PEST MANAGEMENT AND SAMPLING

Overwintering and Comparative Sampling of *Neoseiulus fallacis* (Acari: Phytoseiidae) on Ornamental Nursery Plants

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ABSTRACT The predatory mite *Neoseiulus fallacis* (Garman) is an important biological control agent of spider mites in many agroecosystems, including ornamental nurseries. In this study, sampling methods, tendencies to overwinter among a range of plant types, and the effect of winter protective practices were assessed for *N. fallacis* females. Ten spider mite-infested plant species representing a range of five plant types (conifer, shade tree, evergreen shrub, deciduous shrub, herbaceous perennial) were inoculated with 100 adult female *N. fallacis* in late autumn. Early the following spring, *N. fallacis* was extracted from each plant species by either washing plant parts (leaves, branches, trunks-crowns) in 70% ethanol and filtering contents or placing plant parts into separate Berlese funnels for 5 d. The washing method extracted more *N. fallacis* than the funnel method. When comparing overwintering among a range of plant types, higher densities of *N. fallacis* were extracted from conifers > evergreen shrubs > herbaceous perennials = deciduous shrubs = shade trees. With respect to overwintering location of *N. fallacis* within plants, higher densities of *N. fallacis* were recovered on leaves than trunks-crowns; branches were intermediate. Densities of *N. fallacis* were positively correlated with those of their spider mite prey. Higher densities of *N. fallacis* were collected from plants that were held in a greenhouse versus those left unprotected or under a sheet of polyethylene plastic. Manipulating overwintering sites for the conservation of phytoseiid natural enemies is discussed.

KEY WORDS *Neoseiulus fallacis*, diapause, Tetranychidae, biological control

PREDATORY MITES IN the family Phytoseiidae are important biological control agents of plant-inhabiting pest mites (Helle and Sabelis 1985). Among the Phytoseiidae, only the adult female enters diapause, which in temperate zones is manifested as an arrestment in activity and reproduction (Overmeer 1985). Overwintering sites sought out by diapausing female phytoseiids within plant systems have been assessed often. In early studies, Chant (1959) suggested that overwintering females do not fall with leaves in autumn but move to sites such as splintered twigs, crevices, or under bark. Putman (1959) collected phytoseiids from peach orchards and found that overwintering sites varied among species, ranging from orchard weeds to many locations on trees. Overmeer (1985) suggested that most phytoseiid species show a locational preference for overwintering on a plant. For example, *Typhlodromus pomi* (Parrot) and *Amblyseius umbraticus* (Chant) preferred small twigs (Kinsley and Swift 1971), whereas *Typhlodromus bakeri* (Garman) and *Typhlodromus pyri* Scheuten were found primarily under apple trunk bark.

In this study, we sought to assess tendencies of *Neoseiulus fallacis* (Garman) to overwinter among a range of ornamental plants in temperate regions of western Oregon, U.S. This species is distributed throughout North America and is an important predator of spider mites on many plant types (McMurtry and Croft 1997). In Oregon, inoculative releases of *N. fallacis* are commonly made to reduce spider mites in apple, corn, hops, strawberry, peppermint, and, most recently, nursery plants (Strong and Croft 1995, Morris et al. 1996, Croft and Coop 1998, Pratt and Croft 1998). In Michigan under severe winter conditions, Croft and McGroarty (1977) found that *N. fallacis* overwintered mainly in ground cover of apple orchards. In New York apple orchards under similar conditions, Nyrop et al. (1994) found that *N. fallacis* overwintered on apple twigs and occasionally in ground cover. Morris et al. (1996) found that in more mild, arid areas of Oregon, *N. fallacis* overwintered on peppermint leaves in early winter, but as temperatures decreased the adult females either moved to or only survived in debris near the soil surface.

Knowledge concerning the overwintering behaviors of predator and prey mites is integral to developing integrated pest management (IPM) strategies both within a single season and over several years (Hoy and Flaherty 1970). There are no studies that characterize the suitability of plant types for overwintering of phytoseiids under similar environmental conditions. Many plants occur in multicropping systems such as ornamental nurseries, and certain types (e.g., conifers, herbaceous perennials) may provide better overwintering sites than others. In addition, various cultural practices are employed to minimize plant

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damage from extremes of temperature, wind, and precipitation. For instance, ornamentals may be placed under protective plastic sheets, held in greenhouses, or left out of doors (unprotected) during winter. Therefore, the four specific objectives of our study were as follows: (1) to compare methods of sampling ornamental plants for overwintering *N. fallacis,* (2) to measure frequency of overwintering by *N. fallacis* among 10 species of plants that included five general types, (3) to determine overwintering location of *N. fallacis* within a plant, and (4) to compare effects of plastic coverings, unheated greenhouses and unprotected plants on overwintering success of *N. fallacis.*

Materials and Methods

Ten ornamental species (with eight replicate plants each) ranging from coniferous to herbaceous perennials (Table 1) were obtained from nurseries on 1 October 1997 and placed into a greenhouse (10 by 10 m) at 22:18 ± 5°C, 75 ± 10% RH, and a photoperiod of 16:8 (L:D) h. All plants were potted in plastic containers and watered daily. The spider mites *Oligonychus illicis* (McGregor), *O. ununguis* (Jacobi), or *Tetranychus urticae* Koch were collected from field-grown ornamental plants and reared at 25°C, 60 ± 10% RH, and a photoperiod of 16:8 (L:D) h for at least one generation and then inoculated into each test plant within the greenhouse on 2 October 1997 (Table 1). Specimens of *N. fallacis,* in an inert granular medium, were provided by Biotactics (Grand Terrace, CA). Upon arrival, a subsample of the predatory mites were identified to species (Schuster and Pritchard 1963) and allowed to disperse onto lima bean leaves infested with *T. urticae.* Bean leaves were scanned using a 40× microscope to quantify densities of *N. fallacis,* and multiple leaves containing 100 *N. fallacis* adult females were inoculated into each test plant in the greenhouse on 8 October 1997. To evaluate mite densities after release of *N. fallacis,* we reviewed (without removal from the plant) five leaves or branches (Table 1) per replicate of each plant species with an optical visor at 10× magnification every 7 d for 2 wk. To condition mites to external weather and possibly induce diapause, we incrementally decreased daylength by 2 h every 10 d and adjusted temperatures within the greenhouse until they approached those of the external environment. On 3 November 1997, we removed the test plants from the greenhouse and randomly placed them outside on a gravel substrate (bed) with ≈1 m spaces between plants. Plants were watered below the foliage as needed. We also monitored daily high and low temperatures and rainfall during October–February 1997–98 to compare with the prior 36 yearly averages.

The following two methods of extracting overwintering phytoseiids from plant material were compared: (1) collecting mites from plant materials placed in Berlese funnels (the funnel method) or 2) washing plant materials with ethanol and collecting predators through filtration (the washing method). On 15 February we extracted *N. fallacis* from four randomly selected replicates of each plant species by placing the plant parts, as described in Table 1, or the top 3 cm of soil and associated litter into separate Berlese funnels for 5 d under a 40-W light bulb (Morris et al. 1996). Temperatures were 25 ± 2°C within the funnel. We placed a bouquet of lima bean plants within the base of each funnel, with stems resting in a 0.5-liter jar filled with water. Bean leaves were infested with *T. urticae* and the leaves were arranged to thoroughly cover the funnel base to prevent predators from falling into the water. Bean leaves were scanned every 24 h and each phytoseiid mite was mounted on a glass slide for identification under a phase-contrast microscope at 200× magnification.

The plant washing method also was evaluated on 15 February 1998. The method entailed placing the previously mentioned plant parts (Table 1), but not soil and associated litter, in separate 1-liter jars and adding 300 ml of 70% ethanol (Zacharda 1989, Calkin 1991). Lids were placed on jars and shaken manually for 30 s, left to rest for 1 min, and then shaken again for 30 s. Plant material was removed with forceps and slowly rinsed with 70% ethanol over the jars. The ethanol and associated contents were poured into a Whatman #4 filter paper funnel, gravity filtrated, and scanned for mites within 5 min under a binocular microscope at 40× magnification. All phytoseiid mites washed from plant parts were mounted on glass slides for identification. Spider mites washed from plants also were recorded.

To measure the effect of cultural practices on overwintering *N. fallacis,* we collected 15 plants of either *Viburnum davidii* or *Picea glauca* 'Conica' on 3 October 1998. Plant size and selection, inoculation of spider mites and predators, identification of predators, and conditioning in the greenhouse were performed as before. Plants were removed from the greenhouse on 5 November 1998, and five plants of each ornamental species were randomly placed in either an unheated greenhouse, left unprotected (as in the previous test), or placed under a sheet of protective plastic. The greenhouse consisted of an unlighted polyethylene-enclosed structure (15 by 7 m) with a shade cloth drawn over the roof to reduce solar radiation. Unprotected plants were placed 10 m from the greenhouse in an outdoor nursery bed exactly as in 1997–1998 tests. The protective plastic treatment was similar to the unprotected treatment except that a 3 mil white plastic sheet was drawn over the plants and rested on foliage. The plastic sheet had 0.5-cm holes spaced 0.5 m apart and was held secure with 20-cm stakes at the corners of the plastic. As is consistent with nursery practices, the plastic was removed on days when temperatures exceeded 20°C but was replaced each night. In all cases, ornamental plants were placed directly on a gravel bed and irrigated below foliage once a week. In addition, maximum and minimum air temperatures were recorded three times a week in the greenhouse, under the plastic and near unprotected plants. Only the washing method was used to extract *N. fallacis* from overwintering sites in this test.

Table 1. Overwintering of *N. fallacis* on 10 ornamental plants with comparison of sampling methods

Ornamental species	Plant type	Plant parts	Container size[a]	Plant ht[b]	Plant diam[c]	Pest mite	Spider mite densities on:		Predator densities Avg (SD)	
							10 Oct.	15 Feb.	Washing[d]	Funnel[e]
Picea glauca 'Conica'	Conifer	B, T, (S)	3.8	48	23	*Oligonychus ununguis*	25.3	34.3	19.75 (4.43)a	13.00 (4.97)a
Thuja occidentalis 'Pyramidalis'	Conifer	B, T, (S)	3.8	42	23	*Oligonychus ununguis*	18.5	15.1	10.75 (2.99)ab	6.50 (3.70)ab
Viburnum davidii	Evergreen shrub	L, B, T, (S)	3.8	46	43	*Tetranychus urticae*	37.1	2.7	7.75 (2.63)ab	5.75 (2.75)ab
Ilex crenata 'Convexa'	Evergreen shrub	L, B, T, (S)	3.8	66	48	*Tetranychus urticae*	8.5	1.26	5.25 (4.19)b	2.25 (0.58)bc
Rhododendron 'Ana Kruschke'	Evergreen shrub	L, B, T, (S)	3.8	53	46	*Oligonychus illicis*	16.2	10.1	3.75 (2.22)bc	3.00 (2.16)bc
Hemerocallis hybrid 'Mary Todd'	Herbaceous perennial	C, (S)	3.8	71	56	*Tetranychus urticae*	35.7	0	1.00 (1.15)cd	0.00 (0.00)d
Viburnum opulus 'Sterile'	Deciduous shrub	B, T, (S)	3.8	41	25	*Tetranychus urticae*	10.2	0	0.75 (0.96)cd	0.25 (0.50)cd
Acer x freemanii 'Jeffersred'	Shade tree	B, T, (S)	18.9	229	76	*Tetranychus urticae*	18.3	0	0.25 (0.50)d	0.50 (1.00)cd
Euonymous alatus 'Compacta'	Deciduous shrub	B, T, (S)	3.8	53	36	*Tetranychus urticae*	22.7	0	0.25 (0.50)d	0.25 (0.50)cd
Corylus avellana 'Contorta'	Deciduous shrub	B, T, (S)	3.8	102	30	*Tetranychus urticae*	11.5	0	0.00 (0.00)d	0.25 (0.50)cd
									$P<0.0001$	$P<0.0001$

Plant parts used for extraction of *N. fallacis*; L, leaves; B, branches; T, trunk; C, crown; (S), soil and associated litter, soil was only used in the funnel extraction method. Means followed by different letters are significant at $\alpha = 0.05$ (Tukey HSD).

[a] Plastic plant container (pot) size in liters.

[b] Average height (in cm) of plants ($n = 8$).

[c] Average diameter (in centimeters) of plants at widest point of canopy ($n = 8$).

[d] Average number of *N. fallacis* extracted with the funnel method.

[e] Average number of *N. fallacis* extracted with the alcohol wash method.

Statistical Analyses. To compare sampling methods, we pooled all within-plant samples and $\log(x + 1)$ transformed the number of overwintering *N. fallacis* extracted from each method. We then used a paired *t*-test to compare sampling methods. To graphically compare methods, we subtracted the densities of predatory mites recovered using the funnel method from those of the washing method, and regressed the difference on washing values. To compare overwintering *N. fallacis* among the 10 plant species or among protective cultural practices, we $\log(x + 1)$ transformed all values and used analysis of variance and the Tukey honestly significant difference (HSD) to distinguish differences among treatments. Simple linear regression was used to estimate the correlation of spider and predaceous mite densities collected on 15 February. When comparing *N. fallacis* on different plant types or locations within a plant, conventional parametric statistics are not appropriate because of unequal sample sizes and unknown underlying distributions of mites. One alternative to parametric tests is a randomization test, which Manly (1991) found more powerful than other nonparametric tests. Therefore, using data from the washing technique only, we randomly reordered the numbers of *N. fallacis* found within each plant type or plant part 1,000 times and acquired the mean and standard deviation for respective distributions. We then compared distributions of plant types and among plant parts with a two-sample *t*-test for means (Ramsey and Schafer 1997). We used the Sidak inequality formula to maintain a 0.05 experiment-wise α level when comparing overwintering within and between plants (Jones 1984). Caution should be used when drawing inferences from statistical comparisons among plants because data were not standardized to adjust for differences in plant size, morphology, humidity within plant canopies, or other parameters that may influence overwintering.

Results and Discussion

Winter temperatures for the study period fell within one standard deviation of the average of the previous 36 yr in the Willamette Valley except during November, which was warmer (Fig. 1). Similarly, precipitation fell within one standard deviation of the 36-yr average except for December, which was somewhat lower. These data suggest that *N. fallacis* experienced typical winter conditions for ornamentals grown in the region. With respect to diapause of *N. fallacis,* Rock et al. (1971) found that the critical photoperiod of two strains was between 11.75 and 12 h daylight at 15.6°C, which is within the range of this study. In addition, all predators recovered by either method of collection appeared to be consistent with descriptions of diapausing phytoseiids: pale colored, dorsally flattened females without eggs in their idiosomas (Veerman 1992). We assumed, therefore, that *N. fallacis* had entered diapause during this study.

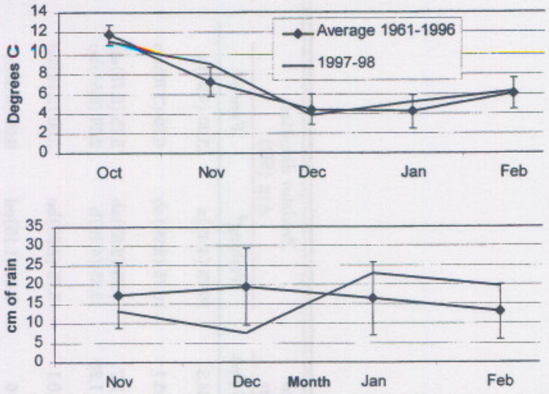

Fig. 1. Comparison of temperature and precipitation for the winter months of 1997–1998 versus the mean of the previous 36 yr in the Willamette Valley, Oregon, U.S.

Washing plants with ethanol extracted more *N. fallacis* than did the funnel extraction method ($t = 2.67$, $df = 39$, $P = 0.005$). When graphically compared (Fig. 2), both methods appeared equally effective at low predator densities, although washing recovered more *N. fallacis* than the funnel method when *N. fallacis* densities were high. These findings are consistent with previous reports that washing and filtering is as effective as visually scanning plant material when sampling phytoseiids (Calkin 1991). In addition, washing recovered spider mite adults and overwintering eggs (Table 1). Therefore, this technique may be well suited for monitoring overwintering pest and predator mite densities on a variety of plant types (Calkin 1991).

Among individual plants, *N. fallacis* was more abundant on *Picea glauca* than all others tested except *Thuja occidentalis* and *Viburnum davidii* (Table 1). Using the washing method, more *N. fallacis* were recovered from *V. davidii* and *Ilex cranata* than *Hemerocallis* and the broad leaf deciduous species. Regardless of sampling method, few *N. fallacis* were recovered from *Hemerocallis* and broadleaf deciduous plants. Also, both sampling methods provided an identical sequence of the

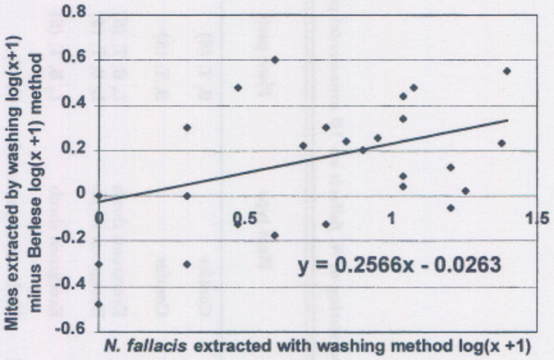

Fig. 2. Comparison among extraction methods of overwintering *N. fallacis* from ornamental plants. The difference of washing method minus funnel method is plotted against the washing method. The positive trend line suggests that washing extracts more overwintering *N. fallacis* than the funnel method as mite densities increase.

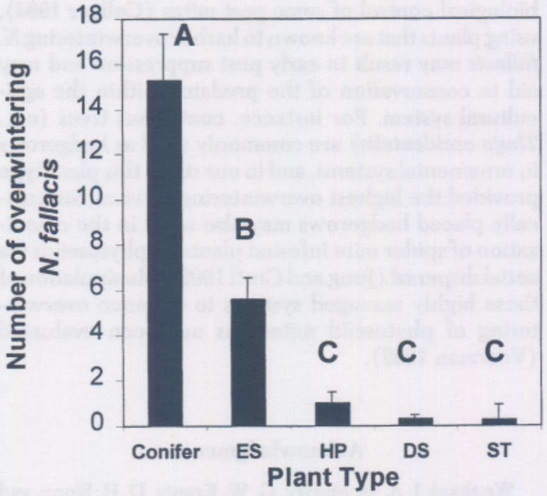

Fig. 3. Number of *N. fallacis* overwintering on four different plant types after inoculation of 100 adult females in late autumn. ES, evergreen shrub; HP, herbaceous perennial; DS, deciduous shrub; ST, shade tree. Columns followed by different letters are significant at $\alpha = 0.05$.

five plant species harboring the most *N. fallacis* (Table 1).

When data from the washing technique were pooled into plant types, *N. fallacis* overwintered most on conifers (Fig. 3). These data were surprising considering that *N. fallacis* is not common on conifers in the region (Hadam et al. 1986, Calkin 1991). However, *N. fallacis* is found in the eastern United States on conifers and can provide adequate control of *O. ununguis* (Boyne and Hain 1983). Broadleaf evergreens harbored more *N. fallacis* than herbaceous perennials and deciduous plants ($t = 5.44$, df = 26, $P < 0.001$; $t = 4.25$, df = 14, $P = 0.001$, respectively). Among broadleaf evergreens, *N. fallacis* were common on *V. davidii* but scarce on *I. cranata* and on rhododendron (Table 1). Herbaceous perennials and broadleaf deciduous plants did not differ in densities of *N. fallacis* ($t = 1.19$, df = 18, $P = 0.25$).

When comparing overwintering locations as collected from the washing technique, more *N. fallacis* were found on leaves than on trunks ($t = 2.89$, df = 46, $P = 0.001$); levels of predators on branches were intermediate (Fig. 4). No *N. fallacis* were extracted from soil and associated litter using the funnel method. This is contrary to reports that *N. fallacis* commonly overwinters in litter or soil (Putman 1959, Morris et al. 1996). One explanation may be that there were different environmental conditions present among these studies, which may have affected the depth of diapause entered into by *N. fallacis* (Veerman 1992). For example, Morris et al. (1996) showed that *N. fallacis* moved from leaves to the soil as temperatures decreased. The effect of temperature on selection and persistence in overwintering sites is unclear.

Densities of overwintering *N. fallacis* collected on 15 February were positively correlated with those of spider mites ($F = 33.87$; df = 1, 8; $P = 0.0004$; Table 1). Highest densities of *N. fallacis* were associated with *O. ununguis*, a species that develops large populations in spring and fall (Calkin 1991). These results may indicate that overwintering survival of *N. fallacis* is related in part to feeding on warm winter days. Insufficient prey densities during warm periods in winter or early spring may result in starvation or dispersal of the predator from plants (Pratt et al. 1998). In other studies of different plants and climates, overwintering sites of *N. fallacis* varied according to severity of conditions and presence of prey. For example, in mild fruit-growing regions of New Zealand, the major winter mortality of *N. fallacis* was thought to be a result of overexploitation of prey and starvation rather than cold temperatures (Penman and Chapman 1980). In the more severe fruit-growing regions of New York, U.S., Nyrop et al. (1994) found that *N. fallacis* overwintered within orchard trees when prey were present but dispersed to ground cover or orchard margins in the absence of prey. Similarly, Morris et al. (1996) showed that overwintering *N. fallacis* were most prevalent when high densities of spider mites also were collected, and as colder temperatures occurred, diapaused mites moved from leaves to more protected sites in debris near the soil. As previously reported, various nonspider mite prey or foods will enhance survival of *N. fallacis* when tetranychid mite densities are low (Pratt et al. 1999). What role these alternative foods play in overwintering survival is unknown.

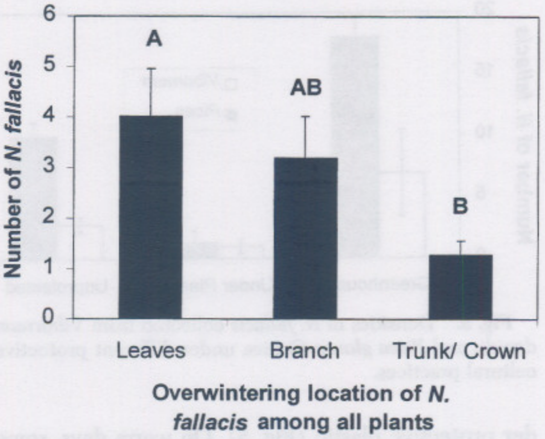

Fig. 4. Overwintering location of *N. fallacis* within 10 ornamental plants after inoculation of 100 adult females in late autumn. Data are from an ethanol washing method only. Columns followed by different letters are significant at $\alpha = 0.05$

Significantly more *N. fallacis* were collected from plants in the greenhouse than under protective plastic or unprotected (Fig. 5). These differences may be the result of less variable and warmer temperatures within the greenhouse (Fig. 6). Also, there was very little wind in the greenhouse and this may have reduced aerial dispersal of mites from plants (Pratt et al. 1998). Lowest levels of overwintering mites were found un-

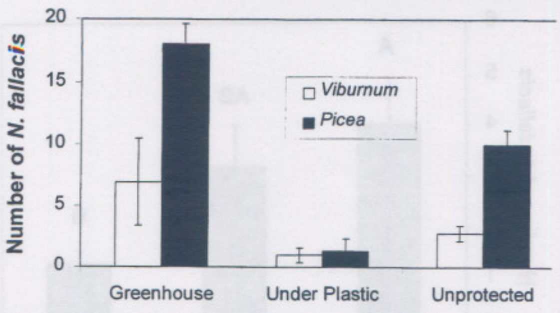

Fig. 5. Densities of *N. fallacis* collected from *Viburnum davidii* and *Picea glauca* Conica under different protective cultural practices.

der protective plastic (Fig. 5). On warm days, some predators that move from foliage to plastic may not return to plants. As described above, warmer conditions may have also caused predators to come out of diapause and feed more on spider mites, thus depleting the food supply and consequently resulting in starvation (Pruszynski and Cone 1973). These findings suggest that covering cold-sensitive plants with protective plastic may negatively affect overwintering of *N. fallacis* in ornamental plants.

In summary, our findings on overwintering survival of *N. fallacis* have relevance to cultural practices, biological control, and overall ornamental IPM. Because increased predator densities in early spring improves biological control of some pest mites (Collyer 1964), using plants that are known to harbor overwintering *N. fallacis* may result in early pest suppression and may aid in conservation of the predator within the agricultural system. For instance, coniferous trees (e.g., *Thuja occidentalis*) are commonly used as hedgerows in ornamental systems, and in our study this plant type provided the highest overwintering survival. Strategically placed hedgerows may also assist in the colonization of spider mite infested plants by phytoseiids via aerial dispersal (Jung and Croft 1999). Manipulation of these highly managed systems to enhance overwintering of phytoseiid mites has not been evaluated (Veerman 1992).

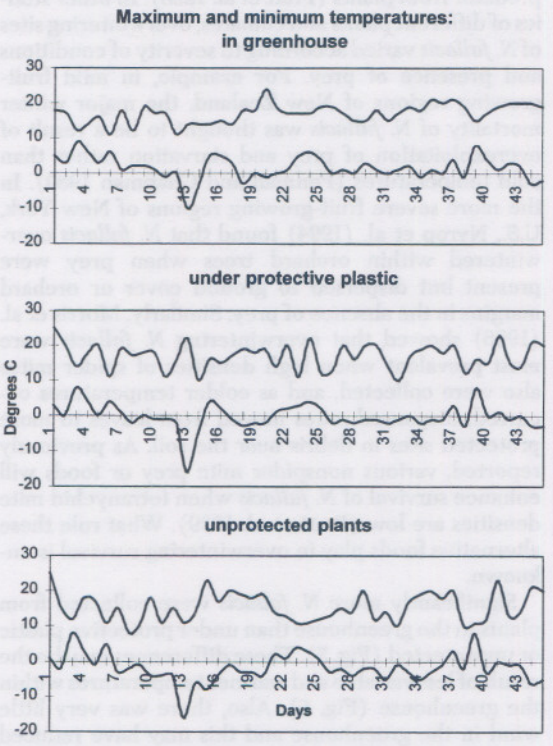

Fig. 6. Maximum and minimum temperatures recorded under three winter cultural practices.

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