

Influences of fertilization on *Aphis gossypii* and insecticide usage

A. Chau¹, K. M. Heinz¹ and F. T. Davies Jr²

¹Department of Entomology, Texas A&M University, College Station, TX, USA; ²Department of Horticultural Sciences, Texas A&M University, College Station, TX, USA

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Abstract: Fertilization levels for ornamental crops may influence pest population dynamics, crop quality, and pest management strategy. We examined the effect of fertilization on population growth and within-plant distribution of melon or cotton aphid, *Aphis gossypii* Glover, on potted chrysanthemum, *Dendranthema grandiflora* (Tzvelev). In terms of pest management implications, we also investigated the effect of fertilization on the number of insecticide applications needed to control *A. gossypii* on potted chrysanthemum. Population growth rate of *A. gossypii* increased with fertilization levels from 0 to 38 ppm N and reached a plateau from 38 to 488 ppm N. Increased fertilization beyond 38 ppm N, 10% of the commercial standard, did not result in higher aphid number. Aphids responded to nutrient availability of plants by distributing themselves in areas with higher level of nitrogen. More aphids were found in the apical and middle strata of the plants than the basal stratum, which had the lowest nitrogen content. Leaf nitrogen content increased with increased fertilization level and was consistently higher in the apical and middle strata than the basal stratum. Increased fertilization from 0 to 375 ppm N did not result in higher number of insecticide applications. All three insecticides (bifenthrin, kinoprene or pymetrozine) were effective in keeping the aphid infestation below a pre-determined level, five aphids per plant, but pymetrozine required the least number of applications. For chrysanthemum, a fast-growing crop and heavy utilizer of nitrogen, increased fertilization shortened the time to flowering, which would allow growers to harvest their crop sooner and reduce the time for aphid population growth. Reduction in time to harvest could result in significant reduction of insecticide usage by reducing the time for aphid population growth. As a result, high fertilization together with minimal runoff may be a useful tactic to an integrated pest management (IPM) programme for managing *A. gossypii* on potted chrysanthemums.

Key words: *Dendranthema grandiflora*, fertilizer input, insecticide application, melon or cotton aphid, population growth rate

1 Introduction

In greenhouse ornamental production, fertilizers are being extensively used to produce high-quality crops. Frequently cited as an unwanted consequence of increased fertilization is an increase in plant nitrate and soluble amino acids (MENGEL and KIRKBY, 2001), which increase a plant's nutritional quality and attractiveness to phytophagous insects (VAN EMDEN, 1966; MINKENBERG and FREDRIX, 1989; BENTZ and LAREW, 1992; BENTZ et al., 1995). Higher levels of leaf nitrogen, in turn, enhance both growth and reproduction of herbivorous insects (DIXON, 1970; MATTSON, 1980; LARSSON, 1989; WARING and COBB, 1992; DOUGLAS, 1993; SLOSSER et al., 1998) and reduce their susceptibility to some insecticides (McKENZIE et al., 1995). Other studies also show that population growth rate and development time of phytophagous insects are influenced not only by plant nutrient levels but also nutrient ratios (BUSCH and PHELAN, 1999; JANSSON and EKBOM, 2002). Even fertilizer source is found to influence ovipositional choice of female *Bemisia argentifolii* Bellows & Perring on poinsettia by varying the soluble nitrogen content in the phloem sap (BENTZ et al., 1995).

Responses by aphids to fertilization vary greatly. Some studies report that nitrogen fertilization does not affect either aphid fecundity or number. ARCHER et al. (1995) showed that the number of Russian wheat aphid, *Diuraphis oxia* (Mordvilko), is not influenced by nitrogen fertilization but is higher on field-grown, non-irrigated wheat than irrigated ones. BETHKE et al. (1998) found that the fecundity of *Aphis gossypii* Glover differs among cultivars of chrysanthemum regardless of irrigation or fertilizer levels. However, studies of *A. gossypii* on cotton show that aphid fecundity increases with increasing nitrogen fertilization (ROSENHEIM et al., 1994; NEVO and COLL, 2001). VAN EMDEN (1966) also showed that reproduction of the cabbage aphid, *Brevicoryne brassicae* (L.), and the green peach aphid, *Myzus persicae* (Sulz.), on Brussels sprout increase with increasing nitrogen fertilization. However, too high a fertilization level can be detrimental to aphids (JANSSON and SMILOWITZ, 1986; BETHKE et al., 1998) as well as the host plants (SCHUCH et al., 1998) because of high salinity. One reason for the variable results may well be that the relationship between aphid growth and fertilization may not be linear. Another

reason may be that fertilizer source and nutrient ratio influence aphid development differently. In addition, all these studies, with the exception of two (ARCHER et al., 1995; NEVO and COLL, 2001), evaluate the effect of fertilization on individual insects within clip cages rather than free populations on plants. The leaves that aphids are confined on may differ in their nutrient quality and could dramatically influence aphid growth rate.

If aphids respond to nutrient availability within plants, then one might expect that the nutrient quality of the foliage would influence how aphids distribute themselves within the plants. Numerous studies have shown that developmental rate and fecundity of aphids are influenced by leaf age (VAN EMDEN and BASHFORD, 1969; WEARING, 1972; JANSSON and SMILOWITZ, 1985). Aphid distribution on plants also varies with host plant and aphid species. For example, JANSSON and SMILOWITZ (1986) showed that most *M. persicae* are found on the lower leaves of potato and their population growth rate is also highest on these leaves. On sugar beet and chrysanthemum, JEPSON (1983) and WYATT (1965) found that *M. persicae* tends to aggregate near the growth point of the plants. *Aphis gossypii*, on the other hand, tends to remain initially on the upper leaves of chrysanthemum but eventually redistributes to lower leaves (VEHRS et al., 1992). In a previous study on chrysanthemum, DAVIES et al. (2004) showed that leaf nitrogen is higher in young and physiologically mature leaves than older, basal leaves.

With production agriculture, environmental concerns and government regulations have prompted the need to reduce point-source runoff. As a result, fertilizer usage for a number of ornamental crops such as roses, poinsettias, and hydroponically grown chrysanthemum has been modified (KAGEYAMA et al., 1991; CABRERA et al., 1993; ROSE and WHITE, 1994). However, strategies for judicious use of fertilizers that maintain plant marketability, minimize aphid population growth, and reduce pesticide usage are needed. A better understanding of the influence of fertilization not only on pest populations but also on pesticide usage may form the foundation for the development of reduced-input crop management practices.

In this study, we used *A. gossypii* and chrysanthemum, *Dendranthema grandiflora* (Tzvelev) cv. 'Charm', as our experimental system. Melon or cotton aphid, *A. gossypii*, is one of the major pests of greenhouse-grown chrysanthemum (FURK and VEDIHI, 1990; VEHRS et al., 1992; GULDEMOND et al., 1994; STORER and VAN EMDEN, 1995). Our two objectives were: (1) to determine the effects of fertilizer input on both the growth and within-plant distribution of *A. gossypii* on chrysanthemum and (2) to examine the combined effects of fertilization and insecticidal input on the control of *A. gossypii* on potted chrysanthemum. To address these objectives, we investigated: (1) the effect of medium to high fertilization on *A. gossypii* abundance, (2) the effect of low to standard fertilization on *A. gossypii* abundance, (3) the effect of fertilization on population growth rate and within-plant distribution of *A. gossypii* under greenhouse conditions, and (4) the effect of fertilizer input on the number of insecticide applications needed to control aphid populations on potted chrysanthemum.

2 Materials and Methods

2.1 Plant, fertilizer and insects

To produce potted chrysanthemum for our studies, we followed the cultural practices recommended to commercial growers (YODER BROTHERS INC., 2001). A soilless potting mix (Sunshine Mix no. 1; Sun Gro Horticulture, Canada Ltd., Bellevue) was used as growing media. Rooted chrysanthemum cuttings were transplanted into pots (15.5 cm in diameter, 14.5 cm in depth; ITML Horticultural Products Inc., Brantford, ON, Canada), with four cuttings per pot. Chrysanthemum require elevated and balanced levels of nitrogen and potassium for proper vegetative growth (CRATER, 1992). To maximize applicability to chrysanthemum growers, a water-soluble, commercially available and complete fertilizer [Peters Professional Peat-lite special, 15-16-17 (15N-6.7P-14.1K); Scotts-Sierra Horticultural Products Company, Marysville, OH, USA] was used as the source of nutrients. The fertilizer used provides 53% N in nitrate form, 20.4% N in ammoniacal form and 26.1% N in urea form. This formulation is recommended by propagators to reduce leaf yellowing and increase longevity (YODER BROTHERS INC., 2001).

The recommended fertilizer rate for pulse or periodic feeding of potted chrysanthemum, based on nitrogen, is 350–400 ppm N (Scotts-Sierra Horticultural Products Company); therefore, we used 375 ppm N as the standard level (100%) for our studies. The amount of phosphorous and potassium in 375 ppm N fertilizer solution is 175 and 354 ppm, respectively. Fertilization began right after transplantation. Plants were fertilized twice a week and watered as needed between fertilizations. Depending on the fertilization levels, 200 ml of reverse-osmosis-treated tap water (for no fertilization) or fertilizer solution (for the other fertilization levels) was applied to each pot.

When there was 2–2.5 cm of new growth on plants, we pinched each plant back to seven laterals. When another 3–3.5 cm of new growth occurred after the pinch, we applied daminozide, a plant growth regulator (B-Nine WSG; Uniroyal Chemical Company Inc., Middlebury, CT, USA), to all pots at the concentration of 3500 ppm to reduce internode elongation (YODER BROTHERS INC., 2001).

Aphids used in the studies were obtained from an *A. gossypii* colony established originally with individuals collected from chrysanthemum grown in research greenhouses at Texas A&M University in College Station, Texas. The colony was maintained in the laboratory at 26°C, 45% relative humidity (RH), and under a 11 : 13 h (light : dark) (L : D) photoperiod on chrysanthemum and periodically augmented with individuals collected from naturally infested chrysanthemum grown in experimental greenhouses at the university.

2.2 Effect of medium to high fertilization levels on *A. gossypii*

We hypothesized that aphid abundance would increase with increased host plant quality. We manipulated host plant quality by manipulating fertilization across a medium to high fertilization range (75–488 ppm N) and determined its influence on aphid abundance. We tested five fertilization levels: 75, 188, 281, 375 and 488 ppm N which is 20, 50, 75, 100 and 130% of the standard level. Two hundred cuttings were transplanted into 50 pots. We used a complete randomized design with 10 replications per treatment with individual pots serving as replicates. The plants were maintained in growth chambers at 24°C day/20°C night, 75% RH. The photoperiod was 16 : 8 h (L : D) for approximately 3 weeks and then switched to 11 : 13 h (L : D) for the duration of the experiment to induce flower production.

On the day after daminozide application, when the plants were approximately 4 weeks old, five apterous adults of *A. gossypii* (6–7 days old) were transferred with a camel's hair brush to the apical region of the plants in each pot. We subsequently visually counted all aphids on each pot at weekly intervals. The experiment was terminated when apterous aphids were found on the bench, away from the pot. Aphid counts were transformed to their natural logarithms and analysed using repeated-measures two-way ANOVA tests (SPSS Inc., 2000) with fertilization level and growth chamber as the main effects. The Greenhouse–Geisser adjustment was used to correct for sphericity (HAND and CROWDER, 1996). Tukey's honestly significant difference (Tukey's HSD) test was used to determine significance between pairs of mean values.

2.3 Effect of low to standard fertilization levels on *A. gossypii*

The previous study assessed aphid responses to fertilization level ranging from medium to high. We reduced the range of fertilization levels in this study in an attempt to identify a response inflection point. Thus, we tested five fertilization levels: 0, 19, 38, 75 and 375 ppm N which is 0, 5, 10, 20 and 100%, respectively, of the standard level. We used the same experimental protocol and statistical analyses as our last experiment, except that there were only six replications (pots) per treatment.

2.4 Effect of fertilization on *A. gossypii* under greenhouse conditions

We examined the effect of fertilization levels on *A. gossypii* abundance and within-plant distribution on chrysanthemum under greenhouse conditions that approximated commercial production in Texas, USA. We hypothesized that aphid abundance would increase with increased fertilization and aphid within-plant distribution would vary among fertilization levels. The experimental design was similar to the previous experiments with a number of modifications. We tested five fertilization levels: 0, 19, 38, 75 and 375 ppm N. The number of replications (pots) was 12 per fertilization level. We enclosed individual greenhouse benches with 285 cm long × 94 cm wide × 107 cm high cages constructed with PVC frames and covered with Reemay® polyester (Reemay, Inc., Old Hickory, TN, USA) to prevent other insects from contaminating our experiments. To examine the within-plant distribution of *A. gossypii*, we divided the plants in each pot into three strata: apical (apical meristem and two newly unfurled leaves), middle (region between apical and basal region where physiologically mature leaves are) and basal (basal post-physiologically mature leaves from the oldest remaining two to three laterals just above the soil line). We randomly selected six pots per fertilization level and inoculated them with aphids as described before. We visually counted all aphids in each stratum on each pot at 5-day intervals instead of weekly intervals. To confirm the effect of fertilization levels on host plant quality, we kept the remaining pots (six per fertilization level) free of aphids for the duration of the experiment. At the end of the experiment, five leaves were randomly taken from each plant stratum in each pot. We oven-dried the leaves at 80°C for 24 h. Nitrogen content in the leaf tissue were analysed using Micro-Dumas Combustion Analysis (Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, Athens, GA, USA). The experiment was conducted from 23 October to 12 December 2001. Temperature and relative humidity inside the enclosed benches were monitored for the

duration of the experiment with HOB0® H8 Pro Series data loggers (Onset Computer Corporation, Bourne, MA, USA) recording at 4-h intervals (daily mean ± SE; 22.15 ± 0.51°C and 61.90 ± 1.76% RH, $n = 52$ days). Daily temperature and relative humidity fluctuations were 7.92 ± 0.60°C and 23.18 ± 2.49% RH (mean ± SE, $n = 52$ d). Day length during this period at College Station (TX, USA) (longitude W96.3°, latitude N30.6°) was calculated from the data on sunrise and sunset provided by the Astronomical Applications Department (U.S. Naval Observatory, Washington, DC, USA) (daily mean ± SE; 10.61 ± 0.04 h).

Aphid counts were transformed to their natural logarithms and analysed using repeated-measures two-way ANOVA tests with fertilization level and bench as main effects. The Greenhouse–Geisser adjustment was used to correct for sphericity. We estimated the population growth rate (r) of *A. gossypii* using the following equation:

$$r = \frac{\ln(N_{x+1}/N_x)}{t}, \quad (1)$$

where N_x is the population size at time x , N_{x+1} the population size at time $x + 1$ and t the difference in days between time $x + 1$ and x . For example, the overall population growth rate was calculated using N_x the population size at the start of the experiment, N_{x+1} the population size at the end of the experiment, and t the duration of the experiment (20 days). Population growth rate between two consecutive sampling dates, e.g. between day 5 and 10, was calculated using N_x the population size at day 5, N_{x+1} the population size at day 10, and t the sampling interval (5 days). One-way ANOVA was used to analyse the effect of fertilization on the overall population growth rate. Tukey's HSD test was used to determine significance between pairs. In addition, repeated-measures one-way ANOVA test was used to analyse the effect of fertilization on population growth rates between sampling dates. Aphid within-plant distribution was first tested with log-linear model test to determine the degree of association between sampling date, fertilization level, and plant strata. Because of a significant three-way interaction between the three factors, G -test of independence for multiway table, often called $R \times C$ test of independence (SOKAL and ROHLF, 1995) were used to test the effect of fertilization level on aphid within-plant distribution for each sampling date. In all cases, G -values were adjusted with William's correction. Leaf nitrogen content was analysed using Scheirer–Ray–Hare two-way ANOVA of ranks test with fertilization level and plant strata as main factors (SOKAL and ROHLF, 1995). The Games and Howell method was used to determine significant differences between pairs.

2.5 Effect of fertilization on insecticides usage for controlling *A. gossypii*

We tested whether the number of insecticidal applications needed to suppress aphid populations varies with the level of fertilization applied to the host plants. We predicted that *A. gossypii* abundance would increase with increased fertilization; consequently, greater numbers of insecticidal applications would be needed to suppress the infestations. In this experiment, we examined the interactive effects of three fertilization levels (0, 75 and 375 ppm N) and three insecticides currently registered for aphid control on ornamentals (bifenthrin, kinoprene and pymetrozine) on populations of *A. gossypii* infesting chrysanthemum grown in the greenhouse. The three insecticides were selected for their different modes of action. Bifenthrin (Talstar®, FMC Corporation,

Philadelphia, PA, USA) is a pyrethroid and a contact insecticide. Kinoprene (Enstar II[®]; Wellmark International, Schaumburg, IL, USA) is an insect growth regulator that also has systemic properties. Pymetrozine (Endeavor[®]; Syngenta Crop Protection, Inc., Greensboro, NC, USA) is a systemic insecticide that acts as a feeding inhibitor. We used a 3 × 3 factorial randomized design with 10 replications (pots) per combination. Three hundred and sixty rooted chrysanthemum cuttings were transplanted into 90 pots. The experiment was conducted from 9 January to 20 March 2003. Temperature and relative humidity inside the greenhouse were monitored for the duration of the experiment with a HOBO[®] H8 Pro Series data logger (Onset Computer Corporation) recording at 4-h intervals (daily mean ± SE; 21.52 ± 2.55°C and 49.58 ± 5.88% RH, $n = 71$ days). Daily temperature and relative humidity fluctuations were 8.07 ± 0.96°C and 21.33 ± 2.53% RH (mean ± SE, $n = 71$ days). Day length during this period at College Station (longitude W96.3°, latitude N30.6°) was calculated from the data on sunrise and sunset provided by the Astronomical Applications Department (daily mean ± SE; 11.11 ± 1.32 h).

Similar to the previous experiments, five apterous adults of *A. gossypii* (6–7 days old) were transferred to the apical region of the plants in each pot on the day after daminozide application. We allowed the aphids to settle and reproduce on the plants for 7 days to simulate an early aphid infestation and visually counted the number of live aphids on each pot at weekly intervals. Tolerance for insect presence and damage is extremely low for ornamental potted plants. We used 20 aphids per pot (five aphids per plant) as our action threshold to determine if insecticidal applications were needed. Aphids were detectable but not conspicuous at this density. Whenever the number of aphids of a pot exceeded 20, we applied a foliar treatment of the assigned insecticide to the pot. Pots were removed for insecticidal treatment and returned to their original location after treatment. We followed the recommended rate on the product label and applied bifenthrin at a rate of 1.56 ml/l, kinoprene at a rate of 1.29 ml/l, and pymetrozine at a rate of 0.19 g/l. Treatment sprays were applied at 414 kPa using a Solo[®] 15.1-l capacity backpack sprayer (Model no. 475; Solo Inc., Newport News, VA, USA) equipped with a standard adjustable hollow cone spray head. Insecticides were applied to upper and lower leaf surfaces until runoff. We terminated the experiment when the flowers of plants receiving 375 ppm N were fully open. The plants were 10 weeks old at the time of flower opening. Numbers of insecticidal applications was analysed using two-way ANOVA tests and followed by Tukey's HSD test to determine significance between pairs of mean values.

3 Results

3.1 Effect of medium to high fertilization levels on *A. gossypii*

The number of aphids did not vary with fertilization levels ranging from 75 to 488 ppm N (repeated measures two-way ANOVA: $F_{4,34} = 0.073$, $P = 0.990$) (fig. 1a). We showed that increased fertilization did not result in higher aphid infestation. There were neither significant treatment effect nor significant interaction between sampling date and treatment effect (repeated-measures two-way ANOVA: $F_{12,102} = 0.415$, Greenhouse–Geisser adjusted $P = 0.928$). Therefore, the

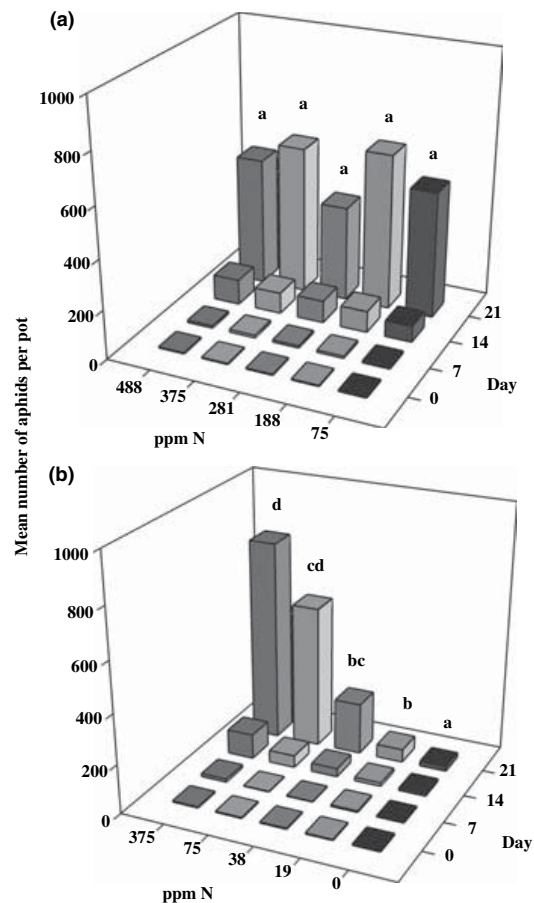


Fig. 1. Mean number of *Aphis gossypii* per pot at weekly sampling intervals (day) on chrysanthemum treated with different fertilization levels. Graph A represents weekly aphid abundance for the medium to high nitrogen range: 75, 188, 281, 375 or 488 ppm N ($n = 10$, except for 281 ppm N, $n = 9$). Graph B represents weekly aphid abundance for the low to standard nitrogen range: 0, 19, 38, 75 or 375 ppm N ($n = 6$). Different letter(s) above the bars indicate significant differences between fertilization levels, pooled across sampling dates, at $P \leq 0.05$ as determined by repeated measures two-way ANOVA (Graph A) or repeated measures one-way ANOVA (Graph B), based on \ln -transformed mean values, and followed by Tukey's honestly significant difference test. Untransformed mean values are presented

aphid population growth rate did not differ significantly among the fertilization levels. Although we detected significant chamber effect (repeated-measures two-way ANOVA: $F_{2,34} = 16.630$, $P < 0.001$) and also significant interaction between sampling date and chamber effects (repeated-measures two-way ANOVA: $F_{6,102} = 19.528$, Greenhouse–Geisser-adjusted $P < 0.001$), the absence of significant interaction between sampling date, chamber, and fertilization suggested that the overall pattern of influence was similar (repeated-measures two-way ANOVA: $F_{24,102} = 0.841$, Greenhouse–Geisser-adjusted $P = 0.651$).

3.2 Effect of low to standard fertilization levels on *A. gossypii*

We found neither significant chamber effect (repeated-measures two-way ANOVA: $F_{1,20} = 0.035$, $P = 0.854$) nor significant interaction between chamber and fertilization level (repeated-measures two-way ANOVA: $F_{4,20} = 1.144$, $P = 0.365$). Therefore, we pooled the data across the chambers for further analyses. The number of aphids varied with fertilization levels from 0 to 375 ppm N (repeated-measures one-way ANOVA: $F_{4,25} = 12.075$, $P < 0.001$). Significantly, more aphids were found on plants fertilized with 375 ppm N (mean \pm SE, 230.50 ± 78.07) than on plants fertilized with 0, 19 or 38 ppm N (mean \pm SE, 9.54 ± 2.62 , 20.08 ± 5.58 and 62.83 ± 29.07 , respectively) (fig. 1b). The number of aphids on plants fertilized with 75 ppm N was not significantly different from those fertilized with either 38 or 375 ppm N (mean \pm SE, 156.83 ± 62.49 , 62.83 ± 29.07 , and 230.50 ± 78.07 , respectively). Furthermore, significant interaction between sampling date and fertilization level (repeated-measures one-way ANOVA: $F_{12,75} = 4.154$, Greenhouse–Geisser-adjusted $P < 0.001$) suggested that the rate of increase among the fertilization levels was not parallel; thereby, aphid population growth rate differed among fertilization levels.

3.3 Effect of fertilization on *A. gossypii* under greenhouse conditions

We found neither a significant bench effect (repeated-measures two-way ANOVA: $F_{2,15} = 0.785$, $P = 0.474$) nor a significant interaction between bench and fertilization level (repeated-measures two-way ANOVA: $F_{8,15} = 0.646$, $P = 0.729$). Therefore, we pooled the data across the benches for all further analyses. Similar to the previous growth chamber experiment, the number of aphids also varied with fertilization levels in the greenhouse (repeated-measures one-way ANOVA: $F_{4,25} = 6.652$, $P < 0.001$) (fig. 2). There were fewer aphids on plants fertilized with 0 ppm N (mean \pm SE, 92.97 ± 24.52) than on plants fertilized either with 75 or 375 ppm N (mean \pm SE, 230.43 ± 67.74 and 251.57 ± 63.70 , respectively). There were no significant differences in aphid number on plants fertilized either with 0, 19 or 38 ppm N (mean \pm SE, 92.97 ± 24.52 , 134.33 ± 36.77 and 175.20 ± 45.11 , respectively).

The overall population growth rate of *A. gossypii* on plants fertilized with 0 ppm N was lower than those on plants fertilized with 38, 75 or 375 ppm N (one-way ANOVA: $F_{4,25} = 5.806$, $P = 0.002$). However, the overall population growth rate of aphids on plants fertilized with 19 ppm N was not significantly different from those on plants fertilized with 0 ppm N nor those fertilized with 38 ppm N or higher ($r = 0.20 \pm 0.01$ on plants fertilized with 0 ppm N, r ranged from 0.22 to 0.26 on plants treated with the other levels). We further compared the population growth rates (r) of *Aphis gossypii* between sampling dates and found that they varied with fertilization levels and time (fig. 3) (repeated-measures one-way

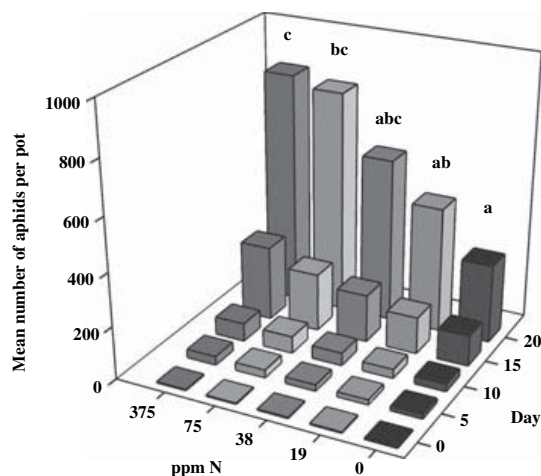


Fig. 2. Mean number of *Aphis gossypii* per pot at 5-day sampling intervals (day) on chrysanthemum treated with different fertilization levels: 0, 19, 38, 75 or 375 ppm N ($n = 6$). Different letter(s) above the bars indicate significant differences between fertilization levels, pooled across sampling dates, at $P \leq 0.05$ as determined by repeated-measures one-way ANOVA, based on \ln -transformed mean values, and followed by Tukey's honestly significant difference test (repeated-measures one-way ANOVA: $F_{4,25} = 6.652$, $P < 0.001$). Untransformed mean values are presented

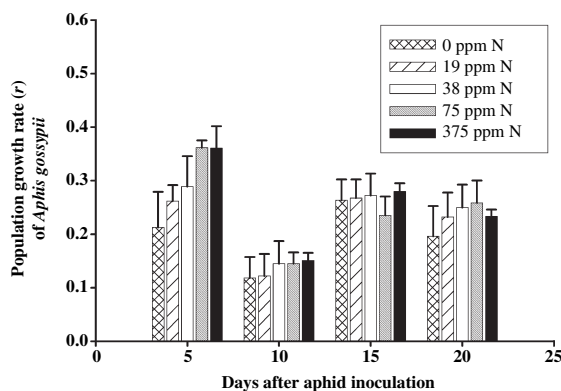


Fig. 3. Mean population growth rate (r) of *Aphis gossypii* (+ SE) on plants fertilized with 0, 19, 38, 75 or 375 ppm N at each sampling interval: 0–5, 5–10, 10–15, and 15–20 d after aphid inoculation

ANOVA: $F_{4,25} = 5.744$, $P = 0.002$, $F_{3,75} = 12.401$, $P < 0.001$, respectively). However, there were no significant interactions between fertilization level and time (repeated-measures one-way ANOVA: $F_{12,75} = 0.513$, $P = 0.900$), which suggested that the slopes of population growth rates among the fertilization levels were parallel.

Our results showed that there was a significant three-way interaction between sampling date, fertilization level, and plant strata ($G_{\text{adj}} = 271.454$, d.f. = 24, $P < 0.001$). A significant three-way interaction indicated that aphid within-plant distribution was

influenced by fertilization level (G_{adj} values range from 65.679 to 466.821, d.f. = 8, all P-values were smaller than 0.001) but the degree of influence differed among sampling dates. Majority of aphids (>40%) were found in the basal region of the plants when they were fertilized with 0 ppm N (fig. 4). With increasing fertilization level, more aphids were found in the apical and the middle regions of the plants.

At the time of harvest, leaf nitrogen content was significantly different among fertilization levels and plant strata (table 1) (Scheirer–Ray–Hare test: $H = 63.477$, d.f. = 4, $P < 0.001$ and $H = 10.203$, d.f. = 2, $P = 0.006$, respectively). However, there was no significant interaction between fertilization levels and plant strata (Scheirer–Ray–Hare test: $H = 1.412$, d.f. = 8, $P = 0.994$). Regardless of plant strata, leaf nitrogen content was highest on plants fertilized with 375 ppm N and lowest on plants fertilized either with 0 or 19 ppm N. Plants fertilized either with 38 or 75 ppm N had leaf nitrogen content in between these two groups (table 1). Leaf nitrogen content was also significantly different among the plant strata and consistently higher in the apical than the basal region of the plant. Leaf nitrogen content in the middle region was not significantly different from the apical region when plants were fertilized with 75 ppm N or higher but was significantly different from the apical region when plants were fertilized with 38 ppm N or lower.

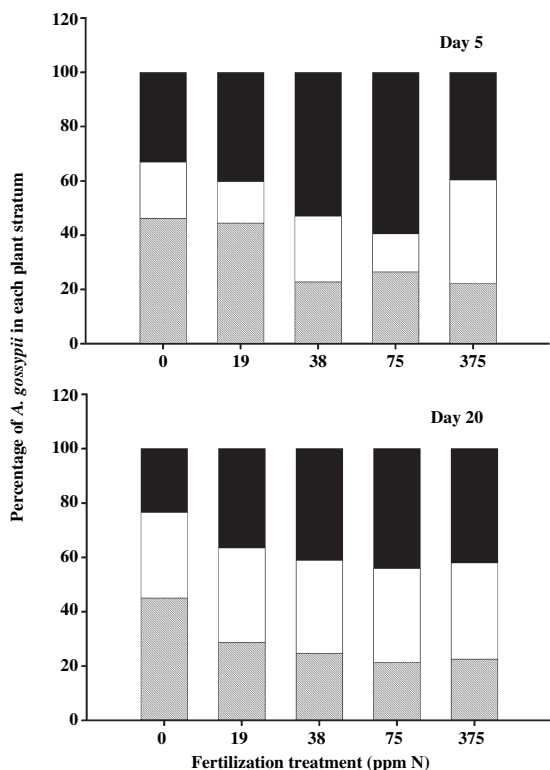


Fig. 4. Percentage of *Aphis gossypii* in each plant stratum: apical (■), middle (□) or basal (▒) of plants fertilized with 0, 19, 38, 75, or 375 ppm N, at day 5 and day 20 after aphid inoculation

3.4 Effect of fertilization on insecticides usage for controlling *A. gossypii*

The number of insecticidal applications was mainly influenced by the type of insecticide (two-way ANOVA: $F_{2,81} = 18.177$, $P < 0.001$) rather than the fertilization level (two-way ANOVA: $F_{2,81} = 0.362$, $P = 0.698$), and no significant interaction was found between the two factors (two-way ANOVA: $F_{4,81} = 0.658$, $P = 0.623$). Pymetrozine, a systemic insecticide, required the fewest applications (fig. 5) to keep the aphid number below the action threshold. Bifenthrin required more applications compared to pymetrozine. Kinoprene, on the other hand, required the most applications; almost double the number for pymetrozine.

4 Discussion

Our studies demonstrated that population abundance of *A. gossypii* was influenced by fertilization levels. The number of aphids increased with fertilization levels from 0 to 38 ppm N and reached a plateau from 38 to 488 ppm N. Aphid populations did not appear to benefit additionally from fertilization beyond 38 ppm N. DAVIES et al. (2004) confirmed that the fertilization levels used for our growth chamber experiment alter plant growth and quality. Plant growth and leaf nitrogen, phosphorous, iron, manganese, boron, and molybdenum increases with fertilization levels, as do chlorophyll and photosynthetic rates.

Numerous studies have investigated the effects of fertilization on aphid growth and performance with variable results. For example, studies of individual *A. gossypii* on cucumber and cotton have shown that body weight, body size, fecundity, developmental rate, and population growth rate are enhanced by nitrogen fertilization (PETITT et al., 1994; ROSENHEIM et al., 1994; NEVO and COLL, 2001). In contrast, BETHKE et al. (1998) found that the fecundity of *A. gossypii* is affected by chrysanthemum cultivar but not irrigation or fertilizer level. These studies focus on the effect of fertilization on the individual level. Our study, however, examined the effect of fertilization on the population level. We found that the positive response of aphid populations to fertilizer level was not consistent across the fertilization range evaluated in this study. The significant effects of fertilization on aphids occur only at very low levels in chrysanthemum and this could partially explain the inconsistent detection of fertilization effects on aphid growth and development in the literature. GULDEMOND et al. (1998) showed that population growth rate of *A. gossypii* on chrysanthemum, estimated at the population level, varies with the developmental stage of chrysanthemum. They found that population growth rate of aphids is higher on flowering plants than young vegetative plants ($r = 0.32$ and 0.27 , respectively). Resource allocation, nutrition quality, physiology and nutrient flow of plants kept in vegetative growth are very different from plants that progress from vegetative to reproductive growth (REEKIE and BAZZAZ, 1987; SAULNIER and REEKIE, 1995). Although the population growth rate of *A. gossypii*

Table 1. Nitrogen content (g/kg) in leaf tissues taken the apical, middle, and basal strata of plants fertilized with 0, 19, 38, 75, or 375 ppm N¹

Fertilization level ²	Plant strata ³			Fertilization effect [mean (SE)] ⁵
	Apical [mean (SE)] ⁴	Middle [mean (SE)] ⁴	Basal [mean (SE)] ⁴	
0	28.49 (0.96) a,A	22.15 (1.21) a,B	23.35 (0.88) a,B	24.66 (0.87) a
19	37.48 (3.32) ab,A	32.47 (4.44) ab,AB	26.62 (2.67) ab,B	32.19 (2.21) b
38	41.41 (1.02) b,A	33.12 (1.65) b,B	30.17 (1.47) b,B	34.90 (1.39) b
75	50.55 (2.84) b,A	46.20 (3.41) b,A	36.15 (2.62) b,B	44.30 (2.18) c
375	68.08 (0.82) c,A	65.57 (0.69) c,A	53.28 (1.69) c,B	62.31 (1.69) d
Plant strata effect [mean (SE)] ⁶	45.20 (2.64) A	39.90 (2.99) AB	33.91 (2.13) B	

¹ An adequate range of nitrogen (g/kg) in leaf tissue is considered to be from 45 to 60 and a deficient (severe to moderate) range is 15 to 30 (modified from LUNT et al., 1964).
² Differences between mean values within column sharing the same letter(s) are not significantly different ($P \geq 0.05$) as determined by Scheirer–Ray–Hare test and followed by Games and Howell method (Scheirer–Ray–Hare test for fertilization levels: $H = 63.477$, d.f. = 4, $P < 0.001$, plant strata: $H = 10.203$, d.f. = 2, $P = 0.006$, and interactions between fertilization levels and plant strata: $H = 1.412$, d.f. = 8, $P = 0.994$).
³ Differences between mean values within row sharing the same capital letter(s) are not significantly different ($P \geq 0.05$) as determined by Scheirer–Ray–Hare test and followed by Games and Howell method (Scheirer–Ray–Hare test for fertilization levels: $H = 63.477$, d.f. = 4, $P < 0.001$, plant strata: $H = 10.203$, d.f. = 2, $P = 0.006$, and interactions between fertilization levels and plant strata: $H = 1.412$, d.f. = 8, $P = 0.994$).
⁴ Each mean and SE is based on a sample size of six.
⁵ The fertilization effect mean values were calculated by pooling across the plant strata. Each mean and SE is based on a sample size of 18.
⁶ The plant strata effect mean values were calculated by pooling across the fertilization levels. Each mean and SE is based on a sample size of 30.

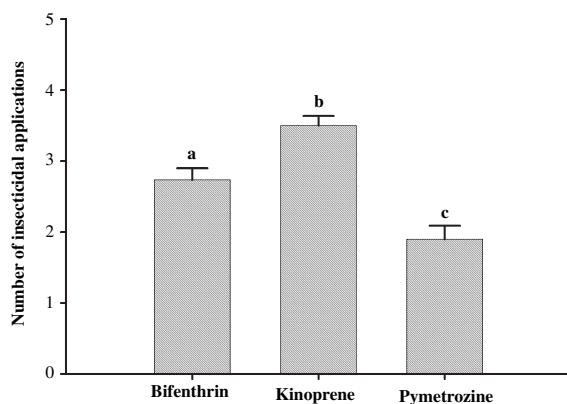


Fig. 5. Mean number of insecticidal applications (+ SE) of bifenthrin, kinoprene or pymetrozine needed to keep *Aphis gossypii* below the threshold of 20 aphids per pot. Bars sharing the same letter(s) are not significantly different ($P \geq 0.05$) as determined by two-way ANOVA followed by Tukey's honestly significant difference test (two-way ANOVA for insecticidal treatments: $F_{2,81} = 18.177$, $P < 0.001$, fertilization levels: $F_{2,81} = 0.362$, $P = 0.698$, and interactions between the two factors: $F_{4,81} = 0.658$, $P = 0.623$)

estimated in our study was slightly lower ($r = 0.26$ on plants fertilized with 375 ppm N), it was a more realistic estimate of aphid population growth as the plants progressed from vegetative to reproductive growth during the production cycle.

Within-plant distribution of *A. gossypii* was influenced by fertilization level. Majority of aphids were found in the apical and middle region than the basal region of most plants except the ones that were fertilized with 0 ppm N. On plants fertilized with

0 ppm N, aphids distributed mostly in the basal region, opposite to the distribution pattern found on other fertilization levels. The pattern of distribution was not random, since a random process would yield aphid numbers in proportion to leaf area. Thus, under a random null expectation, we would expect more aphids in the middle region than the other two regions and more aphids to be found in the basal region than the apical region. We found higher nitrogen content in leaves from both the apical and the middle region of the plants than those from the basal region. These results suggested that aphids were responding to nutrient available in the plants and distributed themselves in areas with higher nitrogen content.

We found that increased fertilization did not result in higher number of insecticide applications. Although the three insecticides were effective in keeping the aphid infestation below the action threshold, pymetrozine required the least number of applications to do so. Our greenhouse study was based on a total of 90 pots. This sample size is small relative to commercial greenhouses that on average produce over 15 000 pots. *Post hoc* sample-size calculations based upon PEARSON and HARTLEY'S (1951) power curves and associated equations (SOKAL and ROHLF, 1995) were applied to insecticide application data to determine how many more replicates might have been needed to detect significant differences among treatments. By setting the power of the *F*-test at 0.90 in our calculations, a difference of 0.07 sprays per pot or greater would be statistically significant with 5229 replicates per treatment. Likewise, 1914 replicates per treatment would have been necessary to find differences of 0.10 sprays per pot or greater with a power of only 0.80. These calculations suggest that on a larger scale, such as on an average production greenhouse, statistically

significant fertilization effects on the number of insecticide applications would be distinguishable. However, our experiment ended when flowers on plants fertilized with 375 ppm N were fully open. At that time, flowers on plants fertilized with 75 ppm N were just beginning to show colour and those on plants fertilized with 0 ppm N were just breaking out from the buds. We suspect that additional insecticide applications would be needed to control aphid infestation on plants grown under reduced fertilization because of longer production time due to delay in flowering.

For fast-growing and nitrogen-demanding crops such as chrysanthemum, increased fertilization shortens the time to flowering. Time to flowering is crucial to growers because shorter production time often means higher profit to cost ratio, faster turn over time, and possibly fewer pesticide input. As a result, increased fertilization together with minimal runoff may help to improve an IPM program for managing *A. gossypii* on potted chrysanthemum by reducing the time for aphid population growth, which could possibly result in reduction of insecticide usage.

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- Author's address:** Amanda Chau (corresponding author), Department of Entomology, Texas A&M University, 2475 TAMU, College Station, TX 77843-2475. Tel: 979-862-3407. Fax: 979-845-6305. E-mail: achau@tamu.edu

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