Neoseiulus fallacis (Garman) (Acari: Phytoseiidae) as a potential biological control agent for spider mites (Acari: Tetranychidae) in Virginia vineyards

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(ABSTRACT)

Outbreaks of spider mites (Acari: Tetranychidae) in vineyards have been increasing with the expansion of the industry in Virginia. Only three effective acaricides are registered on grapes and as resistance begins to occur, control options are limited. Biological control of spider mites by inoculative or inundative releases of predatory mites has been tried on a wide range of crops including grapes. This project examined the feasibility of using *Neoseiulus fallacis (*Acari: Phytoseiidae) as a potential large-scale biological control agent in vineyards. Slide dip bioassays were conducted on *N. fallacis* to determine the toxicity of insecticides, fungicides and herbicides commonly used on grapes in Virginia. In addition to laboratory experiments, commercially obtained *N. fallacis* were released in vineyards with spider mite infestations. The populations of both mites were then monitored on a regular basis to determine dispersal and distribution patterns.

Among the insecticides tested in the laboratory bioassays, carbaryl, azinphos-methyl, phosmet, cyhexatin, and pyridaben all caused significantly higher mortality than the control treatment. Fungicides tested were not toxic to the predator, but three herbicides caused high mortality. Glufosinate caused 100% mortality after 24 hours and both oxyfluorfen and paraquat had adverse effects on *N. fallacis*. The use of materials that were found to be toxic to the predator would not be compatible with inoculative releases of *N. fallacis*.

Field release results were variable. Three releases were made in 1999 and 2000., Recovery of the predator was low following the releases at two of the sites, probably due to lack of prey. At the third site enough predators were recovered to analyze the spatial distribution of the predator and prey populations. It appears that there is no similar aggregation pattern between the predator and prey at the same point in time although there is an indication of the predator spreading is response to the prey distribution. A more complete season of sampling would give more conclusive evidence of this trend. Although the distribution of the two populations were dissimilar, the predator was present throughout the season and did spread through the entire plot indicating that the predator may be able to colonize the vineyard if it successfully overwinter

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Chapter 1

Introduction and Review of Literature

Introduction

The wine grape industry in Virginia has experienced a large expansion in the past 20 years. The 1980 Farm Winery Law, which provides financial benefits to wineries, along with the development of a viticulture and enology research and extension program at Virginia Polytechnic Institute and State University have both been primary factors in the growth of the industry. There are also several organizations in the state (the Virginia Wine Marketing Office, the Virginia Vineyards Association, and the Virginia Winegrowers Advisory Board) that have helped in developing and promoting Virginia wines.

In 1950, the state had only six hectares of grapes, but in 1999 there were 580 bearing hectares and 214.3 non-bearing hectares. Virginia is currently tenth in the nation in tons of grapes produced. The predominant varieties grown in the state are Chardonnay, Cabernet Sauvignon, and White Riesling although growers are experimenting with many different varieties (Virginia Agricultural Statistics Service 2000).

With the expansion of the grape acreage in the state has come increased recognition and concern regarding arthropod pest problems. Major pests that growers face include Japanese beetle, *Popilla japonica* (Newman), grape berry moth, *Endopiza viteana* (Clemens), and yellowjackets, *Vespula* spp. In addition, in the last 10 years there have been increasing problems with spider mites, especially the European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae). 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 spider mites 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, Herron et al. 1994). In most cases the need for acaricides results from the destruction of predators in the system (Parent and Lord 1971).

As an alternative to acaricides, biological control has been attempted on a variety of crops prone to spider mites. Many species of predatory mites in the Phytoseiidae have been used as control agents with varying success. The phytoseiid *Neoseiulus fallacis* (Garman) occurs in temperate humid areas of North America, encompassing the continent from Washington to Nova Scotia and including Virginia (Ballard 1954). This species has been shown to be a major mortality factor of both *Tetranychus* spp. and *P. ulmi* on fruit trees in the eastern United States and Canada (McMurtry and Croft 1997), and has the potential to provide effective biological control for both (Croft and McGroarty 1977).

The objective of this research was to determine the feasibility of using inoculative releases to establish *N. fallacis* in Virginia vineyards and to determine the toxicity of pesticides commonly used in the vineyards to the predator using bioassays. Field releases were also conducted to examine the ability of *N. fallacis* to disperse and establish in the vineyards. Implications of these results for developing a biological control program are discussed.

Review of Literature

I. Biology of *Panonychus ulmi* (Koch)

After WWII, spider mites (Acari: Tetranychidae) became worldwide economic pests mainly due to the increased use of broad-spectrum pesticides (Huffaker et al. 1970). However, the European red mite, *P. ulmi*, first attracted attention in the U.S. in the early 1920's and by the 1930's the species was a persistent enough pest in northeastern orchards to require treatment (Garman and Townsand 1938). The increase in pest status of *P. ulmi* in the 1930's in Connecticut was thought to be a result of dry summers and cold winters or changing grower practices (Garman and Townsand 1938). In Canada it became an important pest after 1945 when DDT began to be used extensively (Parent and Lord 1971). It is currently an economic pest on a wide variety of agricultural crops and significant resources are spent on its control. In the eastern United States and Canada, *P. ulmi* is the major spider mite pest on grapes (Ramsdell and Jubb 1979, Schruft 1985).

The first detailed study of the life history of *P. ulmi* in Virginia was published by Cagle in 1946. Species in the Tetranychidae undergo three immature stages; a six-legged larval stage, and eight-legged protonymph and deutonymph stages. Each of these stages is followed by a quiescent stage known as the nymphochrysalis, deutochrysalis and the teleiochrysalis respectively (van de Vrie et al. 1972). The mites overwinter as eggs on the cane nodes, and in the spring move to the foliage (Pfeiffer and Schultz 1986). Shorter daylength and cool temperatures are the main inducers of diapause (van de Vrie et al. 1972). *P. ulmi* development, survival, and reproduction are driven mainly by temperature and precipitation with lesser effects from food supply and host nitrogen status (Wermelinger et al. 1992).

Generally nine full generations occur on apple in a growing season in Virginia (Cagle 1946). On grapes in Pennsylvania, there were six full generations (Ramsdell and Jubb 1979). Rainfall can have a significant negative impact on spider mites that may be exacerbated by high temperatures (van de Vrie et al. 1972, Simpson and Connell 1973). Dispersal is largely by wind and they can also readily crawl within trees (Garman and Townsand 1938). Mated females will give rise to female or male offspring, and unmated females produce males (Cagle 1946). This production of males as well as the large number of generations and a high reproductive rate are the main reasons for the resistance to pesticides that can build up quickly in *P. ulmi* populations.

Damage to the host is done by both immature and adult mites. They pierce the leaf epidermal cells and extract cell contents which causes chlorosis or a bronzing effect and in severe cases, necrosis (Pfeiffer and Schultz 1986). This damage can result in leaf abscission and small fruit with poor color (Garman and Townsand 1938), although injury caused by *P. ulmi* is not as severe as that by *Tetranychus* spp. (Youngman et al. 1986, Mobley and Marini 1990). However, it is considered the most destructive mite in Michigan apple orchards where it has been shown to affect fruit size, color, number of fruit the following season, and tree vigor (Croft and McGroarty 1977). On grapes in Pennsylvania, *P. ulmi* populations caused heavy foliar bronzing which resulted in significant reductions in juice quality (Jubb et al. 1985).

II. Biology of *Neoseiulus fallacis* (Garman)

Neoseiulus fallacis was first described by Garman (1948) as *Iphidulus fallacis* after collection during a survey of mite species in Connecticut apple orchards. According to a classification of predatory mites based on feeding specialization developed by McMurtry and Croft (1997), *N. fallacis* is a or a selective predator of the spider mite family (Type II). This is opposed to the Type I which are selective predators of *Tetranychus* spp. and Types III and IV which are considered more generalist predators (McMurtry and Croft 1997, Croft et al. 1998). Although Type II predators prefer spider mites, *N. fallacis* has been shown to reproduce on other prey when faced with starvation (Pratt et al. 1999).

Females of *N. fallacis* go through three instars: six-legged larva, and eight-legged protonymph and deutonymph, but the males have no deutonymphal stage (Ballard 1954). Unlike mites in the Tetranychidae, mating is necessary for oviposition (Ballard 1954, Smith and Newsom 1970). The length of the life cycle varies with temperature. The immature period lasted an average of 3.5 days at 26.4ºC and 12.3 days at 13.3ºC (Ball 1980). Mated adult females consumed the most prey of any stage, averaging eight *T. urticae*/day (Ballard 1954). There is no evidence of phytophagy even under starvation conditions (Smith and Newsom 1970) although the species has been found to reproduce on pollen (Pratt et al. 1999). Cannibalism is thought to occur only under instances of extreme starvation (Ballard 1954). Croft et al. (1995) found that *N. fallacis* do not appear to be able to absorb eggs in times of stress but instead cannibalize the eggs possibly as a means of energy conservation. Females laid an average of 3.5 eggs/day at 26.4ºC and 0.9 eggs/day at 13.3ºC, and the total maximum number laid/female were 55 and 45.6 at the same respective temperatures (Ball 1980). The rate of egg production varied depending on the amount of prey consumed (McMurtry et al. 1970).

In apple orchards, *N. fallacis* overwinter as adult females in ground cover and debris and then migrate to trees in the spring after the *P. ulmi* population increases (Johnson and Croft 1976, McGroarty and Croft 1978). Recent studies in Massachusetts indicated that a significant number may actually overwinter on the trees themselves rather than in the ground cover (Nyrop et al. 1994). In ground cover, they are more likely to be found on woody plants or herbaceous forbs than on grasses (Coli et al. 1994). Studies on other phytoseiids have indicated that both the composition and the percent of ground cover may influence mite population dynamics and dispersal (Alston 1994). In peppermint fields in Oregon, augmenting plots with debris increased the overwintering survival of *N. fallacis* while the removal of debris decreased survival (Morris et al. 1996).

When prey is scarce, *N. fallacis* has high powers of aerial dispersal (Johnson and Croft 1981, McMurtry and Croft 1997, Tixier et al. 1998). The predator makes a directional movement to the edge of the leaf and then orients to the airflow and assumes an anteriorly raised stance in order to disperse (Johnson and Croft 1976). Both wind speed and direction have an impact on dispersal (Tixier et al. 1998). In the laboratory, adult females were the 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).

III. Geostatistics

Geostatistical methods were used to determine the predator and prey distribution in commercial vineyards 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. One method used to look at spatial patterns is the variogram. Variograms are commonly used to observe and model spatial dependence and are most easily computed when observations are recorded on a grid (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).

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

There are four main components to a variogram (Figure 1a); the nugget (*Co)*, which represents all unaccounted for spatial variability at a distance less than the sampling distance, the structural variance (*Co*) which is the spatial variability accounted for by the data, the sill (*Co+C*) which is the point at which the sample variance no longer increases, and the range which is the average distance within which the samples remain spatially correlated (Schotzko and O'Keefe 1989, Rossi et al. 1992). As the range becomes smaller, the population distribution becomes correlated at shorter lag distances. Eventually the range becomes so small that the distribution is random.

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 1b illustrates the most common variogram shapes. Random and uniform distributions (Figures 1bI and 1bII) 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 1bIII) or Power model (Figure 1bIV).

In addition to variograms, another common geostatistical technique is kriging. Kriging 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 are used to create density surfaces of the data providing a visual representation of spatial distribution.

IV. Biological control of spider mites by phytoseiids

Phytoseiids have been used in biological control programs in a wide variety of agricultural systems (McMurtry 1982). These crops include apple (Croft and MacRae 1992, Steinburg and Cohen 1992, Croft and Slone 1997), grape (Kinn and Doutt 1972, Duso 1992, Duso and Pasqualetto 1993), nursery citrus (Grafton-Cardwell et al. 1997), strawberry (Croft and Coop 1998), peppermint (Morris et al. 1999) and hops (Strong and Croft 1995). These experiments used a variety of phytoseiids and had varying degrees of

Figure 1a: A Typical Variogram Model

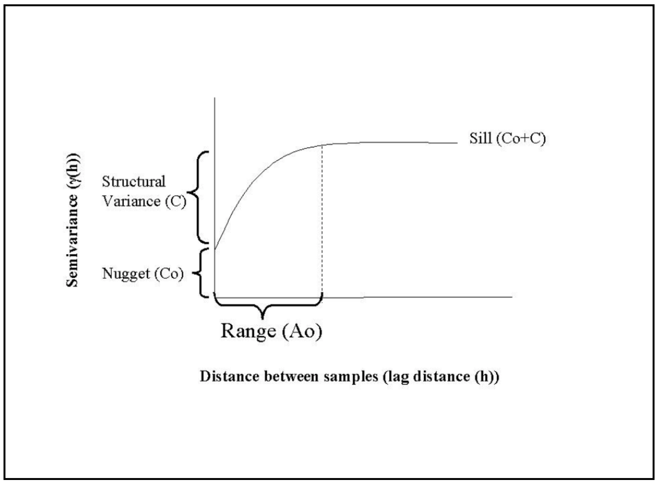

Figure 1b: Four Common Types of Variograms

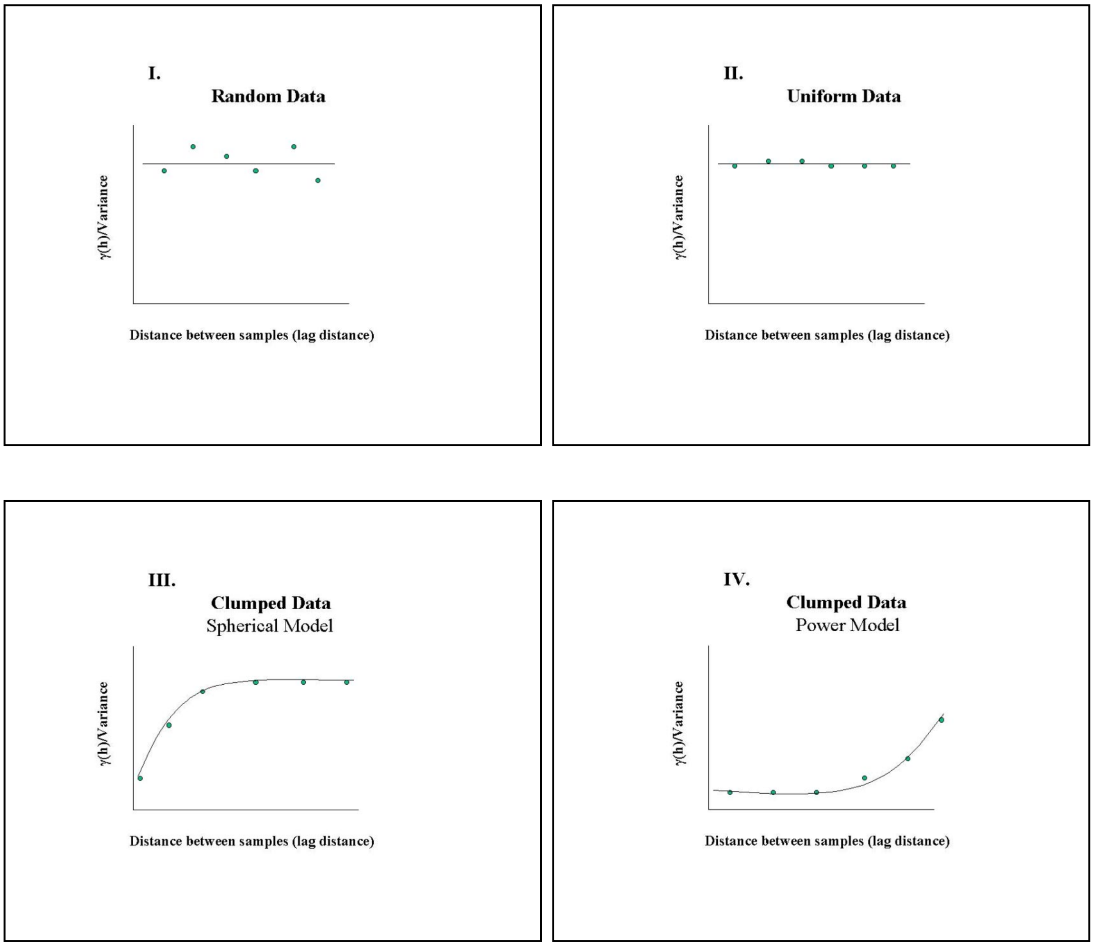

Figure 5b adapted from: Schotzko and O’Keefe 1989 success.

Most of the work has been on single predator/prey systems. Recently investigations have examined use of a greater diversity of predatory mites in biological control programs. In apple, releases of mixed species of phytoseiids gave better control of *P. ulmi* than releases of single species (Croft and MacRae 1992, Croft and Slone 1997). This may be a promising option, but more research should be performed to determine ideal species combinations in various systems.

The use of pesticides in vineyards is one of the main obstacles to establishing a successful phytoseiid population. A strategy that has been used to overcome this problem in other systems is the development and release of strains of phytoseiids that are resistant to various pesticides (Hoy 1982, 1985). Pyrethroid-resistant strains of *Typhlodromus pyri* Scheuten have been successful in controlling *P. ulmi* in Nova Scotian apple orchards (Hardman et al. 2000, Moreau et al. 2000). Croft and Hoying (1975) released strains of *N. fallacis* that were resistant to organophosphates, carbaryl, or both into Michigan orchards. The predators were easily established, but only the organophosphate resistance persisted in the populations.

Most of the work in eastern North America on *N. fallacis* has been done in orchards. There have been no published attempts of releases of *N. fallacis* in vineyards. In California, vineyards have a different predator and prey mite complex. Recently there has been interest in a novel control approach. Vineyards infested with *Tetranychus pacificus* McGregor, the main spider mite pest, have been inoculated with *Eotetranychus willamettei* Ewing, a less damaging pest species. The feeding of the *E. willamettei* causes physiological changes in the vines which make them less susceptible to *T. pacificus* resulting in successful control (Karban and English-Loeb 1990, Karban et al. 1991, Hougen-Eitzman and Karban 1995, Karban et al. 1997). Extensive investigation has also been done on the most common phytoseiid in California vineyards, *Metaseiulus occidentalis* (Nesbitt) (Flaherty and Huffaker 1970).

In Europe, *P.ulmi* is the main spider mite pest on grape, and there is a slightly different complex of phytoseiid mites. In Italy, for example, *Amblyseius aberrans* (Oudemans) was inoculated and established successfully into vineyards with *P. ulmi* infestations (Duso and Pasqualetto 1993, Duso and Vettorazzo 1999). Two to three years after the introduction of *A. aberrans*, they had colonized non-release plots (Duso and Pasqualetto 1993). Releases of another predator native to Italy, *T. pyri*, controlled the spider mites below a damaging level for the first year, but declined thereafter. Possible reasons for the decline included: adverse climatic conditions, release on non-preferred grape varieties, or competition with and predation by anthocorids (Duso and Pasqualetto 1993). With both *A. aberrans* and *T. pyri*, leaf pubescence has been shown to have a positive effect on colonization (Duso 1992, Duso and Vettorazzo 1999). This phenomenon has also been demonstrated in apple where phytoseiids preferred hairier leaves with more pronounced veins that created sheltered areas (Downing and Molliet 1967). Karban et al. (1995) found that the availability of sheltered habitats rather than food availability might be the primary limiting factor for phytoseiid density on grapevines. The shelters may retard desiccation, especially in eggs which are particularly sensitive (Karban et al. 1995). *Typhlodromus. pyri* occurs in the northeastern United States, but has not been reported in Virginia. It has been the focus of biological control research in New York and Nova Scotia orchards, where it has been effective in controlling *P. ulmi* (Nyrop 1988a,b, Hardman et al. 1991, Hardman et al. 2000, Moreau et al. 2000)

V. Potential of *Neoseiulus fallacis* as a biological control agent

The concept of biological control has been defined in many ways. In a broad sense it is the regulation by natural enemies of another organism's population density at a lower average than would otherwise occur (Debach 1974). More specifically, the field of biological control has been defined as the study, importation, augmentation and conservation of beneficial organisms for the regulation of population densities of other organisms (Debach 1964). Biological control has been practiced in entomology and acarology more than in any other field.

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 power of increase greater than the prey, voracity, synchronous distribution with prey, and the ability to resist pesticides (Messenger et al. 1976, Gerson and Smiley 1990, McMurtry 1982). McMurtry and Croft (1997) have suggested that although most studies on the efficacy of phytoseiids as control agents have focused on the rapid rate of increase by the predators, the ability of the predators to regulate spider mites at low equilibrium densities may be just as important. Control agents that regulate 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).

Neoseiulus fallacis possesses many of the characteristics described above. It is a Type II species, a selective predator of tetranychids (McMurtry and Croft 1997, Croft et al. 1998). Type II predators generally have a lower prey requirement than the other feeding types (McMurtry and Croft 1997), indicating that they have high survival rates at low prey densities. McMurtry (1982) rated six phytoseiid species as high, medium, or low on their biological characteristics. He rated *N. fallacis* as high in dispersal power, correlation of distribution in relation to prey, and reproductive potential. He rated the species as medium in voracity, host specificity, and survival ability when prey is scarce. Ball (1980) examined the development of four phytoseiid species. All four had faster developmental rates than their host, *T. urticae*, and *N. fallacis* developed the most rapidly. He suggested that the high rate of increase along with the longevity of the female and its rate of egg consumption imply an ability to quickly suppress prey populations. When tested on 27 different prey types, *N. fallacis* was found to feed and reproduce when prey other than tetranychids was offered, indicating that other less-injurious mites, insects or pollen may enhance survival when favored prey are scarce (Pratt et al. 1999).

Neoseiulus fallacis is associated with a variety of agricultural systems including tree fruits, berries, and field crops (Croft and McGroarty 1977, McMurtry and Croft

1997). Species adapted to these systems generally have the ability to disperse and reproduce rapidly along with an inherent tolerance of or ability to adapt rapidly to changing conditions (McMurtry and Croft 1997). *Neoseiulus fallacis* has been shown to develop resistance to a wide range of pesticides including DDT, organophosphates, and carbamates (Croft 1990). Though typically thought of as a humid-adapted species, *N. fallacis* has also been found to thrive in arid peppermint fields in the Pacific Northwest (Morris et al. 1999), another indicator of adaptability to different habitats.

Because of these attributes, *N. fallacis* has been used in a variety of biological control programs. A program has been established on peppermint in Oregon where inoculative releases were used in establishing *N. fallacis* populations during the first year of mint production (Morris et al. 1999). Also in Oregon, releases of *N. fallacis* provided effective control of *T. urticae* on hops (Strong and Croft 1995). Minimal inoculations 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). Many studies have been performed on the role of biological control by *N. fallacis* in apple orchards (Croft and Hoying 1975, Croft and McGroarty 1977).

The use of *N. fallacis* for biological control in grapes has not been well documented. *N. fallacis* is common in many Virginia orchards but is rarely found in vineyards possibly due to the widespread use of broad spectrum insecticides. A survey in 1987 of Virginia grape growers found that 95% of these growers were using carbaryl, a nonselective pesticide, mainly for Japanese beetle control (Pfeiffer et al. 1990). Carbaryl has been shown to cause 90-100% mortality to *N. fallacis* in laboratory studies (Croft and Stewart 1973, Hislop and Prokopy 1981, Thistlewood and Elfving 1992). In the Pacific Northwest, the use of carbaryl in the 1960s and 1970s for leafhopper control often resulted in mite outbreaks (Kinn and Dout 1972, Cone et al. 1990). In southern Australia, vineyards have only minor to moderate insect problems and as a result are relatively unaffected by pesticides. In this area, large numbers of a complex of phytoseiids are found in association with small non-damaging populations of pest mites (James et al. 1995). Only in vineyards where synthetic pesticides were used were predators absent (James and Whitney 1993). A survey of Greek vineyards also found that phytophagous mites do not cause extensive damage and appear to be well balanced by phytoseiid populations (Papioannou-Souliotis 1999).

The ability of *N. fallacis* to survive and control spider mites on grapes is not documented in the literature. Experiences in other systems with *N. fallacis* indicate that it can be a successful control agent when integrated with a complimentary pesticide program.

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Chapter 2

Relative toxicity of pesticides commonly used in Virginia vineyards to the predatory mite *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) and the implications for establishing a biological control program for spider mites (Acari: Tetranychidae)

Introduction

The European red mite, *Panonychus ulmi* (Koch) (Acari: Tetranychidae), is an economically important pest on a wide variety of agricultural crops. In the eastern United States and Canada it is the most important spider mite pest on grapes (Schruft 1985). Traditionally *P. ulmi* has been controlled using acaricides. However, spider mites have a tendency to build resistance to pesticides (Brader 1977, Dennehy et al. 1983, Herron et al. 1994). Only three acaricides are currently registered for use on grapes in Virginia and resistance to these materials is already evident in some vineyards. As an alternative to acaricidal control, the use of predatory mites as biological control agents is one potential option. The toxicity of pesticides to the predatory mites may be an obstacle to their survival in the vineyard. The toxicity of common pesticides used on grapes to *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae) was investigated.

Neoseiulus fallacis is a predatory mite that occurs throughout North America and especially in the more humid regions of the mid-west and east, including Virginia (Streibert 1981, Welty 1995). It has been shown to be a major mortality factor of both *Tetranychus* spp. and *P. ulmi* on fruit trees in these regions (McMurtry and Croft 1997, Bostanian et al. 1998). *Neoseiulus fallacis* is common in Virginia orchards but rarely seen in vineyards. There are potential explanations for the lack of the predator in the vineyards. First, spider mite outbreaks are a relatively recent phenomenon in vineyards, and the predators may not have colonized the system yet. A second possibility is the use of broad-spectrum insecticides in vineyards to control other pests. The use of these materials, including fungicides and herbicides, often has a detrimental effect on predators of *P. ulmi*. In Massachusetts, experimental and commercial orchards that received pesticides found toxic to *N. fallacis* experienced *Tetranychus urticae* Koch (Acari: Tetranychidae) outbreaks whereas those receiving materials of low toxicity did not exhibit any increase in populations of *T. urticae* or *P. ulmi* (Hislop and Prokopy 1981). The use of pyrethroids in commercial orchards in Ontario caused decreases in phytoseiid populations compared with orchards that had no pyrethroid treatments (Thistlewood 1991). In southern Australia, vineyards have only minor to moderate insect problems and as a result, very few pesticides that would harm predators are used. These vineyards have large numbers of a complex of phytoseiids found in conjunction with small non-damaging populations of pest mites (James et al. 1995).

If a biological control program using *N. fallacis* is to be successful, the pesticides used in the vineyards must not be detrimental to the predator's survival. Most of the materials in use in Virginia vineyards have not been tested for toxicity to *N. fallacis*. We tested a wide range of insecticides, fungicides and herbicides in the laboratory for direct toxicity to *N. fallacis* using a slide-dip bioassay. The results were used and to provide recommendations of compatible materials to use in conjunction with *N. fallacis* released in vineyards.

Materials and Methods

Standard field rates of 18 pesticides were tested for toxicity to *N. fallacis* (Table 1). Materials tested were chosen because they are commonly used on grapes in Virginia and many of the newer materials have not been tested for toxicity to *N. fallacis*. Four separate experiments were conducted. Two of the experiments tested insecticides, one tested fungicides and one tested herbicides for toxicity to *N. fallacis*. After the first insecticide trial several additional materials were tested in a second experiment. All of the testing was done using a slide-dip bioassay developed for use with mites (Anonymous 1968, Hislop and Prokopy 1981). *Neoseiulus fallacis* were obtained from a commercial supplier, The Green Spot Ltd. (Nottingham, New Hampshire), and arrived in a corn grit medium. All testing was done within one to two

Table 1: Pesticides and rates tested for toxicity to *Neoseiulus fallacis* (Garman) in slide dip bioassays.

Insecticides / Acaricides			
Common Name	**Trade Name**	**a.i./100 L**	**Rate/100 L**
dicofol	Kelthane 50WSP	150 g	300 g
fenbutatin-oxide	Vendex 50WP	120 g	240 g
carbaryl	Sevin 80WSP	383.2 g	479 g
cyhexatin	Pennstyl 600 Flowable	150 g	250 mL
azinphos-methyl	Guthion 50WP	120 g	240 g
phosmet	Imidan 70W	168 g	240 g
pyridaben	Pyramite	24.6 g	41 g
Fungicides			
Common Name	**Trade Name**	**a.i./100 L**	**Rate/100 L**
azoxystrobin	Abound Flowable	23.6 mL	103 mL
fenarimol	Rubigan EC	3.7 mL	31 mL
mancozeb	Penncozeb 75DF	247.5 g	330 g
tebuconazole	Elite 45DF	13.5 g	30 g
triflumizole	Procure 50WS	22.5 g	45 g
kresoxin-methyl	Sovran	16.5 g	33 g
Herbicides			
Common Name	**Trade Name**	**a.i./100 L**	**Rate/100 L**
pronamide	Kerb 50W	305.5 g	599 g
oxyfluorfen	Goal 2XL	137.5 mL	625 mL
diuron	Karmex DF	240 g	300 g
paraquat	Gramoxone Extra	115.8 mL	313 mL
glufosinate	Rely	113.3 mL	1000 mL

days of receipt of the mites to ensure viability. Using a fine brush, 10 adult *N. fallacis* were placed on their backs onto Manco® crystal clear packaging tape. The packaging tape was affixed to the ends of 7.6 cm x 2.5 cm glass microscope slides by Scotch® double-sided removable poster tape. Ten slides with ten mites per slide were dipped for each treatment. The slides were immersed and gently agitated for five seconds in a 50-100 mL water suspension of the commercial formulation of the pesticides. The concentrations were based on the recommended field application rates for Virginia as found in the 1999 Horticultural and Forest Crop Pest Management Guide (Pfeiffer et al. 1998), or from manufacturer recommendations for materials not yet registered on grape. Control slides were immersed in tap water. After dipping, the slides were dried on edge for 30 minutes, put into an 20.3 cm x 20.3 cm aluminum cake pan with wet cotton and a clear plastic lid and the pan was placed inside a large clear plastic bag, to maintain high humidity. The slides were kept at 21-24 ^{o}C. Mortality was determined after 24 and 48 hours. Each slide was examined under a microscope and if mites did not move any appendages when prodded with a fine brush they were considered dead.

All results were transformed using an arcsine transformation and analyzed by a one-way ANOVA and Tukey's studentized range test for separating means (SAS Institute Inc. 1997).

Results and Discussion

Insecticides

Both carbaryl and cyhexatin caused significantly higher mortality than the control treatment after 24 and 48 hours (Table 2). Carbaryl is a non-selective insecticide commonly used in the vineyards. Cyhexatin is an acaricide that is being considered for registration on grapes. Neither of the other two acaricides, dicofol or fenbutatin-oxide, were significantly toxic to *N. fallacis*. This is encouraging because if there are no indirect effects as well, these materials could be used in conjunction with *N. fallacis* to lower infestations of *P. ulmi* if the population density is too high for the predator alone to control. Tetranychid mites, including *P. ulmi*, have been shown to develop resistance to

Table 2: Toxicity of insecticides and acaricides to *Neoseiulus fallacis* (n=100) 24 and 48 hours after exposure (November 1999)

Common Name	**Trade Name**	**% Mortality* 24 Hours**	**% Mortality* 48 Hours**
carbaryl	Sevin 80WSP	72.1b	83.7b
cyhexatin	Pennstyl 600 Flowable	87.1b	95.7b
dicofol	Kelthane 50WSP	16.9a	29.6a
fenbutatin-oxide	Vendex 50WP	14.9a	26.3a
Control (water)	-	9.3a	18.5a

* Values followed by different letters are significantly different at alpha=0.05 (Tukey's studentized range test, data transformed using arcsine transformation)

acaricides (Dennehy and Granett 1982, Welty et al. 1987, Herron et al. 1994). However, if used in combination with biological control, they would be applied less frequently and could be rotated to minimize the possibility of resistance development.

The use of carbaryl is one of the main obstacles to establishing *N. fallacis* in vineyards. A 1987 survey of Virginia grape growers found that 95% were using carbaryl, mainly for Japanese beetle control (Pfeiffer et al. 1990). Carbaryl has been shown to cause 90-100% mortality to *N. fallacis* in other laboratory studies (Croft and Stewart 1973, Hislop and Prokopy 1981, Thistlewood and Elfving 1992). In the Pacific Northwest, the use of carbaryl in the 1960's and 1970's often resulted in spider mite outbreaks (Cone et al. 1990). *Neoseiulus fallacis* can develop resistance to organophosphates in commercial orchards (Croft and Stewart 1973, Croft 1977), but carbaryl resistance is not as common (Croft 1977). In fact, when carbaryl-resistant strains were released into orchards, the resistance did not persist (Croft and Hoying 1975). Even if resistance could be developed, the applications of carbaryl in the vineyards may not be repetitive enough for a resistant population to be maintained.

Studies with Japanese beetles on 'Seyval Blanc' vines showed that natural infestation levels failed to significantly affect the fruit quality or quantity, negating the need for insecticide sprays in most seasons (Boucher and Pfeiffer 1989). If carbaryl sprays were eliminated, the survival of *N. fallacis* would be higher.

The effects of two organophosphates and one additional acaricide on *N. fallacis* were tested in July 2000. All three materials caused significantly higher mortality than did the control treatment after 24 hours (Table 3). Pyridaben is a recently registered acaricide on grapes. Because of its high toxicity, pyridaben could not be incorporated into a rotation with dicofol and fenbutatin-oxide without harm to *N. fallacis*.

Phosmet and azinphos-methyl are broad-spectrum materials used against a wide range of vineyard pests. From these results it appears that they would not be compatible with a biological control program using *N. fallacis*. One possibility may be to obtain a strain of organophosphate resistant predators for release into the vineyard, although this was not successful in trials in Massachusetts apple orchards (Prokopy and Christie 1992). Recent restrictions on the use of azinphos-methyl, such as a 21-day reentry interval in grapes, may force growers to increase the use of carbaryl, which is even more toxic to the

Table 3: Toxicity of insecticides and acaricides to *Neoseiulus fallacis* (n=100) 24 and 48 hours after exposure (July 2000).

Common Name	**Trade Name**	**% Mortality* 24 Hours**	**% Mortality* 48 Hours**
azinphos-methyl	Guthion 50WP	57.3a	78.6b
phosmet	Imidan 70W	46.2a	67.5ab
pyridaben	Pyramite	36.3a	50.4a
Control (water)	-	4.72b	12.6c

* Values followed by different letters are significantly different at alpha=0.05 (Tukey's studentized range test, data transformed using arcsine transformation)

predator. Some alternative controls are being researched including the use of mating disruption for controlling grape berry moth. However, these methods are not yet in widespread use. Adoption of alternative controls such as this would make a biological control program more effective.

Fungicides

None of the six fungicides tested caused significantly higher mortality of *N. fallacis* than the control, even after 48 hours (Tables 4). This is consistent with previous tests of the toxicity of fungicides to phytoseiid mites (Hislop and Prokopy 1981, Bostanian et al. 1998). The slide dip method used in this bioassay only shows direct toxicity. There may be effects on fecundity and reproduction that were not examined in these experiments. Although mancozeb does not cause high adult mortality, laboratory tests have shown it to cause a significant decrease in fecundity and egg hatch of *N. fallacis* and other phytoseiids (Ioriatti et al. 1992, Bostanian et al. 1998). Further testing of these indirect effects should be conducted to ensure that these materials would not adversely affect *N. fallacis*. However, the fungicides in use in the vineyards appear to be more compatible with a biological control program than are the insecticides. Overall these results were promising. Because of the humid climate, fungicides are vital to growing grapes in Virginia and it is unlikely that their use could be eliminated for most commercial cultivars.

Table 4: Toxicity of fungicides to *Neoseiulus fallacis* (n=100) 24 and 48 hours after exposure.

Common Name	**Trade Name**	**% Mortality* 24 Hours**	**% Mortality* 48 hours**
azoxystrobin	Abound Flowable	8.0a	12.8a
fenarimol	Rubigan EC	7.3a	17.8a
kresoxin-methyl	Sovran	4.8a	9.4a
mancozeb	Penncozeb 75DF	10.9a	19.7a
tebuconazole	Elite 45DF	9.0a	15.3a
triflumizole	Procure 50WS	10.7a	20.4a
Control (water)	-	4.5a	8.2a

* Values followed by different letters are significantly different at alpha=0.05 (Tukey's studentized range test, data transformed using arcsine transformation)

Herbicides

Although herbicides are not applied directly to the vine, they could still have an effect on *N. fallacis* as this species has been shown to overwinter in ground cover in orchards (McGroarty and Croft 1978). Two of the herbicides tested, oxyfluorfen and glufosinate, were highly toxic to the predator after 24 hours (Tables 5). After 48 hours, paraquat was also found to cause significantly higher mortality than the control. In the laboratory, paraquat has been found to cause as high as 100% mortality to *N. fallacis* after 48 hours (Hislop and Prokopy 1981), and Pfeiffer (1986) showed that field applications of paraquat negatively affect populations of *N. fallacis* and *P.ulmi*. Diuron and pronamide were both of low toxicity. Diuron is rated as a more effective preemergent herbicide than oxyfluorfen in addition to having low toxicity to *N. fallacis*. Paraquat and glufosinate are both postemergent herbicides and both had negative effects on the predator. Further investigation on postemergent herbicides that would be compatible with releases of *N. fallacis* should be conducted.

Generally it has been shown that the predator overwinters in broadleaf vegetation in ground cover or borders of fields and orchards, but with recent research in Massachusetts' orchards it has been suggested that significant overwintering may occur in the trees themselves (Nyrop et al. 1994). If *N. fallacis* did overwinter on the vines the effects from herbicide applications would be less severe. In peppermint fields in Oregon, the removal of ground cover debris in plots decreased the overwintering survival of N. fallacis (Morris et al. 1996), indicating that some ground cover may be essential for survival of the predator. However, the overwintering habits of *N. fallacis* in vineyards in Virginia have not yet been determined.

Table 5: Toxicity of herbicides to *Neoseiulus fallacis* (n=100) 24 and 48 hours after exposure.

Common Name	**Trade Name**	**% Mortality*- 24 hours**	**% Mortality - 48 hours**
diuron	Karmex DF	5.2b	13.1c
glufosinate	Rely	100.0c	100.0a
oxyfluorfen	Goal 2XL	80.5a	96.1a
paraquat	Gramoxone Extra	18.1b	35.5b
pronamide	Kerb 50W	9.2b	13.3c
Control (water)	-	3.8b	11.1c

* Values followed by different letters are significantly different at alpha=0.05 (Tukey's studentized range test, data transformed using arcsine transformation)

Conclusion

From the results of these bioassays, it is evident that there are potential obstacles to developing a biological control program using *N. fallacis* to control *P. ulmi* in Virginia vineyards. The biggest problem is the widespread use of pesticides such as carbaryl and azinphos-methyl, which were both found to cause significant mortality to *N. fallacis*. The high toxicity of several herbicides may also be an obstacle if *N. fallacis* overwinters in the ground cover of the vineyards. Further investigation on overwintering habits of this predator should be conducted.

These bioassays only tested direct toxicity effects. Materials found to be nontoxic by this method could have effects on fecundity, reproduction or longevity. In addition only adult *N. fallacis* were tested. Further testing on nymphal and egg stages would be advisable to ensure that the pesticides truly have no harmful effects. Field trials should also be conducted to confirm laboratory results.

Although there are materials that may have an adverse effect on the predator, results of these laboratory tests indicate that there are many pesticides available to the grower that have little or no direct effect on the predator. Incorporating these materials into an integrated pest management program could improve the survival rate of *N. fallacis* in the vineyards while still protecting this high value crop from other pests.

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