) chlorantraniliprole (May 24), etoxazole (Jun 8) dinotefuran (Jun 23) tolfenpyrad (Jul 6) acetamiprid (Jul 20) DMTP (Sept 6) MEP (Oct 10)	Conventional/groundcover	No data	Conventional/groundcover
Site V	N34° 35’02.3” E133° 39’39.9	3000	permethrin (Apr 18) chlothianidine (May 14) chlorantraniliprole (May 24), etoxazole (Jun 8) dinotefuran (Jun 23) tolfenpyrad (Jul 6) acetamiprid (Jul 20) DMTP (Sept 6) MEP (Oct 10)	Conventional/groundcover	No data	Conventional/groundcover

Table 4.2 Detection of chloroplast sequence of *Paederia foetida* from phytoseiid mites

Species	n[a]	*P. foetida*
N. californicus	19	4
A. eharai	57	9

[a] number of phytoseiid mite samples (*Neoseiulus californicus* and *Amblyseius eharai*) examined.

Chapter Five

General Discussion, conclusion and future prospects

McMurtry and Croft (1997) detailed the types of phytoseiid mites, their life style and their role in biological control. Four major types of phytoseiid mites were proposed; Type I, specialized predators of Tetranychus species represented by the *Phytoseiulus* species; Type II, selective predators of tetranychid mites represented by *Galendromus*, some *Neoseiulus*, and a few *Typhlodromus* species; Type III, generalist predators represented by some *Neoseiulus* species and most *Typhlodromus* and *Amblyseius* species, as well as species in all other genera about which information is available; Type IV, specialized pollen feeders/generalist predators represented by *Euseius* species (McMurtry and Croft 1997). The revised version of the above proposed grouping was later published in 2013 with the four major types reclassified into sub-clusters (McMurtry et al. 2013). Classification of large quantities of phytoseiid mites inhabiting peach orchards is the first step toward biological control of spider mites using phytoseiid mites. However, classification of phytoseiid mite species based on morphological characteristics alone is sometimes difficult (Navajas et al. 1999; Jeyaprakash and Hoy 2002). Sonoda et al. (2012) developed a QS-based method to estimate the phytoseiid mite species composition. The method was developed for five phytoseiid mite species which were observed to have been dwelling in the peach orchards: *N. californicus*, *N. womersleyi*, *A. eharai*, *A. tsugawai*, and *E. sojaensis*.

However, later, additional phytoseiid mite species were detected on peach leaves (*T. vulgaris*) and wild plants (*T. vulgaris*, *S. okinawanus*, and *N. makuwa*) in Kurashiki City, Okayama Prefecture, Japan. Species-specific polymorphic sites for the three species were detected on the 28S ribosomal gene (Fig. 2.1). In this study, henceforth, quadratic equations were newly developed and were used to estimate the phytoseiid mite species compositions on peach leaves and wild plants. Results reveal that three major phytoseiid mites, *N. californicus*, *A. eharai*, and *E. sojaensis*, were occurring in the peach orchards surveyed.

E. sojaensis was observed to be dominant at Site I with no synthetic pesticide use. The dominant existence of *E. sojaensis* was not observed at any other study sites. *E. sojaensis* is a Type IV phytoseiid mite: i.e., generalist phytoseiid mite (McMurtry and Croft 1997). McMurtry (1992) reported that generalist phytoseiid mites play a vital role with the decreased spider mite densities. Ozawa and Yano (2009) showed in the laboratory that *E. sojaensis* can prey on adults of *T. kanzawai* as long as the predator settled on a plant before the prey. The successful suppression of spider mites by the generalist phytoseiid mite at Site I at which no outbreak of spider mite beyond control threshold density might be attributable to the earlier settlement of *E. sojaensis*. No outbreak of spider mite beyond control threshold density was also observed in Site III. On the other hand, in Site II, Site IV, and Site VI, spider mites in some trees were beyond control threshold density. Site III, conventionally managed with pesticides including herbicides had no groundcover, while Site II, Site IV, and Site VI, are conventionally managed by pesticides excluding herbicides had groundcover. *N. californicus* was observed to be dominant in Site III while in Site II, Site IV, and Site VI, in general, *A. eharai* was observed to be dominant. The dominant existence of *N. californicus* might contribute to the successful control of spider mites at Site III. The difference in phytoseiid mite species compositions between Site III and the other three conventional orchards might be attributable to presence or absence of groundcover.

Reportedly, the dominant existence of *N. californicus* is often observed in orchards that received more pesticides applications (Amano et al. 2004; Kishimoto 2002; Kishimoto et al. 2007). However, pesticide practices before harvest period were similar between Site II and Site III where *A. eharai* and *N. californicus* were dominant respectively. Therefore, pesticide practices cannot be the main cause of the large share of *N. californicus* at Site III. Aguilar-Fenollosa et al. (2011) reported that the predatory phytoseiid mites might be affected by increased competition with generalist phytoseiid mites in clementine mandarin orchards with wild groundcover. *N. californicus*, *A. eharai*, and *E. sojaensis*, belonging respectively to Type II (selective predators of tetranychid mites), Type III (generalist predators), and Type IV (specialized pollen feeders/generalist predators) phytoseiid mites (McMurtry and Croft, 1997), were detected at Site II. At Site II, the advantage of *N. californicus* with higher tolerance to pesticides might be diminished by increased competition with the other phytoseiid mites such as *E. sojaensis* and *A. eharai*. However, at Site III with bare ground and less competition with the other phytoseiid mites, the dominance of *N. californicus* might be more stable. The stable dominant occurrence of *N. californicus* might contribute to successful control of spider mites at Site III. Furthermore, although dependence on insecticides and acaricides are deemed a recommended practice for successful control of insect pests, for instance; Site III, development of resistance and the general lack of knowledge or vaguely described mode of actions of known acaricides (van Leeuwen et al. 2013), as well as the negative impacts on the natural flora and fauna are a major concern in pest management systems. Effect of different pesticide practices and ground cover managements on phytoseiid mite species inhabiting in peach orchards must be examined in greater detail in future studies.

There is possibility that wild groundcover could be functioning as origin of spider mites rather than phytoseiid mites at Site II, Site IV, and Site VI (Brandenburg and Kennedy1981; Tedders et al. 1984; Meager and Meyer 1990). Therefore, wild plant species inhabiting the undergrowth vegetation of peach orchards were surveyed to select insectary plants which promote functional roles of phytoseiid mites in spider mite control. Amongst 219 wild plants surveys, *V. persica, O. corniculata, P. foetida* and *P. longiseta* were observed to be harboring larger quantities of phytoseiid mites (Table 3.2). However, *V. persica* contained a large quantity of *T. kanzawai* hence, *V. persica* was excluded from the list of insectary plants. With larger number of phytoseiid mites and less to none of spider mites i.e. *T. kanzawai* and *P. mori* on *O. corniculata, P. foetida* and *P. longiseta*, it could be implied that these plants either suppress the movement of spider mites from groundcover to peach leaves or acts as the means of distribution of phytoseiid mites from groundcover to peach leaves. Evaluation of these groundcover plants harboring numerous phytoseiid mites as insectary plants remains as subject for future investigations.

The phytoseiid mite species composition on four wild plant species (*V. persica*, *O. corniculata*, *P. foetida,* and *P. longiseta*), harboring larger quantities of phytoseiid mites (Table 3.2), at Site I, Site II, and Site IV was examined. The phytoseiid mite species composition on the four wild plant species was varied among peach orchards. However, the phytoseiid mite species composition was similar with that on the peach leaves. *P. harti* is distributed worldwide and are usually found on *Oxalis* spp. (Dubitzki and Gerson 1987), but they have never been observed on peach leaves (Sonoda, unpublished data). To verify that phytoseiid mites move from ground to aerial vegetation, PCR amplification of *P. harti* DNA in phytoseiid mites collected from peach leaves was conducted. Results shows that *P. harti* DNA was detected from phytoseiid mites including *E. sojaensis*, *A. eharai,* and *N. californicus* (Table 3.3). Thus I verified dispersal of the phytoseiid mites occurred from groundcover to peach trees possibly through ambulatory or aerially. In addition to dispersal studies, phytoseiid mite preference of spider mites, i.e. *T. kanzawai* and *P. mori* was conducted. Results reveal that the feeding preference for the three major phytoseiid mite species was more inclined towards *T. kanzawai* rather than *P. mori* in the field. There is however, no data to elucidate the growth stages of spider mites at which phytoseiid mites mostly prey on. Predatory performance of phytoseiid mite species on various growth stages of *T. kanzawai* and *P. mori* remains to be examined to ascertain whether the food preference depends on the food quality or on the defense strategy of the prey stage.

Among the four wild plants, *P. foetida* was an ideal candidate for further examination of its functional significance towards spider mite control as it showed potent with harboring larger quantities of phytoseiid mites during the peach growing season, harbored substantially no spider mites and was occurring during the vital stages of the peach producing season (Table 3.2). Trees with exclusive occurrence of *P. foetida* yielded populations of spider mites less than trees without *P. foetida* at Site V (Fig. 4.3). Laboratory assays on survival and fecundity of *T. kanzawai* showed that *P. foetida* is unsuitable for *T. kanzawai* as a host plant (Fig. 4.5 and Fig. 4.6). By contrast, no such detrimental effects of *P. foetida* were observed for phytoseiid mites (Fig. 4.4). Zheng at al. (2004) reported that *P. foetida* exudes strong sulfur odor when bruised. Hoy et al. (1982) reported that phytoseiid mites are rendered to have a higher tendency to tolerate all formulations of sulfur. It is still unknown why larger numbers of phytoseiid mites are observed when there is no sources of food available in the likes of spider mites present on *P. foetida*. The numerous number of phytoseiid mites with sustained survival trait while lacking food sources in the form of spider mites remains for future investigations.

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List of Publications

David Wari, Jun Yamashita, Yoko Kataoka, Yoko Kohara, Norihide Hinomoto, Hidenari Kishimoto, Shingo Toyoshima, Shoji Sonoda: *Population survey of phytoseiid mites and spider mites on peach leaves and wild plants in Japanese peach orchard*. Experimental and Applied Acarology 03/2014; 63: 313-332. DOI:10.1007/s10493-014-9788-9

David Wari, Ken Funayama, Hidenari Kishimoto, Masatoshi Toyama, Shoji Sonoda: *Molecular verification of dispersal of phytoseiid mites from groundcover plants to tree leaves in Japanese peach orchards*. Biological Control 10/2014; 80:143-155. DOI:10.1016/j.biocontrol.2014.10.002