Experimental and Applied Acarology **33:** 327–339, 2004.

Low-density releases of *Neoseiulus fallacis* provide for rapid dispersal and control of *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae) on apple seedlings

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Key words: Biological control, Dispersal, Equilibrium densities, Inundative release, Predaceous mites, Predator:prey ratios

Abstract. Releases of *Neoseiulus fallacis* (Garman) at 1500–6000 per ha when prey were at 0.1–0.3 per leaf provided seasonal control of *Tetranychus urticae* Koch (all stages) at 1–2 per leaf in an apple seedling rootstock nursery. Predaceous mites (all stages) increased to 0.3–0.4 per leaf after releases and predator prey ratios of $\leq$ 1:3–7 provided pest regulation thereafter. Such low-density releases were thought to be effective because multiple dispersal bouts allowed predators to locate widely distributed spider mites (on 2–6% of leaves). A random-diffusion model simulating predator dispersal (incorporating wind speed and direction parameters) adequately explained movement and pest control patterns. An upright, dense, uniform planting of apple seedlings was an effective producer and recipient for dispersing predators and these attributes seemed to explain why biological control was so effective. Low-density releases of *N. fallacis* for control of *T. urticae* are predicted to be less effective on other crops with less prominent profiles and soil coverage.

Introduction

When introducing a biological control agent to achieve effective pest suppression, economic constraints influence IPM practitioners to use the fewest natural enemies and distribute them with the least effort. If prey are too dense at release time, predators may not give control before severe pest damage occurs. Alternatively, introductions at prey levels that are too low are a common explanation for biological control failure (Beirne 1975; Stiling 1993). With predaceous phytoseiid mites, effective control of tetranychid spider mites can often be predicted based on predator:prey ratios that are sampled at low densities (Croft and Nelson 1972; Croft and McGroarty 1977). For example, a ‘near-equilibrium’ (called ‘equilibrium’, hereafter) predator:prey ratio occurs when predators are about enough to maintain pests from increasing or decreasing much and such ratios occur among natural populations. With *Neoseiulus fallacis* (Garman) and *Tetranychus urticae* Koch, ratios of 1:5–3 can provide such conditions in large apple orchard trees (Croft and McGroarty

1977). A near-equilibrium predator:prey ratio may be difficult to attain because it is either too difficult or costly to release enough predators to almost immediately suppress a pest. Equilibrium predator:prey ratios are affected by many factors such as the plant system for which pest control is sought, environmental conditions and the prey–predator species involved (and especially their dispersal rates; Coop and Croft 1995).

In our preliminary tests, control of *T. urticae* by *N. fallacis* on apple seedlings was based on studies of these mites in Midwestern USA apple orchard (Croft 1976, 1990); however, releases gave more rapid control at lower mite densities in apple seedlings than in apple trees. What made control so different between these different forms of a plant species? An apple seedling stoolbed differs from an orchard in that it is low growing, and has a canopy of contiguous shoots with little exposed soil (Hartmann and Kester 1983; Vasek and Howard 1984). Its profile is much like those of plantings of raspberry, blackberry or mint. We hypothesized that these plant traits might be influencing dispersal success of predators (Jung and Croft 2000; Croft and Jung 2002). In our subsequent studies, we sought to assess if: (1) releases of *N. fallacis* would suppress both moderate and low densities of *T. urticae* in apple rootstock, (2) an individual-based simulation model would describe within-field movements (aerial dispersal) and distributions of *N. fallacis* and *T. urticae*, and (3) apple seedling field dimensions would affect migration of *N. fallacis* as described by the simulation model.

Materials and methods

Predator source, study site and weather

Sources of *N. fallacis* came from Biocontrol Works of Jefferson, Oregon. All *N. fallacis* were produced on lima beans (*Phaseolus lunatus* L.) infested with *T. urticae* under controlled environmental conditions (Strong and Croft 1995). Lima bean plants harboring predators were harvested just prior to release when predators had nearly eliminated all spider mites.

The study site, near Gervais, Oregon (45.6N lat. and 122.5W long.), consisted of apple rootstocks (MM.106 EMLA) cultivated in a 7.3 ha stoolbed field with 400(±22) plants per m^2 and 1 m between each row. Rootstock stoolbeds were rows of established root systems with sawdust drawn up along each row to cover roots and encourage growth of new branches (Hartmann and Kester 1983; Vasek and Howard 1984). Plants emerged from perennial roots in early spring and by May a continuous dense canopy of leaves was created within rows and nearly between rows. Plants were sprinkler irrigated as needed according to soil moisture sensors. In fall, apple rootstocks were harvested, sawdust was replaced and no plant material remained above ground during winter.

During the study period, we gathered wind velocity and direction data at 1 h intervals from a weather monitoring station located 15 km from the study site. Wind direction was categorized into 8 cardinal directions (N, NE, E, SE, S, SW, W, NW). Only wind data for the period of these experiments were used for model simulations.

Small scale releases

Initially, we tested the ability of *N. fallacis* to control *T. urticae* in replicated small plots within the downwind edge of an apple rootstock field (Figure 1). About 10,000 m^2 plots were randomly assigned one of two treatments: (1) release of 210 (±8) adult females of *N. fallacis* or (2) no release of predators (control). We monitored each replicate by removing 50 leaves in an 'X' type pattern across the plot every 14 days. We also monitored a neighboring apple rootstock field of similar size to the release area that served as an external field control. On 27th June, spider mite populations had increased to a moderate density of 0.60 (±0.12) per apple leaf and *N. fallacis* were released into small plots by placing a bean leaf containing three adult females (based on pre-counts) every 6 m along each of 12 rows per replicate (ca. 4000 *N. fallacis* per ha). Leaf samples were placed in an ice cooler, transported to the laboratory

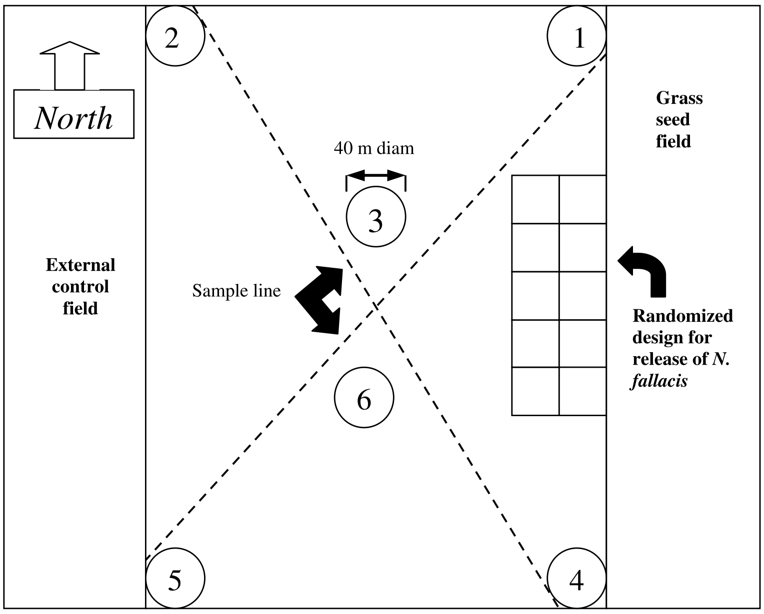

Figure 1. Representation of the 7.3 ha apple rootstock field. Circles 3 and 6: 40 m control sites in 1996; Circles 1–6: 40 m control sites for 1997.

and processed within 24 h. A 40× microscope was used to count pest and predator populations. All predators found on sampled leaves were mounted in Hoyer's solution on glass slides and identified by morphological characteristics (Schuster and Prichard 1963). To normalize population data, we performed a $\log(x + 1)$ transformation prior to analysis. Treatment comparisons were made statistically with a repeated measures analysis of variance (ANOVA) (von Ende 1996).

Releases of N. fallacis *at low prey densities*

We measured biological control of spider mites by *N. fallacis* in a whole field when predators were released at very low prey densities (*T. urticae*). To monitor spider mites within the 7.3 ha field, we removed 400 leaf samples in an 'X' sampling pattern every 14 days and processed these samples as described earlier. Areas of no release of predators were two, 40 m diameter circles, each located near the field center (Figure 1). The diameters of these control circles were chosen according to reported dispersal distances of *N. fallacis* in strawberry fields approximately 10 weeks after initial release (Coop and Croft 1995). To monitor spider mites, 100 leaves were removed from the center of each control circle. When spider mites increased to 0.22 (±0.05) per leaf across the field, *N. fallacis* were released. Release of predators was similar to that made in 1995 with 4000 adult females released per ha in a uniform distribution across the entire field, excluding control circles.

Modeling and validating dispersal of N. fallacis

In low prey density release tests, *N. fallacis* not only suppressed spider mites in release areas but also rapidly dispersed into and suppressed spider mites in control circles. Eventually, higher levels of *N. fallacis* were sampled within the control circles than in the release areas. We questioned if the surprising rate that *N. fallacis* colonized control circles was related to their locations within the field and the prevailing wind direction. We hypothesized that control circles centrally located in a field would have higher densities of dispersed predators due to winds from any direction. Circles near edges of the field would have lower densities according to the prevailing wind and permeability of the boundaries. For instance, downwind control circles would be recipients of many dispersing mites but also loose many predators as they disperse from the field to locations downwind. In contrast, upwind circles would only be recipients of immigrants when the occasional non-prevailing wind occurred. This rationale suggests that centrally located circles would have the highest densities of predatory mites, whereas upwind circles would have the lowest and downwind circles would be intermediate.

To test this hypothesis, we modeled aerial dispersal of *N. fallacis* in the apple rootstock system using the scientific programing package PV-WAVE® (Visual Numerics, Inc., Houston, Texas). The model has three components: (1) The simulated area of predator and prey mite interaction was a two-dimensional matrix filled with a 195 × 375 array of cells, representing a cropland of 195 m × 375 m. (2) The model is individual-based. The predator mites' foraging and dispersal behaviors are modeled individually across the simulated area. The prey population is modeled independently in each cell, following a logistic function with a carrying capacity that is randomly generated between 1–100 and an intrinsic rate of increase of 0.25 (Sabelis 1985). This allowed the population phenomena to be produced solely by local interactions between predators and prey. Initially, each simulation began with 30,000 uniformly distributed predators, with populations developing by preying upon spider mites at local sites of release. A predator will sense the amount of prey in the cell it resides and if the number of prey is not enough for the predator mite's daily needs, it will disperse. (3) Dispersal is determined by wind, including both direction and velocity. To ensure that wind directions and velocities as represented in the model were ecologically reasonable, probability functions were defined as piece-wise linear functions derived from wind data gathered as described previously. Each simulation consisted of 42 time steps that represented 42 days and simulations were repeated 100 times.

To validate the model in the field, inoculative release of the predator, sampling methods, and control circles were exactly as before except that four additional circles were added in extreme corners of the field in a later test (Figure 1). We sampled the center (as described above) of each circle after 6 weeks and ranked circles according to predatory abundance. We then compared validation ranks with simulation model outputs after 42 time steps (days). To measure model accuracy, we analyzed data at three levels of resolution. At a coarse level, we compared only relative abundance of *N. fallacis* in upwind circles (1 + 2 + 3) versus downwind (4 + 5 + 6). Medium resolution compared densities among upwind (1 + 2), central (3 + 6) and downwind (4 + 5) circles, respectively, and finest resolution compared ranks among all six circles. Ranks derived from simulation were considered not different from validation ranks when confidence intervals overlapped.

Assuming that the model accurately predicted dispersal of *N. fallacis*, we also questioned what effect field shape would have on predator emigration. To assess this, we used the individual-based model but adjusted length of upwind border while maintaining the same area. We compared 13 field shapes with upwind borders ranging from 10 to 7312.5 m. Emigration was calculated by subtracting the number of *N. fallacis* present after 42 days from the initial 30,000 predators. Again, overlapping confidence intervals between different lengths of upwind borders were considered not significantly different.

Table 1. Densities of twospotted spider mites (*Tetranychus urticae*) and predaceous phytoseiid mites (*Neoseiulus fallacis*) in apple rootstock release plots (Brooks, OR).

Treatment	Sample dates					
	VII-18	VII-31	VIII-10	VIII-19	VIII-26	IX-6
NF Release						
Pest[1]	0.242 a	0.542 a	1.342 ab	0.483 a	0.742 a	0.042 a
Predators[1]	0.033 a	0.033 a	0.117 a	0.317 b	0.383 ab	0.333 a
Ratio[2]	7.3	16.4	11.5	1.5	1.9	0.1
Control (wi/block)						
Pest	0.600 b	2.033 b	1.783 bc	4.075 c	4.643 b	6.450 b
Predators	0.017 a	0.125 a	0.125 a	0.383 b	0.533 b	1.633 b
Ratio	35.3	16.3	14.2	10.6	8.71	3.9
Control (wo/block)						
Pest	0.267 a	0.400 a	2.300 bc	2.225 b	3.943 b	6.450 b
Predators	0.017 a	0.025 a	0.100 a	0.125 a	0.275 a	1.768 b
Ratio	58.8	16.0	23.0	17.8	14.3	3.6

[1] Expressed as mites (all life stages including eggs) per leaf (based on a sample size of 40 leaves per replicate, three replicates per treatment for 120 leaves per treatment). ANOVA significance at $p < 0.001$; means followed by the same letter within columns for either prey or predator densities are not significantly different at the $p = 0.05$ level (Fishers LSD protected test).

[2] Prey/predator ratio; any value greater than 10:1 (for example, 5:1) is considered to be highly likely to give biological control.

Results

Small scale releases

Introduction of the predator *N. fallacis* into apple rootstock plants significantly reduced populations of *T. urticae* ($p < 0.001$; Table 1). Pest mites were reduced 95% when compared to control plots (Figure 2). In release plots, spider mite population levels peaked in early August at 1.34 (±0.32) per leaf with predator populations peaking at 0.48 (±0.10) per leaf 16 days later. *Tetranychus urticae* levels in control plots reached 6.45 (±0.35) per leaf by early September.

Releases of N. fallacis *at low prey densities*

These releases were highly effective at suppressing *T. urticae* (Figure 3). Spider mites never exceeded 0.90 (±0.12) individuals per leaf in the release treatment as compared to 26 (±4) per leaf in a nearby field of similar size that was without releases of *N. fallacis*. Surprisingly, higher population densities of *N. fallacis* were observed in the control circles (within block controls) than other areas of the field that had been inoculated with the predator (Figure 3b). *N. fallacis* levels peaked at 1.47 (±0.10) in the release areas as compared to 2.20 (±0.12) in the control circles. As a result of the rapid dispersal of *N. fallacis*

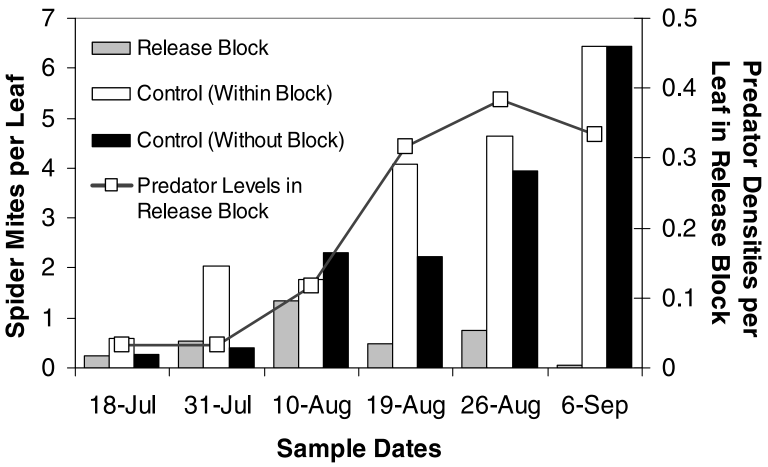

Figure 2. Mite densities after release of *N. fallacis* into apple rootstock seedlings (small scale study, Brooks, OR).

into the control circles, *T. urticae* densities were low and similar in control circles versus release sites.

Wind velocity and direction

Wind direction frequencies during July–September release tests were most from N, NW, and W, with all other combined directions contributing less than 25% of frequencies (Figure 4). Southerly winds increased in 1997 to 17% of total wind direction as compared to previous years (1995 = 9% and 1996 = 8%). No easterly winds were observed during the intervals of interest. When pooling wind speeds for the 3-year study period, southerly winds had the greatest average velocity, with all others except E and SE being about equal (Figure 4).

Modeling dispersal of N. fallacis

The individual-based simulation model accurately predicted the relative density of *N. fallacis* at different locations within a single field at coarse and medium resolutions (Figure 5). When comparing the coarse resolution data, control circles downwind had higher densities than those upwind. At the medium resolution, upwind control circles also had lower densities, while downwind intermediate and centrally located control circles had the highest. In addition, the simulation was accurate at the fine resolution except for circles 3 and 4. The simulation overestimated densities in circle 3 while underestimating those in circle 4.

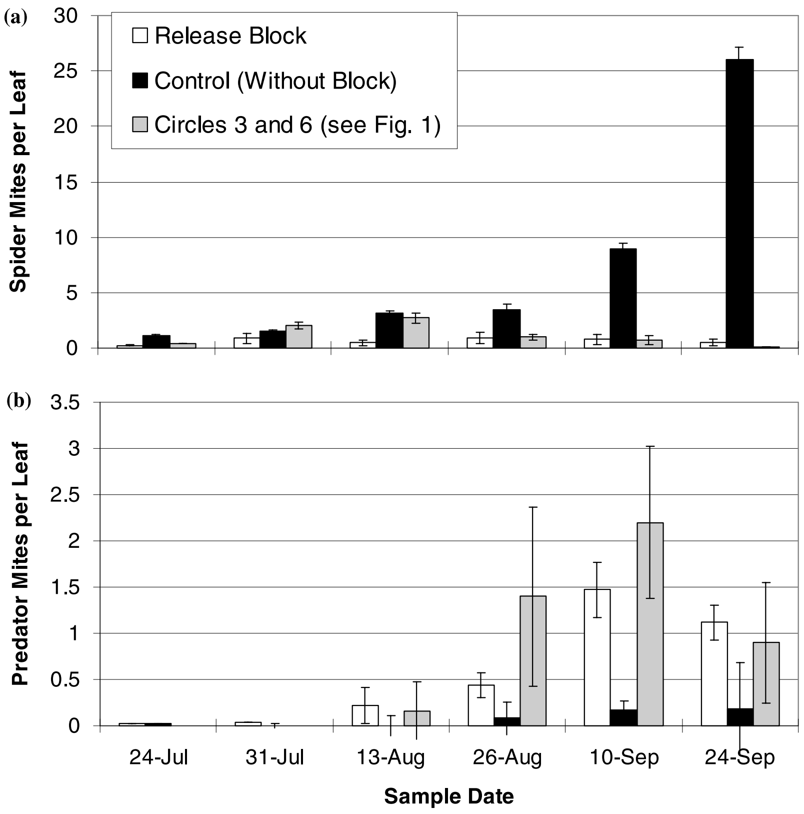

Figure 3. Suppression of *T. urticae* in apple rootstock seedlings after releases of *N. fallacis* into low prey densities (large scale study, Brooks, OR).

Adjusting field shape in the simulation model resulted in the greatest number of *N. fallacis* emigrating from the field when upwind borders were at maximum or minimum lengths (Figure 6). Emigration was reduced as field dimensions approached homogeneity. When upwind borders ranged from 150 to 487.5 m more than 84% of the predatory mite population was conserved within the simulated field. At upwind border lengths of 50 or 1462.5 m only ca. 62% of the mites were conserved.

Discussion

Limited attention has been given to release of natural enemies at low pest densities. One reason for this trend may be that the probability of establishment of a biological control agent is positively correlated to availability of prey (Beirne 1975). In contrast, releasing natural enemies at low prey density may

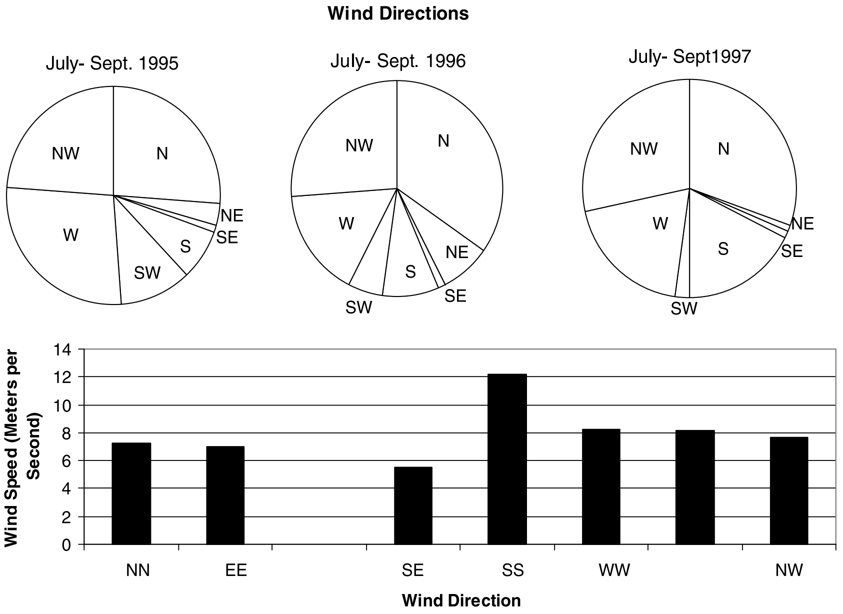

Figure 4. Wind direction and speed for months July–September during 1995–1997 (Brooks, OR).

reduce costs associated with purchasing natural enemies. In addition, taking advantage of the maximum searching and dispersal tendencies of the natural enemy may reduce the need for close release points of the biological control agent (Coop and Croft 1995). In this study, the introduction of *N. fallacis* at low prey densities was successful at reducing densities of the pest mite *T. urticae*. Releases of *N. fallacis* into low prey density in 1996 produced similar levels of control as when with moderate prey densities in 1995.

These data also provide insights into dispersal of phytoseiids. Sabelis and Dicke (1985) demonstrated suppression of long-range dispersal of phytoseiids by spider mite products and kairomones, and rapid dispersal when leaves contained no prey (Pratt et al. 1998). In this study, phytoseiids appeared to be dispersing at high rates when spider mite densities were low but dispersal was terminated once a spider mite colony was located. In 1996, densities of *N. fallacis* were higher in control circles than areas of release. An explanation for this result may be that emigration from control circles was lower than release areas due to higher initial levels of spider mites (Figure 3). Thus, reproduction would be higher in control circles, resulting in higher densities of *N. fallacis*. Later, increased predatory mite densities in centrally located control circles resulted in lower spider mite densities near the end of the test (Figure 3). Another possible explanation is that higher predator densities in the control circles were a result of higher population growth rates there once predators had arrived (the slight delay allowed spider mite densities to get a bit higher), rather

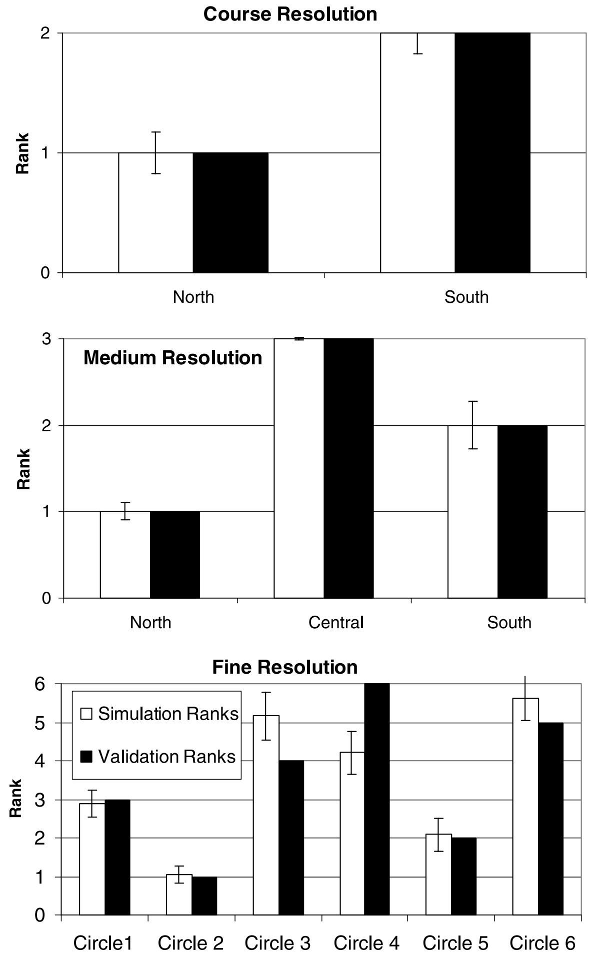

Figure 5. Comparison among validation and simulation data for the dispersal of *N. fallacis* analyzed at three levels of resolution.

than an accumulation of dispersing predators. Monitor data did not indicate such trends, but possibly these density effects were very slight and not easily picked up by sampling methods.

The individual-based model accurately described the dispersal of *N. fallacis* in the apple rootstock system at the coarse, medium and in most locations at fine resolution (Figure 5). These findings suggest that wind direction and velocity are important factors affecting the dispersal of *N. fallacis* in this system (Sabelis and Dicke 1985; Johnson and Croft 1979). Our findings suggest that upwind areas have higher emigration and reduced immigration of *N. fallacis* than downwind areas. Therefore, control of pests by passively dispersed natural enemies would be lowest on upwind field locations. One explanation for

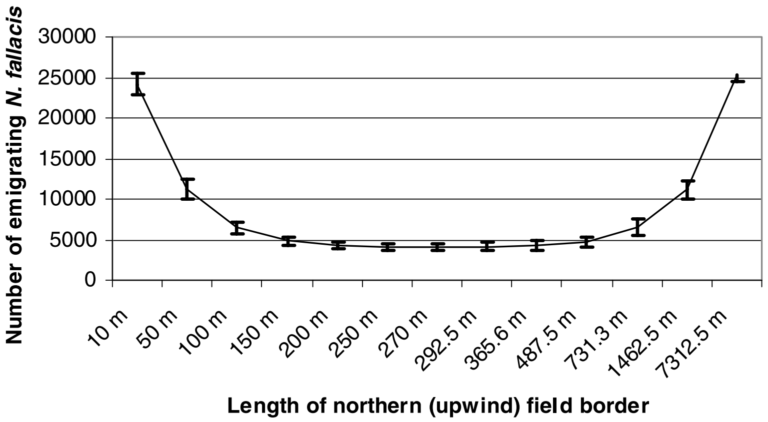

Figure 6. Individual based simulation model output predicting emigration of *N. fallacis* from 13 field shapes, each with a constant area of 7.3 ha (confidence intervals = SD).

the lack of fit among the simulation data and the validation data for control circles 3 and 4 may be that random samples of wind direction and velocity taken at 1 h intervals may not describe actual micro-environmental characteristics of wind movement in the field. Another explanation may be that some predatory mites were able to return to the field after dispersing out of the boundary, which is not incorporated in the current model (Jung and Croft 2000).

Nursery systems represent an extreme in artificial ecosystems. In these systems, spatial and temporal dispersion of plants are structured for uniformity and regularly managed for irrigation, fertilization and pesticide applications. In contrast, adjusting field shapes for improved conservation of natural enemies has rarely been evaluated (Herzog and Funderburk 1986). By adjusting field dimensions in our model we demonstrate that *N. fallacis*, and possibly other passively dispersed organisms, readily dispersed from field boundaries when upwind borders were either extremely short or long (Figure 6). Fields with similar border lengths are predicted to have the highest densities of predatory mites. These findings have relevance to many agricultural systems, including ornamental nurseries that commonly utilize long narrow fields (Pratt and Croft 2000). Such field configurations may be detrimental to conservation of passively dispersed natural enemies like *N. fallacis*.

A final question is how applicable the patterns of effective predator dispersal and pest control as seen on apple seedlings are to other agricultural systems. In this regard, we have studied *N. fallacis* movement on crops with different attributes and have found results to depend on plant profiles, field coverage and prey density conditions (reviewed in Croft and Jung 2002). For example, in a low-profile, but contiguous strawberry field with prey mites at much higher densities, distance of predator spread in time was much less because predators tended to stay in local prey colonies and not disperse repeatedly as in the above experiments. In tall but widely separated hop plants, predator dispersal was not so rapid because while many predators may have dispersed aerially, many fell to the ground and very few hit a slender stem of a few centimeters when foliage was removed at the base of the plant (a cultural procedure used for pathogen control). When a multi-leaf landing pad was left at the plant base then rates of spread of *N. fallacis* between plants was increased. In fields of contiguous mint with moderate profiles and low levels of spider mites, predator dispersal rates were rapid and much like those on apple seedlings, although harvest of the crop in fall greatly disrupted and constrained predator movement into the following spring season (Morris et al. 1996). Although we have not studied other plant systems, we would expect that caneberry (raspberry and blackberry), corn and grapes would be habitats where *N. fallacis* might show similar dispersal efficiencies, although many factors complicate such generalizations (corn is usually a more ephemeral crop than caneberry and grape, the latter two crops are not as dense as are apple seedlings and mint, etc). Clearly, the complex of factors that influences dispersal and biological control on plants with such different morphologies and spatial attributes must be better understood before general models of such relationship can be articulated.

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