

## ABSTRACT

Title of Thesis:       BOTTOM UP EFFECTS OF NUTRIENTS AND WATER ON  
                              BLACK VINE WEEVIL *OTIORHYNCHUS SULCATUS*  
                              (COLEOPTERA: CURCULIONIDAE) AND *HEUCHERA*  
                              *MICRANTHA* “PALACE PURPLE” (SAXIFRAGACEA)

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This thesis investigated: a) the effect of fertilization and water regimes on chemistry and growth of *Heuchera micrantha* and the effect of plant quality on black vine weevil performance b) the effect of black vine weevil larvae on *Heuchera micrantha* growth and c) the effect of fertilization on tolerance of *Heuchera micrantha* plants to different densities of black vine weevil.

I found a positive effect of nitrogen fertilization and water on plant chemistry and growth. Leaf nitrogen, carbon: nitrogen ratio, and phenols were strongly affected by nitrogen fertilization.

Fertilization had no effect on adult and larval survivorship, ovipositional period or feeding preference of black vine weevil adult. Larvae had a strong effect on top and root biomass and this effect increased with increasing levels of fertilizer when water was not limited. Fertilizer did not increase tolerance of *Heuchera micrantha* to adults or larvae of black vine weevil.

BOTTOM UP EFFECTS OF NUTRIENTS AND WATER ON BLACK VINE WEEVIL  
*OTIORHYNCHUS SULCATUS* (COLEOPTERA: CURCULIONIDAE) AND  
HEUCHERA MICRANTHA “PALACE PURPLE” (SAXIFRAGACEA)

by

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## **CHAPTER I**

### **EFFECT OF NITROGEN FERTILIZATION AND WATER REGIMES ON *HEUCHERA MICRANTHA* (ROSALES: SAXIFRAGACEAE) AND *OTIORHYNCHUS SULCATUS* (COLEOPTERA: CURCULIONIDAE)**

#### **Introduction**

In nursery production systems, nitrogen is a key nutrient. For container grown crops, growers apply slow-release nitrogen and supplementary nitrogen at rates of 150 ppm N/week (Davidson et al. 2000). This is considered a necessary practice for increasing plant growth rates and shortening the interval from planting to the time of sale. In addition, nitrogen fertilization has been applied in nursery production as a common strategy for increasing resistance of plants to pests (Smith 1978, Funk 2000, Iles 2000). The belief that fertilizer enhances pest resistance has emerged in the virtual absence of supporting evidence (Raupp et al. 1992, Herms 2001, 2002). Only a few studies conducted in conifers showed that fertilizer increased tree resistance (Oldiges 1959, Schwenke 1960, 1962) but inferences from these studies are limited by lack of statistical rigor (Stark 1965, Herms 2002). Thorough reviews of the literature surrounding the relationship between fertilization and insect attack by Waring and Cobb (1992) and

Harms (2002) provided convincing evidence that fertilization enhanced herbivore performance and decreased resistance of woody plants to most insect pests. Several studies have linked higher levels of leaf nitrogen with increased performance of aphids and adelgids (Mitchel and Paul 1974, McClure 1991, 1992); scales (McClure 1977, 1980); citrus mealybug (Shrewsbury et al. 2004); plant bugs (Holopainen et al. 1995) and sooty copper caterpillars (Fisher and Fiedler 2000). Furthermore, some Lepidoptera such as checkered skipper feed only on leaf blades with the highest nitrogen concentrations (Ravenscroft 1994). Strauss (1987) addressed the question of how members of insect communities responded to nitrogen fertilization in *Artemisia ludoviciana* L., a perennial herbaceous plant. She found that fertilization resulted in greater abundance of phloem- and seed- feeding insects. Performance often declines when herbivores consume nutrient-poor plants or plant parts (Scriber and Slansky 1981, Awmack and Leather 2002, Borowicz 2003).

Plant resistance is genetically based but several studies have shown that environmental factors, including nitrogen fertilization, can dramatically influence expression of these traits (Painter 1951; Bryant et al. 1983, 1987; Harms and Mattson 1992, Harms 2002). The Carbon-Nitrogen-Balance (CNB) hypothesis formulated by Bryant (1983) was explained the effects of nutrients and shade on the expression of traits such as the production of secondary metabolites (Bryant 1983, Harms and Mattson 1992). The Growth-Differentiation-Balance (GDB) provided a more general and complete framework for understanding the effects of extrinsic factors on phenotypic variation in secondary metabolism due to physiological trade-offs between growth processes and the production of secondary metabolites (reviewed by Harms and Mattson 1992, Stamp

2003). These hypotheses along with several others have served as frameworks for investigating the effects of environmental factors on plant defense against the herbivores.

Based on the GDB model (Herms and Mattson 1992), when fertilization and abundant moisture relax sink limitations and stimulate growth, plant resources may be diverted from other processes, including secondary metabolism. Plants maintained with high inputs of water and nutrients have high photosynthetic and growth rates. The GDB model predicts that at moderate to high nitrogen availability most of the carbon produced in leaves goes to supporting new growth and little carbon may be available to support synthesis of carbon-based secondary compounds. In this case, the carbon: nitrogen ratio will decrease in favor of primary production. When plants are grown under conditions that limit sinks such as low nutrient availability, they produce more carbon-based compounds than they can use, and carbon: nitrogen ratio increases, resulting in an increase of carbon-based secondary compounds (Mattson 1980, Bryant et al. 1983, Gerzhanon 1984, Larson et al. 1986, Herms and Mattson 1992).

In addition to inputs of nutrients, the nursery industry relies on irrigation to provide ample soil water to support the rapid growth of ornamental plants (Davidson et al. 2000). Container grown plants are more dependent on water supply than landscape plants, because potting growth media has limited soil moisture capacity and dries out quickly.

Under conditions of water-stress, plant physiology changes. These changes include: decreased stomatal conductance, increased nitrogen available in free amino acid forms, and increased secondary metabolite concentrations in plant tissues (Hsaio 1973, White 1978, Gershenon 1984). Several researchers found that feeding on drought-

stressed plants resulted in improved growth, survival, or reproduction of many herbivorous insects and mites (Mattson and Haack 1987, Waring and Cobb 1992, Koricheva et al. 1989). In several other cases insects performed less well on stressed plants (Koricheva et al. 1989, Huberty and Denno (2004). Huberty and Denno (2004) found that chewing insects, mostly free-living species of moths, sawflies, and beetles, performed poorly when their host plants were water-stressed. A study by Owen et al. (1991) found that damage by *O. sulcatus*, in non-irrigated landscape beds was lower than in irrigated beds.

The black vine weevil, *Otiorhynchus sulcatus*, is an introduced pest in the United States, and originated in the mountains of central Europe (van Toll 2002). *Otiorhynchus sulcatus* is a serious pest in nursery production in Australia, the Netherlands, Germany, Norway and England (Parrella 1984, van Tol 2002). In the USA, *O. sulcatus* was first identified 1835 in Massachusetts (Smith 1932). In a recent regional assessment of needs of the nursery and landscape industries, *O. sulcatus* was identified as the second most important insect pest of ornamentals (Raupp and Hoitink 1996). Total annual crop sales of nursery plants in the USA were \$ 6,177,000,000 (USDA 1998). The root weevil complex reduces the value of hardy ornamentals by approximately 25% in the eastern of United States (<http://pestdata.ncsu.edu>).

The host-plant range of *O. sulcatus* adult consists of one genus in the gymnosperms (*Taxus* spp.) and a broad range of angiosperms but the most preferred plants belong to the order Rosales (Hydrangeaceae, Rosaceae, Ericaceae, and Saxifragaceae) (van Tol 2002). In the last 20 years, growers in the US have expanded production of host plants such as rhododendrons (*Rhododendron* spp.) and yew (*Taxus*

spp.). This has strongly favored population growth of *O. sulcatus* in nurseries (Nielsen et al. 1978). Fertilizer, water, and high plant density are factors that create an optimal habitat for *O. sulcatus* in container plant production. From my surveys in nurseries in Baltimore County, MD in 2001, I found that young plants of *Heuchera* “Palace purple”, *Berginia* “*Berginia cardifolia*”, and *Astilbe* “*Astilbe cardifolia*” suffered more than mature plants from *O. sulcatus* larvae (Bejleri, unpublished data).

In Maryland, *O. sulcatus* adults emerge in late April through early May. Adults begin feeding and laying eggs in spring. Eggs hatch in late May through June and larvae and adults feed during the summer and early fall. Adults remain active until late fall. *Otiorhynchus sulcatus* overwinters as late instar larvae. Adults chew leaf margins causing a notched appearance on leaves. This results in economic damage and significant aesthetic damage. The weevil grubs feed on plant roots and griddle the root crown resulting in stunted plant growth and plant death. Economic damage may result from relatively low larval densities. As few as three larvae per pot caused mortality among one-year-old potted rhododendron (La Lone et al. 1981).

In this study I investigated the effects of three levels of nitrogen fertilization and two water regimes on plant biomass, nitrogen concentration, carbon: nitrogen ratio, and phenolic compounds of *Heuchera* “Palace Purple”. Our model plant *Heuchera micrantha* Dougl. ex Lindl. var. ‘Palace Purple’ (Saxifragaceae), is a perennial herbaceous plant, and one commonly produced. I examined the bottom-up effects of plants maintained in different nutrient and water regimes on *O. sulcatus*. The overall objectives were: (1) to study the effects of nitrogen fertilization and water regimes on the secondary chemistry, nutritional value and growth of *Heuchera micrantha* “Palace

Purple”, and (2) to study the effects of *Heuchera* plants grown under different nitrogen fertilization levels and water regimes on the performance and fitness of *O. sulcatus*.

## Methods

### Experimental design and treatments

Greenhouse and laboratory experiments were performed at the University of Maryland in College Park, Maryland during 2001 and 2002. Study plants, *H. micrantha* ‘Palace Purple’ (Saxifragaceae), were purchased as plugs (Bluemount Nurseries Inc, Monkton, MD) and potted into #1 containers (2.84 L) in a soil-less media (Metro Mix 510, Scott’s Sierra Hort. Products Company, Marysville, OH). A 3x2 factorial design was used in this study with three levels of nitrogen fertilization: 25 mg N / week, 75 mg N / week and 150 mg N / week; and within each nitrogen treatment there were two levels of water (water-stressed and non-water-stressed) (6 treatment combinations, Table 1). Plants from the six treatment combinations were arranged in a completely randomized block.

All plants were placed on drip irrigation, and nitrogen fertilization and water treatments were maintained through the irrigation system. Nutrient solutions were composed of ammonium nitrate, potassium sulfate, potassium phosphate, and potassium nitrate (Fisher Company, Burr Ridge, IL) to obtain nitrogen treatments of low nitrogen (25 mg N/week), moderate nitrogen (75 mg N/week) and high nitrogen (150 mg N/week). A nitrogen level of 150 mg N/week is commonly used in nursery production. Phosphorous and potassium fertilizer were used at the same proportion for all treatments

to balance the nitrogen in the nutrient solution. Nutrient solutions were applied to plants weekly using three Dosatron DI16-11GPM's (Dosatron International, Inc. Clearwater, Florida) to maintain the appropriate nitrogen treatment.

A preliminary experiment was conducted in a growth chamber to determine when *Heuchera* were in a water-stressed versus a non-water-stressed state. Plant water-stress was determined as the point, following soil water saturation, at which stomatal conductance declined significantly. Plant water-stress was estimated using stomatal conductance as suggested by Hsiao (1973). The relationship between stomatal conductance and volumetric soil water was also determined. This allowed daily readings of volumetric soil water to be used as the indicator for when plants needed to be watered to maintain them in water-stressed and non-water-stressed treatments. Ten plants were watered to the water holding capacity of the growth medium. Plants were then placed in a growth chamber. The 24 hour light cycle in the growth chamber was set to simulate light conditions during May as follows: 3 hours at  $300\mu\text{mol}/\text{m}^2/\text{sec}$ ; 3 hours at  $600\mu\text{mol}/\text{m}^2/\text{sec}$ ; 4 hours at  $1000\mu\text{mol}/\text{m}^2/\text{sec}$ ; 3 hours at  $600\mu\text{mol}/\text{m}^2/\text{sec}$ ; 3 hours at  $300\mu\text{mol}/\text{m}^2/\text{sec}$ ; and 8 hours of darkness. Stomatal conductance ( $\text{mol CO}_2/\text{m}^2/\text{sec}$ ) was measured at the same time daily using a LI-COR 6400 Gas Exchange System (LI-COR Environmental, Lincoln, NE). Volumetric soil water (mVolts) was measured daily using a Theta Probe (Type ML 2x, Dynamax, Huston, Texas). Measurements were taken for 6 days and plotted to determine when stomatal conductance declined and its relationship with volumetric soil water.



To ensure that the treatment applications influenced photosynthesis of *Heuchera*, photosynthetic rate was measured on three plants from each treatment combination in June and July 2001 following the treatment initiation.

#### Effect of nitrogen fertilization and water regimes on *Heuchera*

***Plant growth.*** Plants were harvested four months after treatments were applied to determine the effect of nitrogen fertilization and water regimes on plant top and root growth. Plants were harvested just prior to seed set, which often coincides with slowed growth of *Heuchera*. Plant growth was determined by measuring top growth (leaves and petioles), leaf area and root dry weight. Eight plants from each treatment combination (48 plants in total) were brought into laboratory from the greenhouse. Plants were cut at the soil line. Freshly cut leaves were placed flat on a platform and a Canon Re-350 video camera (Canon Inc, Lake Success, New York) was used to capture the leaf images. Total leaf area (cm<sup>2</sup>) was calculated for each plant using CIAS Image Analyses System software (CID Inc, Camas, Washington). Roots were carefully rinsed to remove soil. For each plant, top growth and roots were individually dried in an Isotemp oven at 65 °C for 48 hours prior to weighing on a Denver Instruments Scale model D1-8kD (Denver Instrument, Denver, Colorado).

***Plant chemistry.*** Dried leaf and root samples from the plants were used to measure leaf and root nitrogen and carbon content, and leaf phenolic concentration. Dried leaf and root samples were ground with a Cyclotec 1093 grinder (Foss, Eden Prairie, Minnesota) and stored at -20 °C prior to analysis. Analyses to determine nitrogen and carbon content of leaf and root tissue were performed at the Soils Laboratory,

Maryland Cooperative Extension at the University of Maryland (College Park, MD). Soluble phenolic pigments were analyzed spectrophotometrically. For extraction we used Methanol, H<sub>2</sub>O, HCl (Methanol: H<sub>2</sub>O: HCl in ratio 50: 49: 1). Absorbance was determined using Shimadzu UV-1601 dual beam spectrophotometer (Shimadzu, San Juan, Porto Rico).

*Otiorhynchus sulcatus* preference, performance, and fitness on *Heuchera*  
under varying nitrogen fertilization and water regimes

***Otiorhynchus sulcatus* adult feeding preference.** A laboratory study was conducted in July-August 2001 to determine if *O. sulcatus* adults fed at different rates when given a choice of *Heuchera* foliage from plants receiving varying levels of nitrogen fertilization and water. Feeding arenas were established in petri dishes (125 mm diam.) lined with Styrofoam. Fresh leaves from plants receiving the six treatment combinations were picked each morning and transferred to the laboratory in a cooler to keep them fresh. To avoid any bias of leaf age or toughness on feeding preference, leaves of the same age and whorl location were picked from all treatments. A hole-punch 12 mm in diameter was used to cut leaf discs. All leaves were cut between the mid-vein and a first grade vein. Six leaf discs, one from each treatment plant, were randomized and placed in a circular pattern in the feeding arena. Leaf discs were fixed in place with a small pin.

Fifteen *O. sulcatus* adults were chosen randomly from a colony. Adults were fed foliage from *Taxus* sp. prior to the trial. *Otiorhynchus sulcatus* adults were starved for 24 hours and then one adult was placed in each of 15 feeding arenas. Feeding arenas with *O. sulcatus* adults were placed in an environmental chamber at 25 °C with a 12:12

light: dark regime. An additional feeding arena was set up without a weevil to use as a baseline for leaf disc area. *Otiorhynchus sulcatus* were allowed to feed for 24 hours, after which time, the remaining area for each leaf disc was captured using a Canon Re-350 video camera (Canon Inc, Lake Success, New York). Total leaf area (cm<sup>2</sup>) was calculated for each leaf disc using CIAS Image Analyses System software (CI-400 CIAS, CID Inc, Camas, Washington). Leaf area eaten was calculated as a difference between the leaf disc area from petri dishes without weevils and leaf disc area of petri dishes with weevils for each treatment. This experiment was repeated for 12 days (15 replicates x 12 days = 180 replicates). New adult weevils were used each time the experiment was repeated.

***Otiorhynchus sulcatus* oviposition period and fecundity.** A laboratory study was conducted in April 2001 to determine the effect of eating foliage from *Heuchera* grown under different nitrogen fertilization and water regimes on ovipositional period and fecundity. Pupae of *O. sulcatus* were collected from a field population (Kurt Bluemels Nursery, Inc, Baldwin, MD). Pupae were placed in plastic containers filled with moist (30 – 40% soil water capacity), sterile potting mix and placed in an environmental chamber. To avoid shocking the pupae, the temperature in the chamber was originally set to approximate the outdoor temperature in April (15 °C) and then raised 1 °C every 2-3 days until the chamber reached 25°C. Every morning newly emerged adults were removed from the containers and placed into petri dishes (125 mm diam.) with moistened filter paper (1 adult /125 mm diam petri dish). Every adult was provided with an unlimited amount of fresh foliage from one of the six-nitrogen/water treatments. This study was blocked by time and every 6 adults (1 for each treatment)

were a block. There were 27 replicates of each treatment. Throughout the experiment *O. sulcatus* adults were maintained in an environmental chamber at 25 °C and a light: dark regime of 12:12 hours. Measurements were taken daily on the number of eggs laid for each *O. sulcatus* adult until they died or stopped laying eggs.

***Otiorhynchus sulcatus* larval survival and development.** A greenhouse study was conducted in August 2002 to determine the effect of nitrogen fertilization and water regimes to larval survival and development. To obtain larvae for this study, adults of *O. sulcatus* were collected from a field population (Kurt Bluemels Nursery, Inc, Baldwin, MD) in May 2002 and returned to the laboratory. Adults (50-60) were placed in glass containers (25x25 mm), and held in an environmental chamber at 25 °C and 12:12 (L: D) photoperiod. Adults were fed pesticide free *Taxus* foliage. Food was refreshed every other day. Adults laid eggs on the bottom of container or on top of the leaves. To have enough larvae of the same age, adults were moved to a new container every three or four days. Eggs were collected in large numbers by rinsing leaves and filter papers into a beaker and using a separatory funnel with sucrose gradient to float eggs.

Plastic cups (6.4 X 6.4 cm, Fisher Scientific Pittsburgh, PA) were used for rearing *O. sulcatus* from eggs to second instar larvae. Cups were filled with a sterile media and a piece of fresh, organic carrot was placed in the middle of cup as food for new larvae. Carrots were refreshed every week. Approximately 50 eggs were placed in each cup around the carrot. Six days later cups were gently emptied into petri dishes. Using a dissecting microscope, 10 newly hatched larvae were transferred to new cups and a fresh carrot was provided. Cups with larvae were placed in an environmental chamber at 21 °C darkness regime (after Masaki, 1991). When larvae reached the second instar they were

placed in groups of 10, weighed, and returned to cups. These larvae were used to infest *Heuchera* plants to measure larval survival and development.

On August 26, 2002, eight *Heuchera* plants (replicates) from each treatment were infested with 10 second instars by carefully placing larvae in a trench 2-3 cm deep using a soft brush. After larvae were placed in the trench, they were covered with fine soil. Plants received nitrogen fertilization and water treatments weekly until harvest on October 26<sup>th</sup>, 2002. Larval survival and larval weight were recorded for each treatment.

### Statistical Analysis

Data were analyzed as a 3x2 factorial using the PROC MIXED procedure (SAS Institute 2003). LSMEANS statement was used to compute one and two way means and the expected mean squares for the fixed effects and a random error terms. An *F*-test was computed for fixed effects and interactions. LSD was used as the most sensitive mean comparison method and probability of differences (  $PDIFF \leq .05$  ) as the criteria for significance in the pair wise comparisons of means. CONTRASTS method was used to conduct comparisons of interest. To correct normality and heterogeneity of variances, data were transformed prior to ANOVA. Fecundity data were transformed by taking the square root of the number of eggs. Fecundity was computed as total fecundity over the duration of the study. Log transformation was used to transform larval weight gain to correct for heterogeneity of variances. Larval weight gain was calculated as a difference between larval weights on harvest day with initial weight of larvae at infestation day. Plant growth for both top and bottom dry mass was analyzed as a mean comparison among treatments using the PROC MIXED/LSDIFF procedure.

## Results

### Experimental design and treatments

Stomatal conductance began to drop rapidly after reaching 80-85 mol H<sub>2</sub>O/m<sup>2</sup>/sec (Fig. 1). Below this critical point is when *Heuchera* plants were considered to be water-stressed. A stomatal conductance level of 40-50 mol H<sub>2</sub>O/m<sup>2</sup>/sec was the point just above when plants began to show visual signs of water-stress (wilting) (K. Bejleri, personal observation). These levels of plant stress correspond to volumetric soil water measures of 800-850 mVolts and 400-500 mVolts, respectively. To maintain plants in a non-water-stressed state, plants were watered when volumetric water in the soil reached between 800-850 mVolts. To maintain plants in a water-stressed state, plants were watered when volumetric water in the soil reached 400-450 mVolts. The average volumetric water in the soil of *Heuchera* plants of each treatment was determined each morning. Plants were then watered as necessary to maintain their designated treatment. Photosynthetic rate was significantly higher for plants that received a high level of nitrogen and were non-water-stressed compared to all other treatments (Fig. 2).

### Effect of nitrogen and water regimes on *Heuchera*

***Plant growth.*** There was a significant nitrogen by water interaction for top growth (leaves and petioles) ( $P = 0.0007$ ) (Table 2, Fig. 3) and root growth ( $P = 0.0024$ ) (Table 3, Fig. 4). On non-water-stressed plants, top growth was 76% higher in moderate compared to low levels of nitrogen and 69% higher in high compared to low levels of

nitrogen (Fig. 3). A similar pattern was found for root growth (Fig. 4). However, in water-stressed plants, nitrogen had no effect on top or root growth.

***Plant chemistry.*** There was no significant nitrogen by water interaction or main effect of water on leaf nitrogen content ( $P = 0.279$ ,  $P = 0.125$ ), leaf carbon: nitrogen ratio ( $P = 0.530$ ,  $P = 0.187$ ), or leaf phenolic concentration ( $P = 0.3165$ ,  $P = 0.649$ ), respectively (Table 3). However, there was a significant main effect of nitrogen on leaf nitrogen content ( $P = 0.0001$ ), leaf carbon: nitrogen ratio ( $P = 0.0120$ ), and leaf phenolic concentration ( $P = 0.0194$ ) (Table 2). Leaf nitrogen content in non-water-stressed plants increased as nitrogen level increased (Fig. 5). For plants that received moderate and high levels of nitrogen, leaf nitrogen content was 28% and 48% higher, respectively, compared to leaf nitrogen content for plants that received low nitrogen levels.

A similar pattern followed for water-stressed plants, except there was no significant difference between leaf nitrogen content in plants that received low and moderate nitrogen levels. In both water regimes, the highest leaf nitrogen content was observed in plants that received high nitrogen levels and there was no significant difference between them. There was also no difference in leaf nitrogen content between plants that were water-stressed and received low and moderate nitrogen levels and plants that were non-water-stressed and received moderate nitrogen levels.

The carbon: nitrogen ratio in leaves of non-water-stressed plants that received high nitrogen levels was 23% lower compared to plants that received low nitrogen and 8% lower for plants that received moderate nitrogen levels (Fig. 6). There was no significant difference in carbon: nitrogen ratio between plants that received all three

levels of nitrogen in water stressed plants. However, carbon: nitrogen ratio generally declined with increasing levels of nitrogen.

Leaf phenolic concentration declined as nitrogen level increased for non-water-stressed and water-stressed plants (Fig. 7). In non-water-stressed plants, leaf phenolics were 38% lower in plants that received high nitrogen compared to low nitrogen. However, there was no significant difference in phenolics for plants that received moderate and high levels of nitrogen in the water stress treatment. In water-stressed plants, leaf phenolics did not differ between low and moderate nitrogen treatments, but plants that received high nitrogen had significantly lower phenolics.

There was no significant nitrogen by water interaction on root nitrogen content of *Heuchera* ( $P = 0.4$ ). There was a significant effect of water ( $P < 0.0001$ ) and nitrogen ( $P < 0.0001$ ) on root nitrogen content (Fig. 8). There was also no significant water by nitrogen interaction on root carbon: nitrogen ratio of *Heuchera* ( $P = 0.2766$ ), but there was a significant effect of water ( $P < 0.0001$ ) and nitrogen ( $P < 0.0001$ ) (Fig. 9).

*Otiorhynchus sulcatus* performance and fitness on *Heuchera*  
under varying nitrogen and water regimes

***Otiorhynchus sulcatus* adult feeding preference.** There was no significant nitrogen by water interaction ( $P = 0.6663$ ), or main effect of nitrogen ( $P = 0.9452$ ) or water ( $P = 0.5530$ ) on food choice in *O. sulcatus* (Table 4, Fig. 10). Feeding rates of *O. sulcatus* adults did not differ among the treatments (Fig. 10).

***Otiorhynchus sulcatus* oviposition period and fecundity.** The main effect of water regime on total fecundity was not significant ( $P=0.0912$ ). Therefore, data were



pooled for age to first reproduction across water treatments and I report only nitrogen effects. There were no significant main effects of nitrogen ( $P = 0.2079$ ) on the age to first reproduction for *O. sulcatus* (Table 4, Fig. 11). However, there was a significant nitrogen by water interaction on total fecundity of *O. sulcatus* ( $P = 0.0235$ ) (Table 4) (Fig. 12). When plants were not water-stressed, fecundity increased significantly as nitrogen level increased. The average number of eggs was 40% greater when *O. sulcatus* consumed foliage from *Heuchera* on moderate compared to low nitrogen regimes. Average fecundity was 38% greater when *O. sulcatus* ate foliage from *Heuchera* on high compared to moderate fertilizer regimes. For water-stressed plants, there was no significant difference in fecundity regardless of the nitrogen level the plants received (Fig. 12). In addition, fecundity of *O. sulcatus* on water-stressed plants did not differ from fecundity of *O. sulcatus* on low and moderate nitrogen, non-water-stressed plants. Data for total ovipositional period were pooled across water treatments. Total oviposition period did not differ between three levels of nitrogen ( $P=0.2035$ ) (Table 4) (Fig.15, 16, 17).

***Otiiorhynchus sulcatus* larval survival and development.** Survival of *O. sulcatus* larvae reared on *Heuchera* under varying nitrogen fertilization and water regimes showed no significant nitrogen by water interaction ( $P = 0.5028$ ), or main effects of nitrogen ( $P = 0.4431$ ) or water ( $P = 0.468$ ) (Table 4) (Fig. 13). There was also no interaction ( $P = 0.4050$ ) or main effect of water ( $P = 0.3560$ ) on *O. sulcatus* larval weight gain. However, nitrogen level did significantly affect larval weight gain ( $P = 0.014$ ) (Table 4). Larvae gained the most weight on plants that were non-water-stressed receiving high levels of nitrogen (Fig. 14). This gain in weight was greater than for all other treatments

except water-stressed, high nitrogen plants. Larval weight gain for all other nitrogen and water regimes did not differ.

## Discussion

The GDB hypothesis predicts that at low to moderate levels of resource availability (water and nutrients), rates of net assimilation, growth and secondary metabolism are positively correlated. At moderate to high levels of resource availability, net assimilation rate is constant, relative growth rate and secondary metabolism compounds are inversely correlated, and the physiological trade-off between growth and secondary metabolism is apparent (Herms and Mattson 1992). The GDB hypothesis predicts that at low levels of resource availability such as low levels of nitrogen or water fertilization photosynthetic rate of *Heuchera* would be low, plant growth would be low, and carbon: nitrogen ratio and phenols would be higher compared to plants receiving high levels of nitrogen and water. My results generally support the predictions of the GDB hypothesis. When plants were well watered photosynthetic rate had the lowest values at low and moderate nitrogen levels. In water-stressed plants photosynthetic rates were generally lower and uniform among nitrogen levels. Plants growing with abundant nitrogen and water had the highest photosynthetic rate. As predicted by the GDB model, levels of phenolics declined were nitrogen fertilization increased.

My findings are in agreement with numerous other investigations that have investigated relationships between nitrogen fertilization and plant growth and defense. Bryant et al. (1987) found that nitrogen fertilization resulted in decreased concentrations

of condensed tannins and phenolic glycosides in aspen leaves and increased nitrogen levels in leaves. Concentrations of total phenolics increased directly in response to low nitrogen availability and elevated CO<sub>2</sub> in primary and fascicular needles and in lateral roots of loblolly pine seedling, grown in the greenhouse under four different levels of nitrogen and two levels of CO<sub>2</sub> (Gebauer et al. 1997). Moreover, Hyvärinen et al. (2002) studied the impact of fertilization on phenolic content and growth rate of *Cladina stellaris* (Opiz) and they found a strong negative relationship between nitrogen and phenolic content.

My results also agree with the GDB hypothesis with respect to plant top and bottom growth. *Heuchera* plants that received moderate and high levels of nitrogen in the non-water-stress regime had the highest growth. Plants under water-stress generally had lower growth even when nitrogen was supplied at high levels. It appears that water-stress may constrain the ability of the plant to respond to variation in soil nutrients.

Berger and Glatzel (2001) found that biomass production; number of leaves, leaf area and leaf area per fine-root mass were positively affected by nitrogen fertilization, while leaf productivity decreased (indicated by increasing leaf mass ratios). Stroup et al. (2002) found that nitrogen was more important than water supply as a determining factor in switch grass (*Panicum virgatum* L.) yield and performance. Nitrogen also proved to have a far greater effect on single-leaf photosynthesis rate than water (Stroup et al. 2002). A large body of literature suggests that changes in foliar nitrogen levels, carbon: nitrogen ratios, and quantities of defensive compounds such as phenolics can have important effects on herbivore performance (Waring and Cobb 1992, Mattson 1980, Herms and Mattson 1992, Stamp 2003).

Nitrogen and water availability clearly influenced patterns of resource allocation in *Heuchera*. I hypothesized that performance *O. sulcatus* would be affected by changes in *Heuchera* and they were. Nitrogen is a limiting nutrient for herbivores (Mattson 1980, Scriber and Slanski 1981, Waring and Cobb 1992). Some insect herbivorous preferentially feed and oviposit on high nitrogen plants, with the consequence of higher fecundity, and, for offspring, faster development than those on low nitrogen plants (Raupp and Denno 1983, Minkenberg and Ottenheim 1990, Denno et al. 2002). I expected fecundity of *O. sulcatus* to be strongly affected by levels of nitrogen fertilization. Increasing levels of nitrogen fertilization in the non-water-stress regime resulted in increased average number of eggs laid by *O. sulcatus*. In contrast, nitrogen fertilization did not affected fecundity when water was limited.

I predicted that adults would preferentially feed on leaves with low carbon:nitrogen ratio due to low levels of allelochemicals. However, my results showed that feeding rates across treatments did not differ. This may have resulted because weevils increase consumption rates at low nitrogen levels to increase their nitrogen intake. Several studies have shown that caterpillars, such as Gypsy moth (*Lymantria dispar* L) increase rate of consumption in response to low levels of nitrogen (Mattson and Addy 1975, Mattson 1980).

Survival of *O. sulcatus* larvae did not differ between levels of nitrogen, whereas larval development was affected by nitrogen. The highest larval weight gain resulted for plants under high levels of nitrogen fertilization when water was not limiting. This is an outcome predicted by the GDB hypothesis. Performance of several species of adults and

juvenile insects was enhanced at high levels of resource availability (Bryant 1983, Mattson 1980, Scriber and Slanski 1981, Waring and Cobb 1992, Herms 2002).

Specific attributes of host plant quality such as nutrients directly affect herbivore fecundity (Awmack and Leather 2002). In this study fecundity of *O. sulcatus* may have been positively affected in two ways: from increasing nitrogen levels and decreasing phenolic levels in leaf tissues. Citrus weevil larvae gained the most mass when raised on plants that had high concentrations of total proteins (Borowicz 2003). Heard and Winterton (2000) reported enhanced reproductive performance of the weevil *Neochetina bruchi* (Hustache), developing on plants growing at high water and nutrient concentrations. They had quicker development, higher survival, and higher fecundity.

In a landmark paper White (1984) suggested that some plants incurring stresses such as water deficit would be superior food for herbivorous insects. More recent reviews by Koricheva et al. (1998) and Huberty and Denno (2004) indicate that effects of plant stressors on plants and the subsequent performance of herbivores are complex and influenced by several factors including type of plant growth, growth stage, and rate of development, type and levels of stress, and feeding guild of the herbivore. My results do not support the prediction of the plant stress hypothesis. Water-stress either had no effect or generally reduced performance of *O. sulcatus* adults or larvae relative to treatments where soil water was not reduced. These results agree with one previous study where plant damage by *O. sulcatus* was greater in irrigated compared to non-irrigated landscape beds. (Owen et al. 1991). Huberty and Denno (2004) suggested that intermittent stress might not provide the opportunity for enhanced performance in free-living chewers. Intermittent stress regimes were used in this study. Overall, most empirical evidence and

theoretical arguments indicate that free-living chewing insects are negatively affected by continuous water-stress (Koricheva et al. 1998, Huberty and Denno 2004). Based on my results, plants under water-stress that grew less and were suitable for *O. sulcatus* larvae and adults. Plants without water-stress and exhibiting high levels of growth supported greater performance of *O. sulcatus* larvae and adults.

In conclusion, my research illustrates that nitrogen fertilization and water availability affected photosynthetic rates, top and root growth, leaf and root nitrogen content, carbon: nitrogen ratios and levels of phenolic compound in *Heuchera*. These results support the assumptions and predictions of the GDB hypothesis. Moreover, changes in *Heuchera* photochemistry and growth were related to significant changes in performance of *O. sulcatus*. Fecundity and larval weight gain were enhanced when plants received the highest inputs of resources.

From the perspective of pest management, producers of ornamentals plants such as *Heuchera* may be able to reduce, at least in part, damage caused by pests such as *O. sulcatus* by reducing inputs, particularly nitrogen. Figures 3 and 4 clearly indicate that *Heuchera* grew just as well at intermediate levels of fertilization compared to high levels under both water regimes. However, both adult fecundity and larval weight gain increased significantly when fertilizer was increased from 75 mg N/week to 150 mg N/week. Therefore, by using intermediate levels of fertilizer growers could significantly reduce the growth and reproduction of *O. sulcatus*. This would reduce costs of fertilizer and, likely, the cost of pesticides needed to suppress *O. sulcatus* larvae and adults that perform better at high levels of fertilization.

**Table 1.** Nitrogen fertilization and water treatments applied to *Heuchera micrantha*.

Treatments	
Nitrogen	Water
Low (25 mg/week)	Non water stress
Low (25 mg/week)	Water stress
Moderate (75 mg/week)	Non water stress
Moderate (75 mg/week)	Water stress
High (150 mg/week)	Non water stress
High (150 mg/week)	Water stress

**Table 2.** Summary of *P*-values for the effects of nitrogen fertilization and water regime on *Heuchera micrantha* growth.

Main effect and Interactions	Top growth	Root growth
Water	< 0.0001	0.0003
Nitrogen	< 0.0001	0.0430
Water $\times$ nitrogen	0.0008	0.0024

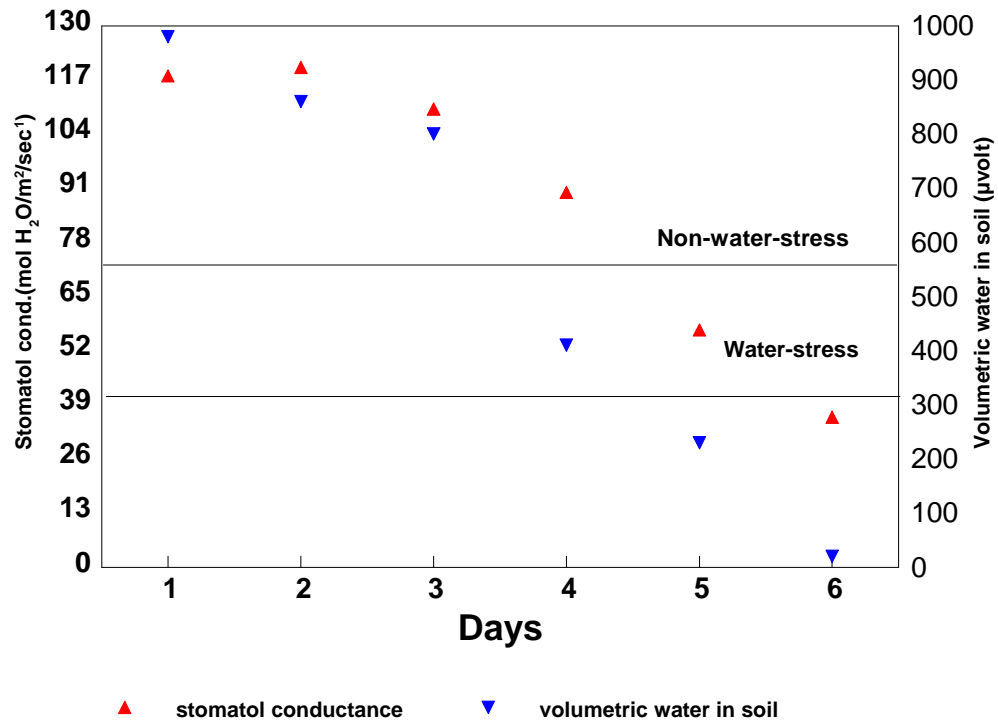


**Table 3.** Summary of *P*-values for the effects of nitrogen fertilization and water regime on *Heuchera micrantha* chemistry.

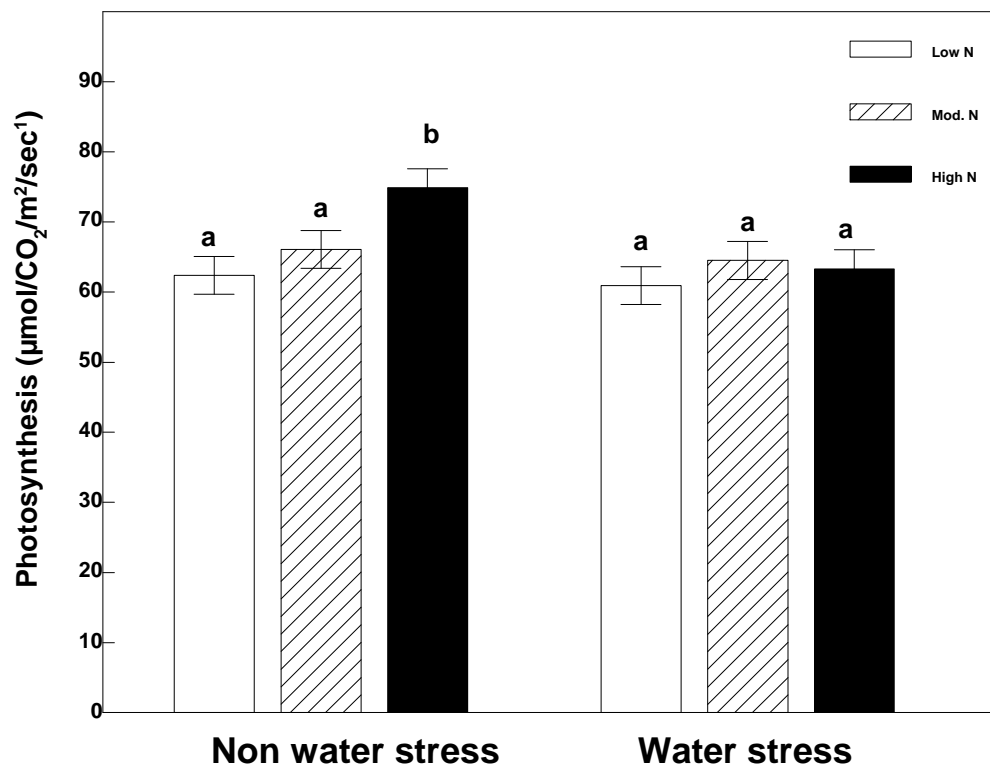
Main effect and Interactions	Nitrogen leaves	CN leaves	Phenols leaves	Nitrogen roots	CN roots
Water	0.125	0.187	0.649	< 0.001	0.001
Nitrogen	< 0.001	0.012	0.019	< 0.001	0.001
Water × nitrogen	0.279	0.530	0.317	0.400	0.277

**Table 4.** Summary of *P*-values for the effects of nitrogen fertilization and water regime on *Otiorhynchus sulcatus* performance.

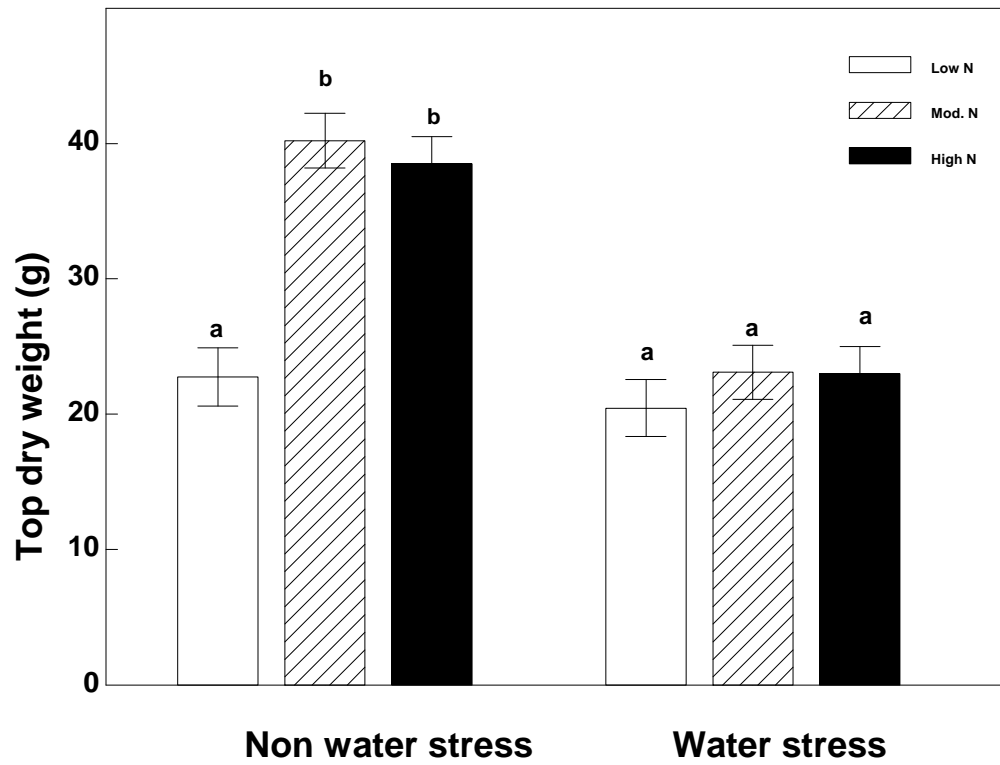
Main effect and interactions	Age to first reproduction	Total fecundity	Feeding preference (adult)	Total fecundity	Larval survival	Larval weight gain
Water			0.553	0.091	0.468	0.356
Nitrogen	0.208	0.203	0.945	0.001	0.443	0.014
Water $\times$ nitrogen			0.666	0.024	0.503	0.405



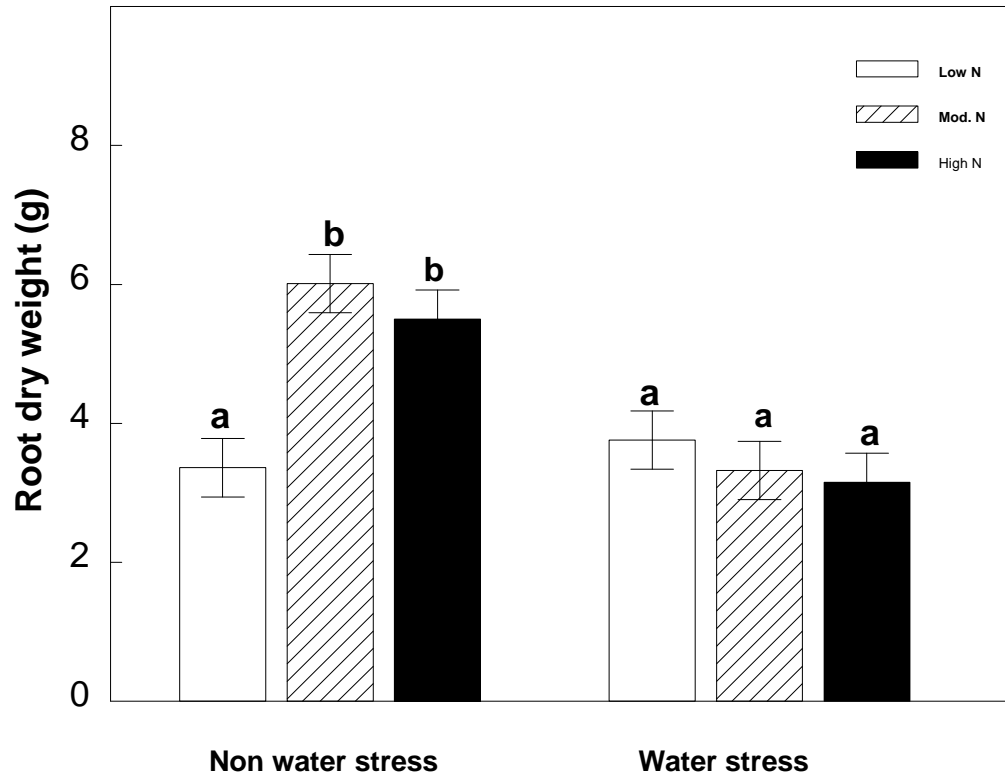
**Fig. 1.** Plot illustrates the relationship of stomatal conductance and volumetric water in soil each day after irrigation to container capacity on day one.



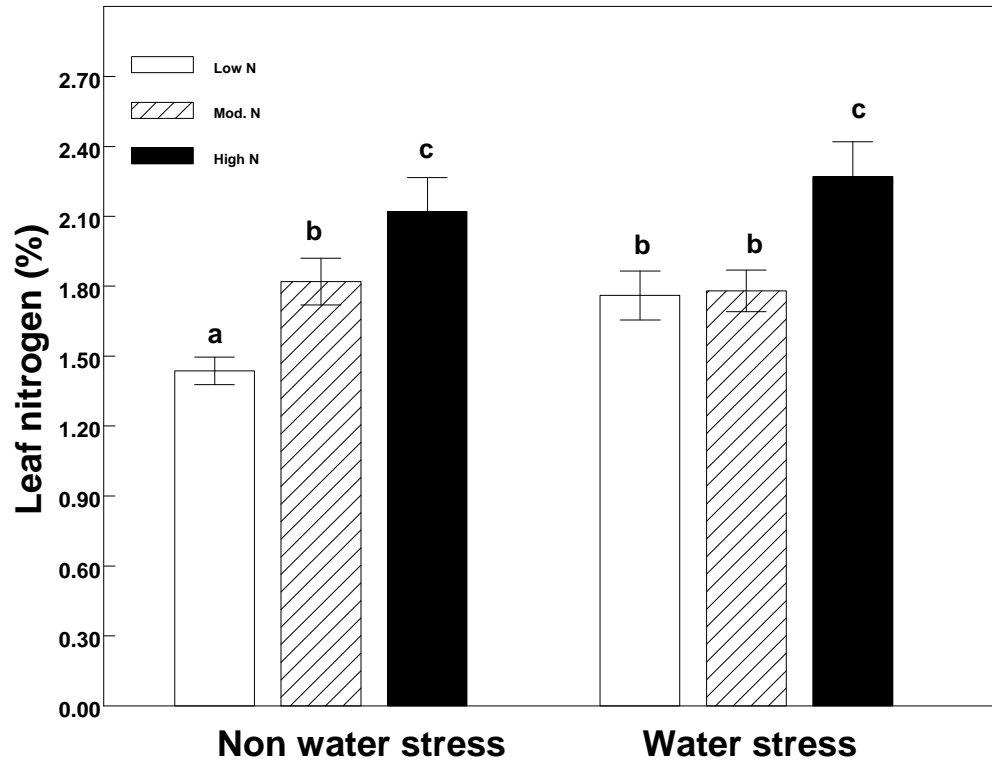
**Fig. 2.** Effect of nitrogen fertilization levels and water regimes on photosynthetic rate of *Heuchera micrantha*. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ )



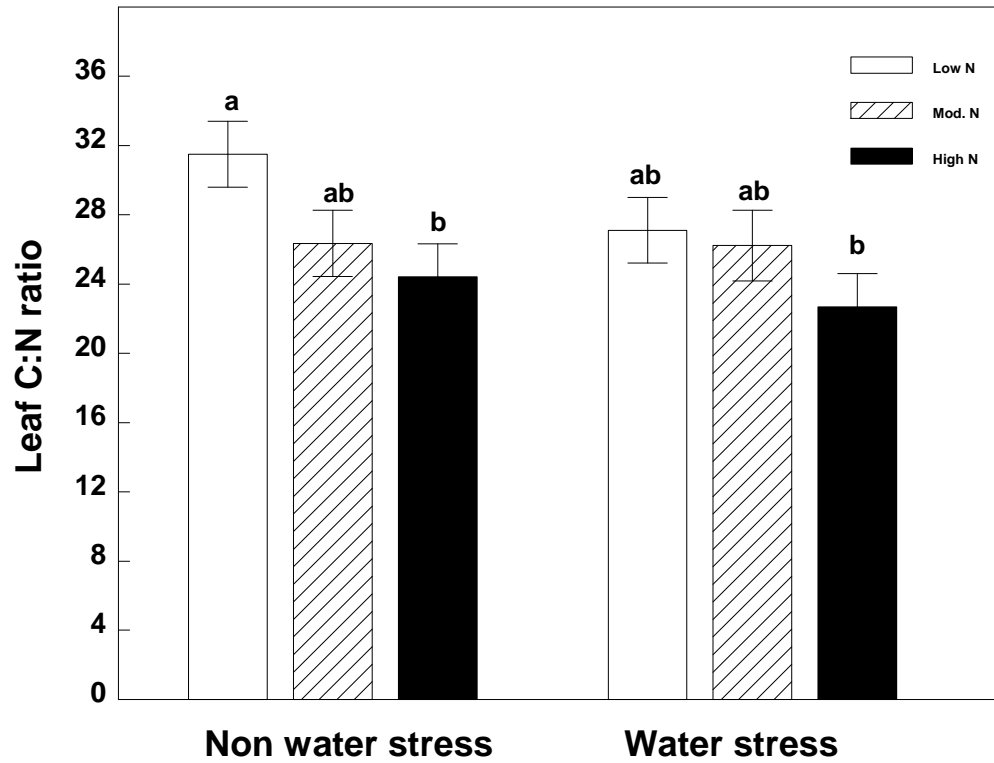
**Fig. 3.** Effect of nitrogen fertilization levels and water regimes on *Heuchera micrantha* top growth. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).



**Fig. 4.** Effect of nitrogen fertilization and water regimes on *Heuchera micrantha* root growth. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ )

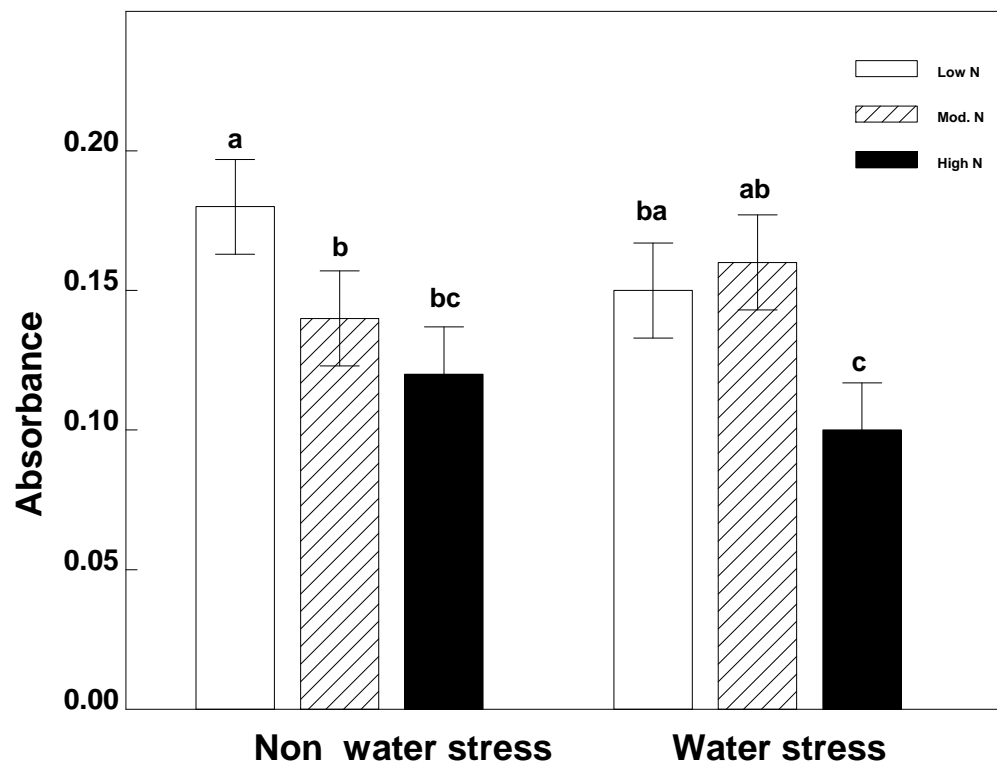


**Fig. 5.** Effect of nitrogen fertilization levels and water regimes on nitrogen concentration of *Heuchera micrantha* leaf tissues. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).

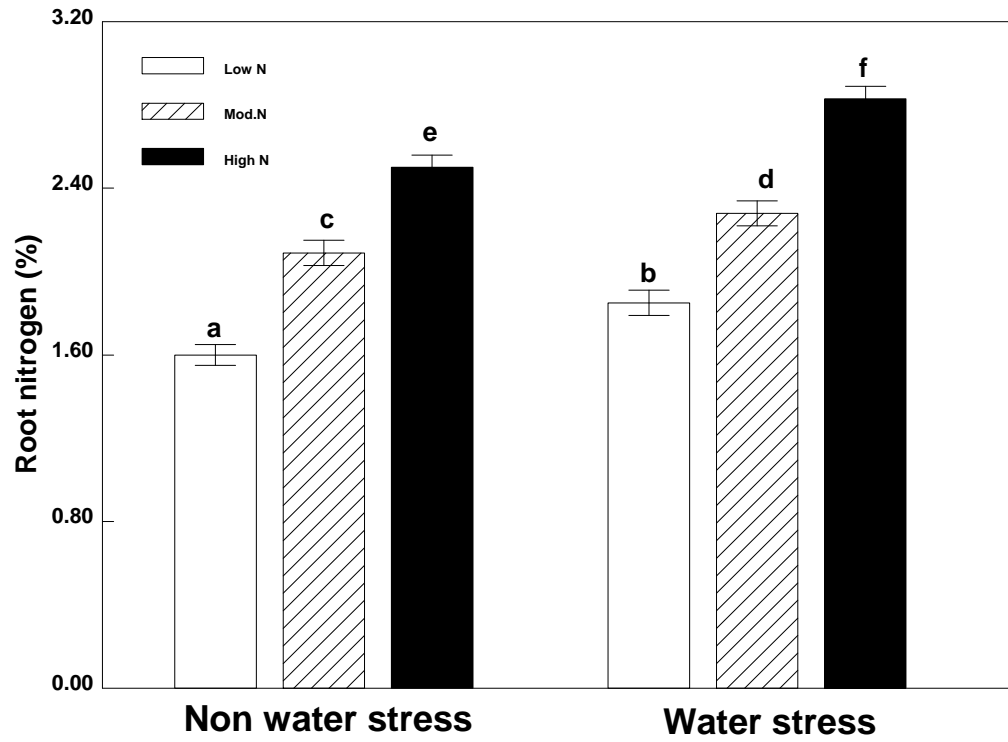


**Fig. 6.** Effect of nitrogen fertilization and water regimes on C:N ratio concentration in leaf tissues. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).

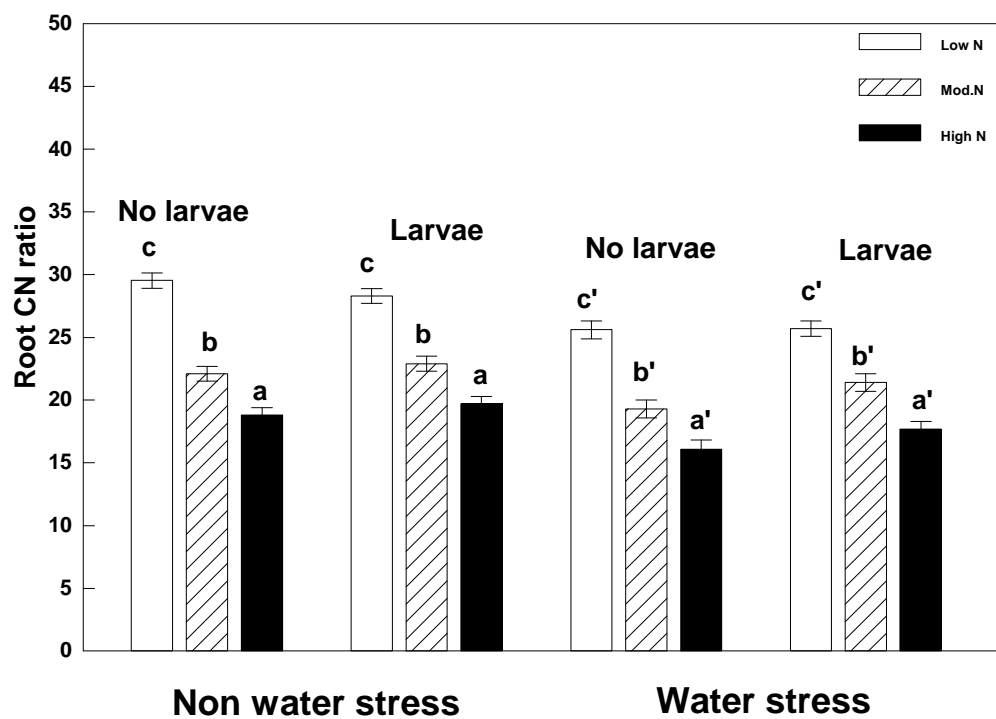




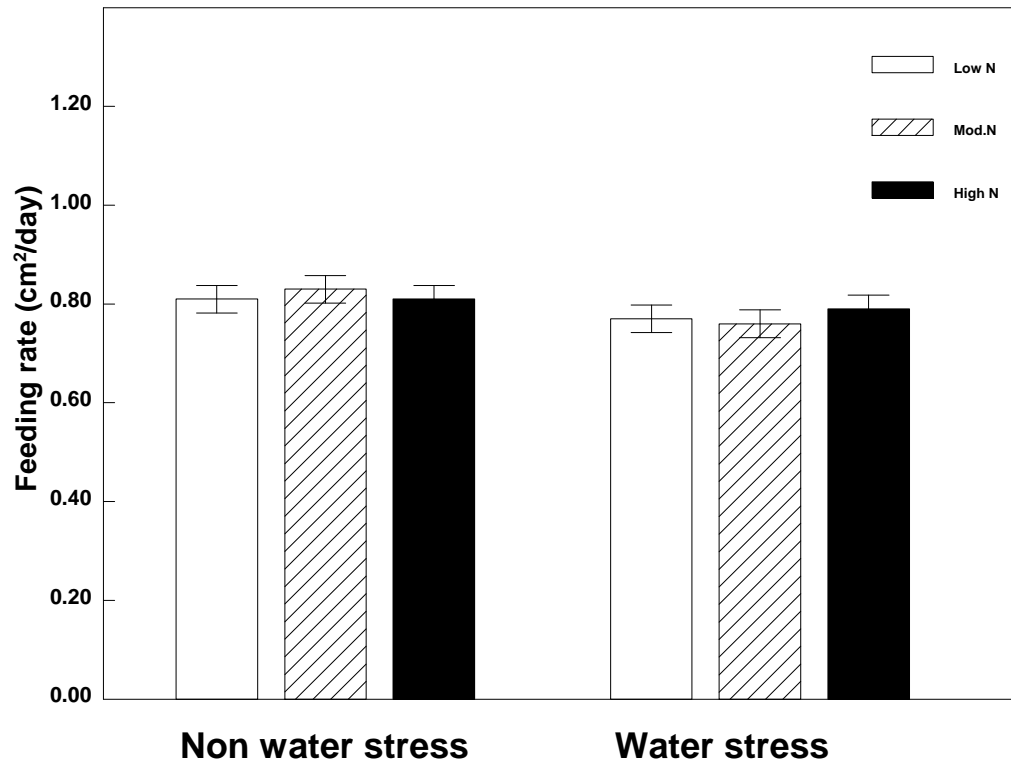
**Fig. 7.** Effect of nitrogen fertilization and water regimes on phenolic concentration in leaf tissues. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).



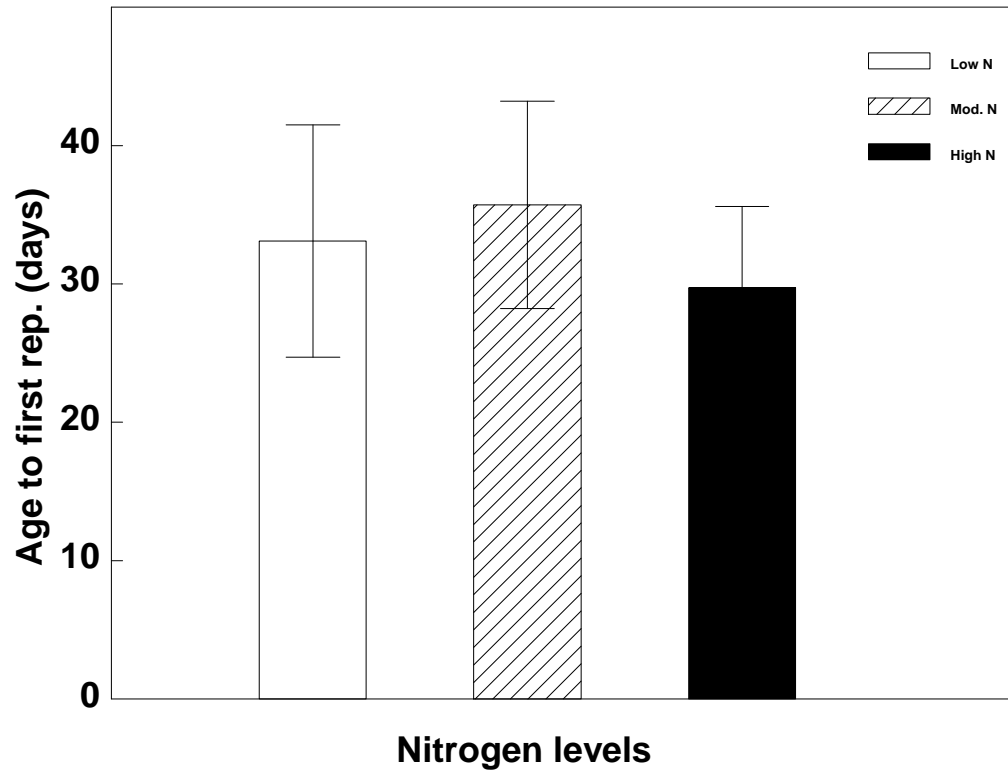
**Fig. 8.** Effect of nitrogen fertilization levels and water regimes on nitrogen concentration of *Heuchera micrantha* root tissues. Means ( $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).



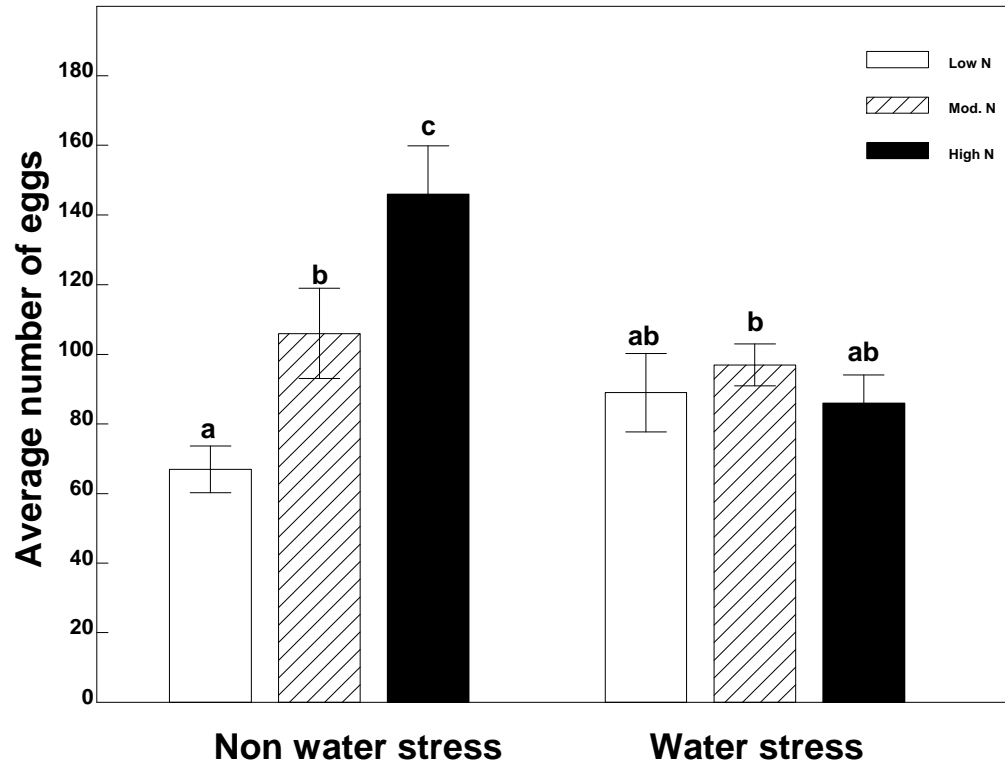
**Fig. 9.** Effect of nitrogen fertilization and water regimes on C: N ratio concentration in root tissues. Means(  $\pm$  SE) with the same letter are not significantly different (LSD  $P < 0.05$ ).



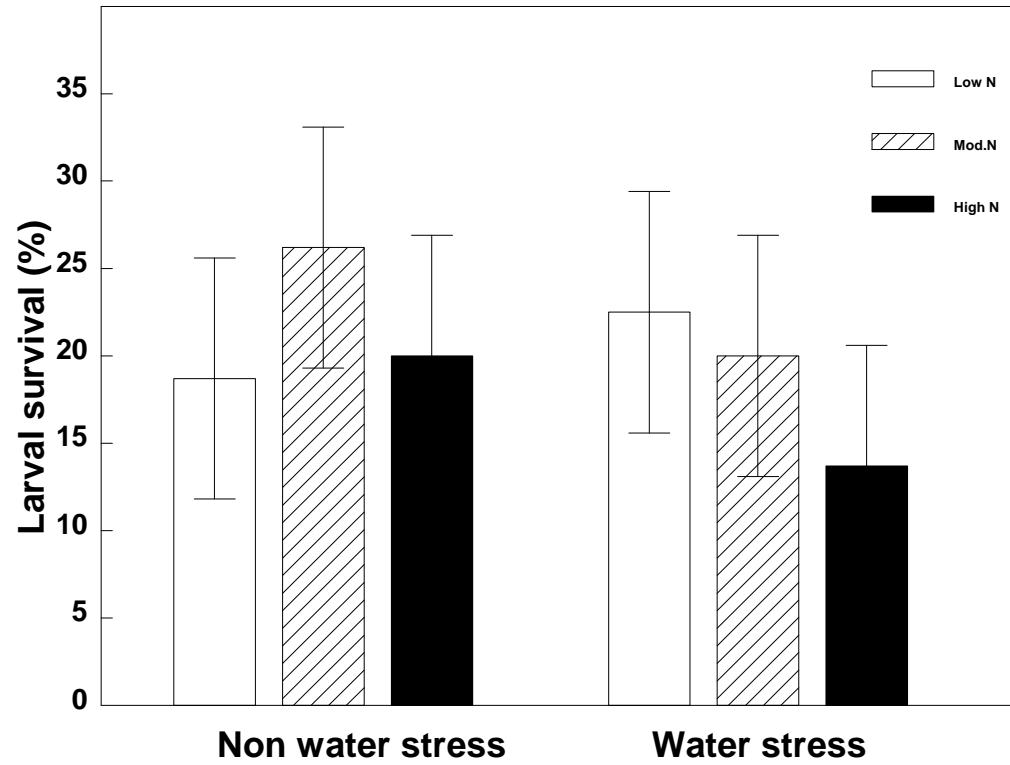
**Fig. 10.** Feeding rate (Means  $\pm$  SE) of *Otiorhynchus sulcatus* adults when given a choice of foliage from *Heuchera micrantha* under varying nitrogen and water regimes.



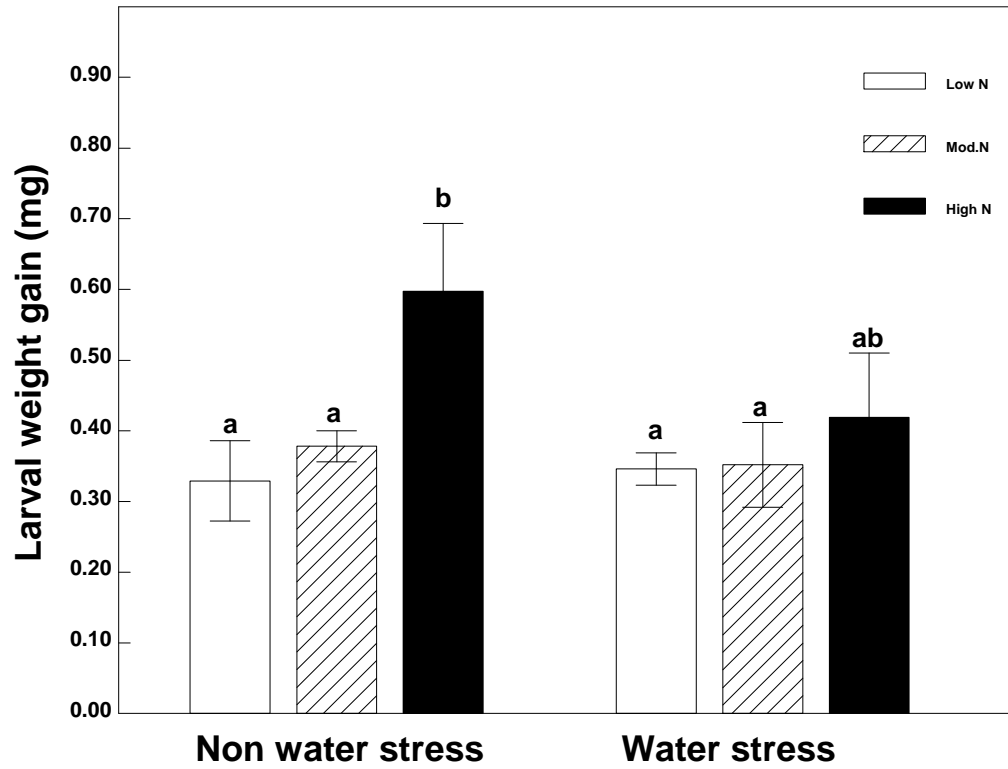
**Fig. 11.** Age to first (Days  $\pm$  SE) reproduction of *Otiorhynchus sulcatus* adults on *Heuchera micrantha* under varying nitrogen regimes. Data pooled across water treatment.



**Fig. 12.** Effect of nitrogen fertilization levels and water regimes on total fecundity (Means  $\pm$  SE) of *Otiorhynchus sulcatus* adults. Means with the same letter are not significantly different (LSD  $P < 0.05$ ).

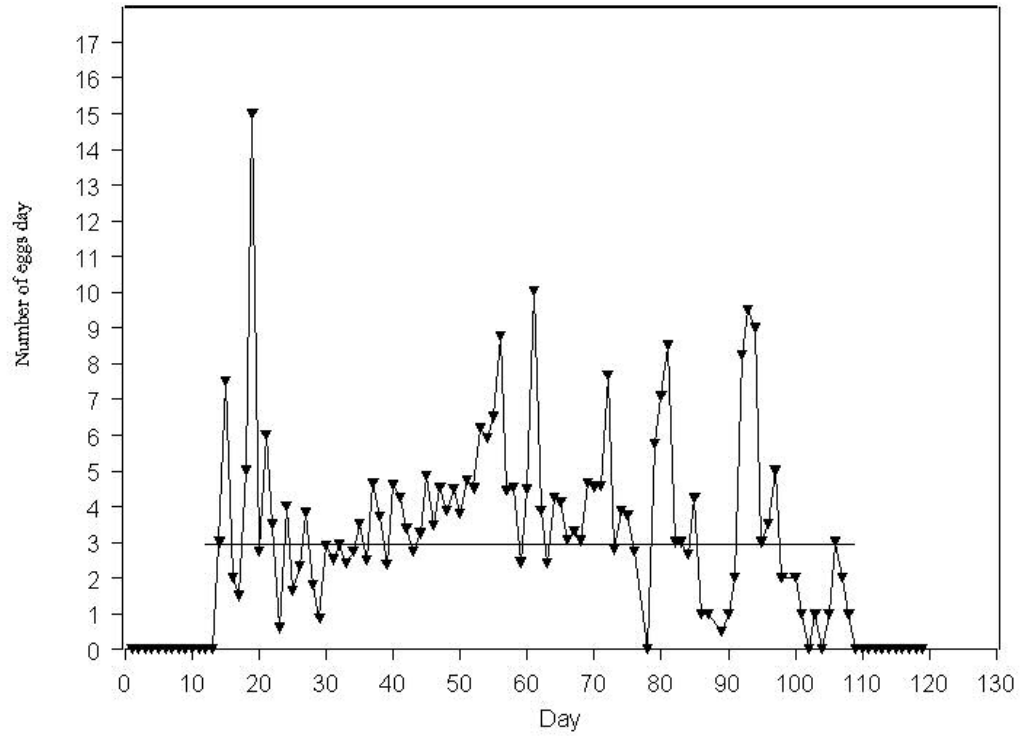


**Fig. 13.** Effect of nitrogen fertilization levels and water regimes on larval survival (Means  $\pm$  SE) of *Otiorhynchus sulcatus*.

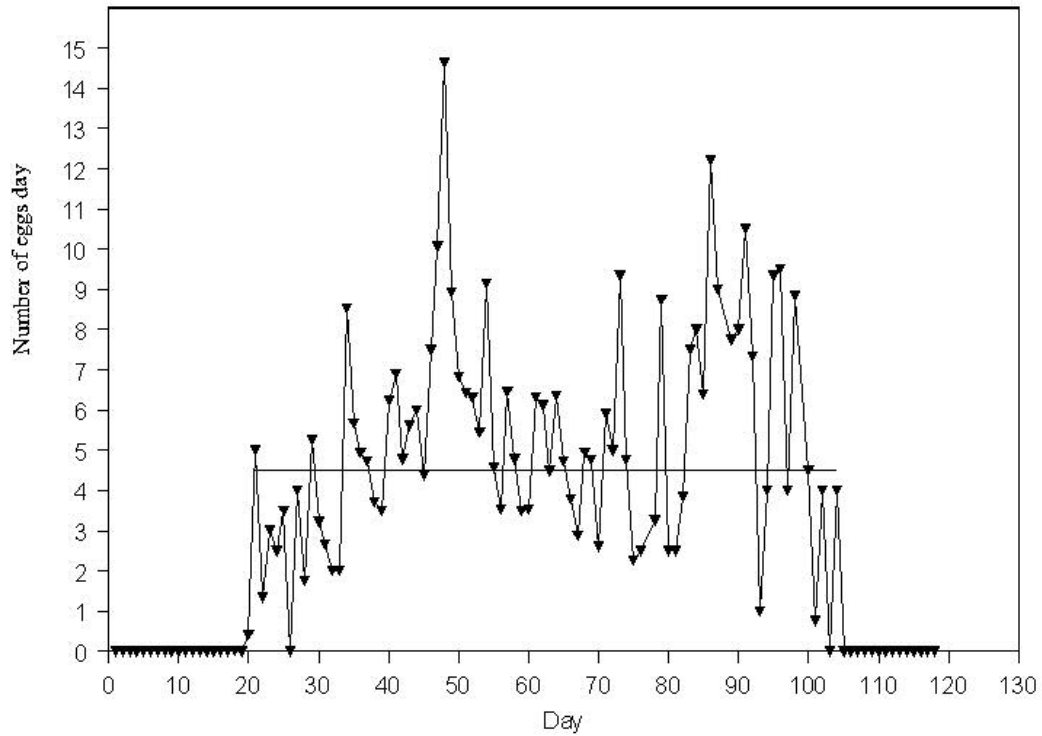


**Fig. 14.** Effect of nitrogen fertilization levels and water regimes on larvae weight gain (Means  $\pm$  SE) of *Otiorhynchus sulcatus* adult. Means with the same letter are not significantly different (LSD  $P < 0.05$ ).

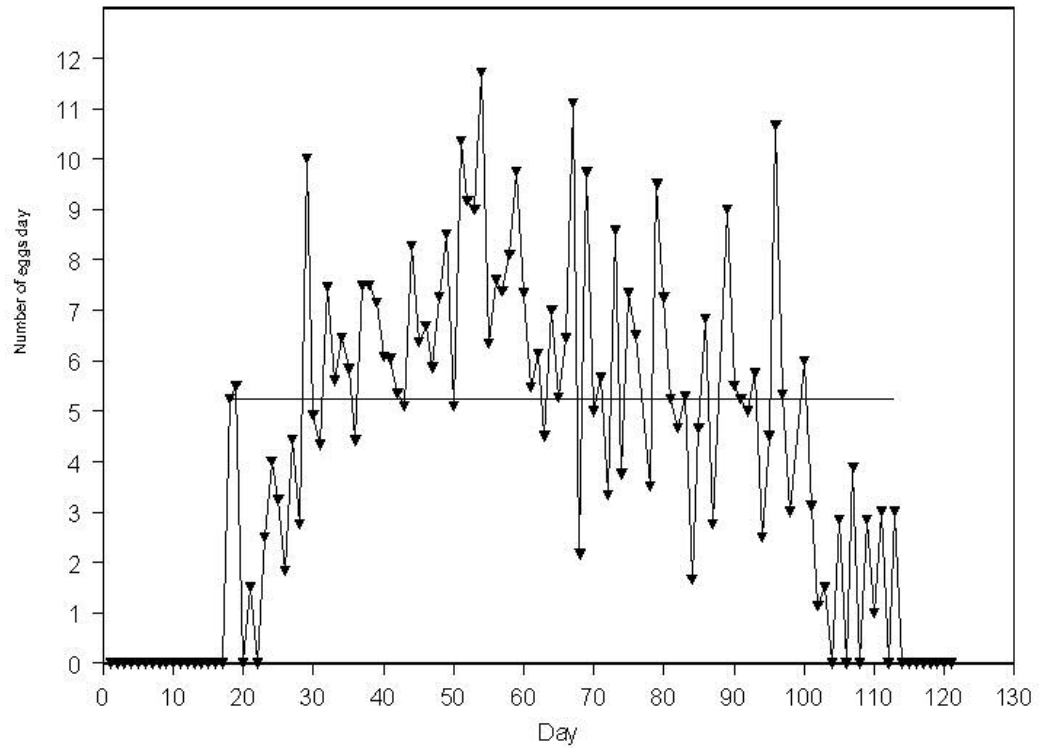




**Fig. 15.** Plot illustrates average number of eggs laid every day from *Otiorhynchus sulcatus* for low nitrogen fertilizer level. Data are plotted for both water regimes. Line shows the average number of eggs laid for each day.



**Fig. 16.** Plot illustrates average number of eggs laid every day from *Otiorhynchus sulcatus* for moderate nitrogen fertilizer level. Data are plotted for both water regimes. Line shows the average number of eggs laid for each day.



**Fig. 17.** Plot illustrates average number of eggs laid every day from *Otiorhynchus sulcatus* for high nitrogen fertilizer level. Data are plotted for both water regimes. Line shows the average number of eggs laid for each day.

## **CHAPTER II**

### **INFLUENCE OF *OTIORHYNCHUS SULCATUS* (COLEOPTERA: CURCULIONIDAE) LARVAE ON GROWTH OF *HEUCHERA MICRANTHA* (ROSALES: SAXIFRAGACEAE) UNDER VARYING NITROGEN FERTILIZATION AND WATER REGIMES**

#### **Introduction**

The plant root system has three major functions: water absorption, nutrient uptake, and anchorage (Harper et al. 1990). Besides these functions, roots may serve for storage of metabolites and photosynthate, for protection of dormant meristematic tissue, and as sites for the initiation of vegetative reproduction (Anderson 1987). More than 50% of net primary production is commonly allocated to below-ground plant parts (Coleman 1976, Fogel 1985). For example, root systems represent a place for storage of 50% of the total soluble sugar content of ryegrass (*Lolium spp.*) (Sutherland 1971). Proteins, carbohydrates, and minerals stored in roots are valuable resources for root feeding herbivores (Sutherland 1971, Andersen 1987).

Root feeding herbivores are serious insect pests in many agriculture systems (Brown and Gange 1990). The agriculture literature contains several examples of damage by subterranean insects resulting in loss of quality, yield, and profit.

Several studies have demonstrated that severe losses by root-feeding insects are common, and may reach 50% or more (Brown and Gange 1990). Of the major root-feeding insect orders Coleoptera, Lepidoptera, Diptera and Homoptera, members of the order Coleoptera are the most numerous and damaging (Anderson 1987). Within Coleoptera, weevils (Curculionidae) are major root-feeding pests and known to cause significant damage (Brown and Gange 1990, Root 1996, Hunter 2001). This is reflected as reduction in growth and development of above ground plants part. Weevil larvae (*Sitona discoideus* Gyllenhal) in Lucerne (*Medicago sativa* L) are known to cause greater damage than the more apparent leaf-chewing adults (Goldson et al. 1985).

Larval feeding on rhizobial root nodules can result in complete destruction of the root mass (Godfrey et al. 1987). Reichman and Smith (1991) found that root loss had a greater effect than leaf loss in red bean (*Tragopogon dubius* L.). Studies conducted with root-feeding herbivores found that they significantly reduced plant height, total biomass, fine root weight, rootstock weight, shoot weight, duration of flowering, and delayed flowering (Notzold et al. 1998). Weevils may also cause changes in root: shoot ratios (Vranjic and Gullan 1990). This occurs due to alterations in carbon and nitrogen allocation, and can result in plant death (Hunter 2001).

The impact of root herbivores is host dependent (Mattson 1980, Herms 2002). Verkaar et al. (1986) and Louda et al. (1990) suggested that plant quality is often a determining factor in herbivore related reductions in plant growth rate, biomass production, and height. Performance of root-feeding herbivores is related to environmental conditions such as nitrogen and water availability (Louda 1990, Owen et al. 1991, Hunter 2001). Root feeding may further exacerbate moisture stress and its

effect on plants and their quality as a food source for herbivores (Gange and Brown 1989).

Very few field studies have documented the effect of root-feeding insects on individual plants. For example, over the last 5 years, *Ecological Entomology* (5 years) and *Agricultural and Forest Entomology* (1 year) have published only four articles on root-feeding insects in comparison to 288 articles on insects that feed on above-ground plant parts (Hunter 2001). There is also a lack of field studies that identify environmental factors such as nutrient, and water and their effects on below-ground herbivores on ornamental plants (Hunter 2001). The lack of information in ornamental systems does not reflect the importance of below-ground herbivores in plant production industries (Brown and Gange 1990, van Tol and Raupp in press). Most of the studies conducted in horticultural systems related to below-ground herbivores focused on the control of weevils, specifically investigating chemicals and pathogens used for controlling them (Zimmermann 1984, Nielson and Roth 1985, Owen et al. 1991, Moorhouse et al. 1993, Cowles 2001, Gil et al. 2001, van Tol and Raupp in press). Hunter (2001) suggested that future studies should focus on relationships between root-feeding herbivores and a variety of environmental factors that affect their performance.

In nursery production systems, nitrogen is considered a key nutrient for plant growth. For container grown crops, fertilization plans used by growers apply slow-release nitrogen and supplementary nitrogen (Davidson et al. 2000). This is considered a necessary practice for rapid plant growth, green color, and increased market value of plants. In addition, nitrogen fertilization has been applied in nursery production as a common strategy for increasing plant pest resistance (Smith 1978, Funk 2000, Iles 2000).

The assumption that fertilizer enhances pest resistance has emerged in the virtual absence of supporting evidence (Raupp et al. 1992, Herms 2001, 2002). In a thorough review of literature surrounding the relationship between fertilization and insect attack, Herms (2001, 2002) provided convincing evidence that fertilization decreased resistance of woody plants to most guilds of insect pests that feed in above-ground plant parts. Our recent studies demonstrated that fertilization increased fecundity and larval performance of *O. sulcatus* (F.) (Bejleri et al. in prep.).

Among insect herbivores in ornamental plant production, black vine weevil, *O. sulcatus* (Coleoptera: Curculionidae) is considered the second most important pest in nurseries in the northeastern United States (Raupp and Hoitink 1996). *Otiorhynchus sulcatus*, also known as the millennium bug (Labuschagne 1999), is a serious global pest in nursery production systems, in Australia, the Netherlands, Germany, Norway and England (Parrella 1984, van Tol 2002). Pest status of this insect has increased during the past 20 years throughout the United States as nurseries have expanded production of favored host plants, including containerized rhododendrons (*Rhododendron* spp.) and yews (*Taxus* spp.) (Elbertson et al. 1997, Nielson et al. 1978). Container production results in greater host density, which facilitates resource location and utilization by weevils. In addition, microhabitat conditions provided by container media and container production areas are apparently optimal for weevil reproduction (Montgomery and Nielson 1979). Therefore, the increased pest status of *O. sulcatus* in nurseries and greenhouses is due to the concentration of favored host plants, the suitability of potting mix, and favorable environmental conditions such as high levels of fertilizer and water (Elbertson et al. 1997).

*Otiorhynchus sulcatus* larvae feed on roots and cause the primary economic damage. Economic damage may occur at relatively low larval densities. For example, La Lone et al. (1981) estimated that three larvae were lethal to one year-old potted rhododendron. Moorhouse (1990) reported that a single *O. sulcatus* larva could kill a *Cyclamen* plant. Established crops are more resistant to weevil damage than young plants and newly transplanted cuttings. Apparently, larger root systems can withstand feeding without adverse effects on growth (Neiswander1953). Surveys of nurseries in Baltimore County, MD (2001), found that young plants of *Heuchera micrantha* “Palace purple”, *Berginia* “*Berginia cardifolia*”, and *Astilbe* “*Astilbe cardifolia*” suffered more damage than mature plants from *O.sulcatus* larvae (Bejleri, unpubl. data).

Little is known about the effects of *O. sulcatus* larvae on the growth of perennial plants. Less is known about the modifying effects of different nutrient and water regimes on weevil damage. The objective of this study was to investigate the effects of *O. sulcatus* larvae on the growth of *Heuchera micrantha* “Palace purple” under different regimes of nitrogen fertilization and water.

## **Methods**

### **Experimental design and treatments**

Greenhouse and laboratory experiments were performed at the University of Maryland in College Park, Maryland during 2002. Study plants, *Heuchera micrantha* Dougl. ex Lindl. var. ‘Palace Purple’ (Saxifragaceae), were purchased as plugs (Bluemount Nurseries Inc, Monkton, MD) and potted into #1 containers (2.84 L) in a



soil-less media (Metro Mix 510, Scott's Sierra Hort. Products Company, Marysville, OH). A  $3 \times 2 \times 2$  factorial design was used in this study, with three levels of nutrients (25 mg N/week, 75mg N/week and 150mg N/week), two water regimes (water-stressed and non-water-stressed), and two levels of weevils (infested or not infested with *O. sulcatus* larvae) (12 treatment combinations, Table 1). Eight replicates of each of the twelve treatment combinations were arranged in a completely randomized block. All plants were placed on drip irrigation, and nitrogen and water treatments were maintained through the irrigation system. Nutrient solutions were composed of ammonium nitrate, potassium sulfate, potassium phosphate, and potassium nitrate (Fisher Company, Burr Ridge, IL). A nitrogen level of 150 mg N/week is commonly used in nursery production. Phosphorous and potassium fertilizer were used at the same proportion for all treatments to balance the nitrogen in the nutrient solution. Nutrient solutions were applied to plants weekly using three Dosatron DI16-11GPM's (Dosatron International, Inc. Clearwater, Florida) to maintain the appropriate nitrogen treatment.

A preliminary experiment was conducted in a growth chamber in 2001, to determine when *Heuchera* were in water-stressed versus non-water-stressed states. Based on our results, non-water-stressed plants in greenhouse received water when water volumetric in soil reached between 800-850 mVolt (Chapter I). This corresponds to stomatal conductance of 80-85 mol H<sub>2</sub>O/m<sup>2</sup>/sec. Plants under water-stress treatment received water when water volumetric in soil reached 400-450 mVolt, which corresponded to stomatal conductance of 40-50 mol H<sub>2</sub>O/m<sup>2</sup>/sec. The average water volumetric in soil for each water treatment was measured every morning using a Theta Probe (Type ML 2x, Dynamax, Huston, Texas).

***Plant infestation.*** On August 26, 2002 second instar larvae from a colony were placed in a container, and taken to the greenhouse in a cooler. One half of the plants in each nitrogen/water treatment were infested with 10, second instar *O. sulcatus* while the other half of the plants were not infested (control). Each cup was picked randomly from cooler and larvae were carefully placed in a trench 2-3 cm deep using soft brush. After larvae were placed, they were covered with fine soil. All plants received nitrogen and water treatments regularly until harvest on October 26<sup>th</sup>, 2002.

***Plant growth.*** Infested and uninfested plants were harvested four months after initiation of treatments to determine the effect of nitrogen fertilization, water regimes, and larvae on plant top and root growth. Plants were harvested just prior to seed set which coincides with slowed growth of *Heuchera*. Plant growth was determined by measuring top growth (leaves and petioles) and root dry weight. Eight plants from each treatment combination (96 plants in total) were brought into laboratory from the greenhouse. Roots were carefully rinsed to remove soil. Tops and roots were individually dried in an Isotemp oven at 65 °C for 48 hours prior to weighing on a Denver Instruments Scale model D1-8kD (Denver Instrument, Denver, Colorado).

***Plant chemistry.*** Dried leaf and root samples were used to measure leaf and root nitrogen and carbon content. Dried leaf and root samples were ground with a Cyclotec 1093 grinder (Foss, Eden Prairie, Minnesota) and stored at -20 °C prior to analysis. Analyses to determine nitrogen and carbon contents of leaf and root tissue were performed at the Soils Laboratory, Maryland Cooperative Extension at the University of Maryland (College Park, MD).

## Statistical Analysis

Data were analyzed as a  $3 \times 2 \times 2$  factorial design using the PROC MIXED procedure (SAS Institute 2003). The factorial analysis of variance carried on the tests of main effects, interactions and all pair wise mean comparisons for all simple effects. The LSMEANS statement was used to compute one and two way means and the expected mean squares for the fixed effects and a random error terms. The MIXED/LSDIFF procedure was used for pair wise comparisons of plant growth, leaf nitrogen, leaf C:N and root C:N means with probability of  $P < 0.005$ .

Prior to ANOVA, the normality and heterogeneity of variances for our data were analyzed and data for larval survival were transformed using a log transformation.

## Results

Survival of *O. sulcatus* larvae ranged from 13.7 % at high nitrogen and water-stressed plants to 26.5 % at high nitrogen and non-water-stressed plants. Survival of *O. sulcatus* larvae reared on *Heuchera* under varying nitrogen fertilization and water regimes showed no significant nitrogen fertilization by water interaction, or main effects of nitrogen fertilization or water (Table 4 Chapter I).

There was no significant water  $\times$  nitrogen  $\times$  larvae interaction on top growth (Table 2). There was no interaction between the presence of larvae and water or nitrogen fertilization on top growth (Table 2). However, all three main effects water, nitrogen fertilization, and larvae significantly affected top growth (Table 2). For non-water-stressed and water- stressed plants, the presence of *O. sulcatus* larvae reduced top growth

in four nitrogen fertilization regimes (Fig. 1). Even in regimes where significance was not detected, there was a trend for reduced top growth when larvae were present (Fig. 1).

There was no significant water  $\times$  nitrogen  $\times$  larvae interaction on root growth (Table 2). There was no interaction between the presence of larvae and water or nitrogen fertilization on root growth (Table 2). However, the water regime and the presence of larvae significantly affected root growth (Table 2). For non-water-stressed and water-stressed plants, the presence of *O. sulcatus* larvae reduced root growth in half of nitrogen fertilization regimes (Fig. 2). As with top growth, even in regimes where significance was not detected, there was a trend for reduced root growth when larvae were present (Fig. 2).

Fertilization generally improved top and root growth in regimes where water was plentiful (Fig. 2). Plants grown under conditions of water-stress did not respond to increased levels of nitrogen fertilization (Fig. 2). At moderate and high levels of nutrient inputs weevil larvae caused greater reductions in plant growth (Fig. 2). There were no significant water  $\times$  nitrogen  $\times$  larvae interactions or two-way interactions between larvae and water and nitrogen regimes on the nitrogen content of *Heuchera* leaves or roots (Table 2). However, leaf and root nitrogen content were significantly affected by water and nitrogen regimes (Table 2). Increasing levels of nitrogen fertilization generally increased leaf and root nitrogen content while increasing the level of water had the opposite effect (Fig. 3 and 5). Leaves and roots of water-stressed plants had higher levels of nitrogen in their tissues (Fig. 3 and 5).

There were no significant water  $\times$  nitrogen  $\times$  larvae interactions on leaf or root C:N ratios (Table 2). However, there was a significant interaction between larvae and

nitrogen regime on the C:N ratio of *Heuchera* leaves and a near significant interaction of larvae and nitrogen regime on C:N ratio of roots (Table 2). At low levels of nitrogen, the presence of larvae tended to reduce C:N ratios but at high levels of nitrogen this trend was reversed (Figs. 4 and 6). Nitrogen fertilization and water regime both directly affected C:N ratios of leaves and roots (Table 2). The C:N ratio of leaves and roots decreased with both increasing levels of nitrogen fertilization and water-stress (Figs. 4 and 6). While larvae had no effect on leaf C:N ratio, their presence tended to elevate C:N ratios of roots but this trend was only marginally significant (Table 2, Fig. 6).

## Discussion

The reduction in root biomass associated with the presence of *O. sulcatus* larvae agrees with findings of other studies (Anderson 1987, Brown and Gange 1990, La Lone et. al. 1981). The feeding of *O. sulcatus* larvae removes stored resources, disrupts roots mechanically and disrupts other physiological functions such as water and nutrient uptake. These results indicate that when water was not limited larvae ate more roots with increasing nitrogen. For water-stressed plants, low nitrogen resulted in a significant reduction in root biomass of about 57%. In moderate and high nitrogen levels, there was no significant reduction in root biomass but there was a clear trend for reduced biomass when larvae were present.

Roots store products of photosynthesis and contain large reserves of amino acids and carbohydrates (Sutherland 1971, Mattson 1980, Andersen 1987). Rich reserves of

amino acids and carbohydrates in roots may be one reason that roots are a suitable resource for herbivores. Nitrogen concentration in *Heuchera* roots varied from 1.6% in low nitrogen treatment and non-water-stress to 2.8% in high nitrogen treatment and water-stress. Leaf nitrogen varied from 1.5% in low nitrogen and non-water-stress to 2.6% in high nitrogen and water-stress treatment. These results provide evidence that *Heuchera* roots are not nitrogen poor tissues and are a good resource for herbivores. This conflicts with Bloom et al. (1985) who suggest that below-ground plant tissues are poor in nitrogen except when storage is involved. My results agree with Mattson (1980), who found that the average nitrogen content of roots was higher than any values tabulated for other plant parts.

Several studies found that changes in root biomass are reflected in alterations in above-ground plant tissues and organs. Impacts of root herbivores include changes in plant architecture (Sheppard et al. 1995) and reductions in plant height and biomass (Hopkins et al 1993). Gange and Brown (1989) found that root feeding by larvae of garden chafer, *Phyllopertha horticola* L, caused symptoms similar to drought stress in *Capsella bursa pasotis* L. My results showed that for non-water-stressed and water-stressed plants larvae reduced plant top biomass. For water-stressed plants this effect was more evident at higher levels of fertilization.

Larvae feeding on roots did not affect nitrogen levels of leaves or their C: N ratios. However, the presence of larvae generally reduced levels of nitrogen found in roots and increased the C: N ratio of roots. This trend is consistent with a shift in allocation of plant resources from growth to carbon-based defenses (Herms and Mattson 1992, Herms 2001, 2002, Stamp 2003, Bejleri et al. in prep). In an earlier work, I

demonstrated that increased C: N ratios in the roots and leaves of *Heuchera* correlated with reduced performance of *O. sulcatus* larvae and adults (Bejleri et al. in prep.).

The implications of these findings to plant growers are as follows. The feeding of *O. sulcatus* larvae significantly reduces both root and top biomass of *Heuchera*. Reductions in root and top growth are exacerbated on well-watered plants under moderate and high levels of nitrogen fertilization commonly used in nursery production systems. When *O. sulcatus* larvae are present, increased levels of nitrogen generally do not compensate for the top or root growth lost due to greater herbivory caused by larvae. For *Heuchera* infested by *O. sulcatus*, growers must eliminate larvae to reap the benefits that increased levels of nitrogen fertilization can provide to plant growth. However, increasing levels of nitrogen fertilization will significantly increase the performance of *O. sulcatus* larvae and adults should plants become infested.

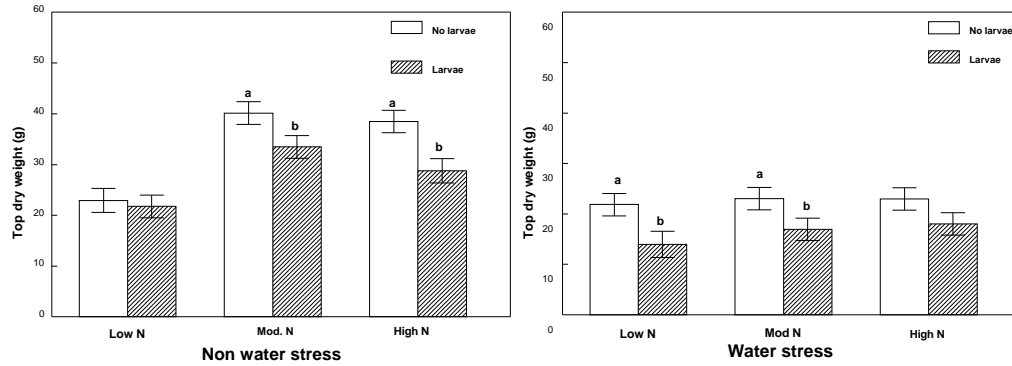
**Table 1.** Nitrogen fertilization and water treatments applied to containerized *Heuchera micrantha* “Palace purple” with and without *Otiorhynchus sulcutus*.

Treatments		
Nitrogen	Water	# Larvae pot
Low 25 mg /week	Non water stress	10
Low 25 mg /week	Non water stress	0
Low 25 mg/ week	Water stress	10
Low 25 mg/ week	Water stress	0
Moderate 75 mg/wek	Non water stress	10
Moderate 75 mg/wek	Non water stress	0
Moderate 25 mg/week	Water stress	10
Moderate 25 mg/week	Water stress	0
High 150 mg/week	Non water stress	10
High 150 mg/week	Non water stress	0
High 150 mg/week	Water stress	10
High 150 mg/week	Water stress	0

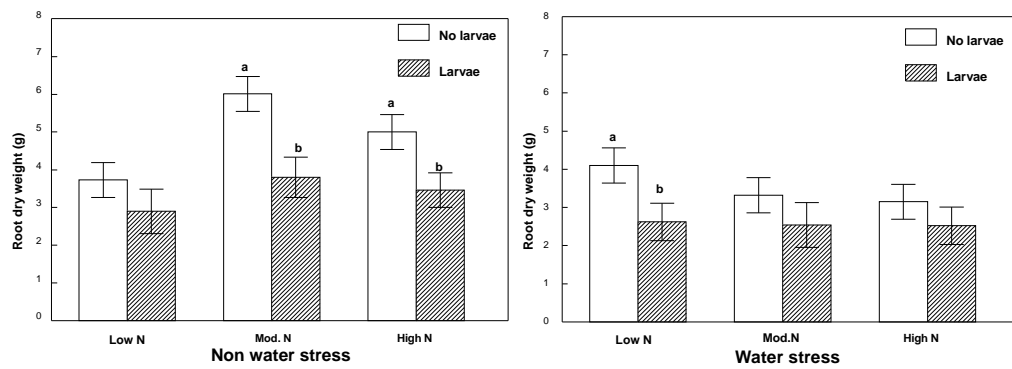


**Table 2.** Results of the ANOVA testing the effect of water, nitrogen fertilization and *Otiorhynchus sulcutus* larvae on top dry weight, root dry weight, nitrogen content and C:N ratio in leaves and roots of *Heuchera micrantha*.

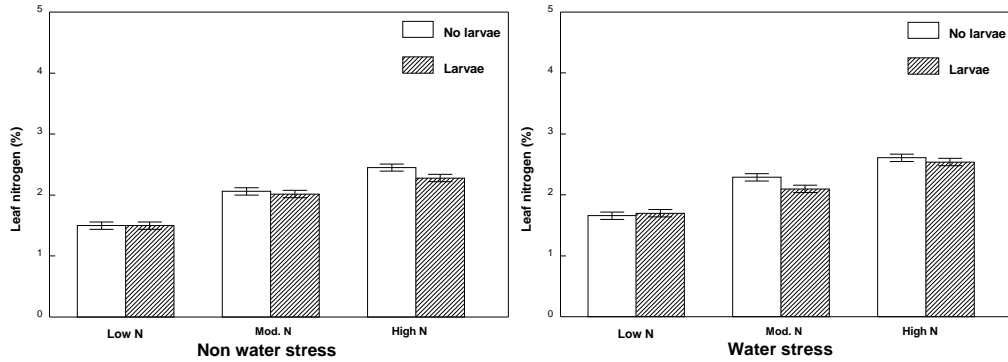
Response	variati	Source of variation	d	F	P
Top dry weight	water		1	62.61	< 0.0001
	nitrogen		2	15.34	< 0.0001
	larvae		1	12.87	0.0006
	water × nitrogen		2	6.63	0.0021
	water × larvae		1	0.23	0.6327
	nitrogen × larvae		2	1.07	0.3485
	water × nitrogen × larvae		2	1.06	0.3516
Root dry weigh	water		1	14.45	0.0003
	nitrogen		2	1.34	0.2669
	larvae		1	19.64	<0.0001
	water × nitrogen		2	5.07	0.0086
	water × larvae		1	0.94	0.3354
	nitrogen × larvae		2	0.20	0.8231
	water × nitrogen × larvae		2	1.16	0.3180
Leaf nitrogen	water		1	19.95	< 0.0001
	nitrogen		2	178.99	< 0.0001
	larvae		1	2.44	0.1217
	water × nitrogen		2	0.29	0.7526
	water × larvae		1	0.12	0.7269
	nitrogen × larvae		2	2.53	0.0853
	water × nitrogen × larvae		2	0.93	0.3971
Leaf CN ratio	water		1	28.83	< 0.0001
	nitrogen		2	206.74	< 0.0001
	larvae		1	0.00	0.9565
	water × nitrogen		2	0.94	0.3936
	water × larvae		1	0.03	0.8644
	nitrogen × larvae		2	4.30	0.0166
	water × nitrogen × larvae		2	0.73	0.4858
Root nitrogen	water		1	37.18	< 0.0001
	nitrogen		2	194.82	< 0.0001
	larvae		1	5.82	0.0182
	water × nitrogen		2	1.80	0.1716
	water × larvae		1	1.25	0.2669
	nitrogen × larvae		2	1.97	0.1468
	water × nitrogen × larvae		2	0.08	0.914
Root CN ratio	water		1	46.32	< 0.0001
	nitrogen		2	219.14	< 0.0001
	larvae		1	3.06	0.0843
	water × nitrogen		2	1.23	0.2970
	water × larvae		1	1.61	0.2087
	nitrogen × larvae		2	2.46	0.0921
	water × nitrogen × larvae		2	0.08	0.9267



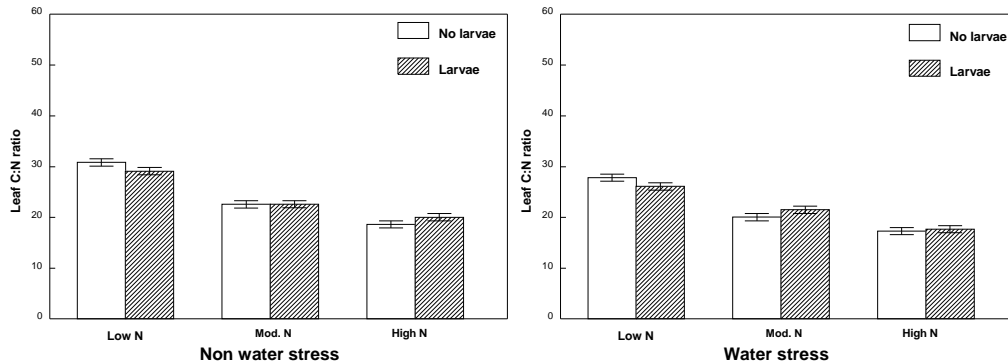
**Fig. 1.** *Otiorhynchus sulcutus* larvae effect on the top dry weight (Mean  $\pm$  SE) of *Heuchera micrantha*. Comparisons were made between infested and uninfested treatments within water regime and for the same levels of nitrogen. Bars with different letters within a water regime and nitrogen level are significantly different ( $P < 0.05$ ).



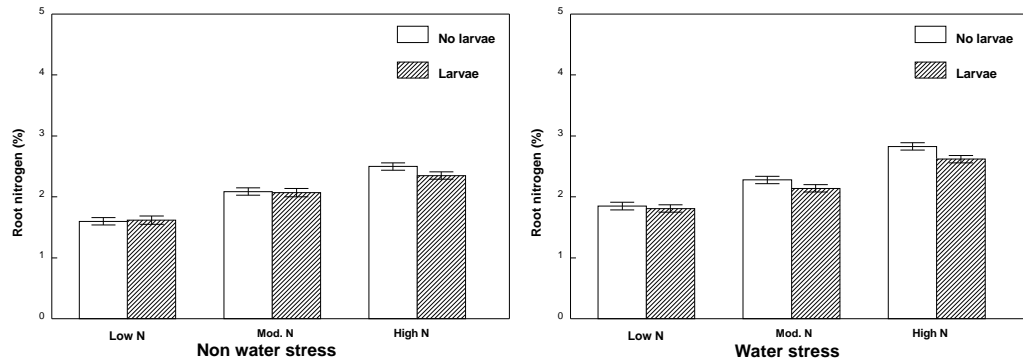
**Fig. 2.** *Otiorhynchus sulcutus* larvae effect on root dry weight (Mean  $\pm$  SE) of *Heuchera micrantha*. Comparisons were made between infested and uninfested treatments within water regime and for the same level of nitrogen. Bars with different letters within a water regime and nitrogen level are significantly different ( $P < 0.05$ ).



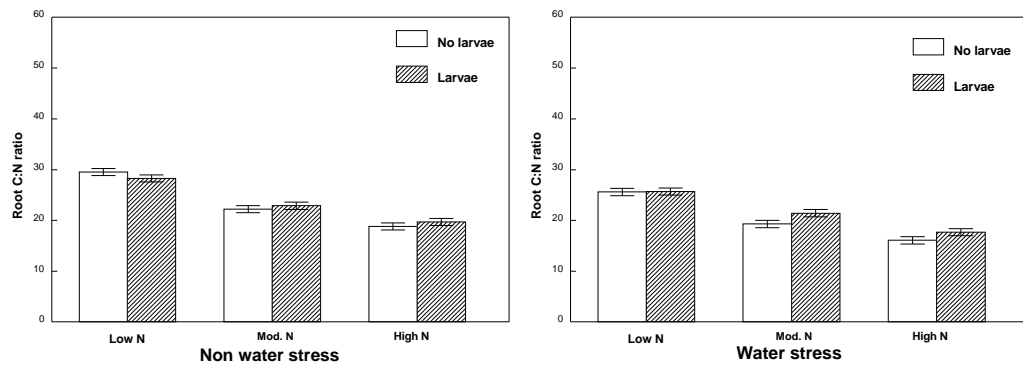
**Fig. 3.** Effect of nitrogen fertilization on leaf nitrogen (Mean  $\pm$  SE) of *Heuchera micrantha* either infested with larvae of *Otiorhynchus sulcutus* or uninfested (control). Comparisons were made between infested and uninfested treatments within water regime and for the same levels of nitrogen.



**Fig. 4.** Effect of nitrogen fertilization on C: N ratio in leaves (Mean  $\pm$  SE) of *Heuchera micrantha* either infested with larvae of *Otiorhynchus sulcutus* or uninfested (control). Comparisons were made between infested and uninfested treatments within water regime and for the same levels of nitrogen.



**Fig. 5.** Effect of nitrogen fertilization on root nitrogen (Mean  $\pm$  SE) of *Heuchera micrantha* either infested with larvae of *Otiorynchus sulcutus* or uninfested (control). Comparisons were made between infested and uninfested treatments within water regime and for the same levels of nitrogen.



**Fig. 6.** Effect of nitrogen fertilization on C: N ratio in roots (Mean  $\pm$  SE) of *Heuchera micrantha* either infested with larvae of *Otiorynchus sulcutus* or uninfested (control). Comparisons were made between infested and uninfested treatments within water regime and for the same levels of nitrogen.

## **CHAPTER III**

### **DOES FERTILIZATION INCREASE TOLERANCE OF *HEUCHERA MICRANTHA* “PALACE PURPLE” (ROSALES:SAXIFRAGACEAE) PLANTS TO *OTIORYNCHUS SULCATUS* (F.) (COLEOPTERA: CURCULIONIDAE)?**

#### **Introduction**

Fertilization is an important practice in nursery production for rapid plant growth and shortening the interval from planting to sale. In addition, nitrogen fertilization has been applied in nursery production as a common strategy for increasing resistance of plants to pests (Smith 1978, Funk 2000, Iles 2000). In ornamental landscapes woody plants are also commonly fertilized, based in part on the rationale that fertilization enhances pest resistance (Herms 2002).

The belief that fertilizer enhances pest resistance has emerged in the virtual absence of supporting evidence (Raupp et al. 1992, Herms 2001, 2002). Numerous studies have found that increased nitrogen fertilization frequently decreases tree resistance to insects by increasing nutrient and/or decreasing allelochemical concentrations (Painter 1951, Bryant et al. 1983, 1987, Herms and Mattson 1992, Herms 2002).

In a thorough review of literature surrounding the relationship between fertilization and insect attack, Herms (2002) provided convincing evidence that fertilization decreased resistance of woody plants to most pest insect guilds. For example, increased leaf nitrogen following fertilization enhanced performance of aphids and adelgids (Mitchell and Paul 1974, McClure 1991, 1992), scales (McClure 1977, 1980), citrus mealybug (Shrewsbury et al. 2004), plant bugs (Holopainen et al. 1995), and several species of caterpillars (Fisher and Fiedler 2000, Dankert 1996, Herms 2001).

Only a few studies conducted in conifers showed that fertilizer increased tree resistance (Oldiges 1959, Schwenke 1960, 1962). However, several of these studies had limitations in experimental design, statistical analyses, or they were confounded by factors such as effects of insecticides (Stark 1965, Herms 2002).

Kyto et al. (1996) emphasized that is important to distinguish between direct effects of fertilization on individual insects and effects of nitrogen fertilization at the population level. For example, Kyto et al. (1996) suggested that fertilization might increase abundance of sucking insects and their attendant ants. Ant activity reduces numbers of herbivores in other guilds. Hence, by increasing members of one guild fertilization may reduce numbers of another. By ignoring the effect of nitrogen fertilization in higher trophic levels results may be misinterpreted.

Plants are attacked above and below ground by insect herbivores. This results in direct and indirect injury to plants. Direct injury consists of removing plant tissues, changing plant architecture, and reducing growth. Indirect effects include changes in metabolic rates, photosynthesis, and levels of chemical defense (Detling et al. 1980,

Karban and Niiho 1995). Defoliation by insects reduces plant height, leaf number, and the number of seeds produced (Brown et al. 1985, Gange et al. 1989, Stamp 1984).

Root feeders are serious insect pests in agriculture systems (Brown and Gange 1990). Damage by subterranean insects results in loss of quality, yield, and income (Brown and Gange 1990). Crop losses of 50% or more to root feeding are not uncommon (Brown and Gange 1990). Several studies have found that performance of root-feeding herbivores and their effect on host plants are related to environmental conditions, such as nitrogen and water availability (Mattson 1980, Louda et al. 1990, Hunter 2001, 2002). To date most studies focused on the role of nitrogen fertilization in plant resistance in deciduous trees (Warning and Pitman 1985, Bryant et al. 1993, Kytö et al. 1996, Nowak and Berisford 2000, Herms 2002).

In managed ornamental systems, such as production nurseries, landscapes, and golf courses, insects and other pests frequently outbreak and cause aesthetic and economic damage to plants (Shrewsbury and Hardin 2003). Insect outbreaks are supported in nursery production because of favorable environmental conditions such as high fertilization, water regimes and optimal temperatures (greenhouse). Moreover, herbivore attack can be more serious in nursery production because most of the plants are young and small (Neiswander 1953). Furthermore, in ornamentals, defoliation is associated not only with reduction in plant growth but also with reduction of marketable value (Sadof and Raupp 1997).

The black vine weevil *Otiorhynchus sulcatus* F., is one of the most important pest species in the USA and Canada, and throughout the world where it threatens several types of herbaceous plants including cranberry, strawberry more than 100 species of

ornamental plants (Smith 1932, van Tol and Raupp in press). Herbaceous ornamental plants are one of the most important components of horticulture in the United States (ERS 1997). The overall objective of this study was to determine if fertilization increased the tolerance of the herbaceous perennial plant, *Heuchera micrantha* “Palace purple” to *O. sulcatus*. In this study I examined the effects of different densities of *O. sulcatus* larvae and adults on the top and bottom growth of *Heuchera micrantha* grown under low and high nitrogen levels.

## Methods

### Experimental design and treatments

Greenhouse and laboratory experiments were performed at the University of Maryland in College Park, Maryland in the summer of 2002. Study plants, *Heuchera micrantha* Dougl. ex Lindl. var. ‘Palace Purple’ (Saxifragaceae), were purchased as plugs (Bluemount Nurseries Inc, Monkton, MD) and potted into #1 containers (2.84 L) in a soil-less media (Metro Mix 510, Scott’s Sierra Hort. Products Company, Marysville, OH). Two studies with similar experimental design were conducted using *O. sulcatus* : one with adults and other with larvae. A  $2 \times 5$  factorial design was used in these studies, with two levels of nutrients (25 mg N/week and 150mg N/week), and five densities of *O. sulcatus* adults or larvae (10 treatment combinations for each study).

All plants were placed on drip irrigation fertilization, and nitrogen and water were maintained through the irrigation system. Nutrient solutions were composed of ammonium nitrate, potassium sulfate, potassium phosphate, and potassium nitrate (Fisher



Company, Burr Ridge, IL). A nitrogen level of 150 mg N/week is commonly used in nursery production. Phosphorous and potassium fertilizer were used at the same proportion for all treatments to balance the nitrogen in the nutrient solution. Nutrient solutions were applied to plants weekly using three Dosatron DI16-11GPM's (Dosatron International, Inc. Clearwater, Florida) to maintain the appropriate nitrogen treatment.

A preliminary experiment was conducted in a growth chamber in 2001, to determine when *Heuchera* were in a non-water-stressed state (Chapter I). Based on our earlier studies, plants received water when the water volumetric readings in the soil reached between 800-850 mVolt. This corresponds to stomatal conductance of 80-85 mol H<sub>2</sub>O/m<sup>2</sup>/sec. The average water volumetric in soil for each water treatment was measured every morning using a Theta Probe (Type ML 2x, Dynamax, Huston, Texas).

***Plant infestation.*** Adult *O. sulcatus* were maintained in laboratory on a diet of *Taxus* (yew) foliage. Larvae were maintained on a diet of organic carrots. On the day of infestation *O. sulcatus* adults and second instars were randomly chosen from colonies. Eight plant replicates from each nitrogen fertilization treatment (low and high) were infested with adults or larvae at densities of 0, 2, 4, 8, and 16 (80 plants total for each study). In the adult study all plants and their containers were covered with mesh inclusion cages to prevent adults from escaping. Larvae were put carefully in a trench 2-3 cm deep using soft brush. After larvae were placed in the trench, they were covered with fine soil. All plants received nitrogen treatments weekly until harvest.

***Plant growth.*** Plants were harvested one month after being infested with *O. sulcatus*. Plants were brought into the laboratory and cut at the soil line. Plant growth was estimated by measuring top (leaves and petioles) and root dry weight. Roots were

carefully rinsed to remove soil. For each plant, top growth and roots were individually dried in an Isotemp oven at 65 °C for 48 hours prior to weighing on a Denver Instruments Scale model D1-8kD (Denver Instrument, Denver, Colorado). Adult and larval survival were recorded for each treatment.

***Plant chemistry.*** Nitrogen was determined for dried leaf and root samples. Dried leaf and root samples were ground with a Cyclotec 1093 grinder (Foss, Eden Prairie, Minnesota) and stored at -20 °C prior to analysis. Analyses to determine nitrogen content of leaf and root tissue were performed at the Soils Laboratory, Maryland Cooperative Extension at the University of Maryland (College Park, MD).

#### Statistical Analysis

Data were analyzed as a 2 × 5 factorial using the PROC MIXED procedure (SAS Institute 2003). The factorial analysis of variance carried out tests of main effects, interactions and all pair wise mean comparisons for all simple effects. The LSMEANS statement was used to compute one and two way means and the expected mean squares for the fixed effects and a random error terms. LSD was computed as the most sensitive mean comparison method and probability of differences (  $PDIFF \leq .05$  ) as the criteria for significance in the pair wise comparisons of means. The Mixed/LSDIFF procedure was used for comparisons of means with a probability of  $P < 0.05$ . To correct for normality and heterogeneity of variances, data were transformed prior to ANOVA. Log transformations were used for adult and larval survival data. The relationship between top and root growth and weevil density (adult and larvae) was examined using PROC REG.

## Results

Survival of *O. sulcatus* adults on *Heuchera* under low and high nitrogen level showed no significant nitrogen by density interaction ( $P=0.9665$ ), or main effects of nitrogen ( $P=0.6364$ ) (Table 1). Adult survival did not differ between nitrogen levels across the range of adult density (Fig. 1). Significant differences in adult density persisted throughout the course of the study (Fig. 1).

No significant nitrogen fertilization by adult density interaction was found. Also, there was no main effect of adult density on leaf nitrogen content (Table 1). However, there was a significant main effect of nitrogen fertilization on leaf nitrogen content ( $P < 0.0001$ ) (Table 1). Leaf nitrogen content was greater at higher nitrogen levels and there was no significant difference across adult densities, except when densities of 0 and 16 were compared (Fig. 2). There was also no difference in leaf nitrogen content among plants that received low nitrogen levels for all adult densities (Fig. 2).

There was no significant nitrogen by beetle density interaction for top growth (Table 1, Fig. 3). Greater top growth resulted from fertilization only for uninfested plants (Fig. 3). At all other densities nitrogen fertilization had no effect on top growth. Top-growth decreased significantly with increasing adult density for the high nitrogen fertilization level:  $r^2=0.30$  and  $P=0.0002$  ( $y = -0.3865x + 32.4$  where  $y$  = top dry weight and  $x$  = adult density) and for the low nitrogen fertilization level:  $r^2=0.21$  and  $P=0.0027$  ( $y = -0.2303x + 31.7$  where  $y$  = top dry weight and  $x$  = adult density) (Fig. 3).

As in adult study, survival of *O. sulcatus* larvae fed *Heuchera* on low and high nitrogen levels showed no significant nitrogen by density interaction (Table 2). There was also no main effect of nitrogen on larval survival (Table 2).

The effect of fertilization on root chemistry followed the same pattern as did leaves. This study revealed no significant nitrogen fertilization by larval density interaction or main effect of density on leaf nitrogen fertilization content (Table 2). However, there was a significant main effect of nitrogen on root nitrogen content (Table 2). The highest root nitrogen content was observed in plants that received high nitrogen levels and there was no significant difference among plants at all larval densities (Fig. 5). There was also no difference in root nitrogen content between plants that received low nitrogen levels for all larval densities (Fig. 5).

Nitrogen fertilization affected root growth in the same way as top growth. There was no significant nitrogen fertilization by larval density interaction for root growth (Table 2, Fig. 6). For non-infested plants root growth between low and high nitrogen was at the border of significance. There was a significant difference in root growth between two nitrogen levels for the densities of zero and eight larvae per pot (Fig. 6). All other comparisons between nitrogen levels within the same larval density were not significant (Fig. 6). The linear trend of root growth followed the same pattern as in adult study. Root growth decreased significantly with increasing larval density, for plants on high nitrogen levels parameters were:  $r^2=0.28$  and  $P=0.0002$  ( $y = -0.076x + 2.554$  where  $y$  = root dry weight and  $x$  = larval density), and for low nitrogen fertilization level:  $r^2=0.13$  and  $P=0.027$  ( $y = -0.0517x + 2.162$  where  $y$  = root dry weight and  $x$  = larval density) (Fig. 6).

## Discussion

The level of nitrogen fertilization did not affect the survival of adults or larvae of *O. sulcatus*. Kyoto et al. (1996) also noted that for many species of herbivorous insects, survival is poorly correlated with the level of nitrogen fertilization. In general survival of larvae was less than that of weevil adults. We observed *O. sulcatus* larvae consuming conspecifics in the laboratory. Cannibalism in beetle larvae has been observed in laboratory conditions in other ground dwelling beetles such as larvae of Japanese beetle, *Popillia japonica* Newman (Regniere et al. 1981). When confined to small containers as they were in this study, larvae may eat each other. This may explain at least in part the high levels of mortality observed in the larvae.

Beetle density did not interact in a significant way with levels of fertilization to affect foliar nitrogen levels. In addition black vine weevil adults and larvae did not effect foliar nitrogen levels. However, fertilization did increase levels of foliar nitrogen in both studies. We have shown that *O. sulcatus* adults and larvae perform better on *Heuchera* plants with higher levels of leaf and root nitrogen. Both adult fecundity and larval weight gain increased significantly when fertilizer was increased (Bejleri et al. in prep). Herms and Mattson (1992) pointed out that phenotypic variation in plant nitrogen content and allelochemical concentrations caused by fertilization affect resistance of plants to insects. Furthermore, then predicting patterns of secondary metabolism in response to environmental variation requires knowledge of the costs and benefits of different allocation patterns in different environments. Several studies have shown that increased leaf nitrogen favors performance of aphids and adelgids (Mitchel and Pael 1974,

McClure 1991, 1992), scales (McClure 1977, 1980), mealy bug (Shrewsbury et al. 2004), plant bugs (Holopainen et al. 1995), and sooty copper caterpillars (Fisher and Fiedler 2000).

The most important finding of this study was the inability of fertilization to increase top or bottom growth of *Heuchera* across a range of beetle densities. *Heuchera micrantha* plants responded in similar ways when fertilized at high and low levels across the range of beetle densities. The only case where fertilization consistently increased plant growth was on *Heuchera* not infested with beetles. At most densities of larvae and adults fertilization did not enhance the ability of *Heuchera* to tolerate beetles. This result agrees with those of others who have measured the effects of fertilization on the ability of plants to tolerate damage by chewing herbivores (Parker 1978, McGraw et al. 1990, Dankert 1996, Houle and Simard 1996). Moreover, Herms (2001) concluded that there is not any convincing evidence that shows fertilization enhances tree resistance or tolerance to insects.

In summary fertilization did not increase tolerance of *Heuchera* to black vine weevil larvae or adults. Also, increasing densities of adults and larvae resulted in reduced top and bottom weight, respectively. The belief that fertilization enhances plant resistance to insects needs to be reexamined in nursery production systems. Fast growth, resulting from fertilization may not be associated with enhanced plant resistance to herbivores. These studies showed that *Heuchera* plants maintained under high nitrogen inputs do not tolerate *O. sulcatus* more than plants receiving low nitrogen rates. Adult and larva damage did not differ between low and high nitrogen levels. In nursery container production fertilization is unlikely to help plants tolerate *O. sulcatus*

infestations. In addition higher levels of fertilizer have been shown to increase the performance of this pest (Bejleri et al., in prep.)

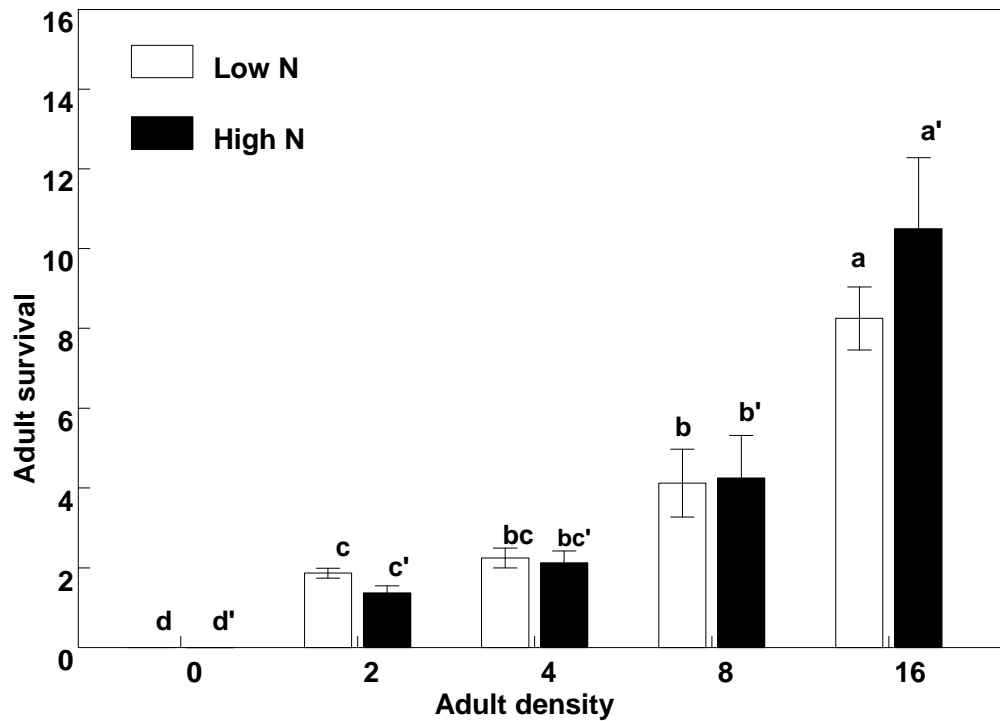
**Table 1.** Results of ANOVA testing the effect of nitrogen fertilization level and *Otiorhynchus sulcutus* adult density on survival, top biomass, leaf nitrogen of *Heuchera micrantha*.

Response variable	Source of variation	df	<i>F</i>	<i>P</i>
Adult survival	nitrogen	1	0.23	0.6364
	adult density	4	45.81	< 0.0001
	nitrogen × density	4	0.14	0.9665
Leaf nitrogen	nitrogen	1	80.44	< 0.0001
	adult density	4	1.44	0.2285
	nitrogen × density	4	0.98	0.4223
Top dry weight	nitrogen	1	2.49	0.8081
	adult density	4	9.02	<0.0001
	nitrogen × density	4	1.44	0.2350

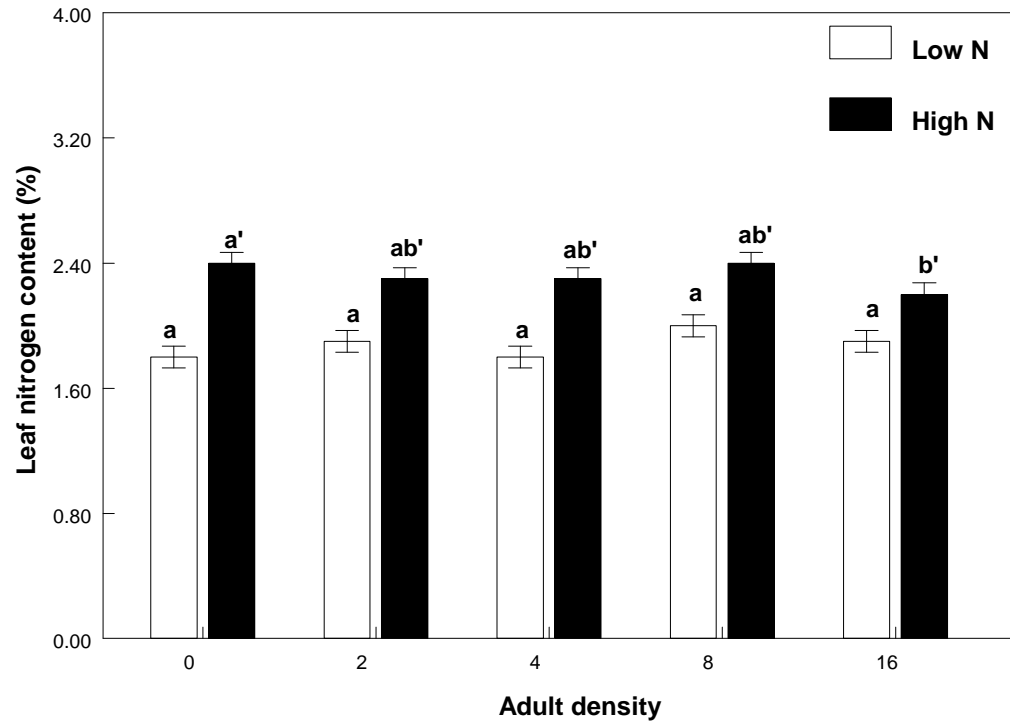


**Table 2.** Results of two way ANOVA testing the effect of nitrogen fertilization level and *Otiorhynchus sulcutus* larval density on survival, root biomass, root nitrogen of *Heuchera micrantha*.

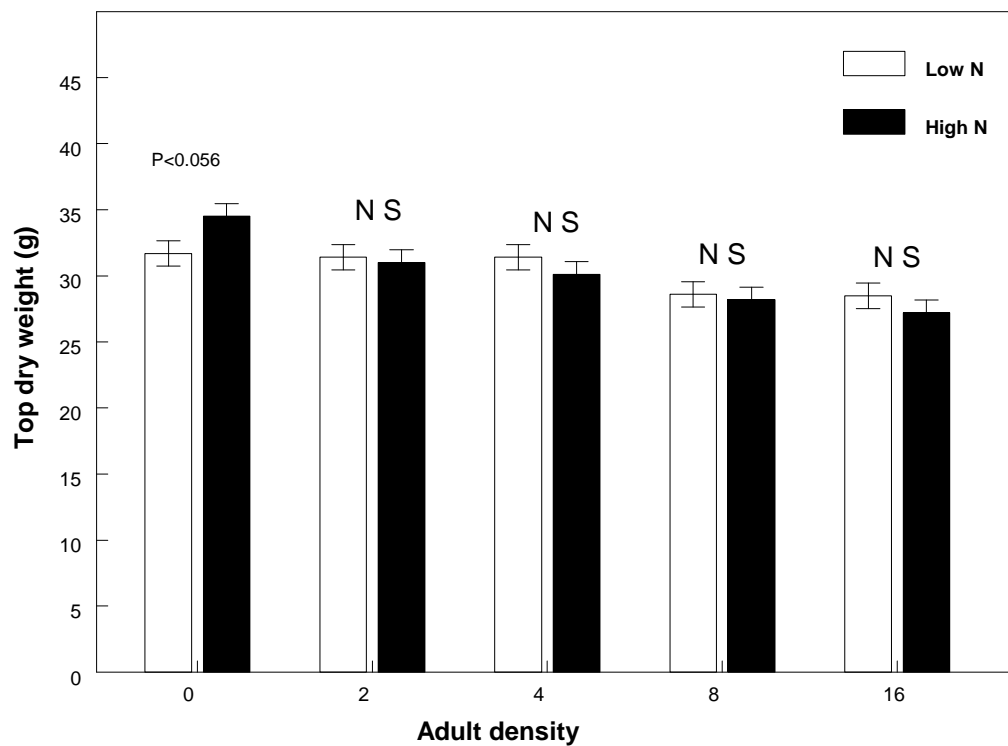
Response variable	Source of variation	df	<i>F</i>	<i>P</i>
Larval survival	nitrogen	1	1.07	0.3055
	larvae density	4	48.69	< 0.0001
	nitrogen × larvae	4	2.30	0.0670
Root nitrogen	nitrogen	1	109.08	< 0.0001
	larvae density	4	0.46	0.7648
	nitrogen × density	4	0.97	0.4314
Root dry weight	nitrogen	1	3.47	0.0670
	larvae density	4	5.98	0.0004
	nitrogen × density	4	1.52	0.2065



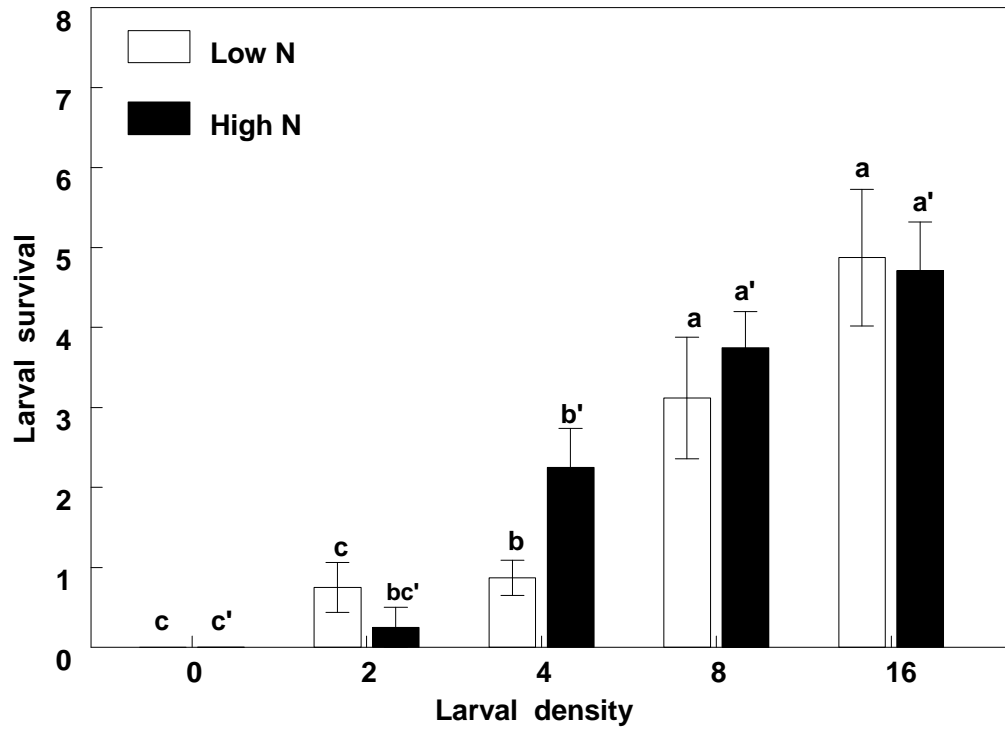
**Fig. 1.** Effect of nitrogen fertilization on survival (Means  $\pm$  SE) of *Otiorhynchus sulcatus* adults at low (25 mg N/week) and high (150 mg N/week) nitrogen levels. Comparisons are made within nitrogen level among five densities. Bars with different letters within nitrogen level are significantly different ( $P < 0.05$ ).



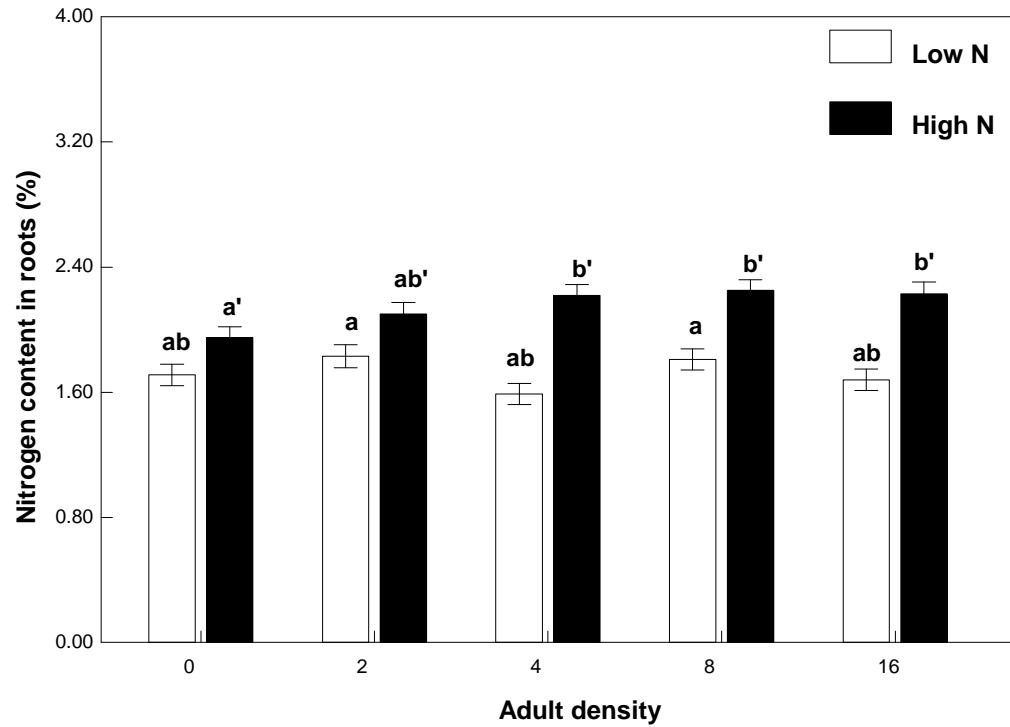
**Fig. 2.** Nitrogen fertilization effect on leaf nitrogen (Means  $\pm$  SE) of *Heuchera micrantha* at low (25 mg N/week) and high (150 mg N/week) nitrogen levels over five *Otiorhynchus sulcatus* densities. Comparisons were made within nitrogen level among five densities. Bars with different letters are significantly different ( $P < 0.05$ ).



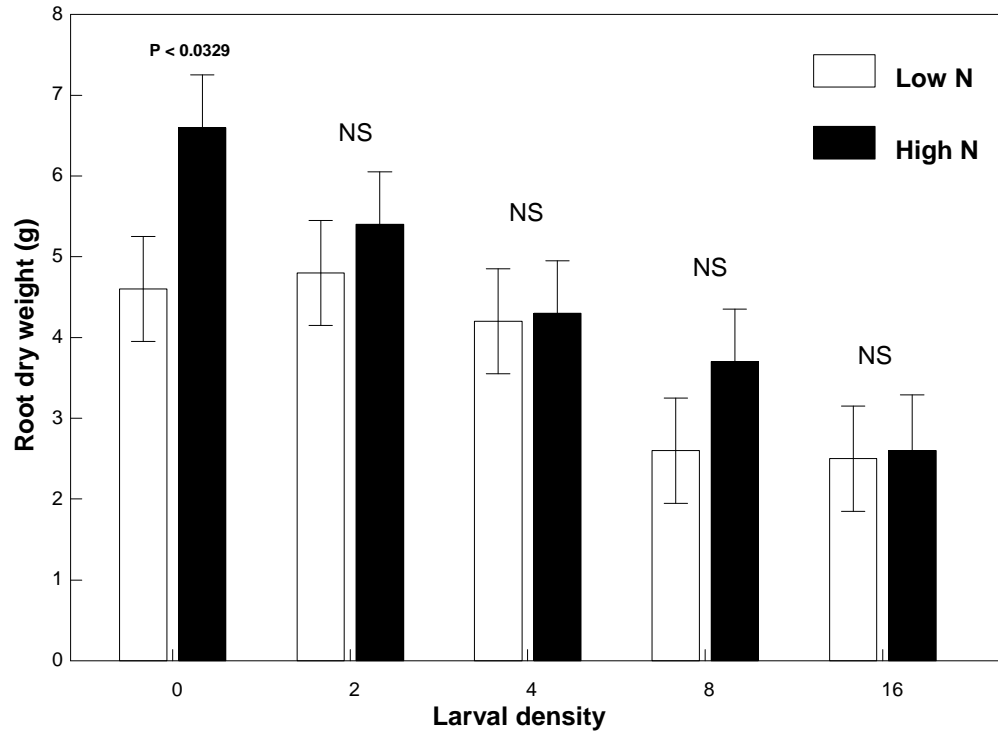
**Fig. 3.** Effect of low (25 mg N/week) and high (150 mg N/week) nitrogen fertilizer and adult *Otiorhynchus sulcatus* density on top dry weight (Means  $\pm$  SE) of *Heuchera micrantha*. Comparisons were made within five densities among low and high nitrogen level. Bars with NS letters are not significantly different ( $P < 0.05$ ).



**Fig. 4.** Effect of nitrogen fertilization on survival (Means  $\pm$  SE) of *Otiorhynchus sulcatus* larvae at low (25 mg N/week) and high (150 mg N/week) nitrogen levels over five densities. Comparisons are made within nitrogen level among five densities. Bars with different letters within nitrogen level are significantly different ( $P < 0.05$ ).



**Fig. 5.** Nitrogen fertilization effect on root nitrogen (Means  $\pm$  SE) of *Heuchera micrantha* at low (25 mg N/week) and high (150 mg N/week) nitrogen levels over five *Otiorhynchus sulcatus* larval density. Comparisons were made within nitrogen level among five densities. Bars with different letters are significantly different ( $P < 0.05$ ).



**Fig. 6.** Effect of low (25 mg N/week) and high (150 mg N/week) nitrogen fertilizer and larvae *Otiorynchus sulcatus* density on root dry weight (Means  $\pm$  SE) of *Heuchera micrantha*. Comparisons were made within five densities among low and high nitrogen level. Bars with NS letters are not significantly different ( $P < 0.05$ ).

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