# Effects of Nitrogen and Potassium Fertilizer on Willamette Spider Mite (*Eotetranychus willamettei*) (Acari: Tetranychidae)

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Abstract

Effects of Nitrogen and Potassium Fertilizer on Willamette Spider Mite

(*Eotetranychus willamettei*) (Acari: Tetranychidae)

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The spider mite family (Tetranychidae) is a well known pest group in agriculture. Within this family, Willamette spider mite (*Eotetranychus willamettei*) causes physical harm and potential damage to grapevines (*Vitis vinifera*) along the central and north coast of California as well as Washington and Oregon. Willamette spider mite prefers cooler climates and feeds by puncturing the plant leaf tissue; therefore removing plant nutrients in the early stages of plant growth. Amending soils with fertilizer is a common cultural practice used in commercial vineyards, but no study has documented the interaction between the effects fertilizer concentrations have on Willamette spider mite. This project consisted of a field study (at Cambria Vineyards & Winery, Santa Maria, CA) and a laboratory study (at a California Polytechnic State University, San Luis Obispo campus greenhouse). The field study tested the effects potassium, nitrogen, and control (unfertilized) treatments had on Willamette spider mite on 'Chardonnay' grapes. Field results showed no significant difference among the three treatments, but suggest a response, given that mite density peaked highest in the potassium treatment and had a second high peak in the nitrogen treatment. In addition, egg density peaked highest in the potassium treatment. The lab study tested the effect four different nitrogen treatments had on Willamette spider mites. Treatments ranged from High N (1500 ppm N fertilizer), Med-High N (1500 ppm N fertilizer), Med–Low N (500 ppm N fertilizer), and Low N

(0-50 ppm N fertilizer). Four parameters were tested: male and female days to maturation, male and female survivorship to adult, adult female longevity and oviposition. Longevity and oviposition lab results indicate that Willamette spider mite has a non-linear response to grape N concentration. Performance was better within the two medium treatments compared to High N and Low N treatments. Survivorship suggests the same as days to maturation although not statistically significant.

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## Introduction

### Background of Willamette Spider Mite

Willamette spider mite (*Eotetranychus willamettei* (McGregor) (Acari: Tetranychidae, the 'spider mites') is a specialist, feeding only on grape (*Vitis* spp.). It is a significant pest of cultivated grapes in viticultural regions in the Western USA, including Oregon and Washington, California's Central Coast and North Coast, the southern San Joaquin Valley, and the Sierra Foothills (Battig, 2004). Feeding by Willamette spider mite can cause leaf injury and potentially, crop damage. Compared to some members of the spider mites which can be found on grapes in the west and which prefer a warmer climate, including Pacific spider mite (*Tetranychus pacificus* McGregor) and McDaniel spider mite (*Tetranychus mcdanieli* McGregor) present in Washington State, Willamette spider mite has a lower optimal developmental temperature. It is therefore often found exclusively in cooler climates, and in warmer climates it begins feeding much earlier in the growing season than the aforementioned spider mite species (Costello, pers. comm.). Willamette spider mite has also been used as an "inoculation" agent, whereby its feeding has induced plant resistance to the more injurious Pacific spider mite (Karban et al., 1997).

Willamette spider mite, like many if not most spider mites, is thought to respond to host plant condition based on cultural practices in the agricultural setting. These include plant water status (English-Loeb, 1990) and plant nutrient status, especially nitrogen (Wood and Reilly, 2000). Cultural practices such as irrigation and fertilization can influence the feeding and reproductive rate of spider mites, and ultimately, their population density (Chen et al., 2007).

Spider mites can also be influenced by chemical applications, such as sulfur dust for control of powdery mildew (*Erisiphe necator* Schwein.) on grapes (Costello, 2007).

## Arthropod Response to Plant Nutrient Concentration

Numerous studies have been done on arthropod pest management, but relatively few have studied the effect of plant nutrients on arthropod populations, and most of these have looked at nitrogen (Mittler, 1953; Mittler, 1958a; Mittler, 1958b; Mittler, 1958c; Myers and Post, 1981; Lightfoot and Whitford, 1987; Nevo and Coll, 2001). The effects of macronutrient deficiencies on arthropod pest density in commercial settings can be difficult to assess, because major nutrients such as nitrogen, potassium, and phosphorus are usually added in surplus (Chen et al., 2007).

Mittler (1958c), studied the giant willow aphid (*Tuberolachnus salignus* Gmelin.) (Homoptera: Aphididae), and investigated the actual amounts of nitrogen ingested, excreted, and absorbed by this insect. Sharp-leaf willow (*Salix acutifolia* Willd.) trees were used as host plants for the aphid, at various stages of leaf development. Mittler (1953, 1958a) had previously shown that the total amino-acid and amide (nitrogen concentration) of the phloem sap of sharp-leaf willows varied substantially with aphid developmental stages. Six willow plants with differing nitrogen concentration levels were used; A and B plants both had actively growing leaves and phloem sap 'rich' in nitrogen (greater than 0.1%, w/v), plant C had advanced leaf growth and 'medium' nitrogen phloem sap (0.05%-0.1%, w/v), and plants D, E, and F had mature leaf growth and 'poor' nitrogen phloem sap (less than 0.05%, w/v). Mittler (1958c) found that 7 to 8 day old aphid nymphs feeding on nitrogen 'rich' willow host plants had an average nitrogen content that was almost ten times greater than the nymphs feeding on nitrogen 'poor' plants. The

amino acid concentration of the honeydew excreted from aphids feeding on plant B was 25 times greater than excretion from aphids on plant D. The aphids on these nitrogen-poor plants did not increase much in size and most did not develop past the 2nd instar. Aphids on the nitrogen rich plants were normal in size and all had reached 3rd instar, and some even reached 4th instar (aphids have four instars). This study was able to physically show that the rate of maturation was more successful on plants that contained a higher nitrogen content.

Lightfoot and Whitford (1987) studied the role nitrogen played on insect density levels on desert creosotebush (*Larrea tridentata* (DC) Coville.). In desert ecosystems, water and nitrogen are limiting factors and nitrogen is known to be an important limiting factor for phytophagous insects. Nitrogen and water were manipulated through a series of fertilizer and irrigation treatments; some treatments simulated rainfall by overhead sprinkler systems (3 plots receiving 6 mm of water once a week and 3 plots receiving 25 mm of water once every 4 weeks; (all plots measuring 5 x 10 m). The other treatments (one-half of each of the plots) received ammonium nitrate fertilizer with the total nitrogen equivalent to 100 kg/ha. The application was made only once during the study, in the month of February. Results found that the fertilized plots significantly increased arthropod densities, especially to phytophagous sap sucking insects such as Hemiptera, Homoptera and Thysanoptera. The increase of insect densities on fertilized host plants had to do with plant to plant movements and phenological adaptations. Alates had the ability to select and move to better quality host plants (those enriched with nitrogen). This study found that increased foliage-arthropod densities were positively correlated with foliage production and foliar nitrogen content. The results also showed that sap sucking insects are more responsive than leaf-chewing insects to desert creosotebush high in nitrogen.

**Spider Mite Response to Plant Nutrient Concentration**

Previous studies have found a positive correlation between high soil nitrogen concentration and density of *Tetranychus* spp. (*T. urticae* or *T. pacificus*) (van de Vrie et al., 1972; Suski and Badowska, 1975; Wermelinger et al., 1985; Wilson et al., 1988; Wood and Reilly, 2000). Wilson (1994) tested the fitness of cotton for the development and reproduction of the two-spotted spider mite. Female mites were reared on 2.5 cm cotton leaf disks and removed once four to five eggs were laid. Once fully mature, female mites were placed with male mites on individual leaf disks and were kept together for 24 hours to ensure mating. Egg production was recorded every two days until adult females died. Five different rearing experiments occurred over the course of three years ([1] October 1988-1989, early squaring; [2] January 1988-1989, peak squaring; [3] March 1989-1990, boll maturation; [4] October 1989-1990, cotyledons; [5] November 1989-1990, pre-squaring). In each experiment mites were reared on a standard host plant that served as the control treatment. Twenty female mites from the control treatment and seventeen mites from each experiment were placed on unsprayed irrigated 'Deltapine 90' cotton. The pre-oviposition period, total fecundity, reproductive period, mean developmental time from egg to adult, and life span were analyzed for each individual mite contained within its own cell case. Immature survival (eggs that developed to adult) was also recorded for both male and female mites. Nitrogen analysis occurred every 14 days. Results showed no significant difference among any of the parameters for experiments 1-3. However, there was significant differences between experiment 4 and 5 and the control treatment for developmental time and fecundity. Mites developed more quickly on control plants and an increased fecundity in experiment 4 compared to experiment 5. Overall, mite developmental time increased in relation to nitrogen content as well as fecundity which was also positively

related to nitrogen. Mites preferred to feed on developing young leaves (cotyledons) more than older leaves. As plants developed and the leaves aged, the nitrogen content decreased.

Chow et al. (2009) studied nitrogen and *T. urticae* on a popular greenhouse ornamental, the cultivated rose. Two-spotted spider mite is a major pest on the genus *Rosa*; a floriculture crop produced world-wide. Chow et al. (2009) wanted to see if spider mite population density on roses would decrease when levels of nitrogen were lowered from recommended cut flower standards (recommended fertilizer rate for constant liquid feeding of roses with nitrogen is between 150 to 200 ppm N) (Dole and Wilkins, 2005). The study set out to determine if reduction of fertilization could be a useful pest management tactic if pest population levels decreased without decreasing crop yield. Host plants were fertilized with nitrogen at 33% (50 ppm N), 50% (75 ppm N), or 100% (150 ppm N). Results showed mean numbers of *T. urticae* instars and eggs per flower shoot to be twice as high on roses fertilized with 100% vs. 33% or 50% N. Total yield was jeopardized with 33% N and 100% N, but was not compromised on roses fertilized with 50% N.

Wermelinger et al. (1985), also studied *T. urticae* and observed the development rate, fecundity, and survivorship of this mite in relation to different levels of nitrogen concentration on host plants of cloned apple trees (*Malus domestica* cv Glockenapfel) and bush beans (*Phaseolus vulgaris* L. cv Gazelle). For the apple trees, they were grown from one original seed in growth chambers, propagated, rooted, and transferred at 4 months (5-10cm high). For each of the concentrations, four trees per one container were used. The four different N concentrations (5 N (5-fold or 1050 ppm), 1 N (Hoagland solution= 15mM $N0^{-3}$ or 210 ppm), 0.2 N (1/5-fold/deficient or 42 ppm) and 0.04 N (1/25-fold/deficient or 8.4 ppm) were based off the Hoagland solution which is a plant nutrient solution commonly used for apples (Epstein, 1973).

There were 32 replications per treatment. One female *T. urticae* was placed on each 2 cm diameter leaf disk (replaced weekly) for each treatment. Egg production was recorded daily and all eggs were removed once the adult female mite died. For the bush beans, four different N concentrations were also used; 5N, 1N (control/standard), 0.1 N (deficient), and 0.02 N (deficient). There were 12 replications per treatment. Fecundity was recorded the same as the apple study. Results of the apple experiment showed a decrease in oviposition the day following the replacement of old leaf disks with newly punched leaf disks. The standard treatment (1 N control/standard) differed significantly from all deficiency treatments but did not differ from 5 N (treatment above standard N application for apples). The 0.2 N treatment caused a prolonged developmental time by 2.6 days and prolonged the pre-oviposition period by 4 days in comparison with the control. Fecundity was strongly correlated to N content ($p<0.0001$), increasing ten times as the N content of the leaves doubled. Results for the bean treatment and oviposition rate were similar to the apple study (the highest rate reached for each experiment was on the third day). Total weight of female mites, total fecundity, and oviposition rates of deficient treatments decreased and was therefore positively correlated to the N content of the leaves ($p<0.01$). The overall results showed that weight, oviposition rate, and fecundity of the mites were higher on beans than on apples (female weight at the lowest N beans was higher than the highest N concentration on apples.) Insufficient bean data suggests further research must be done for accurate results, but the overall study concluded that N deficiency increased the pre-oviposition period and pre-imaginal development time, while decreasing female weight, fecundity, and oviposition rate.

Wermelinger and Delucchi (1990) again studied the two-spotted spider mite on individual leaf discs of apple trees. The host plants (36 seedlings) of Golden Delicious were

planted in 0.91 pots at the 5th leaf stage and fertilized with three different nitrogen levels. The plants were divided into three groups, treatment 'low N' (plain water), treatment 'medium N' (16 mM or 224 ppm) and treatment 'high N' (32 mM or 448 ppm). To ensure the quality of the plants, all received 1mM phosphorus (14 ppm) and 6 mM potassium (84 ppm) after two months. Young spider mite females were placed on leaf disks for each of the three treatments. From the generation of developing eggs that reached adulthood, one newly hatched female and one male was placed per disc. Offspring were subdivided into developmental stages, and if found dead, were replaced with new ones of the same development stage. Offspring sex-ratio, as well as mortality, female fecundity, and leaf nitrogen content were determined. Results showed that a slight increase occurred in the sex-ratio as well as female fecundity for 'high N' treatments. When nitrogen levels ranged between 1.8%-3.0% N, the sex-ratio increased from 0.64 to 0.76. In the first 7 days, mites from 'high N' treatment had a 23% sex-ratio than 'low N' treatments. A relationship between increasing fecundity and higher sex-ratio was also found in the highest N treatment.

Wermelinger et al. (1991) concluded his three part study with this final study focusing on host-plant nutrition. The host plant used was the same apple variety 'Glockenapfel' used in the previous studies, originated from one seed, and was shoot-propagated. Like the previous study (Wermelinger et al., 1985), nutrient solutions were based on the Hoagland solution (Epstein, 1972). The standards were 1N, 1P, and 1K (210 ppm N, 31 ppm P, and 235 ppm K). From this standard, four treatments were factored and formed (5 N or 1050 ppm, 1N or 210 ppm, 0.2 N or 42 ppm, and 0.04 N or 8.4 ppm). For each of the three nutrients (N, P, K), three different experiments were carried out. Each treatment consisted of eight plants, nutrient solutions were renewed monthly, and at the end of the experiment all apple trees were approximately 1.5 m high

(4 ft 11.05 in). Leaf disks (2 cm) were punched from the young apple leaves and 32 total female mite larvae were singularly placed on individual leaf disks for each of the treatments. Leaf disks were checked daily for egg production and mite survival. Eggs were removed daily and leaf disks renewed weekly. Results for the N study showed a significant difference between the control and the two deficiency treatments but only minor differences were found when including all the N treatments. Immature developmental time as well as the pre-oviposition period of the spider mites was prolonged in both deficiency treatments (0.2 N and 0.04 N). The period in which spider mites completed development per day (development rate) was positively correlated with leaf N content. At the lowest N deficiency rate, fecundity was reduced to one-tenth that of the control.

Chen et al. (2007) studied ivy geranium (*Pelargonium peltatum* (L.) 'Amethyst 96') and the two-spotted spider mite in response to six different combinations of nitrogen (2, 8, 16 , 24, and 32 mM (equivalent to 28, 112, 224, 336, and 448 ppm, respectively) and phosphorus (0.08, 0.32, 0.64, 1.28, and 2.56 mM (equivalent to 1.12, 4.48, 8.96, 17.92, and 35.84 ppm, respectively; conversion=1mM=14ppm). This study set out to prove that nutrient management can be used for pest suppression and suggests that any of the three macronutrients (N, P, and K) could benefit the plant health while reducing pest populations. The fertilizer combinations reflect actual ratios used in commercial situations. Based on an initial trial of all combinations, two nitrogen rates (8 or 24mM (equivalent to 112 or 336 ppm, respectively) and three phosphorus rates (0.32, 0.64, and 1.28 mM (equivalent to 4.48, 8.96, and 17.92 ppm, respectively) were used. Results showed that there was no difference of the number of mites per host plant or the amount of plant injury between the two different N fertilization rates. Phosphorus had no effect on the mite population levels until week 8 of the study. Week 8 results

showed that plants fertilized with 0.32 mM/ 4.48 ppm phosphorus were of lower quality (not saleable) than host plants fertilized with 0.64 mM/ 8.96 ppm and 1.28 mM/ 17.92 ppm. Further interpretation suggests that higher rates of phosphorus allow for better compensation for mite feeding injury. Phosphorus on ivy geranium can therefore have a positive effect with tetranychid mites within a certain concentration range and perhaps N or K with further exploration (Chen et al., 2007).

Wood and Reilly (2000) studied a combination of common cultural practices used in agricultural settings to induce different physiological states of pecan orchard trees [*Carya illonoinensis* (Wangenh.) K. Koch]. By doing so, they could then assess the prospective damage inflicted upon the trees by two key arthropod pests of pecan. Cultural practices tested included leaf nitrogen concentration, leaf-water status, and crop load. Black pecan aphid (BPA) and pecan leaf scorch mite (PLSM) were observed and recorded on their response to the three cultural practices. Water availability treatments for this study were either 'Low' (natural precipitation at 140cm/year) or 'High' in which trees received water by drip irrigation. Application schedules were based on average daily evaporation. Trees usually received 170 liters/day from September to October and less during May to August when rainfall was more common. Two different N concentrations were applied in the soil, in a rate of either 'Moderate' (3.15 kg per tree) or 'High' (moderate rate plus 1.47 kg per tree). A mechanical tree shaker was used to create the two treatments for crop load; 'Heavy Crop Load' (standard for a strong alternate-bearing cultivar) or 'Moderate Crop Load' (50% fruit removal when plant terminals exceeded 50%). Leaf N concentration, water availability, and crop load all influenced BPA foliar damage. However, high leaf N and high water availability reduced BPA feeding damage by 30% and BPA populations increased as crop load was reduced by 60%. Pecan leaf scorch

mite damage increased as leaf N concentration increased as well as when water availability increased, causing reduction in pecan leaflets. PLSM caused the greatest foliage damage when high N, high water status, and heavy crop load were combined. Mature trees also experienced greater leaf damage with high N concentrations. This outcome was previously studied on immature trees, but never on mature trees. Cultural practices can therefore influence the outcome of a crop; both positively and negatively and should be considered when developing an improved integrated pest management system.

Wilson et al. (1988) studied Pacific spider mite in a greenhouse environment using four different nitrogen rates that were applied to potted Thompson Seedless grape plants and in the form of ammonium sulfate (21% N), rates of 0.0, 2.0, 4.5, and 9.0 grams per pot were used. Applications were administered in three week intervals and began six weeks after planting. The 0.0 gram treatment was not effective and in order to keep the grape plants alive, the rate was increased to 1.0 grams after six weeks of the study had elapsed. Five replicates of each treatment were used the first year of the study (1985) and four replicates of each treatment were used the following year (1986). The study focused on leaf nitrogen and the position of the leaf in relation to *T. pacificus.* Results found that between lower, middle, and upper leaf position, the upper leaf position contained the highest amount of nitrogen and the lower position contained the least amount of nitrogen. In 1985, results showed that immature development time was shorter and fecundity, longevity, and the length of oviposition period were greater than in 1986. Although there were differences in immature survivorship as well as adult fecundity and adult longevity between the years, there was no significant difference among leaf position. In 1986, immature developmental time decreased ($p<0.05$) as leaf nitrogen increased. As well, adult fecundity

increased as leaf nitrogen increased ($p<0.05$). Adult longevity, immature survivorship, and the duration of oviposition were not affected by leaf nitrogen content.

English-Loeb (1990) is one study that did not find the same response to nitrogen as the above aforementioned studies. In this study, the fertility of the two-spotted spider mite was documented when feeding on bush beans induced with differing nitrogen concentrations. The nitrogen range consisted of zero to 160 ppm and was based off Hoagland's solution. The control therefore represented 105.0 N ppm. The other rates were based off of USDA recommendations and equivalent to half strength Hoagland. Plants assigned to the different ranges of nitrogen received 100 ml of water every six days so that each plant received equal amounts of water but differing amounts of nitrate. All plants were planted in sand and placed in growth chambers. A total of 30 bean plants were used in experiment 1 and had six different nitrogen rates (105.0 ppm, 73.5 ppm, 42.0 ppm, 15.7 ppm, 5.2 ppm, and 0.0 ppm), experiment 2 consisted of 40 bean plants; 20 plants in each chamber were assigned five different rates (157.5 ppm, 105.0 ppm, 52.5 ppm, 10.5 ppm, and 0.0 ppm), and the 3$^{rd}$ experiment consisted of 20 plants assigned to four different nitrogen treatments (105.0 ppm, 78.7 ppm, 31.5, and 0.0 ppm). The majority of all rates were below standard and so this study observed bean plants under nitrogen stress Results found a non linear response, in that the intermediate treatments performed better than the high and low nitrogen concentration treatments.

Nitrogen is more readily used for commercial and production settings even though potassium helps plants resist drought and stress caused by excessive temperatures. Potassium also aids plants in the production of starches, regulate stomata opening and closing, and can help plants resist disease. Potassium has the ability to regulate over 60 different enzyme systems in the plant and aids in the plants overall vigor. Potassium applied in adequate amounts has been

known to promote the growth of plant fibers, creating long and strong shoots. Willamette spider mites feeding in mid to late season have been known to cause yellowish bronzing and potential reddening and defoliation on red varietal grapes. Damage overtime causes a reduction in stomatal conductance. Potassium could therefore favor Willamette spider mites and their survival, by providing a disease free, drought resistant plant. Their feeding presumably does not affect the plants health as quickly as it would without K because of the ability to resist stress. Mite populations are therefore favored by this macronutrient.

## Materials and Methods

*Field Study*

For my field portion of the experiment, the study site was located in the Santa Maria Valley, California, at the Cambria Winery and Vineyards (Degrees Latitude: 34, Minute: 52, Seconds: 8.259, and Degrees Longitude: -120, Minute: 16, Seconds: 21.4068. The temperature for the month of July 2008 averaged 63.6º F (17.5º C) (the highest high at 86.4º F (30º C) and the lowest low at 48.8º F (9.3º C). There was no rainfall in July 2008, although rainfall during the rainy season (from November 2007 until July 2008) totaled 14.41 in (366 mm). The soil type at the site was a Pleasanton gravely fine loam soil, and vines were trained to a bilateral cordon and spur pruned, and trellised with a 1 ft (0.3 m) cross arm with two catch wires (a 'California Sprawl' system). The cultivar was 'Chardonnay' and vine spacing was 12 ft (3.6 m) between rows and 7 ft (2.1 m) between vines within the row.

The duration of this field research project was one complete growing season. Fifteen rows of 'Chardonnay' were used; five rows for the treatments, one buffer row between each treatment row, and three buffer rows on each of the outer ends. The experiment was established as a randomized complete block, with three treatments (nitrogen, potassium, and control) and five replications (Fig. 1). Each treatment row was a block containing one replication of each treatment. Treatment plots consisted of four vines, with two buffer vines between each plot. Treatments within each block began seven vines from the end of the row.

While the control treatment was left unfertilized, vines in the nitrogen treatment received a total of 2.89 lbs (1.31 kg) ammonium nitrate granular form (33.5-0-0) per vine, and was applied for the first three weeks of the experiment. Split into three applications of 0.96 lbs of

fertilizer each, this is the equivalent of 167.5 lbs N/ac (76.13 kg N/ha). Each potassium plot received a total of 3.89 lbs (1.76 kg) potassium (Voyager® Liquid Potassium (0-0-27) per vine. Split into three applications of 1.05 lbs fertilizer each, this is the equivalent of 121.5 lbs K/ac (55.2 kg/ha). Treatment applications began June 24th, 2008 and continued until July 9th, 2008 (once weekly). The only pesticide applied during the study was myclobutanil (Rally®) for control of powdery mildew (*Erisiphe necatar* Schwein.) at a rate of 4 oz/ac (280 g/ha) on June 26th and July 7th.

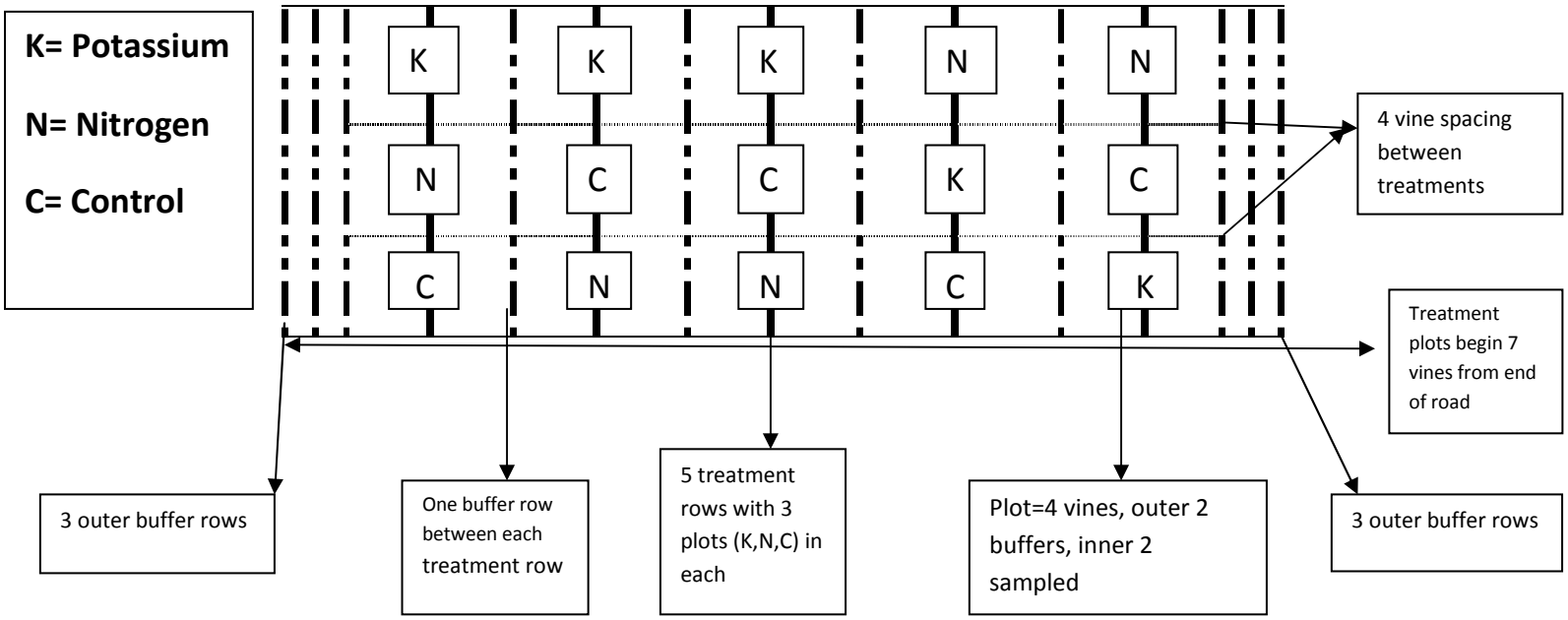


**Figure 1**. Experimental design for field study: Randomized complete block with five replications.

To estimate Willamette spider mite density, weekly leaf samples were taken from July 2 through September 10th. Sample leaves were removed from the low to mid region of the canopy and picked at random. Samples were taken from the inner two vines of the four vine treatments, taking 10 leaves from each plot as a sample. Samples were labeled and transported in brown paper lunch bags in an iced cooler and stored in a refrigerator on the Cal Poly, San Luis Obispo

campus. 50 petioles from each treatment were collected twice on July 16 and August 20. After being dried at 104ºF (40º C), both petiole samples from each date were packaged together and sent to Dellavalle Laboratories Inc. in Fresno, CA to get an estimate of concentration of both the nitrogen and potassium (Table 2).

Once at the lab, leaf samples were run through a mite brushing machine (Leedom Industries, Mi-Wuk Village, CA.) which removed most insects and mites onto a glass plate covered with a sticky substance. Willamette spider mites and their eggs as well as other insects and other mite species were accounted for and recorded weekly. Macmillan (2005) developed a protocol used to estimate the total number of mites per leaf by brushing 10 leaves at a time and counting the number of mites on the center of a grid pattern, representing about 20% of the glass plate area. The raw data was then run through a regression formula (y=[2.269x + 2.28]/10) to estimate average mites per leaf. By using this formula and the number of days the mites were sampled, I could then determine mite-days (multiplying the number of days by the number of mites per leaf). The mite-days for each week were added to mite-days from the previous week for an estimate of cumulative mite-days. Cumulative mite-days were analyzed by analysis of variance (Proc GLM, SAS Institute, 2003), using Tukey's Honestly Significant Different test for mean separation.

*Laboratory Study*

For my lab portion of the experiment, the study site was located in a greenhouse on the Cal Poly campus. The study vines were potted *Vitis vinifera* cv. 'Cabernet Sauvignon', cultivated in five gallon containers (24 containers total). I applied four nitrogen concentration treatments to a group of six vines using a 16-0-0 formulation (Metanaturals Grow® organic

nitrogen). The concentrations were: 1,500 ppm (1.5 ml fertilizer/160 ml water); 1,000 ppm (1.0 ml fertilizer/160 ml water); 500 ppm (0.5 ml fertilizer/160 ml water); and the unfertilized control (zero-50 parts per million of nitrogen applied). 200 ml of each treatment was applied twice weekly for the first month and every other day for the remainder of the study. I will refer to these treatments as High N, Med-High N, Med-Low N, and Low N. To maintain the vitality of the control treatment plants, I applied an all purpose multi-nutrient fertilizer (6-30-30, Monterey Maxi®) at a rate of 0.6g/1 liter water=10ppm. This was administered twice monthly by applying half a liter to all of the 24 plants (all treatments including control) (0.6g x 12 = 7.2 g/12 liters) and therefore divided the 7.2g by the 24 plants equally 0.3g per plant. I took petiole samples on December 8, 2008 for all N treatments including Low N (control) and sampled solely the Low N treatment on September 9, 2009. These samples were dried at 104ºF (40º C) and the petioles sent to Dellavalle Laboratories Inc., Fresno, CA to get an estimate of N concentration in the treatment plants (Table 4).

I established a Willamette spider mite colony from the aforementioned field site in the Santa Maria Valley, CA and placed females from the colony on ¾ in diameter (19mm) leaf disks punched out from plants of the four treatments. Watch-glasses were numbered 1N through 4N to represent the treatments, and the number within the parentheses represented the number of the watch-glass itself and therefore tracked the fertility of the mites. Leaf disks within each watch-glass were numbered 1 through 3. I placed the three numbered leafs disks on wet cotton in the watch-glass, and watered them daily, which maintained the vitality of the leaf disks for up to one week. Once the leaf disk lost its vigor, new leaf disks were cut out from the treatment plant located in the greenhouse, and mites from all developmental stages were carefully transferred to the new leaf disks. I recorded the incidence of molting, and used this information to estimate the

number of days to maturity and survivorship to the three post-larval stages (protonymph, deutonymph, and adult). I also recorded longevity and fertility for adult females. Once an adult female laid an egg on one of the four treatments, and the egg successfully molted into first larval stage (protonymph), the larvae was then carefully transferred to its own fresh leaf disk using a steady hand, a microscope, and a fine haired paint brush. Low N maturation data was insufficient for the first recorded trials, and so I continued the experiment with this Low N treatment the following year (September to October, 2009). I analyzed the data using analysis of variance (Proc GLM, SAS Institute, 2003), using Tukey's Honestly Significant Difference test for mean separation.

## Results

*Field Study*

Average mites per leaf were recorded and plotted on a line graph for each of the three treatments; showing the change in mite density over the course of the field study (Fig 2). All three treatments had two peak density levels (one of the two peaks being the maximum density for that treatment), before permanently declining. Average mites per leaf for the potassium treatment had an increasing mite population density starting July 2nd and continuing for the next three sample dates, before dropping on July 30th. On August 5th, the potassium treatment reached its maximum mite density level, followed by another sudden population drop the following week, before peaking again for the second time on August 20th. After this date, average mites per leaf continued to decline until the last sample date on September 10th. Average mites per leaf for the nitrogen treatment followed a similar pattern of that of potassium. Mite populations rose for the first four weeks, dropped for one week, and drastically climbed again on August 5th. In the nitrogen treatment, mite density peaked on August 5th and again on August 20th, at 84.6 and 87.0 mites per leaf, respectively. Mite density in the potassium treatment peaked on August 5th at 110.6 mites per leaf, and peak density in the control treatment was on August 5th at 71.0 mites per leaf. Although the control's mite numbers were more closely related to nitrogen, the control treatment was similar to potassium in that its maximum peak was on August 5th. However, overall, repeated measures ANOVA showed there was no significant difference among nitrogen, potassium and control treatments ($F=0.60$, $df=2, 8$, $p=0.57$).

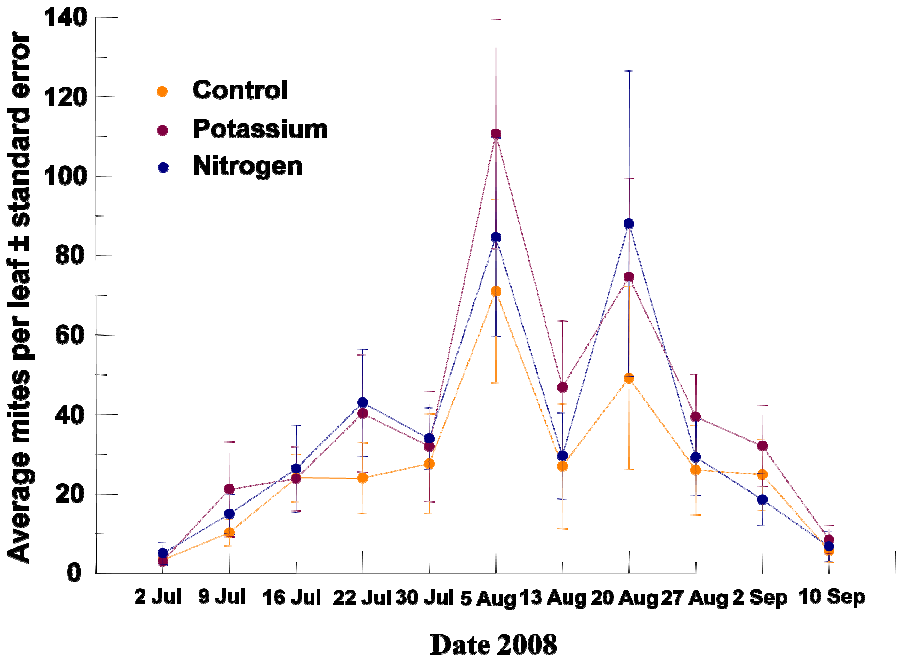


**Figure 2**. Average mites per leaf ± standard error by sampling date for nitrogen, potassium, and control treatments. Repeated measures ANOVA showed no significant difference among treatments, ($F=0.60$, $df=2, 8$, $p=0.57$).

Average mite eggs per leaf were recorded and plotted on a line graph for each of the three treatments; showing the change in mite egg density over the course of the field study (Fig 3). Mite egg density levels for all three treatments had one peak density level on August 5$^{th}$, and in addition, the control had a second peak on August 20$^{th}$. All three treatments began to permanently decline on August 27$^{th}$. The potassium treatment had the highest density amongst the three treatments (60.0 ± 18.0 mite eggs per leaf). The nitrogen treatments density peak was 42.5 ± 13.4 mite eggs per leaf. The control treatment had two peaks, and the lowest absolute egg density among the treatments. On Aug. 5 the control had a level of 28.2 ± 9.3 mite eggs per leaf, and on August 20$^{th}$, the control treatment egg density was 25.3 ± 15.0 mite eggs per leaf.

However, repeated measures ANOVA showed there was no significant difference among nitrogen, potassium and control treatments in egg density (F=1.44, df=2, 8, p=0.29).

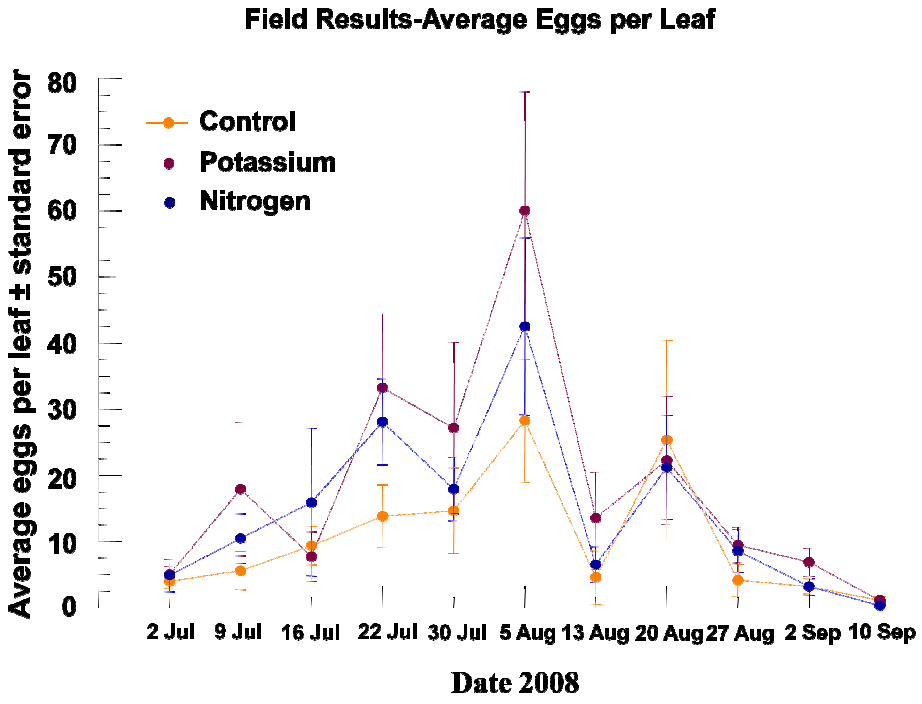


**Figure 3**. Average mite eggs per leaf ± standard error by sampling date for nitrogen, potassium, and control treatments. Repeated measures ANOVA showed no significant difference among treatments (F=1.44, df=2, 8, p=0.29).

In absolute figures, mean cumulative mite-days was highest in the potassium treatment, followed by the nitrogen and finally the control treatments (Table 1.) However, ANOVA showed no statistical difference in mean cumulative mite-days among treatments (F=1.14, df=2, 11, p=0.35)

| Treatment | Mean cumulative mite-days ± standard error |
|---|---|
| Nitrogen | 2619 ± 821 |
| Potassium | 2986 ± 774 |
| Control | 2020 ± 677 |
| ANOVA | P>0.05 |

**Table 1**. Cumulative mite-days for Willamette spider mite and corresponding ANOVA for nitrogen, potassium, and control treatments, field study 2008 (ANOVA F=1.14, df=2, 11, p=0.354).

| **Date** | **Treament** | **$NO_3$-N (ppm)** | **K (%)** |
|---|---|---|---|
| 7/16/2008 | N | 691 | |
| 7/16/2008 | K | | 2.6 |
| 7/16/2008 | Control | 11 | 2.1 |
| 8/20/2008 | N | 1428 | |
| 8/20/2008 | K | | 4.3 |
| 8/20/2008 | Control | 21 | 3.5 |

**Table 2.** Petiole nutrient analysis for field study. Nitrate-N increased approximately 68 fold. Potassium increased approximately 23%. A total of 50 petioles were removed from each treatment at each sample date.

*Laboratory Study*

Mean mite days to maturity, survivorship, longevity (days), and the number of eggs per female for each of the four treatments are shown in Table 2. For days to maturity, the High N (1500 ppm) and Low N (50 ppm) treatments were not significantly different from one another, nor were Med-High N (1000 ppm) and Med-Low N (500 ppm) different from each other. However, collectively the High N and Low N treatments took an average of 2.41 days longer to mature than the Med-High N and Med-Low N treatments ($F=8.76$, $df=3, 35$, $p=0.001$, Table 2). The highest absolute survivorship values were in the Med-High N and Med-Low N treatments at 55% and 54% respectively, compared to 43% for the High N treatment and 32% for the Low N treatment. However, ANOVA found no statistical difference among treatments in survivorship ($F=0.97$, $df=3, 78$, $p=0.41$). Adult mite longevity did not differ significantly among the High N, Med-High N and Med-Low N treatments, although collectively, adult females lived an average 9.12 days longer than the Low N treatment ($F=48.1$, $df=3, 11$, $p<0.0001$, Table 2). The pattern of oviposition from these four treatments mimicked that of days to maturity, in that the High N and Low N were not significantly different from one another and the Med-High N and Med-Low N treatments did not differ from each other. Collectively, females in the Med-High N and Med-Low N treatments laid an average of 12.8 more eggs than the High N and Low N treatments. Med-High N and Med-Low N treatments had a higher survivorship up until the developmental stage molting from deutonymph to adult. Thereafter, Med-High N and Med-Low N treatments had the quickest mortality rate once at adulthood.

| Treatment | Male and Female Days to Maturity | Male and Female Survivorship to Adult | Adult Female Longevity (Days) | Eggs per Female |
|---|---|---|---|---|
| High N (1500 ppm) | 9.50 ± 0.52 a | 0.43 ± 0.11 a | 11.00 ± 0 a | 6.00 ± 1.10 a |
| Med-High N (1000 ppm) | 7.09 ± 0.31 b | 0.55 ± 0.12 a | 15.33 ± 2.33 a | 16.67 ± 1.20 b |
| Med- Low N (500 ppm) | 7.58 ± 0.23 b | 0.54 ± 0.11 a | 13.80 ± 0.86 a | 16.00 ± 2.64 b |
| Low N (0-50 ppm) | 10.00 ± 0.97 a | 0.32 ± 0.11 a | 4.25 ± 0.25 b | 1.00 a |

**Table 3**. Mean Willamette spider mite days to maturity (n=39), percent survivorship to adulthood (n=39), adult female longevity in days (n=15), and oviposition (n=17) (± standard error of the mean) for the four nitrogen treatments. Means followed by the same letter are not significantly different (Tukey's HSD $p>0.05$).

| Date | Treatment | $NO_3$-N (ppm) |
|---|---|---|
| 12/8/2008 | High-N | 164 |
| 12/8/2008 | Med-High N | 123 |
| 12/8/2008 | Med-Low N | 102 |
| 9/9/2009 | Low N | 13 |

**Table 4.** Petiole nutrient analysis for lab study. A total of 50 petioles were removed for each treatment.

## Discussion

Although my field results found no significant difference in Willamette spider mite or egg density among control (unfertilized), potassium, and nitrogen fertilization, there was a suggestion of a response, given that mite density peaked highest in the potassium treatment, with a second peak highest in the nitrogen treatment. In addition, egg density peaked highest in the potassium treatment. And, in absolute figures, cumulative mite days was highest in the potassium and nitrogen treatments, although not statistically significant. My findings are consistent with other studies which have found nitrogen to have a positive influence on spider mite density. For example, Wood and Reilly (2000) studied cultural practices on pecan orchard trees including leaf water stress, crop load, and nitrogen leaf content. Although two important arthropod pests on pecans were analyzed, when nitrogen was at its highest concentration (4.62 kg $NH_4\ NO_3$ per tree) and combined with the highest water status treatment, Pecan leaf scorch mite caused the most foliar damage to the pecan trees. Wilson (1994) assessed the fitness of cotton for the development and reproduction of the two-spotted spider mite. This study found that as the plant develops and leaves age, nitrogen content within the leaves decline. The field preference test was able to document adult female mites and their feeding habits which significantly preferred younger leaf tissue over older leaf tissue. Mite developmental time and fertility had a linear relationship with nitrogen. As the nitrogen content of the leaves increased, mites developed faster and laid more eggs. This study however was able to show that mites are able to evaluate plant quality and change their behavior accordingly. However, this study differed slightly from a previous one by the same lead author (Wilson et al., 1988) with Pacific spider mite as the test mite. In this study, although the developmental time decreased as the rate

of nitrogen increased, mites laid fewer eggs as the nitrogen content increased (Wilson et al., 1988).

Despite the very large difference in nitrogen petiole concentrations between the nitrogen treatment and the control treatment in my field study (68-fold), this had a minor impact on Willamette spider mite density, only being noticeable at the population peaks, and was not statistically significant. The highest absolute Willamette spider mite and egg density was in the potassium treatment, and, although also not statistically different from the control, this is worthy of note given the high potassium concentration in the control treatment (the acceptable range is 1.5-3.0%), and therefore the potassium fertilizer I added to this treatment only increased the concentration of K by 23%. This suggests that Willamette mite is more sensitive to changes in K concentration than N. From my research, I found only one study that has documented a positive correlation with potassium content and spider mites. Fritzsche et al. (1980) reported that population density of *Tetranychus neocaledonicus* (Andre) on various cucurbit varieties increased when K content in the leaves increased. However, Suski and Badowska (1975) found a negative correlation between *T. urticae* rate of population increase on bean leaves and the potassium content of the leaves. In addition, Jesiotr et al. (1979) reported that good potassium supply induced a higher mortality rate in immature two-spotted spider mites. Potassium is currently on the market as a biopesticide (insecticide, fungicide, and miticide). Sil-Matrix® is soluble potassium silicate and is used to suppress Two-spotted spider mite and European red spider mite. The University Of Arkansas Division Of Agriculture sent out a recent brochure discussing spider mites on cotton in the midsouth and stated that growers with potassium deficient soils will be more prone to spider mite infestations.

Results from my laboratory study support other findings that spider mites respond to plant nitrogen concentration (van de Vrie et al., 1972; Suski and Badowska, 1975; Mellors and Propts 1983; Wermelinger et al., 1985; Wilson et al., 1988; Chen et al., 2007). My lab results showed a non-linear response of Willamette spider mite to grape plant nitrogen content. For two of the four parameters analyzed, days to maturity and oviposition, mites in the two mid-level N treatments showed significantly better performance than the high or low N treatments. And, although not statistically significant, the pattern of survivorship was the same as oviposition and days to maturity. One example of a linear response to nitrogen concentration is Wermelinger et al. (1985) who found that when leaf N content was reduced by 50%, two-spotted spider mites on apple leaves had a tenfold reduction in fecundity, the development time was prolonged by 2.6 days (30% higher), the pre-oviposition period was prolonged by 4 days (220% higher), and fertility as well as the rate of oviposition increased 10 times. The results of the 5 N (above standard) treatment did not differ from the 1 N treatment (control) except that the development time between treatments differed. Bean results showed that the threshold for 50% mortality/ longevity of 1 N concentration, mites lived 8 days longer than at 0.2 N and 0.04 N concentration treatments (deficiency treatments). Oviposition and longevity slightly decreased under deficiency treatments as well as above standard N recommendations (5 N treatment). This study differed from my study because total N concentrations were compared where as my study looked at nitrate-N concentrations. There were also differences in the spider mite species used as well as the host plant. Legumes are known for their ability to fix nitrogen within the soil because of a symbiotic relationship with rhizobium bacteria which is found in the root nodules of bean plants. The above standard treatment used in this experiment (5 N) equates to 1050 ppm in which the nitrogen tissue concentration was recorded in total percent nitrogen form. Although this amount

is acceptable for comparison with my lab study because it falls between my Med-High N and High N treatments (1000-1500 ppm), my highest N concentration fell short of its intended range at164 ppm. English-Loeb also conducted an experiment in 1990 that worked with bush beans and the two spotted spider mite. In order to find the average number of eggs produced, the number of offspring at the end of the experiment was calculated by the number of days females were recorded (present and living). Results found a non linear response in that the fertility was highest at the intermediate N levels. This is a direct comparison to my lab results because I also found a non linear response in that my two intermediate treatments for days to maturation and oviposition parameters performed significantly better than my High and Low N treatments.

A negative response to plant nitrogen content by two-spotted spider mite was documented by Chen et al. (2007). The six combinations of N fertilization rates that were later condensed to two combinations were found to not significantly differ from one another with respect to spider mite response. All ivy geranium plants were found to be saleable, and showed that two-spotted spider mite had no direct correlation to the content of nitrogen. However, this study lacked a strong nitrogen gradient range because both N concentration treatments (112 ppm and 336 ppm) differed only by 224 ppm. Even though this study focused on rates below standard recommendations for potted ivy geranium (nutrient deficient), on the whole it is hard to assess and compare our results because only two levels of nitrogen were used. Chen et al. (2007) intended to have low nitrogen values, where as in my study I did not.

Another study that found a negative correlation with nitrogen concentration levels was Wilson et al. (1988), who studied Pacific spider mite and increasing foliar N in Thompson Seedless grapes. Out of all nutrient lab studies researched, this is the only study I found which used *Vitis vinifera* as a host plant. Within their study, mean % N categories consisted of five

ranges (2.0, 2.4, 2.9, 3.5, and 4.2% N). As nitrogen content increased, developmental time decreased and fertility increased. Although both parameters showed a positive response, it's interesting to note that the results showed that the lowest N performed better than the highest N category for immature development. For the other parameters within the study, there was no response because no significant relationship between foliar N and female longevity, immature survivorship, or ovipositional duration was found. In my lab findings, days to maturity and the number of eggs laid had a strong correlation with nitrogen. Wilson et al. (1988) and my study are similar in that both our mite development times had a positive response to nitrogen. However my results also found survivorship and oviposition to perform best in the two medium N treatments while Wilson et al. (1988) found no significant difference in these parameters. This study like Chen et al. (2007) analyzed plant N in total nitrogen concentration, whereas my study looked at nitrate-N concentrations.

Wermelinger and Delucchi (1990) studied Two-spotted spider mite and the effects different leaf N concentrations had on the mite sex ratio on apple plants. Results showed a linear response among the three nitrogen rates and all treatments significantly differed from one another. However there was no significant difference among the three treatments and sex ratio. As well, the ovipositional rate of the high N treatment significantly differed from medium N and low N treatments. There was a slight correlation between the high nitrogen content treatment and female fecundity, and an even greater correlation between female fecundity and sex-ratio. My study like Wermelinger and Delucchi (1990) showed that fertility (eggs laid per female) had a positive response to nitrogen content.

Wermelinger et al. (1991) continued his study (1985, 1990) with Two-spotted spider mites and apples trees. This study however focused on all three macronutrients (N, P, and K).

Because the leaves varied according to the different nutrient solutions, variations of the specific elements affected the leaves differently. The nutritional effects on the spider mites can therefore not be credited completely to one specific nutrient. For the nitrogen portion of the experiment, deficiency treatments significantly differed from control but when including excess nitrogen (5 N), minimal differences occurred. The immature developmental time and the pre-oviposition period were prolonged in both N deficiency treatments. There was also a positive linear response with developmental rate (the portion of development completed in one day) and oviposition rate with N content. Fertility produced the best results with 1 N (standard) and longevity was not affected. This is similar to my lab study because development time and oviposition had a negative response to low levels of nitrogen. Within my results, longevity was also not affected. However, High N, Med-High N, and Med- Low N treatments collectively lived an average of 9.12 days longer than my unfertilized or deficient Low N treatment.

Chow et al. (2009) analyzed Two-spotted spider mite density after fertilizing roses with standard vs. reduced amounts of nitrogen, and found mites and their eggs were twice as dense on roses fertilized with 100% (150 ppm) compared to lower nitrogen concentrations (33% N or 50 ppm and 50% N 75 ppm). Although my nitrogen rates did not reach an acceptable range to compare with my field study, they are similar to this study in that my two medium treatments laid more eggs as well as matured and survived much longer than my lower nitrogen treatment.

Is it possible that a nutrient limit has yet to be reached for the above studies that had a linear response to nitrogen? We can suggest this because arthropods especially spider mites are known to reach a maximum density threshold in which they peak and begin to decline when nutrient levels get too high. Although other factors such as environment (temperature, and water availability) must be taken into account as well as cultural practices, we can somewhat assume

that these studies have yet to reach the capacity in which spider mites begin to be adversely affected by the applied nutrients.

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