Topical Toxicity of Pesticides Used in Virginia Vineyards to the Predatory Mite, *Neoseiulus fallacis* (Garman)[1]

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Abstract Slide dip bioassays were conducted to determine the direct toxicity of insecticides, acaricides, fungicides, and herbicides commonly used in vineyards in Virginia to *Neoseiulus fallacis* (Garman) (Acari: Phytoseiidae), a predatory mite under consideration as a biological control agent for spider mites (Acari: Tetranychidae). Among the insecticides and acaricides tested in the laboratory, carbaryl, azinphos-methyl, phosmet, cyhexatin, and pyridaben caused significantly ($P < 0.05$) higher mortality than the control treatment. None of the fungicides tested were toxic to the predator, but three herbicides caused high mortality. Glufosinate caused 100% mortality after 24 h, and both oxyfluorfen and paraquat had adverse effects on *N. fallacis*. The use of materials that were found to be toxic to the predator may not be compatible with releases of *N. fallacis* into Virginia vineyards. However, incorporating materials that appear to have no direct toxicity to the predator into an integrated pet management program could improve the survival rate of released *N. fallacis* while still protecting this high value crop from other pests.

Key Words *Neoseiulus fallacis*, spider mites, vineyards, bioassay, pesticides

Spider mites (Acari: Tetranychidae) are economically important pests on a variety of agricultural crops. In recent years, they have been controlled using acaricides. However, spider mites have a tendency to develop resistance to pesticides (Baker 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 an 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 Midwest and East, including Virginia (Streibert 1981, Welty 1995). It has been shown to be a major mortality factor of both *Tetranychus* spp. and *Panonychus ulmi* (Koch) on fruit trees in these regions (McMurtry and Croft 1997, Bostanian et al. 1998). *Neoseiulus fallacis* is common in Virginia orchards but rarely is 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

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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, is often detrimental to 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 phytosciid 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 predators. 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 to provide recommendations of compatible materials for 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 in vineyards 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, NH) and arrived in a corn grit medium. All testing was done within 1 to 2 d of receipt of the mites to insure viability. Using a fine brush, 10 adult *N. fallacis* were placed on their backs onto crystal clear packaging tape (Manco®, Avon, OH). The packaging tape was affixed to the ends of 7.6 cm × 2.5 cm glass microscope slides by Scotch® double-sided removable poster tape (3M, St. Paul, MN). Ten slides with 10 mites per slide were dipped for each treatment. The slides were immersed and gently agitated for 5 s in a 50 to 100 mL water suspension of the commercial formulation of the pesticides. The concentrations were based on the recommended field 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 min, placed into a 20.3 cm × 20.3 cm aluminum cake pan with wet cotton and a clear plastic lid. This pan was then placed inside a large clear plastic bag, to maintain high humidity. The slides were kept at 21 to 24°C. Mortality was determined after 24 and 48 h. 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

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

Common name	Trade name	a.i./100 L	Rate/100 L
Insecticides/Acaricides			
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 60WSB	24.6 g	41 g
Fungicides			
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 50WG	16.5 g	33 g
Herbicides			
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

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 h (Table 2). Carbaryl is a non-selective insecticide commonly used in vineyards. Cyhexatin is an acaricide that is being considered for registration on grapes. Neither dicofol nor 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 acaricides (Dennehy and Granett 1982, Welty et al. 1987, Herron et al. 1994). How-

Table 2. Toxicity of insecticides and acaricides in a slide-dip bioassay 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 values are significantly different at alpha = 0.05 (Tukey's studentized range test, data transformed using arcsine transformation).

ever, 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% used carbaryl, mainly for Japanese beetle, *Popillia japonica* Newman, control (Pfeiffer et al. 1990). Carbaryl has been shown to cause 90 to 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 sufficiently repetitive 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). As carbaryl use declines, the survival of *N. fallacis* could improve.

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 h (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 harming *N. fallacis*.

Phosmet and azinphos-methyl are broad-spectrum materials used against a 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 vineyards, 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-d restricted entry interval in grapes, may force growers to increase the use of phosmet, which was

Table 3. Toxicity of insecticides and acaricides in a slide-dip bioassay 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	57.3b	78.6c
phosmet	Imidan 70W	46.2b	67.5bc
pyridaben	Pyramite 60WSB	36.3b	50.4b
Control (water)	—	4.7a	12.6a

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

also found to be toxic to *N. fallacis*. Some alternative controls are being researched including the use of mating disruption for controlling grape berry moth. However, these methods are not yet in general use, although use is increasing because of regulatory restrictions of organophosphates. 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 h (Table 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 insure that

Table 4. Toxicity of fungicides in a slide-dip bioassay 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 50WG	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 values are significantly different at alpha = 0.05 (Tukey's studentized range test, data transformed using arcsine transformation).

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.

Herbicides. Although herbicides are not applied directly to the vine, they could still have an adverse 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 h (Table 5). After 48 h, paraquat also was 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 h (Hislop and Prokopy 1981), and Pfeiffer (1986) showed that field applications of paraquat negatively affect populations of *N. fallacis* and increase populations of *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* (Pfeiffer et al. 1990). 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 this predator overwinters in broadleaf vegetation in ground cover or borders of fields and orchards, but with recent research in New York 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 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.

Conclusions. 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

Table 5. Toxicity of herbicides in a slide-dip bioassay to *Neoseiulus fallacis* (n = 100) 24 and 48 hours after exposure.

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

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

pesticides such as carbaryl and azinphos-methyl, which were both found to cause significant mortality to *N. fallacis*. In addition, some insecticides and acaricides new to the vineyard system are also toxic to this predator. The high toxicity of several herbicides may also be an obstacle if *N. fallacis* overwinters in the ground cover in vineyards. Further investigation on overwintering habits of this predator should be conducted. Pratt and Croft (2000), in a survey of previous toxicity studies toward *N. fallacis*, concluded that insecticides generally showed the greatest toxicity, fungicides the least toxicity, and herbicides were intermediate. Though our results were generally consistent with this conclusion, there was a wide variation in toxicity within pesticide classes.

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 insure that the pesticides truly have no harmful effects. Field trials should also be conducted to confirm laboratory results. Thistlewood et al. (1992) compared topical application (slide-dip) with residual (Petri dish) bioassays for toxicity studies in *N. fallacis*. Slide-dip assays were less sensitive than residual studies in quantifying mortality in mites. Slide-sip studies lend themselves to greater replication, though residual studies allow a more realistic prediction of field mortality. While slide-dip studies suffice for relative comparisons of acute toxicity (as this study), other effects (see sublethal effects discussed by Croft [1990]) are neglected.

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 growers 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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