Effect of Grape-Leaf Potassium Content on Life-History Traits of Willamette Spider Mite, Eotetranychus willamettei (McGregor) (Acari: Tetranychidae)

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Abstract

Vitis vinifera L. cv. Chardonnay vines were fertilized to approximate baseline, low, medium, and high grape-leaf potassium contents, relative to viticultural target values. From those vines, twelve replicates of leaf-discs were made per fertilizer treatment. The leaf-discs were populated with individuals of Willamette spider mite, *Eotetranychus willamettei* (McGregor) and maintained in a plant growth chamber (25°C, 14L:10D h). Data pertaining to maturation time (days), pre-oviposition period (days), lifetime fecundity (eggs / ♀), and longevity (days) of *E. willamettei* was recorded daily. Petiole analysis revealed that the grape-leaf potassium contents were greater than target values. Respective to fertilizer treatment, the actual grape-leaf potassium contents were: 3.43%, 6.28%, 7.39%, and 8.06%. Treatment differences in mean values of maturation time, pre-oviposition period, lifetime fecundity, and longevity of *E. willamettei* were tested for significance by one-way analysis of variance, alpha = 0.05 and were short of statistical significance. However, novel information about several life-history traits of *E. willamettei* was recorded (mean ± SE): maturation = 6.7 ± 0.2 days, pre-oviposition period = 1.5 ± 0.2 days, fecundity = 37.3 ± 3.5 eggs per adult female, and longevity = 22.3 ± 1.7 days.

Keywords: pest, arthropod, reproduction, cultural control, habitat modification

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Vitis vinifera L. is cultivated worldwide for table grapes, raisins and wine (Owens, 2008). In commercial vineyards, fertilization is standard practice. There are thirteen nutrients required for vine and grape development, but generally, nitrogen and potassium are supplied in the greatest quantities, because they are commonly deficient in soil-reserves and significant amounts are removed with grapes at harvest (Brase, 2007). In vineyards, potassium content varies throughout the growing season, so that by harvest, grape berries contain more than half of the total vine-potassium. Late in the season, the range of target values for leaf-potassium content is 1.5-2.0% (Walter-Peterson, 2011).

Although fertilization is needed to support optimal crop development, it also modifies the resources available to pest organisms by altering plant-quality. Examples showing the effects of plant nutrition on pests are available in recent publications (Hofmeester, 1992; Chau, Heinz, & Davies, 2005; Veresoglou, Barto, Menexes, & Rillig, 2013; Veromann *et al.*, 2013), and various effects on vineyard pests have been documented; however, information about how potassium fertilization effects pest mites in vineyards is limited.

Three species of spider mites (Acari: Tetranychidae) cause damage to grape production in California. In order of economic significance, they are: Pacific spider mite, *Tetranychus pacificus* McGregor, Willamette spider mite, *Eotetranychus willamettei* (McGregor), and Two-spotted spider mite, *Tetranychus urticae* Koch. Crop damage due to *T. pacificus* is prevalent in the San Joaquin Valley. Damage from *E. willamettei* generally occurs in coastal and foothill regions, and *T. urticae* rarely damages grape production in California (Bentley *et al.*, 2006).

Generally, spider mites are responsive to changes in leaf nutritional quality. Results of previous studies on how mites respond to plant nutrition imply that crop damages from mite feeding can be mitigated by adjusting fertilizer applications (Sudoi, Khaemba, & Wanjala, 2001; Chen *et al.*, 2007). My goal was to detect whether grape-leaf potassium content affects certain reproductive traits of *E. willamettei* and to determine specific grape-leaf potassium contents that could be used for the integrated management of *E. willamettei* in vineyards.

Literature Review

McNally and Farnham (1985) presented research and a literature review showing that *E. willamettei* can cause significant damage to grape production in California. They established a method of estimating whole-shoot mite densities from mid-shoot leaves, and they confirmed that *E. willamettei* causes economic injury (at ≥50.5 mites per leaf) to Chenin blanc and Zinfandel cultivars of *V. vinifera*. In their research, McNally and Farnham showed that *E. willamettei* feeding decreased grape sugar content even when yield and cluster weight were unaffected.

Research has demonstrated that *E. willamettei* can reduce vine photosynthetic capacity (Welter, Farnham, McNally, & Freeman, 1989), as well as grape soluble-solids content, berry size, and yield (Welter, McNally, & Farnham, 1989). Although, previous experiments found that *E. willamettei* did not effect yield, Welter, McNally, and Farnham (1989) showed that decreases in yield were detectable in a multiple-season trial. Effects on yield varied with canopy structure, environment and cultivar, but reductions in photosynthetic capacity caused by *E. willamettei* were similar to those caused by *T. pacificus* (Welter, Farnham, McNally, & Freeman, 1989).

Later, Welter, Freeman, and Farnham (1991) re-evaluated the economic injury level for *E. willamettei* on perennial crops. Data collected from successive seasons indicated that vine injury from *E. willamettei* persisted for two-years, and accordingly, Welter *et al.* (1991)

recommended that the economic injury level for *E. willamettei* should account for losses that occur during crop recovery. They presented two models to illustrate the hypothetical influence of delayed recovery on economic injury levels.

Karban and English-Loeb (1990) found that inoculating vines with *E. willamettei* reduced subsequent populations of *T. pacificus*. They did not observe an effect on yield, however, soluble-solid content was higher in grapes from inoculated vines.

In a follow up study, Hougen-Eitzman and Karban (1995) compared the effects of early-season vs. late-season inoculations, and they found that only early-season inoculations reduced subsequent populations of *T. pacificus*. In addition, the results of Hougen-Eitzman and Karban (1995) supported the hypothesis that decreases in populations of *T. pacificus* did not result from resource competition but from systemic host-plant resistance induced by *E. willamettei* feeding.

Rodriguez (1953) introduced a method of using detached leaf culture (leaf-discs) to study how phytophagous mites respond to host-plant nutritional quality. Variations of this method are still in use today, though, Xu, Chen, Chen, and Chen (1996) recently modified the method by using a vacuum desiccator to suffuse leaf-discs with nutrient solution. The method proposed by Xu *et al.* (1996) notably supersedes time-intensive work of fertilizing leaf-disc donor plants.

Using detached leaf culture, phosphorus was positively associated with oviposition in *T. urticae* (Rodriguez, 1954) and population densities of *Tetranychus telarius* (L.) (Rodriguez, 1958). Host-plant nitrogen uptake was positively correlated with counts of *Panonychus ulmi* (Koch) and *T. telarius* (Rodriguez, 1958), and later, with the fecundity and population density of *T. urticae* (Rodriguez, Chaplin, Stoltz, & Lasheen, 1970).

Kielkiewicz and van de Vrie (1990) collected data from *T. urticae* on geranium plants to determine their effect on host-plant nitrogen content. Results depended on plant variety and leaf-

tissue age, but Kielkiewicz and van de Vrie (1990) demonstrated that mite feeding primarily depleted nitrogen content of young leaves rather than old leaves.

Wermelinger, Oertli, and Baumgärtner (1991) showed that host-plant nitrogen deficiency can severely limit the population increase of *T. urticae*, and they provided an informative review about the effects of other nutritional components on mites. Research included in that review showed that host-plant phosphorus was variously associated with mite survival and population growth (Wermelinger *et al.*, 1991).

Leaf potassium content increased population densities of *Tetranychus neocaledonicus* André (Sharma & Pande, 1986) and *E. willamettei* (Geddes, 2010). However, Rodriguez (1951) found that potassium uptake could correlate negatively or positively to mite population. Tulisalo (1971) showed that potassium deficiency can enhance the fecundity of *T. urticae*, while Suski and Badowska (1975) found that leaf potassium contents correlated negatively to the intrinsic rate of population increase (R_m) for *T. urticae*. Wermelinger *et al.* (1991) reported lower R_m where potassium supply was excessive or strongly deficient.

Wilson, Smilanick, Hofman, Flaherty, and Ruiz (1988) reported that *T. pacificus* responded to increased foliar nitrogen with enhanced fecundity and shortened developmental time. Also, Wilson (1994) presented a study of *T. urticae* on cotton that indicated a nutritional advantage for mites with nitrogen-rich diets. In the study, young leaves measured significantly higher in nitrogen content, which was correlated positively with fecundity and negatively with developmental time, and female mites tended to prefer young leaves for feeding and oviposition.

Sudoi *et al*. (2001) showed that nitrogen fertilization can be adjusted to mitigate yield losses that result from spider mite damage. Chen *et al*. (2007) found that nitrogen fertilization

had no effect on mite density, but they noted that plants fertilized with high rates of phosphorus compensated better for mite damage.

Materials and Methods

Vitis vinifera L. cv. Chardonnay vines, were obtained from Duarte Nurseries, Inc. (Hughson, CA, USA) and transplanted to steam-sterilized soil in two-gallon pots. Once transplanted, the vines were moved to a greenhouse where they were maintained for the duration of the experiment. Soil moisture was assessed every other day, and in general, irrigation was provided three times per week at approximately 1200mL per plant.

Four plants were randomly assigned to each treatment using the random-number function in a Microsoft Excel® (2004) spreadsheet. According to the assigned treatments, vines were fertilized with a complete fertilizer and progressive concentrations of soluble potassium to approximate baseline, low, medium, and high grape-leaf potassium contents.

Rates of fertilization were selected according to viticultural target values. For one month, all vines were fertilized three times per week with 200mL of a complete fertilizer (24-8-16) at a low concentration (5.66g fertilizer/15L solution). For three months following, select vines were additionally fertilized three times per week with 200mL of a soluble potassium fertilizer (27% K_2O derived from potassium carbonate) at: 0.5% K, 1.5% K and 3.0% K, respective to assigned treatments. After four months of fertilization, leaf-discs were prepared for the experiment.

When the leaf-discs were made, petiole samples were taken per treatment and sent to Dellavalle Laboratory, Inc. (Fresno, CA, USA) for an analysis of leaf potassium contents. At Dellavalle Laboratory, the petiole samples were manually ground and digested overnight in nitric-acid and hydrogen peroxide, and the digested material was filtered and then analyzed using inductively coupled plasma (ICP) spectrometry.

Mites were obtained from Chardonnay vines at Cambria Estate Winery (Santa Maria Valley, CA, USA) where leaves showing signs of mite damage were removed, put in sealable plastic bags (with paper towels) and later stored in a refrigerator. The leaves were examined with a dissecting microscope, and mite infested leaves were used to establish a colony on Chardonnay vines kept in greenhouse cages. Dr. Michael Costello (California Polytechnic State University, San Luis Obispo, CA, USA) confirmed that the collected species was *E. willamettei*, and eggs from the colony were used to populate leaf-discs for the experiment.

To prepare the leaf-discs, fully developed leaves (approximately fifth from terminal) were removed and placed in labeled paper bags. Per treatment, leaves were randomly selected from the bags, and 19mm discs were cut using a cork borer. Twelve replicates were made for each treatment.

The leaf-discs were placed on moistened cotton balls in watch glasses. Multiple eggs were put on each leaf-disc, and all watch glasses were maintained in a plant growth chamber (25°C, 14L:10D h). Moisture in the cotton was maintained daily, and the eggs on the leaf-discs were monitored twice per day until all leaf-discs were populated. Surplus eggs were removed when at least one larval mite was observed, and if multiple mites were present on the same leaf-disc, one mite was left and all others were transferred to leaf-discs within the same treatment.

The leaf-discs with mites were maintained in a plant growth chamber (25°C, 14L:10D h) and monitored daily. Mite development was recorded at hatch and ecdysis by date. Egg casings and molts were removed to avoid erroneous records, and all mites were observed until their death. Eggs from adult females were counted and monitored until hatch, at which time, the egg casings were removed along with second-generation mites.

Data pertaining to maturation time (days), pre-oviposition period (days), lifetime fecundity (eggs / ♀), and longevity (days) were recorded per treatment, and differences in treatment means were tested for significance by one-way analysis of variance, alpha = 0.05 using the analysis tool, Anova: Single Factor, provided in Microsoft Excel® (2004) software. The analysis of variance for each life-history trait indicated that pairwise comparisons would not be appropriate.

Differences in mean values of male longevity and female longevity were tested for significance by one-way analysis of variance, alpha = 0.05 using the analysis tool, Anova: Single Factor, provided in Microsoft Excel® (2004) software. The possibility of bias caused by including male data was tested by comparing means plus or minus one standard error.

Assignment of fertilizer treatments and selection of leaves for leaf-disc preparation were randomized to support an assumption of independence of errors.

Because the treatment effects were small, homogeneity of variance was assumed. However, to test this assumption, the highest calculated variances were compared to the lowest variances per life-history trait, (max-s^2, min-s^2) and when possible, quotient values of maximum and minimum variance (max-s^2/min-s^2) were calculated.

Additivity of treatment effects was assumed, as indicated by results of previous studies of nutritional effects on phytophagous mites. However, to prevent density-dependent treatment variation, larval hatchlings were removed once recorded, so that the number of mites on each leaf-disc was generally limited to one adult plus larval hatchlings between observations.

Results

As measured by ICP spectrometry, the experimental range of grape-leaf potassium contents was substantially higher than the viticultural target range (1.5%-2.0%) suggested by

Walter-Peterson (2011). Respective to fertilizer treatment, the actual grape-leaf potassium contents were: 3.43%, 6.28%, 7.39%, and 8.06%.

On average (mean ± SE), longevity of *E. willamettei* males (11.8 ± 1.7 days) was shorter than that of females (24.4 ± 1.9 days) ($F_{1, 34} = 8.750$, $P = 0.006$, Figure 1), and as a result, longevity statistics that included data from males were negatively skewed. Since the number of males per treatment was unequal, exclusion of male data was considered. However, inclusion of male data did not appear to significantly skew longevity statistics (Figure 2), and since the difference in maturation time between *E. willamettei* males and females also lacked statistical significance ($F_{1, 32} = 0.013$, $P = 0.909$), no data from males was excluded.

The frequency distribution of *E. willamettei* maturation data indicated a normal distribution (Figure 3). Average (mean ± SE) maturation time was shortest (6.6 ± 0.2 days) in the low potassium (0.5% K) treatment and longest (6.9 ± 0.4 days) in the high potassium (3.0% K) treatment; however, treatment differences in mean values of maturation time were not statistically significant ($F\text{-}critical_{3,30} = 2.922$, $F_{3,30} = 0.115$, $P = 0.950$, Table 1). The null hypothesis regarding maturation time data, H_0 = no treatment differences in maturation time, was not rejected.

Evidence supporting an assumption of normal distribution was lacking in *E. willamettei* pre-oviposition period data; however, as an alternative non-parametric method, the Kruskal-Wallis Test was considered inappropriate due to the number of repeated ranks entailed. Instead, a normal distribution was assumed for a one-way analysis of variance. The average (mean ± SE) pre-oviposition period was shortest (1.0 ± 0.0) in the low potassium (0.5% K) treatment and longest (1.6 ± 0.4) in the medium potassium (1.5% K) treatment; however, treatment differences in mean values of pre-oviposition period were not statistically significant ($F\text{-}critical_{3, 23} = 3.028$,

$F_{3, 23} = 1.274$, $P = 0.307$, Table 1). The null hypothesis regarding pre-oviposition period data, H_0 = no treatment differences in pre-oviposition period, was not rejected.

Distribution of *E. willamettei* lifetime fecundity data in a stem-and-leaf plot indicated a normal distribution (Figure 4). Average (mean ± SE) lifetime fecundity was greatest (44.7 ± 8.3) in the medium potassium (1.5% K) treatment and least (26.0 ± 5.8) in the high potassium (3.0% K) treatment; however, treatment differences in mean values of lifetime fecundity were not statistically significant ($F\text{-}critical_{3, 23} = 3.028$, $F_{3, 23} = 1.452$, $P = 0.254$, Table 1). The null hypothesis regarding lifetime fecundity data, H_0 = no treatment differences in lifetime fecundity, was not rejected.

Distribution of *E. willamettei* longevity data in a stem-and-leaf plot indicated a normal distribution (Figure 5). Average (mean ± SE) longevity was longest (23.1 ± 4.1) in the medium potassium (1.5% K) treatment and shortest (20.8 ± 3.6) in the high potassium (3.0% K) treatment; however, treatment differences in the mean values of longevity data were not statistically significant ($F\text{-}critical_{3, 32} = 2.901$, $F_{3, 32} = 0.084$, $P = 0.968$, Table 1). The null hypothesis regarding longevity data, H_0 = no treatment differences in longevity, was not rejected.

Quotient values for the maximum and minimum variance (max-s^2, min-s^2) of pre-oviposition period data (1.7, 0) could not be computed due to a zero value; however, variance quotients (max-s^2/min-s^2) for maturation time (1.3/0.3 = 4.67), lifetime fecundity (487.6/139.8 = 3.5), and longevity (154.9/83.6 = 1.9) were close to one, and therefore did not indicate a violation of assumed homogeneity of variance.

Discussion

Vines that were fertilized with the high potassium (3.0% K) treatment showed symptoms of toxicity and decreased vigor. However, life-history traits of *E. willamettei* were unaffected by

differences in grape-leaf potassium content within the experimental range. Hanna, Wilson, Zalom, Flaherty, and Leaviti (1996) previously reported that *E. willamettei* was less responsive than *T. pacificus* to differences in vine vigor. Yet, results showing the effect of macronutrient content on related mite species suggest a different response. For example, Rodriguez (1958) showed that phosphorus content correlated positively with counts of *Tetranychus telarius* (L.) below 0.20% but negatively when greater than 0.20%, and similarly, Wermelinger *et al.* (1991) reported lower R_m where potassium supply was either strongly deficient or excessive.

Furthermore, along with excessive leaf potassium content, other changes in host-plant nutritional quality are likely. In earlier nutritional studies, the influence of potassium on availability of other nutrients was considered. Rodriguez (1951) showed that excessive potassium supply interfered with plant uptake of magnesium, calcium, boron, and manganese, and Wermelinger *et al.* (1991) emphasized that, "herbivore performance is more likely [a] response to the overall physiological state of the plant".

A lacking response of *E. willamettei* to a range of leaf potassium contents that includes toxic levels for an important host-plant hints at phenotypic plasticity or adaptation. However, the actual mechanism resulting to such phenotypic plasticity or adaptation is unclear. Since typical leaf potassium contents for other host-plants are unknown, the frequency that *E. willamettei* has been exposed similar leaf potassium contents cannot be estimated, and as a result, even a simple model of natural selection would be speculative. Instead, the author suggests that an analogous experiment having less variance within treatments is needed before phenotypic plasticity or adaptation is assumed.

Although the results of this experiment were not statistically compared to previous data, mean values of the pre-oviposition period and longevity of *E. willamettei* were consistent with

life-history information developed by Stavrinides and Mills (2011); average lifetime fecundity was found to be slightly higher than indicated (Stavrinides & Mills, 2011), and information regarding the maturation time of *E. willamettei* was not previously available.

Conclusion

Research on *E. willamettei* is warranted by the continued economic importance of grape production in California along with the demonstrated potential of *E. willamettei* to cause damage to that industry. Future studies regarding the effect of leaf nutrition on *E. willamettei* could utilize the method introduced by Xu *et al*. (1996) to better manage leaf-disc potassium contents and could limit the experimental range of grape-leaf potassium content to viticultural target values or levels of deficiency, since leaf potassium contents that are relevant for the integrated management of *E. willamettei* are unlikely to be discovered at levels greater than viticultural target values, *i.e*. 1.5-2.0%. Whenever feasible, quantitative data concerning the interactions of potassium fertilizer with other components of mite nutrition should also be considered.

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Tables

Table 1

Leaf-potassium contents and associated mean values (mean ± SE) of maturation time, pre-oviposition period, lifetime fecundity, and longevity for E. willamettei reared at 25°C

Treatment	Leaf	Mite			
Fertilizer (%)	K (%)	Maturation (days)	Pre-ovip. (days)	Fecundity (eggs / ♀)	Longevity (days)
3.0 K	8.06	6.9 ± 0.4 [8]	1.6 ± 0.4 [7]	26.0 ± 5.8 [7]	20.8 ± 3.6 [9]
1.5 K	7.39	6.7 ± 0.4 [9]	2.0 ± 0.5 [7]	44.7 ± 8.3 [7]	23.1 ± 4.1 [9]
0.5 K	6.28	6.6 ± 0.2 [8]	1.0 ± 0.0 [7]	39.0 ± 6.9 [7]	22.4 ± 3.7 [9]
24-8-16	3.43	6.7 ± 0.3 [9]	1.5 ± 0.3 [6]	39.8 ± 4.8 [6]	22.9 ± 3.0 [9]
All	//	6.7 ± 0.2 [34]	1.5 ± 0.2 [27]	37.3 ± 3.5 [27]	22.3 ± 1.7 [36]

Note. Sample size is provided in square brackets. Differences in mean values were tested by one-way analysis of variance and were short of significance, alpha = 0.05%.

Figures

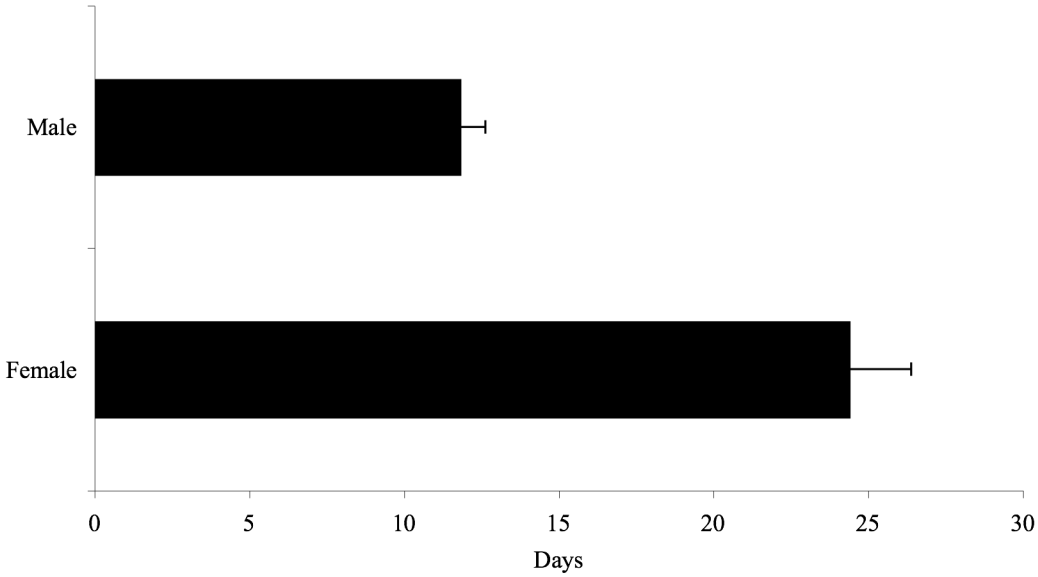

Figure 1. Bar chart comparing mean values of male vs. female longevity for *E. willamettei*

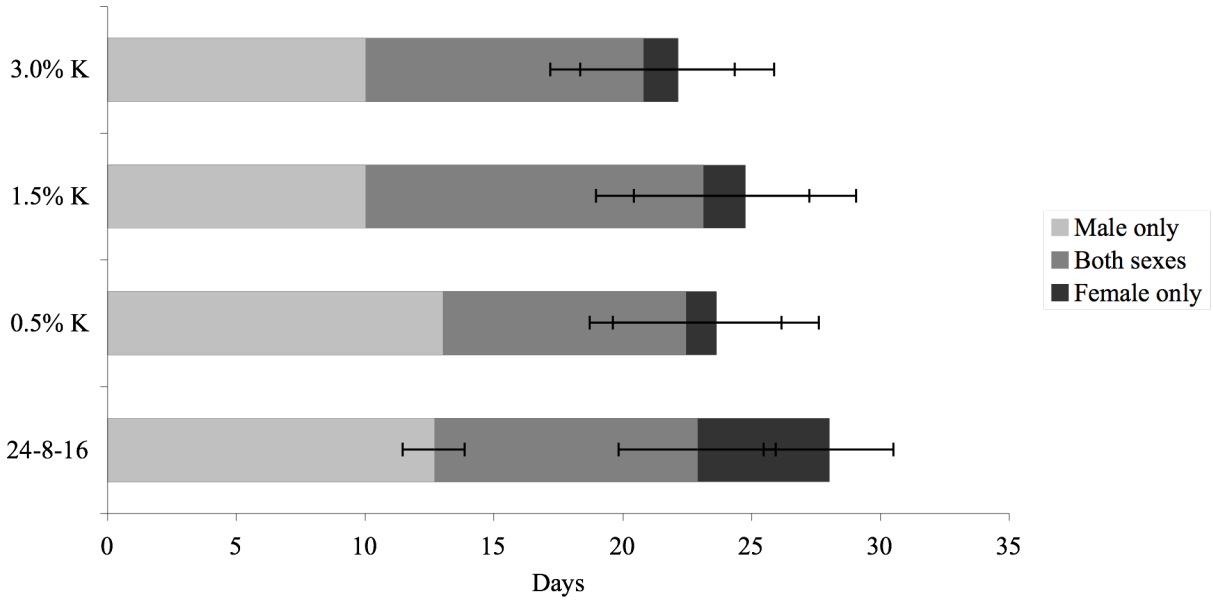

Figure 2. Bar chart comparing mean values of sex-based longevity vs. collective longevity for *E. willamettei* per fertilizer treatment

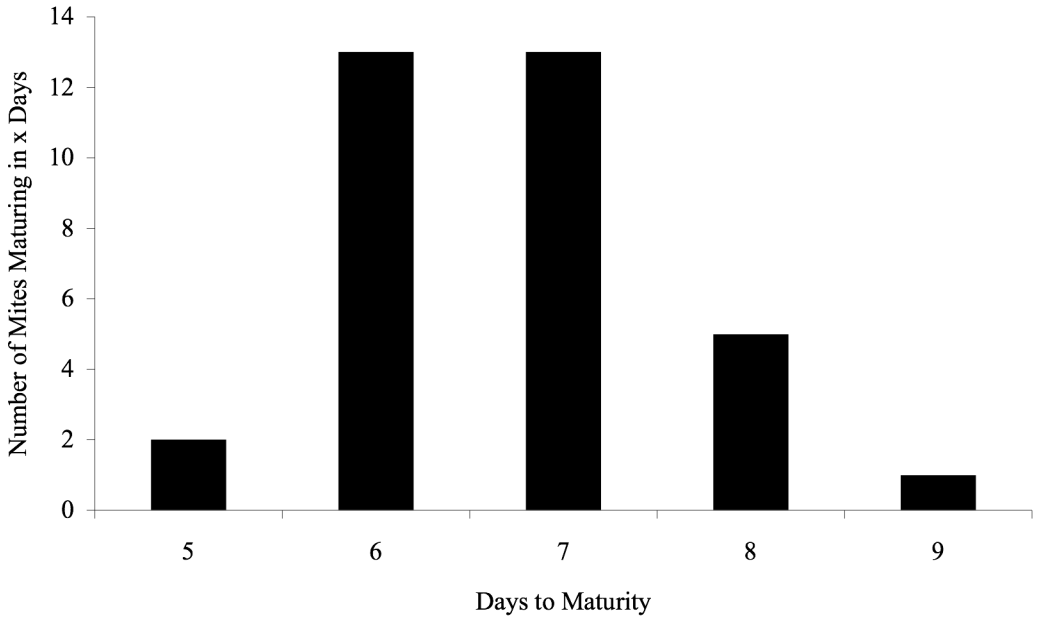

Figure 3. Bar chart showing the frequency of *E. willamettei* maturing in *x* days (n=34)

2	0	56
2	1	23
4	2	2239
9	3	345556799
4	4	1569
3	5	336
2	6	66
1	7	7

Figure 4. Steam-and-leaf plot showing number of eggs laid per *E. willamettei* female (n=27)

3	0	178
11	1	00122235567
11	2	00012556799
9	3	001245556
2	4	02

Figure 5. Stem-and-leaf plot showing number of days lived per *E. willamettei* individual (n=36)