Chapter 3
Dispersal and establishment of *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) after inoculative releases into Virginia vineyards

Introduction

I. *Neoseiulus fallacis* as a biological control agent

Spider mites (Acari: Tetranychidae) have become a persistent problem in Virginia vineyards. Currently there are only three acaricides registered for use on grapes. Acaricide resistance in spider mite populations has already been observed in some vineyards and the tendency of tetranychids to develop resistance to a wide range of pesticides has been well documented (Croft and McGroarty 1973, Dennehy and Granett 1982, Dennehy et al. 1983, Helle 1985, Welty et al. 1987, Croft 1990, Herron et al. 1994).

Biological control has been attempted as an alternative to acaricides on a variety of crops prone to spider mites. Many species of phytoseiids have been used as control agents with varying success. *Neoseiulus fallacis* (Garman) occurs in temperate humid areas of North America (Ballard 1954), and is associated with a wide variety of agricultural systems including tree fruit, berries and field crops (Croft and McGroarty 1977, McMurtry and Croft 1997). Species adapted to these systems generally have high dispersal rates, reproductive rates and tolerance to rapidly changing conditions (McMurtry and Croft 1997). Ball (1980) suggested that the predator's high rate of increase along with the longevity of the female and its rate of egg consumption implies an ability to suppress prey populations quickly. When tested on 27 different prey types, *N. fallacis* was found to feed and reproduce on various pests indicating that other less-injurious mites, insects or pollen may enhance survival when preferred prey are scarce (Pratt et al. 1999). In addition to it's ability to reproduce on a variety of prey, *N. fallacis* has been shown to develop resistance to commonly used pesticides including DDT, organophosphates and carbamates (Croft 1990).

Because of these desirable attributes, *N. fallacis* has been used in a variety of biological control programs. A program has been in place on peppermint in Oregon where releases of *N. fallacis* resulted in successful establishment of the predator during the first year of mint production (Morris et al. 1999). In addition, in Oregon, *N. fallacis* provided effective control of *Tetranychus urticae* Koch (Acari: Tetranychidae) on hops (Strong and Croft 1995). Releases of relatively low numbers of *N. fallacis* (1500 mites/2.5 ha) in strawberries have also led to control of *T. urticae* and the development of guidelines for growers to use in a practical control program (Coop and Croft 1995, Croft and Coop 1998).

The use of *N. fallacis* for biological control in grapes has not been well documented. *Neoseiulus fallacis* is common in many Virginia orchards, but have not been seen in vineyards, possibly due to the widespread use of broad-spectrum insecticides (Pfeiffer and Metzger, unpublished). In order to determine the feasibility of establishing *N. fallacis* in vineyards as an alternative control method for spider mites, inoculative releases were made in three commercial vineyards during the 1999 and 2000 seasons. The plots were monitored to determine the distribution of predator and prey as well as the dispersal capabilities of *N. fallacis*.

II. Dispersal of *Neoseiulus fallacis*

Neoseiulus fallacis has high powers of aerial dispersal in response to low prey density (Johnson and Croft 1976, Johnson and Croft 1981, McMurtry and Croft 1997, Tixier et al. 1998) which can be impacted by both wind speed and direction (Tixier et al. 1998). In the laboratory, adult females were the life stage most likely to respond to air currents and actively disperse (Johnson and Croft 1976). In field situations, *N. fallacis* dispersed at least 72 m from release points within one month (Johnson and Croft 1981). A related species, *Metaseiulus occidentalis* (Nesbitt), dispersed at least 800 m during a three-year period, and was shown to move at least 200 m in a two week period with winds of ca. 100-200m/minute (Hoy et al. 1985). In addition, within one year, *M. occidentalis* had dispersed throughout a 32-hectare almond orchard from a single release point (Hoy 1982). Greenhouse studies on *Phtyoseiulus persimilis* Athias-Henriot showed that this mite dispersed 15 m in one week by locomotion alone (van de Vrie

1985). *Phytoseiulus persimilis* is one of the most active predatory mites and this may be the maximum dispersal possible by locomotion. Therefore, alternate methods are probably used to disperse hundreds of meters (Sabelis and Dicke 1985).

A predator still needs to locate prey patches once aerial dispersal has occurred. Hislop and Prokopy (1981) demonstrated that searching behavior of *N. fallacis* is affected by kairomones produced by spider mites as well as by predator-emitted marking pheromones. These chemicals may assist the predator in locating or staying in patches of prey (Zhang and Sanderson 1997). Predators are not abiotically dislodged, but can decide when to start aerial dispersal (Sabelis and Dicke 1985). If kairomones are perceived it may be more beneficial for them to postpone aerial dispersal and continue searching for spider mites which they can detect at distances of at least 1 meter (Sabelis and Dicke 1985).

A study of the spatial distribution of *N. fallacis* and its prey in vineyards is important in order to optimize the number of release sites necessary, determine the optimum time for release, and to find the best spots at a site for the releases. Spatial dispersal is also vital to establishing viable field sampling methods (Taylor 1984).

III. Geostatistical Methods

Geostatistical methods were used to determine the predator and prey distribution in a commercial vineyard following the release of *N. fallacis*. Geostatistics is defined by Rossi et al. (1992) as "a branch of applied statistics that focuses on the detection, modeling, and estimation of spatial patterns." These methods measure the relationship among values as a function of distance or the degree to which values in one place are similar to values in another place (Midgarden et al. 1993). These techniques therefore can be used to determine the type of distribution of predator and prey, as well as, their spatial relationships.

Materials and Methods

Neoseiulus fallacis were obtained from The Green Spot, Ltd., Nottingham, New Hampshire, a commercial supplier of biological control materials. They were shipped on

bean leaves along with a small number of twospotted spider mites to provide food during shipping. The mites were stored in a refrigerator until transport to the field. All of the releases were carried out within 24 hours of receipt of the live shipment to ensure viability. *Neoseiulus fallacis* were released into three commercial vineyards during 1999 and 2000. The vineyards were all located in north-central Virginia, the main grape-growing region of the state (Figure 1).

Site 1: Landwirt Vineyard

Landwirt vineyard is located in Rockingham County, Virginia, in the Shenandoah Valley (Figure 1). The study was conducted within a block containing 'Riesling' variety planted at 1500 vines per hectare in a north/south row orientation.

Predators were released into a 0.2 ha experimental plot in the block consisting of 12 rows by 25 vines. One hundred and fifty *N. fallacis* were released on one vine in each of the middle six rows (Figure 2). Vines chosen for release were staggered so that they were not directly across from one another. This was done to allow observations on dispersal of mites both along and across rows. A total of 900 mites was released in the plot. This rate was chosen by modifying a release program developed by Croft and Coop (1998) for strawberries in California, where they received satisfactory dispersal and control by releasing 1500 *N. fallacis* into a 2.5 hectare field. The rate was multiplied by six to approximate the difference in plant height between grapevines and strawberry plants. In the laboratory,150 mites on bean leaves were counted into each of 6 containers. The containers of mites were transported to the field in a cooler to maintain a constant environment. At the field site, the leaves were paper clipped into the middle of several shoots closest to the trunk.

An initial population count (5 leaves per vine) of *P. ulmi* was taken before the release, which was made in the late afternoon on 13 May 1999. The vineyard was sampled weekly for four weeks and then every other week until 3 August 1999. Sampling was conducted in a grid pattern throughout the release plot, with every third vine in each row sampled for a total of 96 vines. Five leaves on each vine were also

Figure 1: Commercial vineyard field sites in Virginia

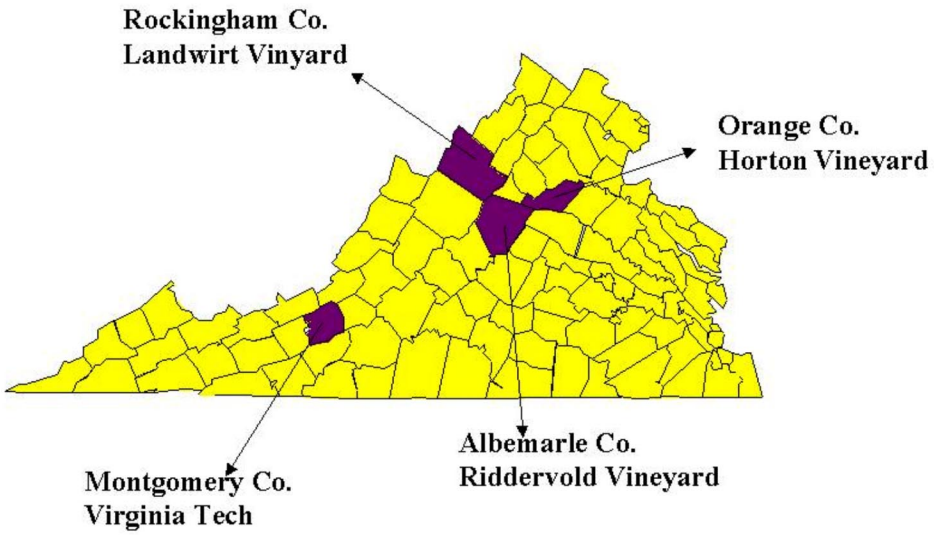
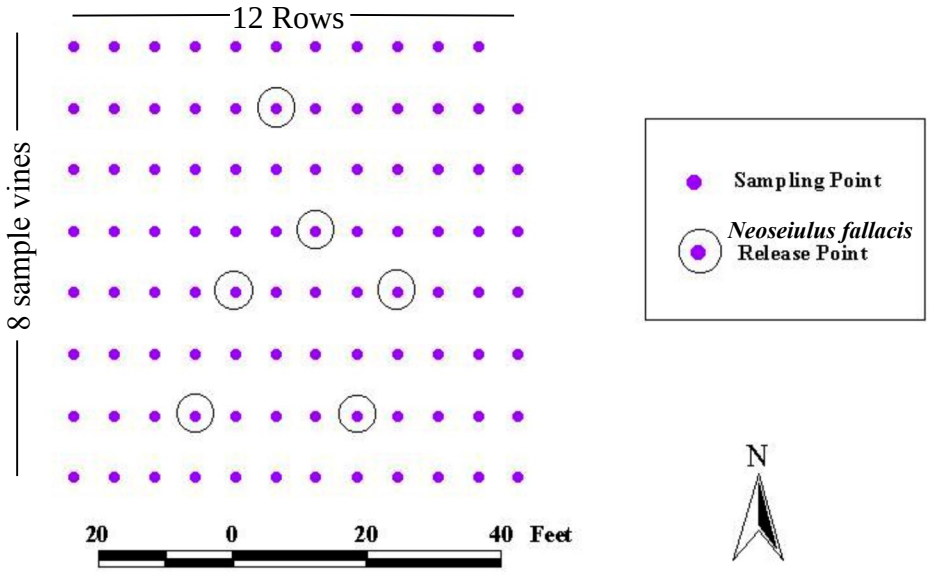


Figure 2: Landwirt Vineyard Release Plot

Rockingham Co., Virginia



examined for number of *N. fallacis* and number of *P.ulmi* using a 2.75X binocular magnifier(General Hardware MFG Co., Inc., New York, NY).

Site 2: Horton Vineyard

Horton vineyard is located in Orange County, Virginia (Figure 1). The variety at this site was a red variety, 'Malbec', planted at 1000 vines/hectare in a north/south row orientation. Prior to release, the vines surrounding the experimental plot were treated with an acaricide

The experimental plot was 0.1 hectares, and contained 5 rows with 12 vines and 5 posts per row. In this vineyard, posts, which are evenly spaced in the vineyard and support the trellis, were used as sampling points in order to provide a more consistent sampling grid. This eliminated problems of dead or missing vines. Because of the plot size, mites were released at five points rather than six at a rate of 900 per 0.1 hectares and 180 per release point (Figure 3). The release was performed in the early afternoon on 3 August 2000. Pre-release samples were taken back to the lab for counting because of the weather. Sampling was carried out weekly for ten weeks, until the leaves of the vines senesced.

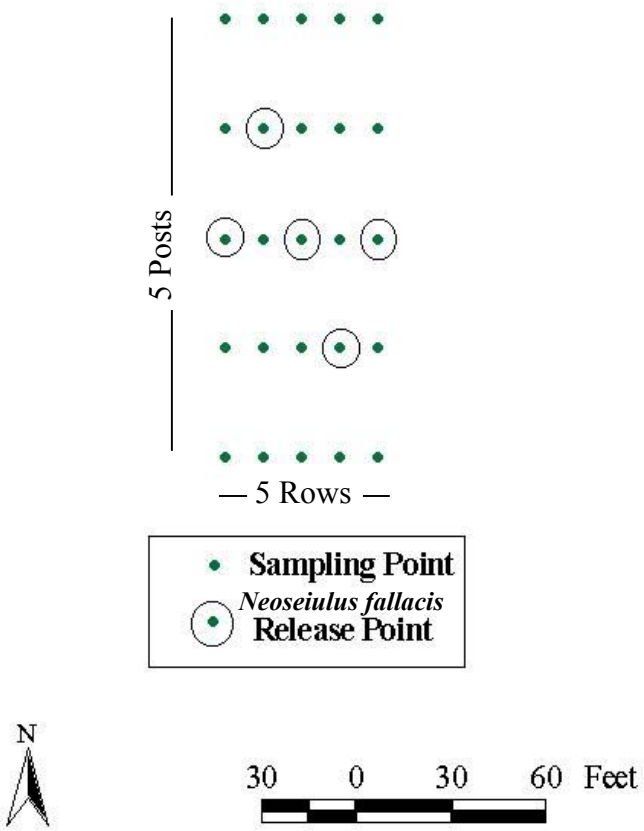
This vineyard had an infestation of twospotted spider mite rather than European red mite. *Tetranychus urticae* outbreaks are unusual in Virginia vineyards, although the past two summers they were more prevalent than usual in apple orchards around the state. Both *T. urticae* and *N. fallacis* were counted and recorded for five leaves at each post for a total of 25 samples (Figure 3).

Site 3: Riddervold Vineyard

The Riddervold vineyard is located in Albemarle County, Virginia (Figure 1). The release was done within a block containing 'Riesling' grapes, planted at 1500 vines/ hectare in an east/west row orientation.

The experimental plot was 0.1 hectares, 8 rows with 18 vines and 9 posts per row. Posts were used for sample points rather than vines as at site 2. Samples (5 leaves per post) were taken at each post for a total of 72 sample points. Mites were released on the

Figure 3: Horton Vineyard Release Plot, Albemarle Co. Virginia



middle six rows in a staggered pattern (Figure 4). The release rate was doubled from the first release to 900 mites/0.1 hectares so that 150 mites were released at each of the six points.

The release was made at midmorning on 3 August 2000. It was overcast and raining which hindered the initial population survey. The release was completed before heavy rain began.

Sampling was conducted weekly for ten weeks until the leaves were no longer viable due to several hard frosts. Both the number of *N. fallacis* and *P. ulmi* were recorded for five leaves at each post.

Analysis

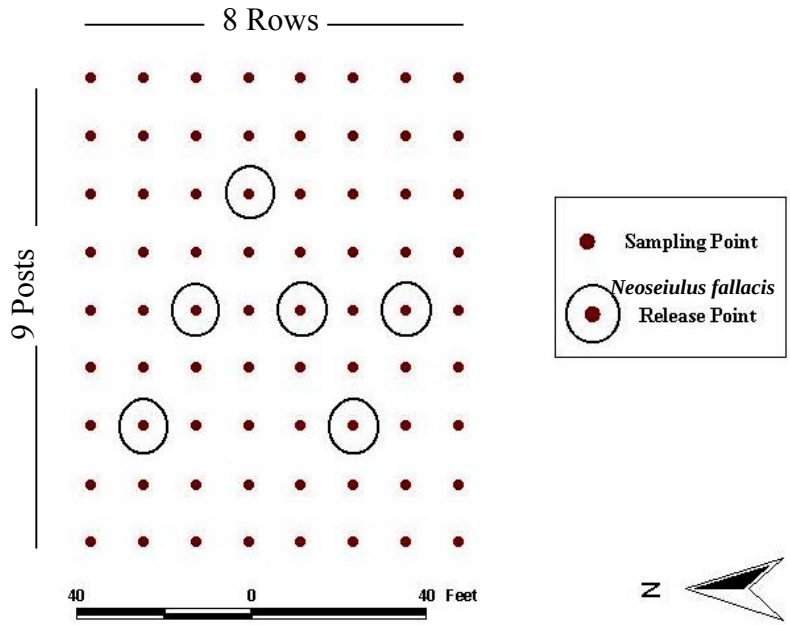
There were not enough predators recovered at the Landwirt and Horton sites to perform geostatistical analysis. General trends in the prey and predator populations were described using site maps produced in ArcView GIS v3.2 (ESRI Redlands, CA).

Riddervold vineyard had better recovery of predators and therefore the data were analyzed for spatial dynamics of predator and prey in the system. Both variograms and kriging were used to elucidate the distribution patterns of both populations throughout the season. Variograms are commonly used to observe and model spatial dependence and are most easily computed when observations are recorded on a grid as they were in this study (Hohn et al. 1993). Variograms model the average degree of similarity between values as a function of the separation distance (Rossi et al. 1992). For N sample pairs, h is the lag distance between two sampling points (x_i and x_i+h), and $z(x_i)$ and $z(x_i+h)$ are the population density at a point x_i and that point plus the lag distance h (Ellsbury et al. 1998)(1).

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2 \qquad (1)$$

Variograms were calculated for both predator and prey spatial distribution for different sampling dates during the season. The variograms were standardized by dividing each variogram value by the population variance. This allowed spatial dependence comparisons to be made between the predator and prey, which had different

Figure 4: Riddervold Vineyard Release Plot, Albemarle Co., Virginia



levels of spatial variability (Rossi et al. 1992). All analysis was done in GS+ for Windows (version 2.3, Gamma Design Software, Plainwell, MI). Models for the variograms were expressed as Gaussian functions (2).

$$\gamma(h) = Co + C[1 - \exp(-h^2 / Ao^2)] \qquad (2)$$

In this model, h is the lag distance, Co is the nugget variance>=0, C is the structural variance>= Co, and Ao is the range parameter (Figure 5a). $Co+C$ is equal to the sill which is the point at which the sample variance no longer increases (Schotzko and O'Keefe 1989). The nugget represents all unaccounted for spatial variability at a distance less than the sampling distance, and the range is the average distance within which the samples remain spatially correlated (Rossi et al. 1992).

The shape of the variogram plot defines the type of spatial structure and the range of spatial dependence in the populations (Schotzko and O'Keefe 1989). Figure 5b illustrates the most common variogram shapes. Random and uniform distributions (Figures 5bI and 5bII) are linear, but the random distribution will have a low r (correlation coefficient). Variograms for clumped distributions can often either be fitted to a spherical (Figure 5bIII) or Power model (Figure 5bIV). Most of these distributions were observed in this analysis and will be discussed further below.

The variogram models created were used to produce kriged estimates of the predator and prey population densities at 12-ft intervals in GS+ v2.3. Kriging is a geostatistical technique that estimates weighed averages of values from nearby locations for use in interpolating two-dimensional data (Hohn et al. 1993, Leibhold et al. 1993). The kriged estimates were used to create density surfaces for both populations using GS+.

Results and Discussion

Site 1: Landwirt Vineyard, Rockingham Co.

In 1999, Virginia experienced a severe drought. Although spider mites generally thrive in hot and dry conditions, the climate may have been too extreme for their survival. There were very few outbreaks across the state in either orchards or vineyards. Consequently, finding field sites to release *N. fallacis* was difficult. Landwirt vineyard

Figure 5a: A Typical Variogram Model

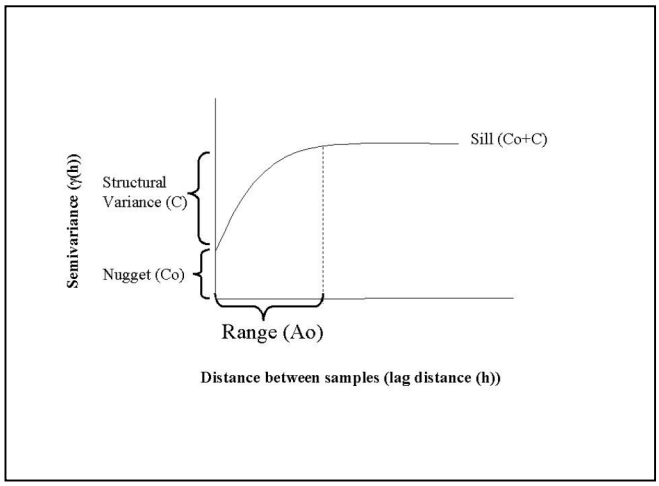


Figure 5b: Four Common Types of Variograms

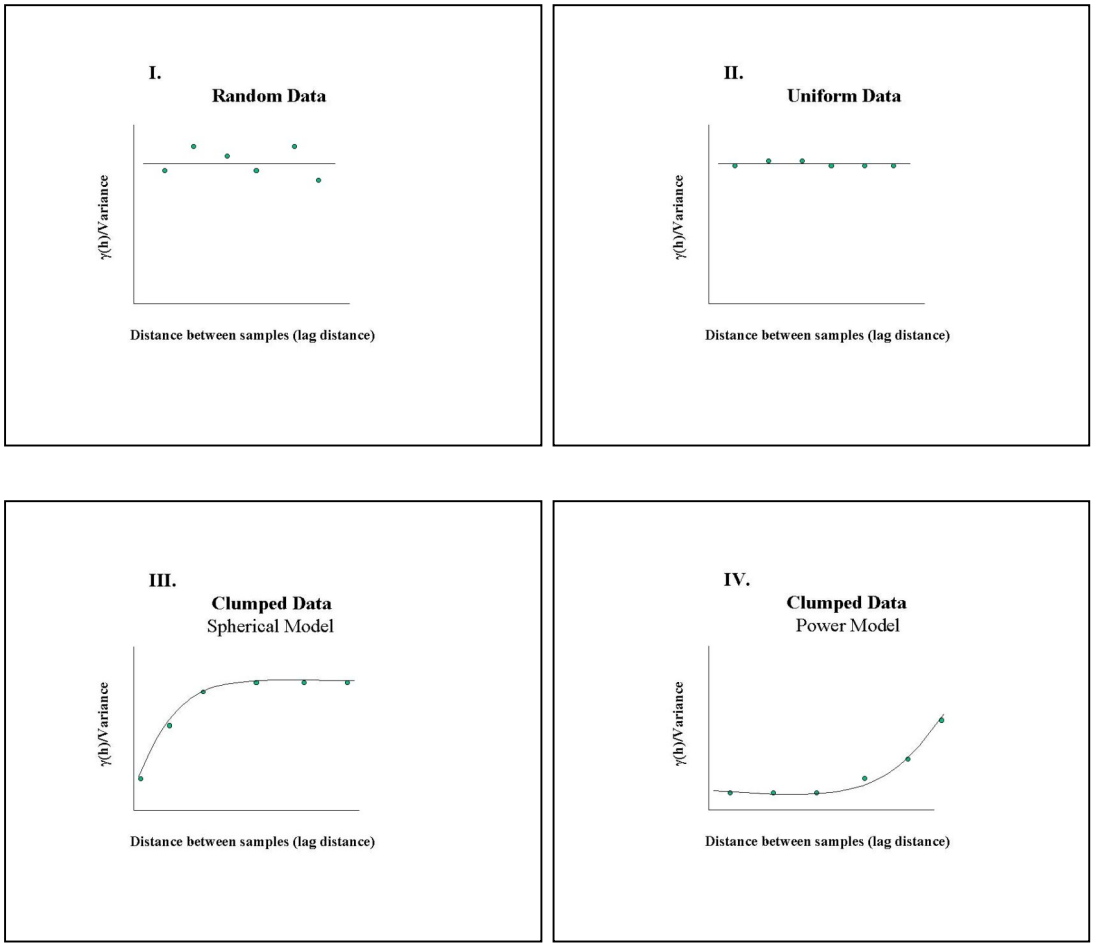


Figure 5b adapted from: Schotzko and O'Keefe 1989

had an infestation of *P.ulmi* in late spring. Early season infestations are rare, but can potentially be more damaging than late summer outbreaks because the mites are concentrated on fewer leaves. The initial population of *P. ulmi* was not above the economic threshold of 10 mites/leaf, but did have an average of 2.3 mites/leaf with as high as 11 mites/leaf on one vine. The evening after the release the vineyard experienced a severe thunderstorm. There was a decrease in the *P. ulmi* population one week after the initial count, and the population never returned to the initial density during the remainder of the season (Figure 6). The first week after the release, three predators were observed, all located on vines where the releases were made. During the following two weeks, two predators were observed on each date, again on release vines. For the remainder of the season , no *N. fallacis* were found (Figure 6). Figure 7 illustrates the points in the plot where predators were observed during the season. The overall distribution of prey in the system was basically uniform and the population was small to nonexistent for most of the season. (Figure 8)

There are a variety of factors that could have contributed to the lack of predators recovered. The severe drought and crash of the prey population both could have adversely affected the predator population. In addition, the thunderstorm the night of the release may have caused significant mortality to the predators. Finally, the application of pesticides toxic to the predator may have played a role.

Site 2: Horton Vineyard, Orange Co.

The 2000 season was very wet compared with 1999. Despite the poor weather conditions, two field sites were found with late summer spider mite infestations. At Horton vineyard, a rather unusual situation occurred. Rather than a *P. ulmi* infestation, the vineyard had an outbreak of *T. urticae*. In 1998, the same plot in the vineyard had *P. ulmi* present but no *T. urticae*. It appears that *T. urticae* has become a pest in the past two years, possibly displacing *P. ulmi*. This outbreak is particularly a cause for concern because *T. urticae* has been shown to be more damaging than *P. ulmi* in both almonds and apple (Youngman et al. 1986, Mobley and Marini 1990). This species is rare on grapes in California, but has been reported as serious in the former Soviet Union, South Africa, and Australia (Flaherty and Wilson 1999). The threshold of 10 mites/leaf that has

Figure 6: Population densities over time of *Panonychus ulmi* (Koch) and *Neoseiulus fallacis* (Garman) in Landwirt Vineyard, Rockingham Co. Virginia 1999

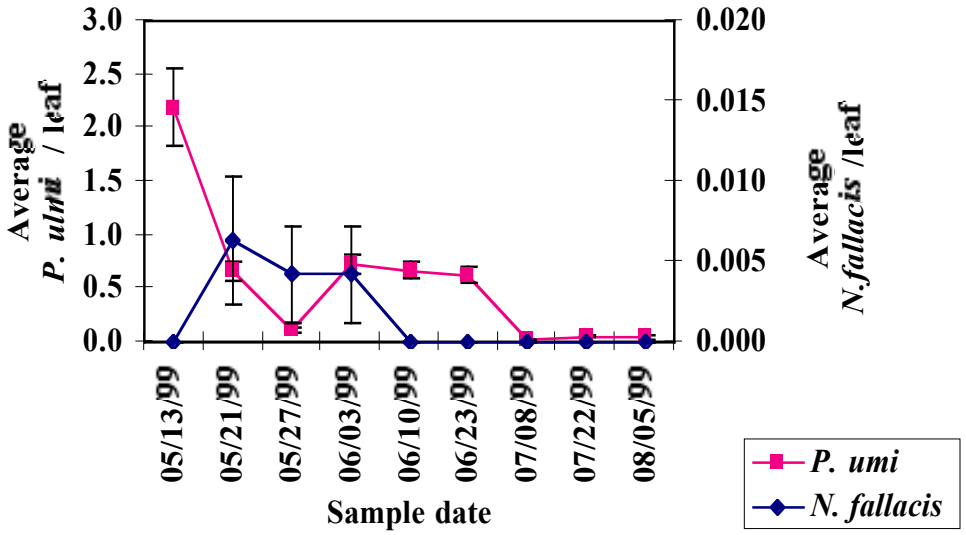


Figure 7: Distribution of *Neoseiulus fallacis* (Garman) in Landwirt Vineyard Release Plot 1999

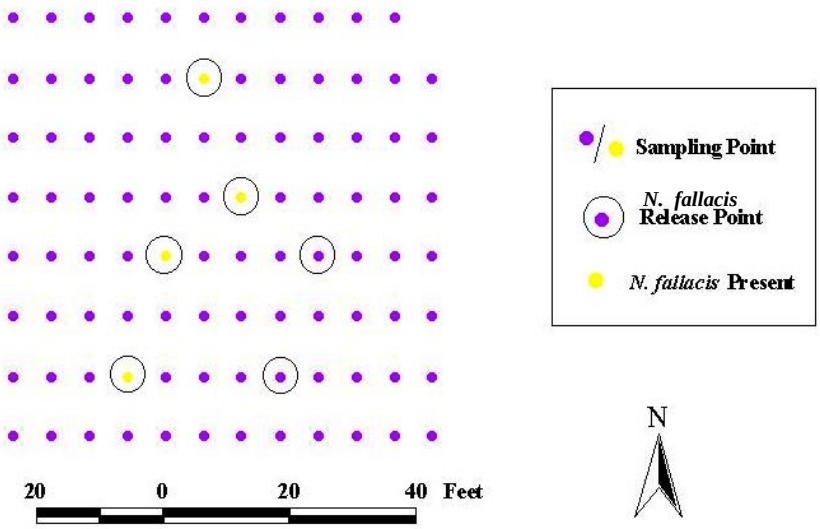
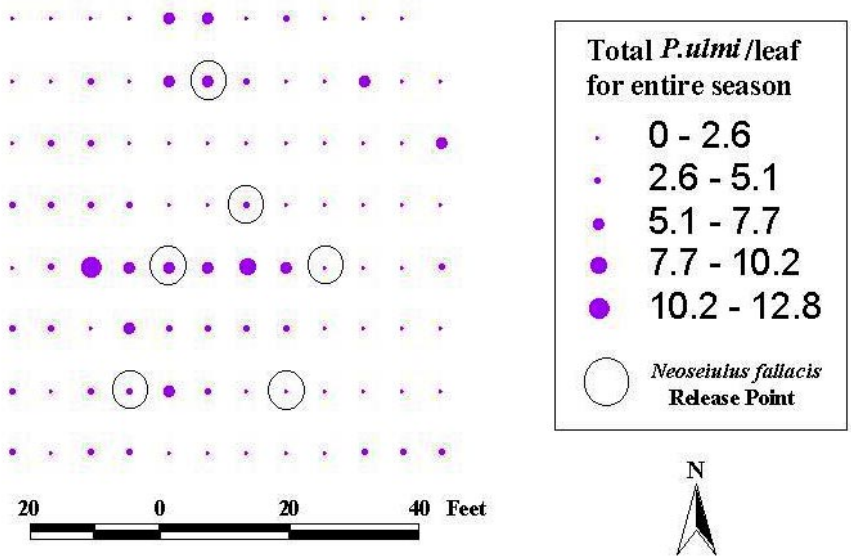


Figure 8: *Panonychus ulmi* (Koch) Population Density in Landwirt Vineyard Release Plot 1999



been established for *P. ulmi* may be too high for *T. urticae,* however, further research of their effect on grapevines is needed. *Tetranychus urticae* is actually considered the native prey of *N. fallacis* (Croft and McGroarty 1977). Releases of *N. fallacis* on both hops and peppermint in the western U.S. have been successful in controlling *T. urticae* (Strong and Croft 1995, Morris et al. 1996). When tested in the laboratory on 27 different prey-foods, survival, reproduction and development were highest on *T. urticae* (Pratt et al. 1999). Although the original objective of this research was to examine the potential of releasing *N. fallacis* into vineyards infested with *P. ulmi,* it was decided to release here as well to observe results in a vineyard infested with *T. urticae*.

Preliminary observations the week before the release indicated populations of *T. urticae* greater than 10/leaf at least in some parts of the plot. However, on the day of the release, the population had dropped to an average of 3.7 mites/leaf. Conditions were overcast and damp on the release date. Figure 9 shows the weekly average predator and prey populations for the season. After the release, the prey population dropped and then leveled off to between 0.3-1 mite/leaf for the remainder of the season. The distribution of *T. urticae* in the field was irregular (Figure 10). The south end of the plot appeared to have a slightly higher population than most of the rest of the field. This part of the plot is bordered by a grass alley and a dirt road. Spider mite populations in California have been shown to increase in areas exposed to dust from dirt roads (Flaherty et al. 1982). However, even the sampling points with the highest densities of *T. urticae* had only a total of 14.28-16.8 mites/leaf over the entire season (Figure 10).

The lack of prey in the Horton vineyard was probably the main reason for the disappearance of predators from the system. However, there were predators present at very low numbers throughout the season. Next spring the plot should be scouted to determine if predators survived the winter and established in the vineyard even at low numbers

Site 3: Riddervold Vineyard, Albemarle Co.

Of the three release sites, Riddervold had the highest infestation of spider mites. In the week prior to the release, the population was over 20 *P. ulmi*/leaf in some areas of the plot. The day of the release, pre-release population sampling was hindered by rain.

Figure 9: Population densities over time of *Tetranychus urticae* Koch and *Neoseiulus fallacis* (Garman) in Horton Vineyard, Orange Co. Virginia 2000

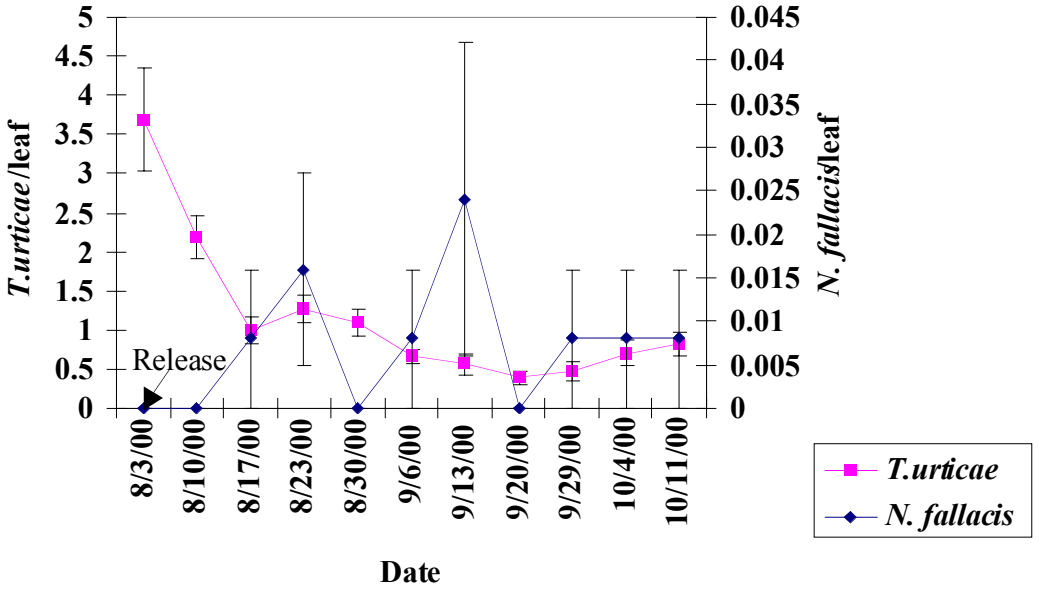


Figure 10: *Tetranychus urticae* Koch Population Density in Horton Vineyard Release Plot 2000

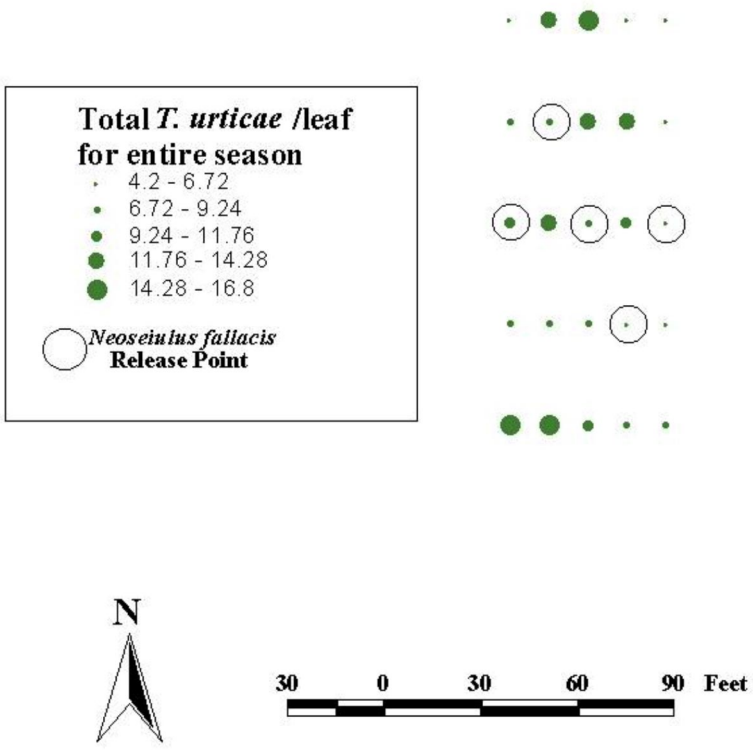
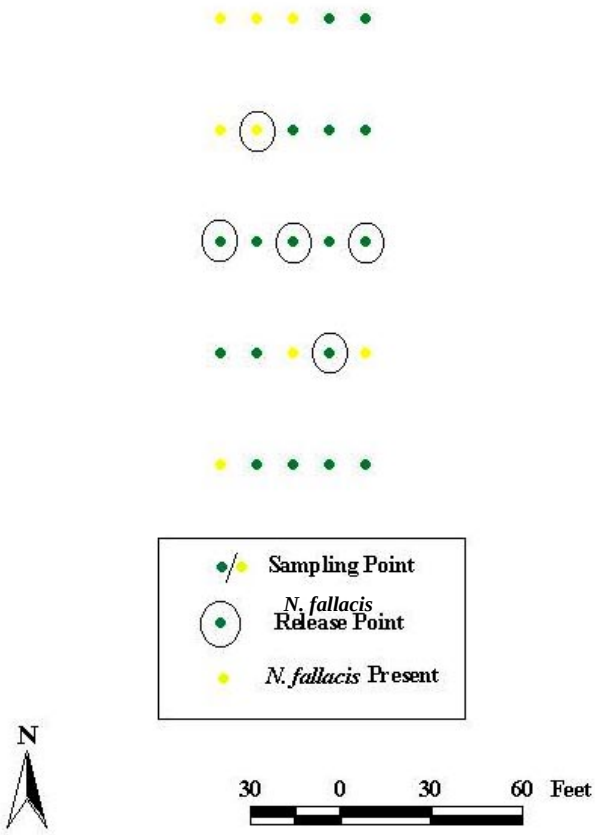


Figure 11: Distribution of *Neoseiulus fallacis* (Garman) in Horton Vineyard Release Plot 2000



Only a small subset of samples was taken before it began to rain. The population in this small sample averaged 23.8/leaf. There were no predators observed on either of these dates. Figure 12 shows the average population of both *P. ulmi* and *N. fallacis* over the course of the season.

The preliminary samples showed that the highest prey density peak for the season occurred prior to the release. It appears that the week of the release was near the peak population of prey, and the population declined after that point to less than one mite/leaf on the final sampling date. On 17 and 23 August, conditions were cloudy with light rain. It is very difficult to accurately sample mites under these conditions. This may explain the drop in population for those two weeks. Rain also has a negative impact on spider mite populations, which may also partly explain the decline (Simpson and Connell 1973). The predator population was very low for the first three weeks, but later increased, peaking at 0.12 mites/leaf on 20 and 27 September 2000. The *N. fallacis* population on 17 and 23 August may also have been underrepresented for the reasons mentioned above. On the last sampling date there were still predators present in the system. Figure 13 shows the total population distribution of *P. ulmi* for the season and Figure 14 shows the points where *N. fallacis* were found indicating that they had spread to almost all parts of the plot.

The spatial distribution of the predator and the prey were examined in detail for weeks 6-9 of the sampling period using geostatistical methods. This time period was chosen because of the rain effects of the earlier weeks as well as the low number of predators sampled. Figure 15a-d shows variograms and kriged surfaces for each of these weeks. Each variogram plots the semivariance (_(h))/variance relative to the lag distance for both predator and prey. The visual representation of the plots is shown in the kriged surfaces where the x-axis indicates the north/south direction of the plot, the y-axis is the east/west direction along which the posts were sampled, and the z-axis is the population level of either *N. fallacis* or *P. ulmi*.

The data collected during weeks 5 and 6 were similar in distribution therefore only week 6 is depicted (Figure 15a). At distances less than 50 feet, *P. ulmi* densities were correlated spatially, but the distribution became random at greater lag distances. That is, sampling points closer together were more likely to have similar numbers of prey

Figure 12: Population densities over time of *Panonychus ulmi* Koch and *Neoseiulus fallacis* (Garman) in Riddervold Vineyard, Albemarle Co. Virginia 2000

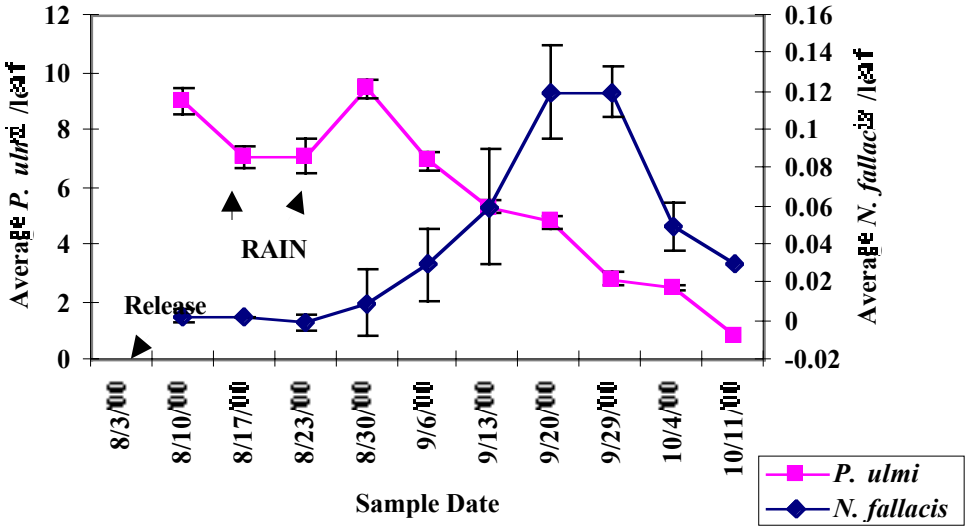


Figure 13: *Panonychus ulmi* (Koch) Population Density in Riddervold Vineyard Release Plot 2000

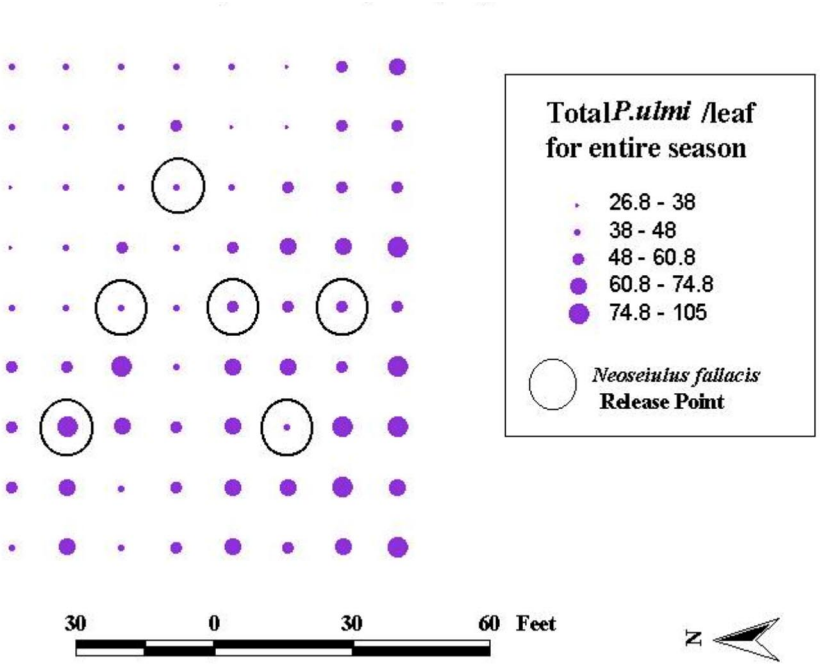


Figure 14: *Neoseiulus fallacis* (Garman) Distribution in Riddervold Vineyard Release Plot 2000