

## ABSTRACT

WILLIAMS, JAY LOGAN. Predatory Mites (Acari: Phytoseiidae) in Fraser fir Christmas Tree Plantations in the Southern Appalachians of North Carolina. (Under the direction of Fred P. Hain).

Experiments were conducted to evaluate the potential use of phytoseiid (Acari: Phytoseiidae) mites as control agents for Spruce Spider mite (*Oligonychus ununguis* Jacobi) and Rosette Bud Mite (Acari: Phytoptidae), pests of Fraser fir (*Abies fraseri* (Pursh) Poiret)), in Christmas tree production areas of North Carolina. The predatory mite community structure in Fraser fir plantations was characterized. Three predator families in the Prostigmata were collected, Anystidae, Bdellidae, and Cunaxidae. The most abundant phytoseiid mites collected were *Arrenoseius morgani* (Chant) (56.4%), *Typhlodromips sessor* (DeLeon) (21.1%), and *Proprioseiopsis solens* (DeLeon) (5.1%). Ninety four phytoseiids were collected on trees infested with spider mites. Sixty four phytoseiids were collected from uninfested trees. Phytoseiid abundance on different Fraser fir size classes was follows: 1.2-1.5 m (n=61), 1.8-2.1m (n=34), and 1.5-1.8 m (n=27). Eighty six phytoseiids were collected from plantations with a diverse ground cover. Clover and bare-ground each yielded 17 phytoseiids while grass ground cover yielded 14 phytoseiids.

The occurrence of a phytoseiid mite *Typhlodromalus peregrinus* (Muma) (Acari: Phytoseiidae), overwintering in rosette bud mite galls (Acari: Phytoptidae) was investigated. Twenty-nine phytoseiid mites were found in 14 galls. There was an average of 2 phytoseiid mites per gall and the highest number of phytoseiid mites in any one gall was 4. Gall heights, width, cavity depth, and aperture size (n=121) averaged 7.4 mm, 7.4 mm, 2.1 mm and 2.0 mm, respectively. The height, width, depth, and aperture size of galls containing *T.*

*peregrinus* (n=14) averaged 7.2 mm, 7.5 mm, 2.9 mm, and 1.6 mm, respectively. *Typhlodromalus peregrinus* was observed feeding on all stages of the rosette bud mite.

The overwintering sites and seasonal abundance of phytoseiid mites in North Carolina Fraser fir plantations was assessed. Plantations with ground cover consisting of white clover (*Trifolium repens* L.) were compared to plantations with bare ground. A total of 414 Phytoseiidae with 12 different species were collected during this study. The phytoseiids collected, in order of abundance, were *Arrenoseius morgani* (Chant) (160), *Typhlodromips sessor* (DeLeon) (156), and *Typhlodromalus peregrinus* (Muma) (34).

Mixed grass ground cover samples and Fraser fir needle samples contained 174 and 158 phytoseiids, respectively. Mixed grass samples also had the greatest diversity of phytoseiids. Fraser fir branches collected from plantations with a white clover groundcover yielded a greater abundance of phytoseiid mites than Fraser fir branches collected from plantations with a mixed species herbaceous groundcover.

A total of 512 Phytoseiidae were collected from plantations in a comparison of bare ground and clover ground cover influences on phytoseiid mites. *Typhlodromips sessor* (DeLeon), *Typhlodromalus peregrinus* and *Arrenoseius morgani* were the most abundant species collected from Fraser fir with either clover or bare ground cover crops. There were no statistical differences in the effects of the clover ground cover and bare ground analyzed on phytoseiid mite abundance.

Vegetation samples were collected in Laurel Springs and Waynesville, North Carolina from 4 ground cover crop species: white Dutch clover, mammoth red clover, birdfoot trefoil, and fescue. The most abundant phytoseiid mites were *Typhlodromips sessor*, *Arrenoseius morgani*, *Proprioseiopsis solens* (DeLeon), and *Typhlodromalus peregrinus*.

There were no statistical differences in the effects of ground cover vegetation analyzed on phytoseiid mite abundance.

Predatory Mites (Acari: Phytoseiidae) in Fraser fir Christmas Tree Plantations  
in the Southern Appalachians of North Carolina

by  
Jay Logan Williams

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APPROVED BY:

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John Frampton

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John Monahan

---

David Orr

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Fred P. Hain  
Chair of Committee

## **DEDICATION**

To my family,

Terri, Logan, Sarah, Mom and Dad.

## **BIOGRAPHY**

Jay Logan Williams was born October 31, 1957 in Raleigh North Carolina. He received his elementary and secondary education in Raleigh graduating from Millbrook High School in 1976. He received the Bachelor of Arts degree with a major in Philosophy from North Carolina State University in 1981. In 1982 he graduated from North Carolina State University with an Associate of Applied Agriculture degree with a major in Agricultural Pest Control. He received the Master of Science degree in Entomology from North Carolina State University in 1994. In 2001 he was admitted to North Carolina State University to begin studying for the Doctor of Philosophy in Forestry degree. He has been employed with the State of North Carolina for 24 years. The author is married to the former Teresa Joseph, and they have two children, Sarah and Logan.

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## **CHAPTER 1**

### **Introduction and Literature Review**

Growing Christmas trees in North Carolina is a popular and lucrative commercial endeavor which attracts both part-time and professional growers. A majority of these growers are part-timers, having less than 10 acres of Christmas trees in production in North Carolina. Christmas tree growers produce any of a number of species depending on the desired tree characteristics and location within the state. Eastern white pine (*Pinus strobus* L.), Virginia pine (*Pinus virginiana* Miller), red cedar (*Juniperus virginiana* L.), Leyland cypress (*X Cupressocyparis leylandii* (Jacks & Dallim.) Dallim.), and Fraser fir (*Abies fraseri* (Pursh) Poiret) are frequently grown by Christmas tree producers in North Carolina (McKinley 1997 b). Approximately, 18% of the nation's Christmas tree crop is grown in North Carolina. North Carolina ranks second in the nation behind Oregon in tree harvesting, shipping about 5.5 to 6 million trees per year (North Carolina Department of Agriculture and Consumer Services 2008).

Growing Christmas trees in North Carolina has increased in popularity over the last few decades. Roughly 95% of the trees in production are Fraser firs (North Carolina Department of Agriculture and Consumer Services 2008). North Carolina ranks first in the nation in Fraser fir production. At the present time, there are approximately 1,500 Fraser fir Christmas tree farmers in western North Carolina. An estimated 42 million trees are grown on 12140 ha in the northwestern portion of the state. Ashe, Avery, Alleghany, Watauga and Jackson counties produce 88% of North Carolina's Christmas trees (North Carolina Department of Agriculture and Consumer Services 2008).

Successful Christmas tree production requires an enormous commitment on the part

of the grower, as it is extremely labor intensive to produce a lucrative, marketable, and quality crop (Division of Forest Resources 1988). It also requires a considerable investment of capital and time (McKinley 1997a). Approximately 90 man-hours of labor per .40 ha per year are required to produce a marketable product (Division of Forest Resources 1988).

Why is growing Fraser Fir Christmas trees in North Carolina so popular, considering the expense and labor involved? One potential answer is related to the location of the state. The climate and geography of western North Carolina are conducive to the growth of Fraser fir. This species occurs naturally on some of the highest mountain peaks in the state at an elevation of 1,310 to 1,524 m or more. However, it also grows well in plantations at lower elevations, making it a popular choice for Christmas tree growers (McKinley 1997a). Western North Carolina is in a profitable region for small agribusinesses like farmers and Christmas tree producers. The state is located near some of the largest cities in the southeast. This strategic location contributes vastly to the marketability of North Carolina's agricultural and forest resources products, including Fraser fir (McKinley 1997a.). An estimated 23 to 28 million people in the United States sought natural Christmas trees during the 2003 holiday season. This was an increase of 6 million tree shoppers over 2002 (Associated Press 2003). When compared to other tree species marketed as Christmas trees, Fraser fir is considered the Christmas tree of choice among consumers. The color and texture of its foliage, pleasant aroma, needle retention, and shape all contribute to this popularity (McKinley 1997a.). These traits are largely dependent on the health and vigor of the tree, which are influenced by soil fertility, planting site and pest control (Division of Forest Resources 1988). Factors to consider when selecting a planting site include “topography, surface condition, elevation, exposure, internal drainage, and accessibility to roads” (Division of Forest Resources 1988).

The use of good stock, properly planted and the control of weeds, insects and diseases also contribute to successful Christmas tree production.

### **Problem Statement**

There are several common pests of Fraser fir Christmas trees. These include the spruce spider mite (*Oligonychus ununguis* Jacobi) (Boyne and Hain 1983a), rosette bud mites (*Trisetacus* sp.) (McKinley 1997a), balsam twig aphids (*Mindarus abietinus* Koch) (Nettleton and Hain 1982), *Cinara* aphids (*Cinara* sp.) (McKinley 1997a), balsam woolly adelgid (*Adelges piceae* Ratz.) (Arthur and Hain 1984), and white grubs (*Phyllophaga* spp., *P. fusca*, and *Polyphylla comes*) (Kard and Hain 1987). Traditionally, the Fraser fir Christmas tree industry has relied heavily on the use of agricultural chemicals to control these pests. Recently, however, there has been a trend by growers to use fewer pesticides and fertilizers in order to protect beneficial organisms and preserve soil and water resources (Sidebottom 2001).

The majority of North Carolina's Christmas trees are grown in close proximity to the New, Yadkin, and Watauga River headwaters. Intensive agricultural production in these areas has the potential to generate non-point source pollution, which may have serious consequences for sensitive aquatic resources. It is therefore important that agricultural production near mountain creeks and rivers avoid management practices that may contribute to significant non-point source pollution, either from agricultural chemicals, sediment loading, or both.

Herbicides such as atrazine can have serious environmental consequences when over used or not used properly. Triazine herbicides including atrazine and simazine have been found in ground and surface water in western North Carolina. A study conducted by the

North Carolina Cooperative extension Service during 2000-2001 found that 10.6% of 178 well samples detected levels of triazine. Samples, collected in Avery and Watauga Counties did not exceed the USEPA current Maximum Contaminant Level of 3 ppb. (Sidebottom 2006). Ecological damage from agricultural chemicals is not limited to humans. Atrazine at levels as low as 0.1 parts per billion (ppb) has been demonstrated to cause hermaphroditism in male American leopard frogs (*Rana pipiens* complex), suggesting that atrazine and other endocrine disrupting agricultural chemicals may play a role in amphibian declines (Anonomous 2003). The use of atrazine has decreased among North Carolina Christmas tree producers in recent years and is being rapidly replaced with chemical mowing techniques utilizing herbicides such as Roundup (Sidebottom 2001).

Cover crops may decrease runoff and sedimentation from agricultural areas and ultimately lead to a reduction in the grower dependence on herbicides such as atrazine. However, a small percentage of growers continue to use herbicides in order to eliminate all cover vegetation (Sidebottom 2001).

Insecticide application is also commonly used to address the recurring pest problems that plague many conventional Christmas tree farms. One example is the spruce spider mite. If pesticides for control of the spruce spider mite can be reduced or eliminated, then more biological control agents may survive to feed on other pests, such as balsam twig aphid and balsam wooly adelgid.

## **Objectives**

The purpose of this paper is to evaluate Fraser fir Christmas tree production in North Carolina and to determine if it provides a suitable environment for a conservation based IPM program for *O. ununguis* Jacobi.

## **Spruce Spider Mite Overview**

Spruce spider mite was first collected and described from spruce trees in Germany in 1905 (Peterson and Hildahl 1969). It is found on conifers throughout the world including Japan, Canada, United States, England, and Finland (Akita 1971, Boyne and Hain 1983a, Garman 1923, Loyttyniemi 1970, Peterson and Hildahl 1969, Ryle 1923.) In the North Temperate region a number of conifers are host to the spruce spider mite including: arborvitae, cypress, fir, false cypress, hemlock, incense cedar, larch, juniper, redwood, pine, yew, Douglas-fir and spruce (Charlet and McMurtry 1977, Jeppson et al. 1975, Johnson and Lyon 1994).

Spruce spider mite is regarded as one of the most serious mites attacking conifers (Johnson and Lyons 1994). It is especially troublesome in plantation and ornamental plantings (Peterson and Hildahl 1969, Rose and Lindquist 1977, Johnson and Lyon 1994). Lehman (1982) conducted surveys on the conifers of Pennsylvania and found that *O. ununguis* Jacobi was the most frequently encountered tetranychid collected from 43 conifer species in a survey of nurseries, cemeteries, and ornamental and roadside plantings. Loyttyniemi (1970) provides a complete review of the literature prior to 1970. This publication also provides a comprehensive study of the spruce spider mite's biology in Finland.

## **Spruce Spider Mite Biology**

The biology of the spider mite family Tetranychidae has been investigated by a number of workers (Carey 1982, Carey and Bradley 1982, Congdon and Logan 1983, Hanna et al. 1982, Landwehr and Allen 1982, Perring et al. 1984, Takafuji and Kamibayashi 1984, Saito 1986, 1987). *Oligonychus ununguis* Jacobi has five life stages including egg, a six-

legged larva, and eight-legged protonymph, deutonymph, and adult, and its life cycle is comparable to other mites in the family Tetranychidae (Jeppson et al. 1975). Eggs are 0.1 mm in width, have a slightly flattened globular shape and a thin hair or stripe extending from the top of the egg. The egg color is grayish brown when first laid, but later changes to orange brown (Jeppson et al. 1975).

The larvae are pink to orange when they first hatch but change to orange as feeding begins. Larvae have three pairs of legs (Peterson and Hildahl 1969) but, the nymphal stages have four pairs. Both the protonymph and deutonymph stages are similar in shape; the deutonymph however, is larger. The color of the nymphal stages varies from light to dark green (Peterson and Hildahl 1969).

Adult mite coloration varies from dark green to dark brown or black (Johnson and Lyon 1994). Female mites are about 0.5 mm long and oval. Males tend to be smaller with longer legs and a more pointed abdomen than females (Peterson and Hildahl 1969). Both adult and immature spruce spider mites spin fine webbing that they use for protection (Stewart and Peterson 1960). This webbing is spun between needles and in heavy infestations may cover the ends of branches (Stewart and Peterson 1960).

The time for development from egg to adult in spruce spider mites is from 8 to 24 days (Kielczewski 1966). Mated females produce both male and female offspring, however, the eggs of unfertilized females develop into males (arrhenotoky) (Loyttyniemi 1970). Larval development requires around 3 days and development of nymphs is usually completed in 6 days (Johnson and Lyons 1994). There are typically 3 or more generations produced at intervals of 2-3 weeks (Johnson and Lyons 1994).

Larvae and adults can be dispersed by the wind (Johnson and Lyon 1994). Most spider mites have a habit of covering leaves, shoots, and flowers with very fine silken webbing, produced from a pair of glands near the mouth. The silk strands aid dispersal by allowing the mites to spin down from infested to non-infested leaves, and to be blown by wind currents. (Rose and Lindquist 1977). Various sex ratios have been reported and range from 50% to 90 % female (Loyttyniemi 1970, Jeppson et al. 1975 and Saito 1979). The spruce spider mite overwinters as eggs in diapause (Johnson and Lyon 1994).

Eggs are usually laid on the bark of small branches at the base of the needle but not on the main trunk (Loyttyniemi 1970, Richmond and Shetlar 1996). Richmond and Shetlar (1996) reported that overwintering eggs were laid on the top, middle and bottom of the tree in an even distribution. Johnson and Lyon (1994) report that overwintering eggs are deposited under bud scales in the axils of needles. They are also found under webbing on branches and stems. Oviposition may begin in late summer if crowded conditions exist (Shinkaji 1975). The overwintering diapausing eggs can withstand a temperature of  $-40^{\circ}$  C (Loyttyniemi 1970).

In general, spider mite populations are influenced by weather, with warm, dry conditions generally favorable to survival and reproduction (Rose and Lindquist 1977). However, spruce spider mite is considered a “cool season” mite because it generally reaches peak populations during the spring and fall (Leman 1998). Hatch occurs in March to early April under favorable temperatures. Populations generally peak in June then taper off in mid summer. However, a secondary increase may occur in September and October (Kielczewski 1966). In North Carolina Fraser fir production areas, this mite can cause damage to Fraser fir anytime from March through November (Sidebottom 2002).

Research has been conducted on the effects of temperature, rainfall and humidity on the dynamics of this mite (Boyne and Hain 1983a). Mite populations grow best and build-up rapidly at temperatures around 26<sup>0</sup> C, with an RH of around 50-60 % and complete a generation every 15 days (Boyne and Hain 1983a). Egg production was 50% less at an RH of 88-90%. When mites were exposed to 20<sup>0</sup> C, a generation was completed in 23 days and the adults lived twice as long. Females exposed to the cooler temperature deposited an average of 39 eggs during their lifetime, while the mites exposed to the warmer temperature only deposited 29 eggs (Boyne and Hain 1983a). In laboratory experiments, eggs exposed to temperatures exceeding 29<sup>0</sup>C failed to survive due to the excessive temperatures (Boyne and Hain 1983a).

Rainfall can limit mite populations. In fact, the use of a steady stream of water sprayed on landscape plants has been suggested as a control measure for spruce spider mite (Stewart and Peterson 1960).

### **Predatory Mites Overview**

It has been demonstrated that natural enemies such as mites in the family Phytoseiidae (Subclass Acarina: Order Parasitiformes) are major predators of tetranychid mites and can be successfully used in biological control programs (Jeppson et al. 1975, McMurty et al. 1970a). Gerson and Smiley (1990) provides keys and an introduction to the families of mites with potential for use in the biological control of pests. Gerson, et al. (2003) discusses in detail the 34 acarine families with mites useful for the control of insects, mites, weeds, and nematodes pests. In addition, they provide an illustrated key for identification.

The taxonomy of this group is unsettled. Many workers place the whole family of Phytoseiidae into one genus, while others recognize more than 50 genera in the family (Hoy 1982). Some works have taken into account biological, morphological and ecological differences between the taxa (Muma and Denmark 1970). Chant (1959) characterizes the biology of seven species of phytoseiid mites in England and also provides a taxonomic review of the family describing 38 new species. The most up to date information on the family is a comprehensive review provided by Chant and McMurtry (1994; 2003a, b; 2004a, b; 2005a, b).

### **Predatory Mite Biology**

McMurtry and Croft (1997) reviewed the life styles and feeding habits of the Phytoseiidae and their relative importance to biological control. They divided the family into four different feeding guilds: Type I) specialized predators of *Tetranychus* like *Phytoseiulus*; Type II) selective predators of tetranychid mites like *Galendromus*, *Neoseiulus* and some *Typhlodromus* species; Type III) generalist predators including *Typhlodromus* and *Amblyseius* species and others; and Type IV) specialized pollen feeders/generalist predators like *Euseius*.

Helle and Sabelis (1985) provide a great deal of information on the biology of phytoseiid mites. In general, phytoseiid mites can be described as oval or pear shaped in appearance. They are approximately 0.3-0.5 mm long and 0.3 mm wide. Coloration may vary with the type of food consumed but is generally opaque yellow to red. There are five stages in the life cycle, including egg, larva, protonymph, deutonymph, and adult (Jeppson et al. 1975). Mated females are the overwintering stage (McMurtry et al. 1970). Eggs are oval in shape with an opaque color. Larvae have three pair of legs, while both nymphal stages as

well as the adult stage have four pairs of legs. They usually develop from egg to adult in six to seven days; however, this is influenced by temperature and food availability (Jeppson et al. 1975). Most phytoseiid mites are predaceous or at least partially predaceous, although some species feed on pollen (Jeppson et al. 1975).

Several species of predatory mites have been shown experimentally to reproduce when given pollen and fungi as a food source (Smith and Papcek 1991; McMurtry 1992; Karban et al. 1995). This ability of phytoseiid mites to feed on alternative food sources like pollen and fungi may be an important attribute to consider when developing a Christmas tree IPM program, as this characteristic may allow the predators to thrive and increase in numbers in the absence of spider mite prey (McMurtry and Rodriguez 1987). When spider mite populations are low, specialist predatory mites may either emigrate from the system or perish from lack of a food source (Burnett, 1979; Strenseth 1985). Emigration and starvation among specialist predators within an agricultural ecosystem can lead to instability in predator-prey interactions (McMurtry 1992, Nyrop et al. 1998, Jung and Croft 2001a).

*Neoseiulus fallacis* (Garman) is a selective (Type II) predator of the spider mite family Tetranychidae (McMurtry and Croft 1997). This species will feed and reproduce on pollen (Pratt et al. 1999) and cannibalism has been observed during times of starvation (Ballard 1954). Croft et al. (1995) reported that *N. fallacis* has a moderate tendency to move within host plants, high reproductive rate and readily disperses in search of spider mite outbreaks. However, because it has high energy needs, it is frequently displaced by more generalist predators when prey levels are low. The life cycle of *N. fallacis* was studied and it was determined that only the females enter a deutonymphal stage while males develop from protonymph directly to adult (Ballard 1954). Females mate approximately one hour after

they mature into the adult stage. Males consume an average of 3.9 prey each day and females eat approximately 8.0 per day. Cannibalism occurred following several days of starvation (Ballard 1954). Additional information on the biology of this species is given by Ball (1980), Furr and Shaw (1977), Smith and Newsom (1970), and Tanigoshi et al. (1975).

The feeding behavior of hatching larvae of *N. fallacis*, *Metaseiulus occidentalis* (Nesbitt), *Amblyseius andersoni* Chant and *Typhlodromus pyri* Scheuten were evaluated by holding them in containers without food at 95% RH and 20<sup>0</sup> C. Unfed *M. occidentalis* quickly starved within 2-3 days, but the other three species lived for 12 to 14 days. Some of these developed into adults by cannabilizing and scavenging dead bodies (Croft and Croft 1993). Overwintering habitats were evaluated for *N. fallacis* (Garman) in peppermint fields. It was found that 37% overwintered in dead leaves, 45% in debris below the leaves, 3% in hollow stems, and 15% on live foliage (Morris et al. 1996). Of these, 95% of the *N. fallacis* were on new foliage and feeding on *Tetranychus urticae* Koch by April (Morris et al. 1996). It was found that augmenting plots with debris contributed to overwintering survivorship of the predators. Conversely, debris removal decreased survival of the predators (Morris et al. 1996).

Predatory mites in the genus *Euseius* are considered specialized pollen feeders/generalist predators (McMurty and Croft 1997). The nutritional value of some types of pollens may provide an important function by sustaining and increasing the population of predatory mites like *Euseius finlandicus* Oudemans in the absence of prey (Broufas and Koveos 2000). In a laboratory experiments, the predatory mite *Euseius tularensis* (Congdon) was fed pollen from several different field grown legumes in order to compare survival, sex ratio, and reproduction. The results of the study indicate that this species develops and

reproduces on pollens from various species of legumes. The results of field experiments demonstrated that *E. tularensis* can be successfully mass-reared in cover crops and by taking cuttings from the cover crop and placing them in young citrus trees to increase predator densities significantly (Grafton-Cardwell and Ouyang 1999).

Several works have been compiled which explore the biology of *Metaseiulus occidentalis* (Hess and Hoy 1982, Hoy 1979, Laing 1969, and Lee and Davis 1968). In a Utah study *M. occidentalis* was found to overwinter under bark and in other concealed places (Lee and Davis 1968). Larval and deutonymphal stages of prey mites as well as pollen were consumed by larval *M. occidentalis*. Adult males consumed on average 31.2 prey while the females devoured approximately 76.3 (Lee and Davis 1968). Females lay approximately 33.7 eggs. On average males lived approximately 26 days, while females lived 38.7 days. Further investigations determined that emerging adults soon mated, sometimes more than once, and cannibalism occurred only in the immature stages. In addition, adult females oviposited for 15.9 days with an average of 2.2 eggs per day (Laing 1969). A number of symbiotic as well as pathogenic microorganisms have been found associated with *M. occidentalis* (Hess and Hoy 1982). Research has demonstrated that *M. occidentalis* locates its prey by seeking out chemical markers left by the prey (Hoy and Smilanick 1981). Berstein (1984 ) found that there was a greater tendency for predators to disperse from the plant when prey densities are low. Thus, the chemical markers initiate a particular search behavior in the predator. Berstein (1983) found that predator females would seek out shaded places and that females deprived of food tended to live longer with increased relative humidity. Further, the predator tends to move from plant to plant more quickly than the prey. The copulatory behavior and egg production of the phytoseiid predators

*Amblyseius potentillae* (Garman) and *Typhlodromus pyri* Scheuten were compared (Overmeer et al. 1982). A single mating was sufficient for total egg production by *A. potentillae*, while *T. pyri* required several copulations. In addition, *T. pyri* is capable of maturing and surviving at low prey densities due to its ability to survive on other food sources in the absence of prey (Hayes and McArdle 1987).

The intrinsic rate of population increase for three phytoseiids, *Amblyseius longispinosus* Evans, *Amblyseius deleoni* Muma and Denmark, and *Amblyseius paraki* Ehara was studied. It was determined that *A. longispinosus* and *A. deleoni* increase their populations when prey numbers increase while the intrinsic rate of natural increase of *A. paraki* was almost equal to its prey, *T. urticae* (Saito and Mori 1981).

### **Pest control with Predatory Mites**

Several researchers have evaluated the effectiveness of predators in spruce spider mite control (Boyne and Hain 1983b, Hoy 1982, Jeppson et al. 1975 and McMurtry et al. 1970). Boyne and Hain (1983b) determined that *N. fallacis* was indeed an effective control agent for spruce spider mite on Fraser fir seedlings in a laboratory experiment. They also evaluated the effect of humidity on *N. fallacis* survival, development and fecundity. All three parameters were similar for three relative humidities at 26<sup>0</sup> C although none of the predators reached maturity at 60-65% relative humidity. Boyne (1980) noted that a significant reduction in a field population of spruce spider mite coincided with a dramatic increase in *N. fallacis*.

The mites *Proprioseiopsis temperellus* (Muma and Denmark), *Phytoseiulus macropilis* (Banks), *N. fallacis*, and *Galendromus longipilus* (Nesbitt) were evaluated as predators of *T. urticae* (Ball 1980). Prey consumption, fecundity and development were

investigated at 13.3 and 26.4<sup>0</sup> C. The results of the study indicated that each of the predator species developed more quickly than did the prey (Ball 1980). Further, *G. longipilus* and *N. fallacis* effectively devoured *T. urticae* within 11 days of exposing them to the prey. *Neoseiulus fallacis* was effective at controlling the prey due to its relatively high oviposition rates 3.5 eggs per day at 26.4<sup>0</sup> C (Ball 1980). Two predatory mites, *Metaseiulus occidentalis* and *N. fallacis* have revealed some promise in the control of spruce spider mites in Fraser fir plantations (Mangini and Hain 1991). In hot, dry summer months however, populations of *N. fallacis* may not increase fast enough to control the rapid population build-up of spruce spider mite (Kramer and Hain 1989).

Phytoseiid mites may play an important role in controlling spider mites on crops, including Christmas trees in Oregon. A total of 1,209 phytoseiid mites with 11 species represented were collected during the survey (Hadam et al. 1986). West and DeAngelis (1993) surveyed Douglas fir, noble fir and grand fir Christmas tree plantations in western Oregon, and found that, *Typhlodromus americanus* Chant and Yoshida-Shaul was the most common mite preying on the spruce spider mite *O. ununguis*. Also present but to a lesser degree were *Amblyseius andersoni* (Chant) and *Typhlodromus rhenanoides* Athias-Henriot.

Release strategies in relation to dispersal of *N. fallacis* were evaluated to determine its effectiveness as a biological control agent of *T. urticae* Koch in a strawberry field. Releases were made in April and in 6 to 12 weeks the predators had effectively controlled the pests and dispersed downwind up to 20-30 m (Coop and Croft 1995). Dispersal was influenced by temperature, wind direction, spider mite density and mowing. Strategies suggested for the field releases include releasing ca. 100 female mites per 1 to 2 m of row prior to 1 July. In addition, releases should be made on the upwind side of the field and 50 m

apart (Coop and Croft 1995). Shrewsbury and Hardin (2003) evaluated four commercially available predatory mites (*Galendromus occidentalis* Nesbitt, *G. annectans* De Leon, *G. helveolus* (Chant), and *N. fallacis* for use in augmentive releases to control spruce spider mites naturally occurring on container grown junipers. They found that because of the inaccuracy and underestimation of spider mite densities using beat sampling methods often recommended in extension publications, predator prey ratio rates were under estimated and too low to provide adequate control of spruce spider mites in augmentive release trials. They also found that augmentive predator releases were not economically feasible. Gough (1991) found that *Phytoseiulus persimilis* Athias-Henriot effectively controlled *T. urticae* Koch on rose hedges in southern Queensland.

Modification of cultural practices is often necessary to enhance the effectiveness of predatory mites in some agricultural systems. For instance, *A. fallacis*, a predator of *T. urticae*, overwinters below the soil surface in the crown of hops. Foliage is removed from the base of the plant and dirt is piled around the base by the growers. This cultural practice actually reduces the overwintering populations of predatory mites the following spring (Croft et al. 1993).

In perennial cropping systems, generalist predatory mites tend to persist in the absence of the prey species more readily than specialist predators (Nyrop et al. 1998). This ability to feed on pollen, fungi, and other mites, has led to the successful use of *Typhlodromus pyri* for the biological control of European red mite in apple orchards (Walde et al. 1992).

In a general survey of the literature and discussions with experts, Nyrop et al. (1998) came to some tentative conclusions regarding the general use of predatory mites as biological

control agents in perennial cropping systems in North America. The first and most important conclusion is that biological control of spider mites in perennial cropping systems is indeed possible. Second, for effective control to occur, the predatory mites must be maintained in close proximity to the pest mites. This may involve several strategies, including the reduction of pesticide use to conserve generalist predators. In the case of specialized predators, alternate prey may be required when the pest species is unavailable. Third, certain mites are better adapted to survive on specific types of vegetation than others. Fourth, some species are more adapted to certain geographical regions, climates or even microclimates. Fifth, there is one dominant species of specialist predator and one dominant species of generalist predator present in a given cropping system. Finally, there are only a few species of predatory mites which have been used successfully and this is probably related to the pesticide tolerance levels of certain predatory mite species. Therefore, a great deal is yet to be learned about the dynamic interactions of plant pest mites and their predators.

### **Predator-Prey Interactions**

Age and nutritional history of predators influence prey consumption and functional response. When the predator is exposed to one density of prey for an extended period, gaps in predation rate may occur when the prey density is changed (Eveleigh and Chant 1981a). *Phytoseiulus persimilis* and *Amblyseius degenerans* were compared in terms of prey required for development, survival, and oviposition (Eveleigh and Chant 1981b). Both species increased their fecundity in response to the number of prey consumed. Eveleigh and Chant (1982) found that an increase in prey density increased fecundity of *P. persimilis* and had no effect on *A. degenerans*. Prey aggregation has an effect on predator searching success and functional response in *P. persimilis*; however it does not provide the same effect as in *A.*

*degenerans*. Moraes and McMurtry (1981) studied the biology of *Amblyseius citrifolius* (Denmark and Muma). They found that relative humidity did not affect the egg stage of this species, and the best food for survival was *T. pacificus* and the pollen of *Malephora crocera* (Jacq.). The effects of temperature on development, survival, and fecundity of *Amblyseius tetranychivorus* (Gupton) was evaluated at 25, 30 and 35<sup>0</sup> C. The optimal temperature was found to be 30<sup>0</sup> C, and the highest temperature actually reduced the fecundity and ovipositional period for this species (Krishnamoorthy 1982).

### **Ground Cover and Border Vegetation Management for Predatory Mites**

Cover crop management may prove useful in the control of certain agricultural pests (Bugg and Ellis 1990). Picket and Bugg (1998) provide a comprehensive review of improvements to biological control using ground cover management. Cover crop management has been suggested as useful in attracting and providing a food source for certain insects. Research conducted in Massachusetts recorded 20 species of Ichneumonidae (Hymenoptera) feeding on extra floral nectaries of faba bean (*Vicia faba* L.) (Bugg and Ellis 1989). Several species of these insects were reported to attack lepidopterous pests in forest and agricultural systems (Bugg and Ellis 1989).

In some situations cover crops can also attract and harbor pest arthropods. For example, hybrid vetches and crimson clover supported tarnished plant bug (*Lygus lineolaris*). The use of cover crops to manage natural enemies has been evaluated for sustainable vegetable production. Bugg (1992) evaluated the dispersal of arthropods from cover crops to vegetable crops. He found that cover crop manipulation may prove useful in management of natural enemies however; much more research in these systems is required.

A number of studies have evaluated the potential of mites as useful predators of spider mites. Cover crops provide important habitat for phytoseiid mites (McMurtry 1982). Muma (1961) found a greater number of phytoseiid mites in citrus trees with established ground covers around them. Populations of *Tetranychus pacificus* (McGregor) were reduced when grasses were maintained in vineyards (Flaherty et al. 1972). Flaherty (1969) found that *Eotetranychus willamettei* was more effectively kept at low levels by the predator *Metaseiulus occidentalis* because the predator was feeding on alternative prey dwelling in the grasses. Mangini and Hain (2007) compared phytoseiid diversity in 6 different cover crops to a multispecies feral ground cover in North Carolina Fraser fir plantations. They found 9 different species of phytoseiid mites in the 6 different cover crops and 15 different species in the multispecies cover crops (Mangini 1988).

Cover crops not only provide overwintering habitat for natural enemies but may also provide suitable refuge for pest species in certain systems. For example, Karban et al. (1997) found that the Willamette mite (*Eotetranychus willamettei* Ewing, Acari: Tetranychidae) overwinters along with its predator *M. occidentalis* in the bud scales of grapes. Another pest species, the two-spotted spider mite (*T. urticae*), was also found overwintering in cover crops planted in the vineyards.

Dispersal of the predatory mite *N. fallacis* in ground cover, on tree trunks and the canopy of Michigan apple trees was investigated by Johnson and Croft (1981). They found that adult females overwintered in the ground cover under the apple trees (Johnson and Croft 1981). In spring, *N. fallacis* fed on phytophagous mites in the ground cover, increasing their populations and then departed from ground cover and walked up tree trunks (Johnson and Croft 1981). Coli et al. (1994) found that this species was found more frequently on wood

plants and forbs than in grasses. *Metaseiulus occidentalis* and *N. fallacis* overwinter and feed in ground cover in apple orchards prior to migrating to trees in the spring (Lee and Davis 1968, McGroarty and Croft 1978, Tanigoshi et al. 1983). Tanigoshi et al. (1983) found that vegetation at the base of the apple trees aided in the survival of *N. fallacis* during the winter. However, Nyrop et al. (1994) found that in Massachusetts apple orchards, significant numbers of *N. fallacis* overwinter on the trees. *Neoseiulus fallacis* also shows a strong tendency to disperse aerially when its prey is in short supply (Johnson and Croft 1981, McMurtry and Croft 1997, Tixier et al. 1998). Hardman et al. (2005) found that outbreaks of *T. urticae* Koch in ground cover were most effectively controlled by specialist phytoseiid such as *Amblyseius fallacis* Garman rather than generalist predators and modified herbicide use.

Physical characteristics of the cover crop plants may play an essential role in the enhancement of phytoseiids in crop plants. Mites utilize microhabitats formed by leaf structures (Grostal and O'Dowd 1994). Several researchers have demonstrated that the presence or absence of leaf pubescences and leaf domatia may influence phytoseiid mite abundance (Downing and Moilliet 1967; Overmeer and van Zon 1984; Duso 1992; Karban et al. 1995; Walter and O'Dowd 1992ab; Walter and O'Dowd 1995; Walter 1996). Given the results of these studies it is apparent that the physical attributes of the cover crop leaf structure are important factors influencing the presence of predatory mites.

Border vegetation or the trees adjacent to agricultural fields may also have a positive effect on the abundance and species diversity of predatory mites. Soloman (1981) discusses the importance of hedge-row windbreaks as an important source of wind blown predatory mites for apple orchards under an integrated pest management program. The composition

and physical structure of vegetation bordering French vineyards was shown to influence species richness and abundance of predatory mites (Tixier et al. 1998). They found that plants with dense pubescence on the leaves and environments with tall and dense vegetation bordering grape fields yielded higher densities of predatory mites than fields bordered by low growing or shrubby vegetation. Tuovinen and Rokx (1991) and Tuovinen (1994) noted that vegetation adjacent to apple orchards in Finland had an effect on phytoseiid densities and species composition. Tuovinen (1994) postulated that low growing shrubs may not be as important as larger trees that provide a more suitable environment for aerial dispersal of mites into the orchards. The aerial dispersal of predatory mites from vegetation growing in close proximity to agricultural crops is important for the biological control of spider mites and is a primary means by which crop plants are colonized by predaceous mites (Hoy et al. 1985, Duso 1989, Dunley and Croft 1990, Tixier et al. 1998, 2000, Jung and Croft 2001b).

Variation in pollen abundance in Nova Scotia apple orchards was strongly correlated with surrounding vegetation and to some extent, on mowing schedules. The availability of early spring pollen was thought to have a greater influence in the population increases of the predatory mites, *T. pyri* and *Zetzellia mali* than the abundance of the prey mite *Aculus schlechtendali* (Addison et al. 2000). On natural hedgerows consisting of elderberry and hornbeam located adjacent to vineyards in Italy, Duso et al. (2004) demonstrated a potential relationship between pollen availability and phytoseiid abundance. Croft et al. (1990) conducted an analysis of 9 different factors suspected of influencing the abundance of *M. occidentalis* and *T. pyri* in Oregon apple orchards. They found that vegetation surrounding the apple blocks and pesticide use were the most important factors.

## Effects of Pesticides on Predatory Mites

The ability of phytoseiid predators to tolerate pesticide usage in perennial crops is very an important consideration in biological control programs (Nyrop et al. 1998). Often, different species of phytoseiid mites will have completely different tolerances to certain pesticides and this information can be very useful in selecting pesticides for use in IPM programs on perennial crops (Watve and Lienk 1975)

Pratt and Croft (2000) investigated the toxicity of several general pesticide classes used in the nursery industry on *N. fallacis* and 3 other phytoseiid mites. In general, insecticides were the most toxic to phytoseiid mites followed by herbicides, which were intermediate in their toxicity. Fungicides tended to be the least toxic of the agricultural chemicals evaluated. Many of the pesticides evaluated are currently registered for use in North Carolina Fraser fir production. In surveys of treated and untreated apple orchards in North Carolina, Farrier et al. (1980) collected 81 species of mites. There were twice as many species collected from the untreated trees compared to the trees treated with pesticides. The dominant species of phytoseiid collected from sprayed orchards was *N. fallacis*. Autumn carbofuran applications eliminated *N. fallacis* from overwintering sites and actually contributed to an increase in *T. urticae* population levels in peppermint fields (Morris et al. 2000).

A total of four species of phytoseiid mites were collected from *Picea abies* (L) Karst. and *Abies nordmanniana* (Stevens) Spach Christmas tree plantations in England (Fitzgerald and Soloman 2000). They found that plantations that had been chemically treated had low populations of phytoseiid mites. Conversely, those Christmas tree plantations that had not been treated had high populations of phytoseiid mites.

Pesticide resistance in phytoseiid mites contributes greatly to the overall success of a conservation biological control program for perennial crops (Nyrop 1998). Hadam et al. (1986) surveyed phytoseiid mites on commercial crops in Oregon and revealed that the most commonly collected phytoseiid mite collected from apple, peach, prune-plum, cherry, filbert, grape, strawberry, raspberry, corn mint, and Christmas trees was *T. pyri*. Using a slide-dip technique, they found that *T. pyri* was moderately resistant to azinphosmethyl and was highly resistant to carbaryl and parathion. Vidal and Kreiter (1995) evaluated 2 strains of *T. pyri* for physiological and genetic patterns of resistance to several different insecticides. They identified resistance to a range of insecticide classes including carbamates, organophosphates and pyrethroids. They found that resistance to parathion methyl was not linked to ester-link hydrolysis or oxidative degradation of the insecticide. However, oxidative degradation and hydrolytic reactions do serve as mechanisms of resistance to the pyrethroid insecticide, fenvalerate. Survivorship of 1 susceptible and 2 synthetic pyrethroid-resistant strains of *A. fallacis* Garman was compared with an indigenous population following permethrin and fenvalerate applications (Whalon et al. 1982). Two applications of permethrin and 1 application of fenvalerate eliminated the susceptible and indigenous populations however, 2 of the synthetic pyrethroid-resistant strains survived the permethrin treatments while only 1 survived the fenvalerate applications. The overwintering survivorship of the synthetic pyrethroid-resistant strains was demonstrated using microelectrophoresis and LC<sub>50</sub> experiments. The ability of phytoseiid mites to develop pesticide resistance naturally is a slow and unpredictable process (Croft 1979; 1990).

The ability of phytoseiid mites to tolerate a certain amount of pesticide use may be a key factor in whether or not they can be used successfully to control spider mites in perennial cropping systems (McMurtry et al. 1970; Amano and Chant 1990; Croft 1990).

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## CHAPTER 2

### **The predatory mite (Acari: Mesostigmata, Prostigmata) community in northwestern North Carolina Fraser fir (*Abies fraseri* (Pursh) Poiret) Christmas tree plantations**

#### **Abstract**

Surveys were conducted in Ashe and Watauga Counties of North Carolina to characterize the predatory mite (Acari: Mesostigmata, Prostigmata) community structure in northwestern North Carolina Fraser fir (*Abies fraseri* (Pursh) Poiret) Christmas tree plantations and to evaluate their potential application for biological control of the spruce spider mite. To characterize the predatory mite communities in Fraser fir plantations, branch samples were collected from Fraser fir trees on privately owned Christmas tree plantations. Common Fraser fir ground cover plants and border vegetation were also sampled. Three potential spider mite predator groups in the prostigmata were collected, Anystidae, Bdellidae, and Cunaxidae. The most abundant phytoseiid mites collected were *Arrenoseius morgani* (Chant) (56.4%), *Typhlodromips sessor* (DeLeon) (21.1%), and *Proprioseiopsis solens* (DeLeon) (5.1%). Only two species were collected from ground cover and border vegetation, *T. sessor* and *A. morgani*. The total number of phytoseiid mites was greater on the trees infested with spider mites (94 phytoseiids) compared to the uninfested trees (64 phytoseiids). Phytoseiid abundance on Fraser fir size classes was as follows: 1.2-1.5 m (n=61 phytoseiids), 1.8-2.1m (n=34) and 1.5-1.8 m (n=27). A total of 86 phytoseiids were collected from Fraser fir plantations with a diverse ground cover. Clover and bare ground each yielded 17 phytoseiids while grass ground cover yielded 14 phytoseiids. The mean number of phytoseiids collected from Fraser fir with either a diverse, grass, clover or bare ground were 3.58, 2.80, 8.50 and 3.40 respectively.

**Key words:** Tetranychidae, Phytoseiidae, *Abies fraseri*

## **Introduction**

The spruce spider mite (*Oligonychus ununguis* Jacobi) (Acari: Prostigmata) is regarded as one of the most serious mites attacking conifers (Johnson and Lyons 1976). Damage from this pest is particularly problematic in plantation and ornamental plantings (Peterson and Hildahl 1969, Rose and Lindquist 1977).

Predatory mites in the family Phytoseiidae (Acari: Mesostigmata) are especially useful in the control of tetranychid mites (McMurtry et al. 1970). Numerous studies have investigated the importance of predatory mites in perennial agricultural systems such as apples, grapes, and strawberries (Amano and Chant 1990, Coop and Croft 1995, Downing and Moilliet 1967, Duso 1992, Karban et al. 1995). The predatory mite communities associated with Christmas tree plantations has been studied on *Picea abies* (L.) Karst. and *Abies nordmanniana* (Stevens) Spach. in England (Fitzgerald 2000). However, relatively little information is available on the predatory mites communities associated with Fraser fir (*Abies fraseri* (Pursh) Poiret) Christmas tree production. Several studies have investigated the feasibility of using phytoseiid mites as components of biological control programs for the management of spruce spider mite in Fraser fir production (Boyne and Hain 1983, Kramer and Hain 1989, and Mangini and Hain 1991). Fifteen species of phytoseiid mites were found during a survey of ground cover crops used in North Carolina Fraser fir production (Mangini and Hain 2007). However, this survey did not compare the cover crop predatory mite fauna to the communities actually found on Fraser fir trees.

The goal of this research was to determine the structure of predatory mite communities associated with Fraser fir production areas of northwestern North Carolina in

order to identify mites with potential for an integrated pest management program emphasizing conservation. Conservation meaning the manipulation of environmental factors with the purpose of protecting and enhancing natural enemies (DeBach 1964). The influence of ground cover vegetation, field elevation, and tree size were also investigated.

### **Materials and methods**

Studies of beneficial mite (Acari: Parasitiformes, Acariformes) communities were conducted during 2005-2006 in 30 privately owned Fraser fir plantations in Ashe and Watauga Counties, North Carolina. Field sizes averaged approximately 2. - 4 ha in size with trees planted in rows 1.5 m apart. Fraser fir tree sizes ranged from .3 - 2.4 m in height. Ground cover crops were visually assessed and recorded as either diverse (4 dominant species) or sparse: fields with a monocultural ground cover such as fescue (*Festuca* spp.), clover (*Trifolium* spp.), or bare- ground.

Elevation and longitude/ latitude were recorded at the edge of each field using a hand held Garmin GPS 72 Global Positioning Device (GPS). Size classes (height) of the Fraser fir were visually estimated and recorded. Predatory mite diversity and evenness were determined for phytoseiid mites collected at various elevations using the Shannon's index (Vandermeer 1981). A simple linear regression was calculated to determine if there was a statistical relationship between the Shannon's index and elevation (SAS Institute, 2007).

A total of 7 plantations were sampled in 2005. Samples were collected on August 9, 20, 23, and September 7. Ten trees were sampled by cutting 1 randomly selected Fraser fir 14 cm branch every 6.0 m along a single transect. Samples were placed in 7.56 L plastic

bags, transported to the lab in coolers, and processed by washing branches in isopropyl alcohol, then filtering the alcohol through a No. 325 mesh, cement wet washing sieve (52 x 76 mm) to remove mites.

In 2006, a total of 23 plantations were sampled. Sampling dates were on May 2, July 12, 27, 28, August 2, 18, 23, and September 7, 2006. Each plantation was sampled 1 time each except for 4 of the plantations which were sample twice. Mite samples were collected by beating 10 trees, one every 6 m, per field in a single transect. In order to sample more plantations, the sampling procedure was streamlined by beating branches from the top, center and bottom over a white plastic tray 32 x 45 cm in size. A fine camel's hair brush moistened with 70% ethanol was used to collect mites from the tray, these were then placed in a 2-dram vial filled with 70% ethanol and a drop of glycerin and transported back to the lab.

Data on ground cover vegetation was collected at 28 of the 30 sites. The threat of thunderstorms prevented us from sampling 2 of the sites. Some plantations were sampled less frequently because the distance between fields with different ground cover types made some plantations impractical to survey. A total of 16 plantations with diverse ground covers were sampled. Eight plantations were sampled twice. Two plantations with white clover (*Trifolium repens* L.) ground cover were sampled twice each. Five plantations planted in grass (*Festuca* spp.) and 5 plantations without established ground cover vegetation (bare ground) were sampled 1 time each. Predatory mite diversity and evenness were determined for phytoseiid mites collected from trees growing in plantations with diverse, clover and grass ground covers and from plantations with bare ground using the Shannon's index.

Common Fraser fir ground cover plants and border vegetation were sampled on August 9, 23, September 20, 2005, and July 12, 2006. Three species of herbaceous plants were selected based upon their dominance in the plantation. One plant of each species was cut at the soil line, stored in 7.56 L plastic bags, and placed in coolers to transport back to the lab. Trees bordering Fraser fir plantations were observed for dominance (most frequently observed) and sampled by removing 30 leaves from each of 10 trees, placing them in 7.56 L plastic bags and transporting them back to the lab in coolers. Conifers were sampled by cutting three 14-cm branches from 5 trees. Samples were processed as described for Fraser fir branches. All samples were processed on the same day they were collected.

Arthropods were sorted using a 10-50x-stereomicroscope. Predatory mites were removed and mounted with Hoyer's medium on glass microscope slides and covered with a 12 mm diameter cover slip. Slides were dried for 48 h in a 50° C drying oven (Krantz 1978). Generic and specific identifications were made by the primary author at 40-100x with a phase contrast microscope. A representative number of slides were verified by Gilberto de Moraes (Universidade de Sao Paulo, Piracicaba, Brazil) and Cal Welbourn (Florida Department of Agriculture and Consumer Services).

## **Results**

A total of 258 specimens representing 4 different families and 10 different genera of potential spruce spider mite predators were collected (Table 2.1). Three potential spider mite predator families in the Prostigmata were collected, Anystidae, Bdellidae, and Cunaxidae. The most abundant species collected was *Anystis baccarum* (L) (Table 2.1).

Seven phytoseiid species were collected from Fraser fir (Table 2.1). The most abundant were *Arrenoseius morgani* (Chant) (56.4%) and *Typhlodromips sessor* (DeLeon) (21.1%). Only two species were collected from ground cover and border vegetation, *T. sessor* and *A. morgani* (Table 2.2). Only mature female phytoseiid mites were identified. Males, immatures and damaged mites were recorded as phytoseiidae and left undetermined. Fraser firs at 16 of the 30 plantations sampled were infested with spruce spider mites. *Arrenoseius morgani*, *Neoseiulus fallacis* (Garman), *T. sessor*, *Typhlodromalus peregrinus* (Muma), *Amblyseiella setosa* Muma, and *P. solens* were all collected from trees infested with spruce spider mites. The total number of phytoseiid mites was greater on the trees infested with spider mites (94 phytoseiids) compared to apparently uninfested trees (64 phytoseiids). Phytoseiids were most abundant on Fraser fir size classes 1.2-1.5 m (n=61 phytoseiids), 1.8-2.1m (n=34) and 1.5-1.8 m (n=27). However, these three size classes were also sampled at a greater frequency (Table 2.3).

A total of 86 phytoseiids were collected from Fraser fir plantations with a diverse ground cover. Plantations with a grass ground cover yielded a total of 14 phytoseiids while clover and bare ground plantations each yielded 17 phytoseiids. The mean number of phytoseiids collected from Fraser fir with either a diverse, grass, clover or bare ground cover were 3.58, 2.80, 8.50 and 3.40 respectively (Table 2.4). Shannon's diversity (H) and evenness (E) values for phytoseiids collected from Fraser fir were: diverse (H 0.517, E 0.612), grass (H 0.380, E 0.797), clover (H 0.541, E 0.774) and bare ground (H 0.157, E 0.523).

Elevations were taken at 29 of the 30 sites and ranged from 863 to 1257 m ASL. Phytoseiid mites tended to be evenly distributed among the 22 elevations where they were found with the exception of 1 site at 897 m ALS which contained 46 phytoseiid mites. Twenty- two of the 29 elevations where samples were collected contained phytoseiid mites. The greatest Shannon's diversity indices were found at 910 m (0.474) and 960 m (0.452) elevation (Table 2.7). The statistical relationship of the Shannon's diversity index and elevation was small and insignificant ( $F = 0.08$ ,  $df = 1$ ,  $P < 0.782$ ).

## **Discussion**

While the 2005 surveys employed a more intensive sampling and reliable method (alcohol washing branches) (Shrewsbury and Hardin 2003), it did not result in greater species diversity (Shannon's Diversity, 0.444) than the 2006 Method (beat sampling) (Shannon's Diversity, 0.568). This is perhaps because of the limited number of sites sampled during 2005 or perhaps due to fluctuating populations of spruce spider mites at these sites.

Beat sampling abundance data may be misleading. In one study beat sampling underestimated spider mite populations and led to insufficient release rates during augmentative releases of *Neoseiulus fallacis* on juniper (Shrewsbury and Hardin 2003). Washing plant material in alcohol then collecting mites through filtration is an effective method of monitoring phytoseiid populations (Pratt and Croft 2000). We used the beat sampling technique because it is the standard method used by Cooperative Extension personnel in North Carolina to monitor populations of predatory mites. In addition, in most cases we were sampling trees scheduled for the current season's market and were concerned about the use of a more destructive sampling method.

It would be difficult to arrive at any conclusions regarding the effects of elevation, or size class of Fraser fir in terms of the influences these parameters may or may not have on phytoseiid diversity and abundance under field conditions. There are far too many uncontrolled variables in a study of this nature.

The Shannon's diversity index is a mathematical measure of species diversity in a community. This diversity index considers the number of species in a community, the total number of individuals within a species, and their relative abundances. The relative abundance is the proportion of the number of individual species to the total number of individuals in a community. The Shannon's diversity index number increases when the number of species or species evenness increases. Shannon's evenness is a numerical method to demonstrate the equality of species in a community, (Vandermeer 1981). Shannon's diversity (H) and evenness (E) values for phytoseiids collected from Fraser fir in diverse ground covers and in clover ground covers were considerably higher than grass or bare ground. For example, Table 2.5 compares 4 different sites where the abundance and diversity of phytoseiids was greatest during this study. Phytoseiid abundance at one site was 45 individuals representing three different species. The ground cover was diverse and spider mites were present. However, we also collected relatively high numbers and greater species diversity from a plantation with a monocultural ground cover of white clover and no spruce spider mites. It is likely that the beat sampling technique failed to detect low level populations of spruce spider mites (Shrewsbury and Hardin 2003) and this trend may have had a profound effect on the results of this survey.

A few observations can still be made regarding the potential of using predatory mites as components of a Fraser fir IPM program in North Carolina. Even though *Anystis*

*baccarum* was the second most abundant predatory mite collected in this survey, the usefulness of this species as a biological control agent for spruce spider mite is questionable. *Anystis baccarum* is a general predator living in diverse habitats, is known to feed on various arthropods including tetranychids, and has been found in association with several perennial crops (Sorenson et al. 1976). This species is not considered a practical biological control agent for tetranychids, particularly for inundative releases. It has a long generation time, there is prey size discrimination by later stages, and colonial webbing inhibits feeding ability (Sorenson et al. 1976).

Several species of bdellid mites were associated with conifers in Pennsylvania (Leman 1982). These are common predators of various arthropods including Willamette mites (Kinn and Douth 1972), oribatid mites, Collembola (Wallace and Mahon 1973) and Tetranychidae (Snetsinger 1956). Immature *Cunaxoides* sp. feed on spruce spider mite (Leman 1982). While these predatory mites likely serve as important generalist predators, this study concentrates more on the phytoseiid diversity in an effort to develop a conservation based IPM strategy for spruce spider mite.

Most of the phytoseiids collected in this study appear to be common inhabitants in perennial cropping systems in North Carolina. For example, *A. setosa*, *A. morgani*, *N. fallacis* and *T. sensor* were all found in N.C. apple orchards (Farrier et al. 1980). Nine species of phytoseiid mites were found in 6 different monocultural cover crops, and 15 different species from mixed cover crops associated with Fraser fir production areas (Mangini and Hain 2007). However, during this study we found only four of these on the foliage of Fraser fir, *N. fallacis*, *T. sensor*, *A. setosa* and *A. morgani*.

*Amblyseiella setosa* has previously been found associated with ground covers used in Fraser fir production in a previous study (Mangini 1988), and been reported on citrus in Florida (Muma 1964). This mite likely feeds on saprophagous, fungivorous and possibly phytophagous mites (Muma 1971), however, it has previously been collected along with spruce spider mites on conifers (Lehman 1982).

When fed a diet of *Tetranychus urticae* Koch, *Typhlodromalus. peregrinus* demonstrated the shortest generation time, greatest female longevity and highest mean fecundity when compared to those on diets of pollen and immature stages of *Panonychus citri* (McCregor) (Fouly et al. 1995 and Muma 1971). Further life history studies are needed to determine if this species would be a suitable predator of spruce spider mites in a Fraser fir IPM program.

*Typhlodromips sessor* is a common species with a wide distribution along the Atlantic coastal states of the USA (Sciarappa et al. 1977). It is considered a generalist and consumes both plant and animal matter (Sciarappa and Swift 1977, McMurtry and Croft 1997). This species is commonly found on herbaceous plants with pubescent leaves and/or thorns (Sciarappa et al. 1977). This mite is well adapted to early successional plant communities and was commonly found in 1- 5 year old communities while other phytoseiid species did not reach sizeable populations (Sciarappa et al. 1977). Even 10 year-old communities had a relative abundance of 72.3% for *T. sessor*. Since Fraser fir Christmas trees are grown on a short rotational schedule, these fields have similarities to early successional plant communities. Given this information and the apparent abundance of *T. sessor* observed during this study, research efforts should continue towards utilizing this species as a component of a conservation based IPM in Fraser fir production.

*Arrenoseius morgani* was previously reported to be commonly associated with spruce spider mite (Leman 1982), and this trend was confirmed by the results of this survey. Unfortunately little or no research has been conducted with this species or others of the same group (Moraes, personal communication). Additional research is needed on this species, particularly since it was found in “good” numbers along with spruce spider mite. The food preference for members of the *P. dorsatus* group, of which *P. solens* is an affiliate, is likely to be phytophagous mites (Muma 1971).

Predatory mites can be classified based on their feeding specialization (McMurtry and Croft 1997). Most of the phytoseiid mites collected during this survey are classified as Type II and Type III predators (Table 2.6). Type I predators are selective predators of *Tetranychus* spp., while Type II predators are selective predators with a preference for mites in the family Tetranychidae (McMurtry and Croft 1997). These mites are typically associated with prey species that construct dense webbing. Pollen consumption under field conditions has not been demonstrated for Type II species (McMurtry and Croft 1997). *Neoseiulus fallacis*, a Type II predator, has been shown to feed on prey other than spider mites when starved (Pratt et al. 1999). Type III are generalist predators including some *Neoseiulus*, *Typhlodromus* and *Amblyseius* species, but also other genera where information about food preferences exists (McMurtry and Croft 1997). Type IV are specialized pollen/generalists predators in the genus *Euseius* (McMurtry and Croft 1997).

In perennial agroecosystems, especially stable systems like tree crops, generalist phytoseiid species tend to be the most dominant (McMurtry 1992). We noticed this trend during our survey. Generalist species are defined as those feeding on a wide range of food substances including pollen. These generalists have a lower reproductive potential than

predators selective on Tetranychids. Since they utilize a variety of food sources, they are less likely to emigrate to new sites in search of food (McMurtry 1992). This may account for the low abundance of *N. fallacis* collected during our survey, given that it likely has a greater tendency to emigrate in search of food when spider mite populations are low.

There is growing evidence to suggest that generalist phytoseiids can control populations of tetranychid mites in some situations, especially in perennial cropping systems. This may occur where predator- prey ratios are high, up to 1:1 or greater for some species, and the prey densities are low (McMurtry 1992). In support of this argument, field experiments have demonstrated that when generalist predatory mites are present in great enough numbers before the prey populations are high, they can limit spider mite population increases on grapes (Prischmann et al. 2006).

*Typhlodromips sessor*, a good example of a Type III generalist predator, is a common component of the predatory mite fauna in and around North Carolina Fraser fir production areas. This species along with other Type III generalist predators like *peregrinus* may have the potential to play an important role in spruce spider mite control. *Neoseiulus fallacis*, a Type II predator, while present, did not appear to be widespread in this study.

Table 2.6 summarizes the potential for developing conservation based IPM program for spruce spider mite in Fraser fir production areas of North Carolina based on the phytoseiid mites collected during this survey. It is apparent, given what is currently known about the biology of the phytoseiids collected, two have potential for use in a conservation based IPM program for Fraser fir.

This research was an important first step in the development of an integrated pest management strategy for spruce spider mite in Fraser fir production areas. Such an IPM

program should encompass balsam woolly adelgid (BWA) because controlling BWA with pesticides creates a worse spruce spider mite problem by reducing the number of predators (Potter et al. 2005). Through understanding the species composition of predatory mites in this agriculture ecosystem we can begin to develop cultural methods that may lead to enhancing or sustaining predatory mites within the system that may prove valuable for control of the spruce spider mite. A rational next step for this investigation is to better understand the overwintering biology of the phytoseiid mites commonly encountered during the Fraser fir growing season. Other research should be conducted to develop better methods of monitoring phytoseiid mite populations under field conditions.

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**Table 2.1 List of predatory mites collected from Fraser fir in 2005-2007.**

Family, Species	Number Specimens
<b>Phytoseiidae</b>	
<i>Amblyseiella setosa</i> Muma	6
<i>Arrenoseius morgani</i> (Chant)	88
<i>Neoseiulus fallacis</i> (Garman)	7
<i>Typhlodromips sessor</i> (DeLeon)	33
<i>Proprioseiopsis solens</i> (DeLeon)	8
<i>Typhlodromus</i> ( <i>Anthoseius</i> )	1
<i>Typhlodromalus peregrinus</i> (Muma)	2
Undetermined phytoseiids	11
<b>Anystidae</b>	
<i>Anystis baccharum</i> (L.1758)	84
<b>Bdellidae</b>	
<i>Bdella sp.</i>	6
<b>Cunaxidae</b>	
<i>Cunaxoides sp.</i>	12
<b>Total Predatory Mites</b>	<b>258</b>

**Table 2.2 Fraser fir (*Abies fraseri* (Pursh ) Poiret) border and ground cover vegetation sampled for phytoseiids**

Plant	No. sampled	Phytoseiid species.	No. coll
<i>Acer rubrum</i>	1	<i>Typhlodromips sessor</i>	1
<i>Achillea millifolium</i>	1		
<i>Ambrosia artemisiifolia</i>	1		
<i>Betula lenta</i>	1	<i>T. Sessor</i>	1
<i>Chenopodium album</i>	1		
<i>Daucus carota</i>	1		
<i>Juglans nigra</i>	1		
<i>Kalmia latifolia</i>	1		
<i>Lindera benzoin</i>	1	<i>T. Sessor</i>	1
<i>Liriodendron tulipifera</i>	2		
<i>Nyssa sylvatica</i>	1		
<i>Pinus</i> spp	7	<i>T. sessor</i> , undetermined phyto	10 1
<i>Phytolacca americana</i>	1		
<i>Prunus serotina</i>	1		
<i>Quercus alba</i>	1		
<i>Q. coccinea</i>	2	<i>T. sessor</i>	1
<i>Q. montana</i>	1		
<i>Q. rubra</i>	3	<i>T. sessor</i> undetermined phyto	7 2
<i>Q. veluntina</i>	2		
<i>Rhus typhina</i>	1		
<i>Robinia psuedoacacia</i>	2		
<i>Rosa multiflora</i>	1	<i>T. sessor</i>	1
<i>Rubus argutus</i>	2	<i>T. sessor</i> undetermined phyto	4 3
<i>Solidago canadensis</i>	1	undetermined phyto	1
<i>S. Rugosa</i>	1	<i>T. sessor</i> undetermined phyto	3 5
<i>Sambucus canadensis</i>	1		
<i>Solanum carolinense</i>	1	<i>T. sessor</i> undetermined phyto	2 1
<i>Tsuga canadensis</i>	1	<i>Arrenoseius morgani</i>	2
<i>Vitis</i> sp.	2	undetermined phyto	2

**Table 2. 3 Number of phytoseiid mites collected on various Fraser Fir (*Abies fraseri* (Pursh) Poiret) size classes**

Size class	No .sites Sampled <sup>1</sup>	F. Fir/ site sampled	Total Trees sampled/size class	No. SSM Infested sites	Total Phytoseiids Coll.	Mean Phytoseiids /tree
1 (.3-.6 m)	3	10	30	1	2	0.06
2 (.6-.9 m)	3	10	30	2	6	0.02
3 (1.2-1.5 m)	2	10	20	1	61	3.0
4 (1.5-1.8 m)	8	10	80	0	27	0.33
5 (1.8-2.1 m)	13	10	130	6	34	0.26
6 (2.1-2.4 m)	2	10	20	1	2	0.1
7 (2.4-2.7)	5	10	50	2	15	0.3

1. A composite sample was collected from 10 trees/site.

**Table 2. 4 Total phytoseiids collected from field with various ground covers**

Ground cover	No. samples	Total phytoseiids coll.	Mean phytoseiids/sample
Diverse	24	86	3.58
Grass	5	14	2.80
Clover	2	17	8.50
Bare ground	5	17	3.40

**Table 2.5 Comparison of four sites with high abundance of phytoseiids**

Elevation	Tree size (m)	Ground cover	Phyto sp.	Total	SSM
2994	1.2-1.5	Diverse	AM,NF,PS	45	Yes
3299	1.8-2.1	Bare ground	Am	15	Yes
3152	1.2-1.5	White clover	AM,NF,PS,TS	16	No
2913	1.5-1.8	Diverse	AM,TS,TP	7	No

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AM=*Arrenoseius morgani*

NF=*Neoseiulus fallacis*

PS=*Proprioseiopsis solens*

TS=*Typhlodromips sessor*

TP=*Typhlodromalus peregrinus*

SSM=Spruce spider mite

**Table 2.6 Life-styles of phytoseiid mites collected and potential IPM applications**

Phytoseiid Species	Life-style Type	Type of Crop	IPM strategy
<i>Amblyseiella setosa</i> Muma	x	x	x
<i>Arrenoseius morgani</i> (Chant)	x	x	x
<i>Neoseiulus fallacis</i> (Garman)	II	T, Sh, L	C,A,Im
<i>Typhlodromips sessor</i> (DeLeon)	III	T	x
<i>Proprioseiopsis solens</i> (DeLeon)	x	x	x
<i>Typhlodromus</i> ( <i>Anthoseius</i> )	II	T	X
<i>Typhlodromalus peregrinus</i> (Muma)	III	T, Sh	C, Im

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(Adopted from McMurtry and Croft 1997)

T, Tree, Sh, Shrub, L, low growing

X, no information available

C, conservation, A, augmentation, Im, importation and establishment

Type II-selective predators of tetranychid mites

Type III-generalist predators

**Table 2.7 Shannon's diversity (H) and evenness (E) values for phytoseiid mite fauna collected from Fraser fir trees at various elevations in western North Carolina.**

Elevation (m)	Shannon's H	Shannon's E
863	0.00	0.00
866	.301	1.0
868	0.00	
879	.276	.918
887	.301	1.0
888	.346	.725
890	0.00	0.00
896	0.00	
897	.230	.483
900	.233	.488
907	0.00	
910	.473	.787
911	.276	.918
914	0.00	
922	0.00	0.00
928	.346	.725
934	0.00	0.00
945	.276	.918
953	.301	1.0
957	0.00	0.00
960	.452	.751
965	0.00	
968	0.00	0.00
989	.301	1.0
1005	0.00	
1256	.244	.811

## CHAPTER 3

### Association of a predatory mite with galls of Rosette Bud Mite (Acari: Phytoptidae)

#### Abstract

The occurrence of a phytoseiid mite *Typhlodromalus peregrinus* (Muma) (Acari: Phytoseiidae), over wintering in rosette bud mite *Tricetacus fraseri* Amrine (Acari: Phytoptidae) galls is documented along with parameters, including aperture size and morphological features, influencing the occupation of rosette bud mite galls by this predator during the winter months. Observations are made on winter diapause and utilization of *T. fraseri* as a winter food source for *T. peregrinus*. This paper also reports on the overwintering stage of *T. fraseri*. Studies were conducted in a single commercial Fraser fir tree plantation in Ashe County, N.C. during the winter of 2006-7. Twenty-nine phytoseiid mites were found in 14 galls (10.6%). There was an average of 2 phytoseiid mites per gall and the highest number of phytoseiid mites in any one gall was 4. Gall heights, width, cavity depth, and aperture size (n=121) averaged 7.4 mm, 7.4 mm, 2.1 mm, and 2.0 mm, respectively. The height, width, depth, and aperture size of galls containing *T. peregrinus* (n=14) averaged 7.2 mm, 7.5 mm, 2.9 mm, and 1.6 mm, respectively. *Typhlodromalus peregrinus* was observed feeding on all stages of the rosette bud mite.

**Key words:** rosette bud mite, *Tricetacus fraseri* Amrine, Fraser fir, *Abies fraseri*

#### Introduction

The rosette bud mite (RBM) *Tricetacus fraseri* Amrine (Acari: Phytoptidae) is a pest of commercially grown Fraser fir (*Abies fraseri* (Pursh) Poiret) Christmas trees. Typically, it is found in the Appalachian regions of North Carolina, Tennessee, and southern Virginia.

In North Carolina, it generally occurs at elevations above 836 m above sea level but has also been recorded in plantations at lower elevations.

Eriophyid mite feeding often induces abnormal plant growth including stunting of a bud, leaves and shoots, erineae, and galls (Jeppson *et al.*, 1975). These sites provide refuge or shelters for eriophyid mites and may aid in preventing desiccation, providing food niches, and possibly escape from predation (Sabelis 1996). *Trisetacus fraseri* feeding produces a condition known as rosette bud that results in the terminal bud having a more rounded appearance than a noninfested bud. The infested bud does not break in the spring or it breaks to form several weakened shoots (Sidebottom 2002). The quality of the Christmas tree is reduced because of gaps in foliage, flat sides, and weak bottoms which reduces the value and grade of the trees (Sidebottom 2002).

Larger trees (1.7 m or greater) generally have higher numbers of rosette buds than smaller trees and the buds are found at significantly higher numbers on the lower portion of trees and inside the canopy. Rosette buds are found on all sides of the tree (O'Reilly 1998). Infested trees with as many as 100-150 rosette buds are generally clumped within a stand with several infested trees surrounded by uninfested trees. Rosette buds open in the spring around May and mites disperse on wind currents completely vacating the bud within 2 weeks (O'Reilly 1998).

Several authors have reported finding phytoseiid mites associated with erineae and galls formed by eriophyid mites (Keifer, 1946; Sabelis, 1996). Phytoseiid mites have been found inside eriophyid induced galls on inkbush (*Suaeda fruticosa* Forsk)(Sabelis 1996), paper birch (*Betula papyrifera* Marsh) (Beer, 1963), large-tooth aspen (*Populus grandidentata* Michx) (Beer 1963), and willow (Prischmann *et al.* 2005). In some cases, there is evidence

that the predatory mites are utilizing the eriophyid mites as a food resource (Sabelis, 1996). For example, *Kampimodromus* (= *Amblyseius*) *aberrands* (Oudemans) has been found in filbert (*Corylus* sp.) galls feeding on eriophyid mites (McMurtry and Croft 1997; Sabelis, 1996).

Certain phytoseiid mites have been observed over wintering with their prey species, including eriophyids, spider mites, tarsonemids, and tydeids (Knisley and Swift, 1971; Veerman 1992). However, while *Euseius. finlandicus* often over winter in association with tydeid mites in peach orchards, they apparently do not feed on the prey as indicated by the clear guts in individuals collected during the winter (Broufas et al. 2002).

There continues to be little information available on the predation risks to gall inhabiting eriophyid mites (Sabelis 1996). The purpose of this study is to report on the occurrence of a phytoseiid mite *Typhlodromalus peregrinus* (Muma) (Acari: Phytoseiidae), over wintering in rosette bud mite galls, and we suggest possible parameters, including aperture size and morphological features, influencing the occupation of rosette bud mite galls by this predator during the winter months. Observations are made on the utilization of *T. fraseri* as a winter food source for *T. peregrinus*.

## **Methods**

Most of the studies were conducted in a single commercial Fraser fir tree plantation in Ashe County, N.C. during the winter of 2006-7. The first sample was collected on December 3, 2006. Trees were 2.1-2.4 m in height and planted in rows 1.5 m apart. There were several trees in the plantation that were 7.6-9.1 m tall. Ten galls with perforated tops (apertures) were collected from 3 randomly selected trees in the center of the Fraser fir plantation. On subsequent sampling dates (January 3 and January 19, 2007) randomly selected samples of 5-

6 opened galls (galls with an aperture located at the terminal end of the bud) per tree from 24 different trees were collected from the center of the field. Galls were removed with hand pruners and placed in 3.7 L plastic bags. The galls were transported to the lab in coolers then stored in a refrigerator for up to 7 d. Each gall was dissected to expose the *T. fraseri* feeding cavity and the numbers of phytoseiids within the gall were counted using a binocular microscope.

Measurements of gall height, width, cavity depth, and aperture size were taken using mm calipers. Data were statistically analyzed using SAS (SAS Institute, 2007). A Poisson regression model was proposed where the number of mites  $y(i)$  for sample (gall)  $i$  follows a Poisson distribution with rate parameter (mean and variance)  $m(i)$ . Two measurements on the galls, depth and opening were used as covariates to explain the variation in the log of mean:  $E(y(i)) = m(i) = \text{expected number of mites in sample } i$   $\log(m(i)) = b_0 + b_1 * \text{depth}(i) + b_2 * \text{opening}(i)$ .

In order to observe feeding and oviposition, galls were sliced longitudinally into two sections and observed with a compound microscope. If phytoseiid mites were observed they were removed by wetting a fine paintbrush in distilled water and then carefully transferring the mite onto 1 cm diameter circles punched from poster board painted black with Krylon Ultra Flat Interior Exterior spray paint. The disks were floated on distilled water in Polystyrene standard sterile 100 x 15 mm Fisher brand covered dishes. To provide a resting or oviposition site for the phytoseiid mite, a few strands of Organic Essentials cotton from virgin staple fiber was placed on each disk and covered with a 12 mm X 12-mm square cover slip. These culture plates were kept at 17°C-18°C with a 10L/14D photoperiod.

Seven sites in Avery County with known RBM infestations were surveyed on February 12, 2007, in an attempt to locate additional sites with apertures to examine for phytoseiid mites. The buds with apertures could not be located at any of these plantations. Two additional plantations were found which contained rosette buds with perforation. These were located in Watauga and Ashe Counties. Thirty buds were removed from 15 trees at each site. These were dissected as described above.

Phytoseiid mites were fed a diet of either adult, immature or eggs of bud mites every 2-5 d. Rosette bud mite galls were dissected with a razor and live eriophyid mites and eggs were placed on the disk using a fine paintbrush. Observations were made periodically. Following the feeding, phytoseiid mites were observed for 20 minutes with a 10-50 x-stereomicroscope. Any phytoseiid eggs observed were recorded along with the developmental stage of phytoseiid mites. Observations were made from January 20, 2007 when the mites were first transferred to the disk until March 13, 2007.

A representative number of predatory mites were removed from the galls and mounted with Hoyer's medium on glass microscope slides and covered with a 12 mm diameter cover slip. Slides were dried for 48 h in a 50° C drying oven. (Krantz 1978). Generic and specific identifications were made by the primary author at 40-100x with phase contrast microscope. Representative slides were verified by Gilberto de Moraes (Universidade de Sao Paulo, Piracicaba, Brazil).

Rosette buds (n=204) were collected on February 28, 2007, dissected as already described and the overwintering developmental stage of *T. fraseri* was observed using a 10-50 x-stereomicroscope.

## Results

On December 3, 2006 a total of 30 galls were examined for the presence of phytoseiid mites. Ten percent of the galls collected during the initial survey contained individual *Typhlodromalus peregrinus* (Muma). A total of 121 galls collected on January 3, and January 19, 2007 were examined. Galls were collected from the original site where phytoseiids were observed over wintering and a site in Watauga County. Twenty-nine phytoseiid mites were found in 14 galls (10.6%). Each gall examined also contained *T. fraseri* eggs and either larvae or adults. There was an average of 2 phytoseiid mites per gall and the highest number of phytoseiid mites in any one gall was 4. All phytoseiid mites mounted on the microscope slides were identified as *T. peregrinus*.

Gall heights, width, cavity depth, and aperture size (n=121) averaged 7.4 mm, 7.4 mm, 2.1 mm, and 2.0 mm, respectively (Table 1). The height, width, depth, and aperture size of galls containing *T. peregrinus* (n=14) averaged 7.2 mm, 7.5 mm, 2.9 mm, and 1.6 mm, respectively. There was a statistical relationship between the opening size and the depth of the cavity and the number of *T. peregrinus* observed. (Table 2). Smaller aperture sizes (1.6 mm) and larger cavity depth (2.9 mm) contained *T. peregrinus* more frequently than galls with greater aperture sizes or more shallow cavity depths. No phytoseiid mites were found in rosette bud mite galls at the Watauga County site.

Rosette bud galls collected on February 28, 2007 (n=204) did not contain phytoseiid mites. Gall heights, width, cavity depth, and opening sizes averaged 8.1 mm, 6.7 mm, 2.2 mm, and 1.3 mm respectively. In the rosette bud galls with various sized openings 1-4 mm (n=174), larval or adult eriophyid mites and eggs were present in 7.4 % of the galls

examined. Eggs only and larvae/adult only were present in 5% each. The 93% of unopened galls (n=30) contained eggs.

The original phytoseiid mite that was transferred to the disk on January 20 was observed feeding on adults and eggs of rosette bud mites. One immature phytoseiid was observed on January 31. On February 2, two eggs were observed attached to cotton strands. Two new immature mite were observed on February 5. On February 24 a new immature phytoseiid mite was observed. Two new phytoseiid mite eggs were observed on March 1. These two eggs had developed into 2 additional immature phytoseiids by March 4.

## **Discussion**

*T. peregrinus* is a widely distributed species in eastern North America (Moraes et al. 2004, Pena et al. 1989, Villanueva and Childers 2005). Abundances of phytoseiid mites and their tree locations vary seasonally (Muma 1967, Villanueva and Childers 2005). The absence of *T. peregrinus* from the galls collected during the March 1, 2007 sampling date may suggest a similar seasonal movement trend in North Carolina Christmas tree plantations.

Phytoseiid mites are known to frequent domatia and tomenta on plant leaves that provide microclimates suitable for oviposition, development, and protection from predation (Walter 1996). Relative humidity, temperature, and light intensity may also contribute to phytoseiid activity. *T. peregrinus* is active at night and seeks refuge in shaded leaves during the day (Muma 1967, 1975).

In early summer a cavity between the outer bud scales of the gall and next years' developing bud forms as a result of rosette bud mite feeding (Sidebottom 2002). Then it is likely that as the bud continues to age and dry an aperture large enough for *T. peregrinus* to enter develops on some galls. Phytoseiid mites are able to enter galls as they dry and the

aperture in the gall expands enough to allow entry by the predator (Keifer 1946, Prischmann et al. 2005). The statistical relationship of the aperture size and the cavity depth suggests that *T. peregrinus* may select galls for refuge that provide adequate protection, and favorable microclimates, and an available food source and do not occupy less favorable locations. It also suggests that there are significant aperture sizes preferred by *T. peregrinus* presumably because these opening provide optimal microhabitat conditions within the gall while still large enough for the predator to enter yet small enough to provide refuge for overwintering *T. fraseri*.

Phytoseiid mites are known to move about constantly in search of food (Sabelis 1985). Eriophyid mites are well known prey of many species of phytoseiid mites (Sabelis 1996, Overmeer 1985). Eriophyid mites are particularly vulnerable to phytoseiid predation because of their small size and limited mobility when they are on the leaf surface (Sabelis 1996).

Mites in the family Eriophyidae are an inadequate food source for *T. peregrinus* (Muma 1971). Food sources were determined to be inadequate if the predator lived for no more than 1 to 2 weeks. Food sources that allowed the predators to survive for extensive periods of time, and where oviposition occurred regularly and immatures completed development, were classified as adequate (Muma 1971). Observations reported in this paper suggest that *T. peregrinus* is utilizing *T. fraseri* as a food source during the winter months in western North Carolina.

This paper provides the first report of *T. peregrinus* entering the galls of rosette bud mite. Additional research is needed to further explore the interactions of *T. peregrinus* and

rosette bud mite. Future research should be conducted to determine the total impact of the predator on the bud mite populations, and if this phenomenon leads to a greater abundance of phytoseiid mites the next spring when spruce spider mites are present.

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**Table 3.1. Mean gall heights, depth, width and aperture size influencing the presence of *Typhlodromalus peregrinus* in rosette bud mite galls (*Trisetacus fraseri* Amrine)**

Variable	Buds with <i>Typhlodromalus peregrinus</i> (n=14)	Total Galls Characterized (n=121)
Height	7.5 mm	7.4 mm
Cavity depth	2.9 mm	2.1 mm
Width	7.4 mm	7.4 mm
Aperture size	1.6 mm	2.0 mm

**Table 3.2. Results of Poisson regression model on depth and opening size of rosette bud mite galls (*Trisetacus fraseri* Amrine) (n = 121)**

Parameter	DF	Estimate	Standard Error	Wald	95% Confidence limits	Chi Square	Pr > Chi Square
Intercept	1	-4.1612	0.9173	-5.9590	-2.3634	20.58	< .0001
Depth	1	1.9011	0.9173	1.2601	2.5420	33.80	< .0001
Opening	1	-1.1796	0.2221	-1.6148	-0.7443	28.21	< .0001
Scale	0	1.0000	0.0000	1.0000	1.0000		

## CHAPTER 4

### Seasonal distribution of phytoseiid mites (Acari: Phytoseiidae) in North Carolina Fraser fir (*Abies fraseri* (Pursh) Poiret) Christmas tree plantations

#### Abstract

The overwintering sites and seasonal abundance of phytoseiid mites in North Carolina Fraser fir Christmas tree plantations were assessed. Potential overwintering sites include Fraser fir branches, Fraser fir bark, surface litter composed of Fraser fir needles, a mixture of fescue grass (*Festuca* spp) and nimblewill (*Mhlenbergia schreberi*), a mixture of herbaceous debris from the previous growing season, and a monocultural cover crop of white clover (*Trifolium repens*). Twelve different species of mites (n= 414) in the family Phytoseiidae were collected during this study. The most abundant phytoseiids collected were *Arrenoseius morgani* (Chant) (n=160), *Typhlodromips sessor* (DeLeon) (n=156), and *Typhlodromalus peregrinus* (Muma) (n = 34).

During the spring and summer, Fraser fir plantations with a ground cover consisting of white clover (*Trifolium repens*) were compared to plantations with bare ground in order to assess the abundance and species composition of phytoseiid mites. A total of 512 Phytoseiidae representing 8 different species were collected. *Typhlodromips sessor* (DeLeon), *Typhlodromalus peregrinus* (Muma), and *Arrenoseius morgani* (Chant) were the most abundant species collected from Fraser fir with either clover ground cover or bare ground. Phytoseiid mite abundance was not statistically different between clover ground cover and bare ground plantations.

**Key words:** phytoseiid, ground cover

## Introduction

The spruce spider mite (*Oligonychus ununguis* Jacobi) (Acari: Tetranychidae) is considered one of the most detrimental mites attacking conifers (Johnson and Lyons 1976). This pest has a wide host range and is frequently encountered on nursery crops (Lehman 1982). It is especially troublesome in plantation and ornamental plantings (Rose and Lindquist 1977, Johnson and Lyon 1994). It is also an important pest of Fraser fir (*Abies fraseri* (Pursh) Poiret) grown for Christmas trees in North Carolina (Sidebottom 2002).

Predatory mites in the family Phytoseiidae (Acari: Mesostigmata) are significant predators of phytophagous mites in the family Tetranychidae (McMurtry et al. 1970). Many species of phytoseiid mites have been studied extensively for their application in biological control of mite pests (Moraes 2004). Phytoseiid mites have been observed in North Carolina Fraser fir plantations infested with spruce spider mite (Boyne and Hain 1983). An apparent decline in a spruce spider mite population on Fraser fir following an increase in *Neoseiulus fallacis* (Garman) abundance has also been observed (Boyne 1980). These observations and subsequent research have suggested that Fraser fir production areas may be well suited for a pest control program utilizing phytoseiid mites to control spruce spider mite (Kramer and Hain 1989, Mangini and Hain 2007).

The seasonal dynamics of natural enemy populations are a vital component of efficacious biological control programs (Khan and Fent 2004). Phytoseiid mites are known to overwinter in a variety of locations on trees and in debris located in close proximity to orchards where perennial crops are grown (Pratt and Croft 2000; Chant 1959; Putman 1959; Leetham and Jorgensen 1969; Knisley and Swift 1971; Broufas et al. 2002). The predator *N. fallacis*, for example, overwintered in ground cover debris in Michigan apple orchards (Croft

and McGroarty 1977). However, this species was found in greater densities overwintering on apple twigs than in ground cover debris in New York apple orchards (Nyrop et al. 1994). Several authors have reported on the overwinter sites of the phytoseiid *Typhlodromus pyri* Scheuten (Chant 1959, Zacharda 1989) and noted that they spend the winter in bark crevices. *Typhlodromus pyri* overwintered in cracks and crevices of older apple tree twigs (Khan and Fent 2004). These and other studies suggest that ground covers may have a questionable influence on population densities of some species of phytoseiid mites in at least some perennial crops (Stanyard et al. 1997).

Nonetheless, ground cover management is an important component of the Fraser fir Integrated Pest Management program in North Carolina (Sidebottom 1997). Ground covers may decrease runoff and sedimentation and influence fertilizer uptake in these agricultural areas (Sidebottom 1997), but a small percentage of growers continue to use herbicides in order to eliminate all ground cover vegetation (Sidebottom 2001). Experiments on the effect of ground cover management in Fraser fir production areas have been conducted, comparing phytoseiid mite diversity in several different ground cover species to mite diversity in naturally occurring ground covers (Mangini and Hain 2007). However, there remains relatively little information on phytoseiid mite diversity in Fraser fir trees, and there are no published reports of the overwintering habitats of phytoseiid mites in North Carolina Fraser fir production areas.

The goal of this project was to evaluate potential overwintering refuges of phytoseiid mites in commercial Fraser fir plantations, and to compare phytoseiid mite diversity during the spring and summer on Fraser fir with a white clover cover crop to trees grown on bare

ground. These mites may be potentially useful in a biological control program for spruce spider mites in Fraser fir plantations.

## **Methods**

### *Overwintering Study*

Overwintering studies were conducted between December and March 2006-2007 in Ashe, Alleghany and Watauga counties in North Carolina. Samples were collected from a total of 37 privately owned Fraser fir plantations. Twenty of the sampled sites had a mixed herbaceous and grass ground cover during the previous fall and were sampled 1 time each. Seventeen plantations were sampled on a single day, February 28, 2007 and had a ground cover consisting of white clover (*Trifolium repens*). Plantation sizes averaged approximately 2.023 ha (5 acres) in size. Fraser fir tree sizes ranged from .304 m (1 ft) to 2.438 m (8 ft) in height, and were planted in rows 1.524 m (5 ft) apart.

The following potential overwintering sites were sampled from mixed herbaceous and grass ground cover sites: Fraser fir bark (n=9), Fraser fir needle surface litter (n = 13), a mixture of fescue grass (*Festuca* spp) and nimblewill (*Mhlenbergia schreberi*) (n = 21), a mixture of herbaceous debris from the previous growing season (n=10), and white clover (*Trifolium repens*) (n=17).

The mixed species ground cover and mixed grasses were each collected separately from 3 randomly chosen fixed surface areas (20 x 20 cm) located between the rows of Fraser fir. The mixed ground cover consisted of multiple species of naturally occurring ground cover vegetation debris and was carefully gathered by hand. Mixed species of grasses (Poaceae) were clipped at the soil line from fixed surface areas (20 x 20 cm). Samples from

each treatment were combined and placed in a plastic bag to make a single composite sample of each ground cover type from each plantation. No attempts were made to identify the ground cover debris left from the previous years' growing season. No living vegetation debris was collected. The debris tended to be composed of woody stems and leaves.

Fraser fir needles were carefully gathered by hand from 3 randomly chosen fixed surface areas (20 x 20 cm) located directly beneath trees, and also placed in plastic bags. Fraser fir branches were collected (n=15) from fields which had a mixed herbaceous and grass ground cover during the previous fall. We also collected (n=17) Fraser fir branches from plantations with a white clover cover crop.

Foliar samples were collected from 10 trees at each plantation by cutting 1 randomly selected Fraser fir 14-cm branch every 20 feet (6.0 m) along a single transect. Samples were placed in plastic bags, and transported to the lab in coolers. Bark samples were collected by cutting 20-30 strips 30 cm long x 2.4 wide from 5 trees that appeared to have crevices suitable as habitat for phytoseiid mites.

Samples collected from plantations planted in clover consisted of Fraser fir branches collected as previously described and 3 samples of white clover from a fixed area (20 x 20 cm) from each plantation. Clover ground cover samples were collected from under the trees. A plug of soil (A horizon) and the attached clover was removed by cutting into the soil 3 cm deep and gently removing the plug by hand. Two Fraser fir cones were picked by hand from 5 trees and combined into 1 composite sample.

Arthropods were extracted from the branches and from cover crop samples using

Tullgren funnels outfitted with a 40 w incandescent bulb for drying. A cup with 70% ethanol and a few drops of glycerin was placed under each funnel to collect mites. Samples were left in the funnels for 5 d. Funnels were washed and wiped clean between uses to avoid cross contamination.

Arthropods were sorted using a 10-50x stereo-microscope. Predatory mites were removed and mounted with Hoyer's medium on glass microscope slides and covered with a 12 mm diameter cover slip. Slides were dried for 48 hours in a drying oven set at 50° C (Krantz 1978). Generic and specific identifications were made at 40-100x with a phase contrast microscope. The primary author performed all identifications. Only adult females were identified during the study. A representative number of slides were verified by Gilberto de Moraes (Universidade de Sao Paulo, Piracicaba, Brazil). Shannon's diversity and evenness values were calculated to evaluate phytoseiid overwintering sites in Fraser fir plantations (Vandermeer 1981).

#### *Clover ground cover and bare ground comparison*

Fifteen sites were each sampled on the same days, 4 April, 1 June, 2 July, 2 August, and 4 September, 2007 and were located in Ashe and Alleghany Counties, North Carolina. Ten of these plantations had a ground cover crop consisting of white clover, but in 5 bare ground plantations, the ground cover had been eliminated by herbicides. Fraser fir trees were sampled at each plantation following the same methodology as described in the overwintering study. A white clover sample was also collected at each of the ten plantations that used it as a cover crop. The clover was collected from 3 randomly chosen locations at

each site and placed into a 7.5 L bag to form a single composite sample from each site. Samples were collected from between rows of Fraser fir by cutting the clover at the soil line.

An analysis of variance (ANOVA) test was used to determine if there was a difference in the abundance of phytoseiid mites in the Fraser fir trees growing in fields with a clover ground cover versus trees that were growing on bare ground (Proc GLM, SAS 2007). The analysis was conducted by site, day, and ground cover. Shannon's diversity values were calculated to evaluate predatory mite diversity and evenness on Fraser fir trees growing in fields with a clover ground cover and for trees that were growing on bare ground (Vandermeer 1981).

## **Results and Discussion**

### *Overwintering Study*

Twelve different species of phytoseiid mites were collected during this study (Table 4.1). The most abundant phytoseiids collected were *Arrenoseius morgani* (Chant) (n = 160), *Typhlodromips sessor* (DeLeon) (n = 156), and *Typhlodromalus peregrinus* (Muma) (n = 34). The mixed grass ground samples and Fraser fir needle samples yielded the greatest numbers of phytoseiid mites, with 174 and 158 specimens, respectively. Mixed grass samples had the greatest diversity of phytoseiids. The Fraser fir branches collected from plantations with a white clover ground cover yielded more phytoseiid mites than the Fraser fir branches collected from plantations with a mixed species herbaceous ground cover (Table 4.1). Shannon's diversity was greatest (0.451) in mixed grass overwintering sites and in Fraser fir trees (0.379) growing in plantations with a white clover cover crop (Table 4.2).

These data lend support to previous research, which found that certain species of phytoseiid mites overwinter in trees as well as in ground cover crops (Nyrop et al. 1994). *Neoseiulus fallacis* overwintered in greater densities on ornamental conifers than on evergreen shrubs, herbaceous perennials, deciduous shrubs or shade trees (Pratt and Croft 2000); however, we only collected 1 specimen of *N. fallacis* from Fraser fir branches during our winter sampling. Our study found that this species is found in both mixed grass ground covers, as well as mixed ground cover debris, and on the foliage of Fraser fir during the winter. *Typhlodromalus peregrinus* was collected in the greatest density from the Fraser fir branches; it is considered a generalist predator that feeds on pollen and small arthropods, including spider mites (McMurtry and Croft 1997). There is evidence to suggest that generalist predators more readily occupy microhabitats on trees throughout the season than specialist predators, which must seek out new prey populations when food availability is scarce (McMurtry 1992). For example, when prey mite populations were low on apple trees, *Typhlodromus pyri*, a generalist predator, did not migrate as readily as the specialist predator *T. occidentalis* (Dunley and Croft, 1990).

#### *Clover ground cover and bare ground comparison*

A total of 512 Phytoseiidae representing 8 different species were collected during the spring and summer sampling (Table 4.3). *Typhlodromips sessor*, *Typhlodromips peregrinus*, and *Arrenoseius morgani* were the most abundant species collected from Fraser fir with either a clover ground cover or with the trees on bare ground. *Neoseiulus fallacis* was found in greatest abundance on Fraser fir branches with a clover cover crop, and three species of phytoseiid mites were found in the clover samples. The most abundant species in the clover,

*Neoseiulus vagus* (Denmark) was never collected from Fraser fir foliage. However, *A. morgani*, and *T. sessor* were both collected in the clover samples and were common on Fraser fir branches. Shannon's diversity (H) and evenness (E) values were greatest (H 0.625, E 0.894) on Fraser fir trees growing in fields with a clover ground cover (Table 4.3). Phytoseiid mite abundance was not significantly different in clover ground cover vs. bare ground, by ground cover type ( $P>.788$ ), date ( $P>.116$ ) or site ( $P>.140$ ) (Table 4.4).

There is sufficient biological information on four of the species collected during these seasonal studies (*N. fallacis*, *T. sessor*, *T. peregrinus*, and *Typhlodromus (Anthoseius) claudiglans* (Schuster)) to suggest that they could be useful in a biological control program on Fraser fir trees (McMurtry and Croft 1997). Both *N. fallacis* and *T. sessor* were found in the Fraser fir foliage. *Typhlodromus (Anthoseius) claudiglans* was found only in bark crevices during the winter, and not on foliage. *Typhlodromalus peregrinus* was the most abundant phytoseiid mite collected on Fraser fir foliage during the winter, while *T. sessor* was found in greater abundance during the spring and summer. *Arrenoseius morgani* was previously reported to be commonly associated with spruce spider mite (Leman 1982), unfortunately little or no research has been conducted with this species or others of the same group (Moraes, personal communication). Additional research is needed on this species to investigate its potential as a predator of spruce spider mite.

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**Table 4.1. Phytoseiidae collected from overwintering sites in Fraser fir plantations, in Ashe, Watauga, and Avery Counties, North Carolina**

Phytoseiidae Species	Clover	Bark	Mixed grasses	Mixed ground cover	F.fir branches <sup>1</sup>	F.Fir branches <sup>2</sup>	F.fir needles <sup>2</sup>	F. fir cones <sup>2</sup>	Total Phyto.
<i>Amblyseiella setosa</i> Muma								2	2
<i>Arrenoseius morgani</i> (Chant)			5	1			154		160
<i>Chelaseius vicinus</i> (Muma)			2						2
<i>Neoseiulus fallacis</i> (Garman)			6	1	1				8
<i>Neoseiulus vagus</i> (Denmark)	1		24	4					29
<i>Proprioseiopsis clause</i> (Muma)			3						3
<i>Proprioseiopsis solens</i> (DeLeon)					6				6
<i>Proprioseiopsis okanagensis</i> (Chant)			3				2		5
<i>Tenorioseius gracilisetae</i>			3						3
<i>Typhlodromalus peregrinus</i> (Muma)			2	1	29	2			34
<i>Typhlodromus</i> ( <i>Anthoseius</i> ) <i>claudiglans</i> (Schuster)		6							6
<i>Typhlodromips sessor</i> (DeLeon)	2		126	20	5	1	2		156
Total Phytoseiids/ground cover	3	6	174	27	41	3	158		414
No. samples collected	15	9	21	10	17	15	13	1	
Mean Phytoseiids/sample	.2	.66	8.28	2.7	2.4	.2	12.1	.5	

1. Collected from Fraser fir with a white clover cover crop. 2. Collected from Fraser fir with a mixed herbaceous and grass ground cover.

**Table 4.2. Shannon's diversity (H) and evenness (E) values for phytoseiid mite overwintering sites in Fraser fir plantations, in Ashe, Watauga, and Avery Counties, North Carolina 2006-2007**

Overwintering sites	Shannon's diversity (H)	Shannon's evenness (E)
Clover	0.276	0.918
Bark	0.000	
Mixed grasses	0.451	0.472
Mixed ground covers	0.378	0.541
Fraser fir branches <sup>1</sup>	0.379	0.630
Fraser Fir branches <sup>2</sup>	0.276	0.918
Fraser fir cones	0.000	
Fraser fir needles	0.059	0.123

1. Collected from Fraser fir with a white clover cover crop. 2. Collected from Fraser fir growing on bare ground.

**Table 4.3. Phytoseiidae collected from Fraser fir in plantations with either a white clover cover crop or bare ground.**

Phytoseiidae Species	Fraser fir branches <sup>1</sup>	Fraser Fir branches <sup>2</sup>	White Clover	Total Phytoseiidae
<i>Amblyseiella setosa</i> Muma		6		6
<i>Arrenoseius morgani</i> (Chant)	66	14	7	87
<i>Neoseiulus fallacis</i> (Garman)	7	2		9
<i>Neoseiulus vagus</i> (Denmark)			26	26
<i>Proprioseiopsis solens</i> (DeLeon)	45	4		49
<i>Typhlodromalus peregrinus</i> (Muma)	68	24		92
<i>Typhlodromina conspicua</i>		1		1
<i>Typhlodromips sessor</i> (DeLeon)	93	63	15	171
Unidentified males	23	11	3	37
Unidentified immatures	10	24		34
Total Phytoseiids	312	149	51	512
No. samples collected	50	20	50	
Mean Phytoseiids/sample	6.2	7.4	1.0	
Shannon's Diversity	0.625	0.564	0.424	
Shannon's Evenness	0.894	0.667	0.889	

1. Collected from Fraser fir with a white clover cover crop. 2. Collected from Fraser fir growing on bare ground.

**Table 4.4. ANOVA test results for the influence of clover ground cover and bare ground on phytoseiid mite abundance on Fraser fir trees.**

Source	DF	SS	MS	F	P
Model	22	4980	226	1.44	0.159
Site	17	4061*	238	1.52	0.140
Day	4	1248*	312	1.99	0.116
Ground cover	1	11.4*	11.4	0.07	0.788
Error	37	5813	157		

\*Type III SS

## CHAPTER 5

### **The influence of four different ground cover vegetation types used in North Carolina Fraser fir (*Abies fraseri* (Pursh ) Poiret) Christmas tree plantations on abundance and species composition of phytoseiid mites (Acari: Phytoseiidae)**

#### **Abstract**

Vegetation samples were collected in Laurel Springs and Waynesville, North Carolina from 4 ground cover species: White Dutch clover (*Trifolium repens*), Mammoth Red Clover (*Trifolium pratense*), birdfoot trefoil (*Lotus corniculatus*), and Fescue, (*Festuca* spp.). The most abundant phytoseiid mite species found during the study were *Typhlodromips sessor* (DeLeon), *Arrenoseius morgani* (Chant), *Proprioseiopsis solens* (DeLeon), and *Typhlodromalus peregrinus* (Muma). There were no statistical differences in the effects of any of the ground cover vegetation analyzed on phytoseiid mite abundance.

**Key words:** Phytoseiidae, Fraser fir, *Abies fraseri*

#### **Introduction**

Ground covers provide many benefits to the landscape, including erosion control, weed control, the reduction of surface water pollution, and improved soil structure (Hartwig and Ammon 2002). Ground cover habitats also enhance or conserve natural enemies of arthropod pests in agricultural settings (Landis et al. 2000) by providing food and refugia for the natural enemies of certain pests (Bugg and Waddington 1994). Among the beneficial arthropods that utilize these habitats are predatory mites in the family Phytoseiidae (Acari: Mesostigmata) (McMurtry 1982), whose density is enhanced in ground cover associated with certain perennial crops (Muma 1961).

Phytoseiid mites are predators of phytophagous mites in the family Tetranychidae (McMurtry et al. 1970), including the spruce spider mite (*Oligonychus ununguis* Jacobi)

(Acari: Tetranychidae), a pest of the Fraser fir Christmas trees (Boyne and Hain 1983). There is variation among ground cover species in their ability to provide appropriate harborage for the phytoseiids that prey on tetranychid mites (Flaherty 1969), due to specific requirements of the predators (Barbosa 1998) such as temperature and humidity tolerances (Kramer and Hain 1989). Physical characteristics of ground cover plants, e.g., the microhabitats formed by leaf structures (Grostal and O'Dowd 1994), also may play an essential role in the enhancement of phytoseiids in perennial cropping systems. Several researchers have demonstrated that the presence or absence of leaf hairs (“acrodonti”) may influence phytoseiid mite abundance (Kaban *et al* 1995; Walter and O’Dowd 1992a; Walter and O’Dowd 1992b; Walter 1996). Pollen availability may also be a factor, as many phytoseiid mites feed on pollen as well as their prey (Chant 1959, Chant and Fleschner 1960, McMurtry and Croft 1997), and pollen from leguminous cover crops is known to serve as a carbohydrate source for certain phytoseiids (Grafton-Cardwell *et. al.* 1999).

The goal of this study was to gather information relating to the appropriate ground cover vegetation to use in managing the natural enemies of spruce spider mite in Fraser fir production areas in North Carolina. Four different ground covers were evaluated to determine: 1) their impact on species abundance or diversity of phytoseiid mites, either on the trees or in the ground cover; 2), their influence on the abundance of phytoseiid mites known to be natural enemies of spruce spider mite; and 3) their effect on the species composition of the phytoseiid mites found in the ground cover compared to those found on the adjacent Fraser firs.

## Methods

The study areas were located in western North Carolina at the Upper Mountain Research Station located near Laurel Springs, Ashe County, and the Mountain Research Station located near Waynesville, Haywood County. The study consisted of three ground cover treatments and one control: white Dutch clover (*Trifolium repens* L.), mammoth red clover (*Trifolium pratense* L.), birdfoot trefoil (*Lotus corniculatus* L.), and fescue (*Festuca* spp.) as the control. Our goal was to simulate actual growing conditions a tree farmer may encounter when minimal herbicide applications are used. Therefore, there was encroachment of several species of herbaceous vegetation into the plots through out the growing season. The fescue treatments tended to have less encroachment of herbaceous vegetation because it out competed other species. The treatments were chosen because these species are currently being used as ground covers in North Carolina Fraser fir plantations. Ground covers and Fraser fir trees were established in eight, 9.1 x 13.7 m blocks in a complete randomized block design replicated twice in each study location. Fraser trees were between 30.5 cm and 48.3 cm tall at the time they were planted. Trees were planted in 24 rows with 19 trees per row for a total of 456 trees per location. Trees were planted 1.5 m apart. The number of trees per block varied from 50 to 57. Both the ground covers and the Fraser fir transplants were planted in March 2005 at each location. Each site was mowed once per year, usually during mid summer. Periodic herbicidal treatments were applied to the ground covers in a 2 ft band along the rows of trees in order to eliminate competition with the trees.

Samples were collected monthly from both sites between April and September 2007: May 19, June 16, July 4, August 2, and September 4 at Laurel Springs; May 19, June 11, June 25, August 11 and September 1 at Waynesville. Foliar samples were collected from

each block by cutting one randomly selected 14-cm branch from each of 10 Fraser fir trees. Samples were placed in 7.5 L plastic bags, and transported to the laboratory in coolers. Three randomly chosen fixed surface area (20 x 20 cm) samples were collected from the ground cover vegetation in each plot by cutting the ground cover at the soil line between rows of Fraser fir and placing the sample into a 7.5 L plastic bag.

Arthropods were extracted from the branches using Tullgren funnels outfitted with a 40 w incandescent bulb for drying. A cup with 70% ethanol and a few drops of glycerin was placed under each funnel to collect mites. Samples were left in the funnels for 5 d. Funnels were washed and wiped clean between uses to avoid cross contamination.

Arthropods were sorted using a 10-50x stereo-microscope. Phytoseiid mites were removed and mounted with Hoyer's medium on glass microscope slides and covered with 12 mm diameter cover slips. Slides were dried for 48 hours in a drying oven set at 50° C (Krantz 1978). Generic and specific identifications were made using a phase contrast microscope at 40-100x. The primary author performed all identifications. A representative number of slides were verified by Gilberto de Moraes (Universidade de Sao Paulo, Piracicaba, Brazil).

We pooled the data from the two stations in order to address site specific differences in encroaching vegetation which could potentially influence phytoseiid abundances. An analysis of variance (ANOVA) test was used to determine if there was a difference among ground covers with respect to their influence on the abundance of phytoseiid mites both in the Fraser fir and/or in the ground cover vegetation (Proc GLM, SAS, 2007). The analysis was conducted by site, day and ground cover. Shannon's diversity and evenness values were

calculated to compare phytoseiid diversity/evenness among the different ground cover vegetation plots (Vandermeer 1981).

## **Results and Discussion**

Ten different species were identified from the phytoseiid mites collected during the study. The most abundant species found were *Typhlodromips sessor* (DeLeon) (n=257), *Arrenoseius morgani* (Chant) (127), *Proprioseiopsis solens* (DeLeon) (n=92) and, *Typhlodromalus peregrinus* (n=52) (Muma).

A total of 678 phytoseiid mites were collected from the Fraser fir trees (Table 5.1) and 58 were collected from the ground covers (Table 5.2). The Fraser fir trees yielded the greatest diversity of phytoseiid mites with nine different species collected, while five species were collected from the ground covers. *Chelaseius floridanus* (Muma) and *Neoseiulus vagus* (Denmark) were collected exclusively from ground cover. Mite species diversity on the Fraser fir trees was similar in all ground cover treatments. The exceptions were *Phytoseius* sp., collected just once, and a small number (n = 13) of *Typhlodromina conspicua* (Garman).

Neither phytoseiid mite abundance nor diversity were statistically different among ground cover treatments in newly established Fraser fir plantations (Table 5.3). Our results have significance for cultural practices currently used in Fraser fir IPM programs, as the influence of ground cover management on certain species of phytoseiid mites, including *Neoseiulus fallacis*, has been questioned by some researchers (Nyrop et al. 1994).

Certain species of phytoseiid mites may favor the structure of Fraser fir plantations independent of the ground cover type. *Typhlodromips sessor*, for example, is a common species with a wide distribution along the Atlantic coastal states of the USA (Sciarappa et al.

1977). It is considered a generalist and consumes both plant and animal matter (Sciarappa and Swift 1977, McMurtry and Croft 1997). This species is commonly found on herbaceous plants with pubescent leaves and/or thorns (Sciarappa et al. 1977), which may account for its lack of abundance in the species of ground covers we evaluated in this study. However, it is well adapted to early successional plant communities and can be common in 1- 5 year old fields (Sciarappa et al. 1977). Since Fraser fir Christmas trees are grown on a short rotational schedule, these plantations are similar to early successional plant communities. It is therefore not surprising that we found *T. sessor* to be the most abundant species on the Fraser fir trees during this investigation, independent of the ground cover type.

*Typhlodromalus. peregrinus* is a generalist predator known to feed on spider mites and pollen (Muma 1971). The food preference for members of the *P. dorsatus* group, of which *P. solens* is an affiliate, is likely to be phytophagous mites (Muma 1971). *Arrenoseius morgani* was previously reported to be commonly associated with spruce spider mite (Leman 1982). Unfortunately little research has been conducted with this species or others in the same group (Moraes, personal communication). Further life history studies are needed to determine if these species would be suitable predators of spruce spider mites in a Fraser fir IPM program. Future research should also evaluate a wider range of ground cover species.

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**Table 5. 1. Total number and Shannon’s diversity and evenness values of Phytoseiidae collected from Fraser fir trees from plots with four different ground cover crops at Upper Mountain Research Station and Mountain Research Station**

Phytoseiid Species	Fescue	White clover	Red clover	Birdfoot Trefoil	Total phytoseiids
<i>Arrenoseius morgani</i> (Chant)	38	20	31	38	127
<i>Neoseiulus fallacis</i> (Garman)	23	5	8	1	37
<i>Neoseiulus vagus</i> (Denmark)					0
<i>Proprioseiopsis solens</i> (DeLeon)	13	44	13	22	92
<i>Proprioseiopsis okanagensis</i> (Chant)	1	1	1		3
<i>Phytoseius</i> sp.				1	1
<i>Typhlodromina conspicua</i> (Garman)	4		5	4	13
<i>Typhlodromalus peregrinus</i> (Muma)	6	23	22	1	52
<i>Typhlodromips sessor</i> (DeLeon)	108	11	97	41	257
Unidentified males	20	5	9	13	47
Unidentified immatures	14	6	8	9	37
Unidentified phytoseiids	3	4	1	4	12
Total Phytoseiids/ground cover	230	119	195	134	678
Shannon’s Diversity	0.563	0.627	0.589	0.570	
Shannon’s Evenness	0.666	0.805	0.697	0.674	

**Table 5. 2. Total number and Shannon’s diversity and evenness values of Phytoseiidae collected from four different ground cover crops at Upper Mountain Research Station and Mountain Research Station**

Phytoseiid Species	Fescue	White clover	Red clover	Birdfoot Trefoil	Total phytoseiids
<i>Arrenoseius morgani</i> (Chant)	1		1		2
<i>Chelaseius floridanus</i> (Muma)		1			1
<i>Neoseiulus fallacis</i> (Garman)				1	1
<i>Neoseiulus vagus</i> (Denmark)	4	8	23	2	37
<i>Proprioseiopsis solens</i> (DeLeon)					0
<i>Proprioseiopsi okanagensis</i> (Chant)		10	1		11
<i>Phytoseius</i> sp.					0
<i>Typhlodromina conspicua</i> (Garman)					0
<i>Typhlodromalus peregrinus</i> (Muma)					0
<i>Typhlodromips sessor</i> (DeLeon)	2	3			5
Unidentified males					0
Unidentified immatures	1				1
Unidentified phytoseiids					
Total Phytoseiids/ground cover	8	22	25	3	58
Shannon’s Diversity	0.217	0.372	0.145	0.276	
Shannon’s Evenness	0.722	0.780	0.304	0.918	

**Table 5.3. ANOVA test results for 4 different ground covers on phytoseiid mite abundance**

Source	DF	SS	MS	F	P> F
Model	12	131	10.9	0.83	0.621
Site	1	0.00	0.00	0.00	1.00
Day	8	71.4	8.92	0.68	0.70
Ground cover	3	34.4	11.4	0.87	0.46
Error	27	356	13.2		

\*Type III SS

## CHAPTER 6

### Overall Conclusions and Recommendations for Future Research

The overall objective of this research was to evaluate Fraser fir Christmas tree production in North Carolina and to determine if it provides a suitable environment for a conservation based Integrated Pest Management (IPM) program for spruce spider mites (*O. ununguis* Jacobi). “Conservation based” IPM are actions that can be taken by the grower to conserve or protect natural enemies (Barbosa 1998), in this case predatory mites in the family Phytoseiidae. While the objective was met there remain a number of questions that should be answered to fully implement an effective IPM program, especially as it pertains to ground cover management and its influence on phytoseiid mites.

In this study we identified the common predatory mites inhabiting the typical Fraser fir plantations in the northwestern mountains of North Carolina. It is apparent that *Arrenoseius morgani* (Chant), *Typhlodromips sessor* (DeLeon), *Proprioseiopsis solens* (DeLeon), and *Typhlodromalus peregrinus* (Muma) are very common inhabitants of Fraser fir plantations in the region studied. These species were present in northwestern North Carolina Fraser fir plantations throughout the year. Future research should be conducted to determine the level of influence these species have on spruce spider mite population dynamics. Since these predators are generalist, their numerical response to a spruce spider mite build-up and their ability to prevent or contain an outbreak should be examined. Another area of research would be to evaluate the potential to rear the predatory mites under laboratory conditions.

*Neoseiulus fallacis* (Garman), a commercially available spider mite predator, was also found in some plantations but it was far less abundant and not as widely distributed as the

above-mentioned species. There should be research conducted on this species to determine if augmentation releases will increase the abundance of this mite in Fraser fir plantations and to evaluate the effects these releases have on spruce spider mite populations. These experiments should be designed to evaluate the compatibility of augmentation releases with typical Fraser fir management practices.

The influence of ground cover on phytoseiid mite species richness and population dynamics in Fraser fir plantations continues to be unknown and there are a number of questions that remain unanswered. In this study, we focused our research on ground covers that are typically recommended in Fraser fir IPM programs. The plants studied included white Dutch clover (*Trifolium repens*), mammoth red clover (*Trifolium pretense*), birdfoot trefoil (*Lotus corniculatus*) and fescue (*Festuca* spp.) as the control. Phytoseiid mite diversity and their abundances on the Fraser fir trees growing with these four ground covers were similar, with no statistical differences. Phytoseiid species composition in the four plots was consistent with other studies we conducted in managed Fraser fir plantations. However, the species found in the ground covers were far less numerous, and the species, *Neoseiulus vagus* and *Proprioseiopsis okanagensis*, were different from the species we found in the Fraser fir trees. This suggests that the most abundant species found on Fraser fir trees, *A. morgani*, *T. sessor*, *P. solens* and *T. peregrinus* were not utilizing the ground cover vegetation in the plots during the course of this study.

The ground cover plants studied did not appear to have a negative influence on the abundance or species composition of phytoseiid mites on the Fraser fir trees. All the ground covers, especially red clover, had an abundance and diversity of phytoseiids in the Fraser firs

similar to that of the controls (fescue). The most common species of phytoseiids found in this study were also found to be common in managed Fraser fir plantations in the region. Future research of this kind should focus on different species of ground cover plants, perhaps vegetation that provides more suitable microhabitats required by phytoseiid mites (Karban et al. 1995), however, care must be taken to insure the ground covers chosen are compatible with Fraser fir production practices.

In order to evaluate the success of a Fraser fir IPM program, a method to quickly estimate phytoseiid mite abundances and capture them for identification is needed. A simple trap made from vinyl tape, a piece of Velcro and woolen yarn has been used to evaluate the seasonal dynamics of phytoseiid mites on pear trees in Japan (Koike et al. 2000). It is recommended that research be conducted using similar traps fastened to the branches of Fraser fir trees. If successful, phytoseiid mite traps could greatly enhance the grower's ability to scout for natural enemies in Fraser fir plantations.

During this study we found that *T. peregrinus* will overwinter in the galls of rosette bud mite and feed on the bud mites. Future research should be conducted to determine the total impact of the predator on the bud mite populations, and if this phenomenon leads to a greater abundance of phytoseiid mites the next spring when spruce spider mites are present. In addition, research should be conducted to evaluate the interactions of *T. peregrinus*, rosette bud mites and spruce spider mites. Given that all stages of phytoseiid mites have been found in phytoseiid traps mentioned above, including eggs, immatures and adults it is apparent that artificial structures can also provide oviposition sites (Koike et al. 2000). Research should be conducted on phytoseiid mite traps as a means of predicting phytoseiid abundance.

It is recommended that to the extent possible, growers allow the growth of native vegetation surrounding plantations for overwintering sites and for recolonization of plantations following pesticide applications. Plant diversity will provide greater opportunities for plants that are compatible with phytoseiid mite biological requirements to grow in close proximity to the plantations. Similarly, within plantation herbicide use should be minimized when possible, to protect existing ground covers. If possible, herbicides should be applied to limited areas around the tree allowing for the growth of vegetation between rows. Given the soil conservation and nitrogen fixing properties of legumes, white and red cover and birdfoot trefoil should be encouraged when possible.

In summary, northwestern North Carolina Fraser fir Christmas tree plantations provide suitable environments for a conservation based IPM program for spruce spider mite. The role of ground cover vegetation on species composition and abundance of phytoseiid mites on Fraser fir trees will need further investigation before a species composition list can be developed. New and better techniques for monitoring phytoseiid mite populations are in need of investigation.

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