Fertility Affects Susceptibility of Chrysanthemum to Cotton Aphids: Influence on Plant Growth, Photosynthesis, Ethylene Evolution, and Herbivore Abundance

Fred T. Davies, Jr., and Chunajiu He

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133.

Amanda Chau and Kevin M. Heinz

Department of Entomology, Texas A&M University, College Station, TX 77843-2475

Andrew D. Cartmill

Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843-2133

ADDITIONAL INDEX WORDS. *Aphis gossypii*, best management practices, *Dendranthema grandiflora*, insect herbivory, integrated pest management, melon aphid, nutrition, plant stress

ABSTRACT. This research details the influence of fertility on plant growth, photosynthesis, ethylene evolution and herbivore abundance of chrysanthemum (*Dendranthema grandiflora* Tzvelev ‘Charm’) inoculated with cotton aphids (*Aphis gossypii* Glover). We tested five fertility levels that consisted of 0%, 5%, 10%, 20%, and 100% (375 ppm N) of recommended nitrogen levels. Aphid abundance was greatest at high fertility. Fertility affected the vertical distribution of aphids. A higher population of aphids were observed in physiologically mature and older leaves at low fertility, whereas at high fertility young leaves had 33% more aphids than older, basal leaves. Aphids depressed plant vegetative and reproductive growth, and altered carbohydrate partitioning at high fertility. Aphid-inoculated (AI) plants at high fertility had increased specific leaf area (SLA), i.e., thinner leaves and greater leaf area than aphid-free (NonAI) plants. Aphids caused greater ethylene production in reproductive buds and young leaves of high fertility plants, but had no effect on ethylene evolution in physiologically mature or older, basal leaves. Plant growth, leaf nitrogen (N), phosphorus (P), iron (Fe) and manganese (Mn) increased at higher fertility, as did chlorophyll and photosynthetic rates. Leaf N was highest in young and physiologically mature leaves compared to basal leaves. Aphids decreased leaf N and P. Aphids reduced photosynthesis in young leaves of high fertility plants, whereas physiologically mature and older leaves were unaffected.

The cotton or melon aphid (*Aphis gossypii* Glover) is a serious economic pest of greenhouse crops such as chrysanthemum (*Dendranthema grandiflora* Tzvelev ‘Charm’) (Blackman and Eastop, 2000; Storer and Van Emden, 1995). High densities of cotton aphids can occur throughout the chrysanthemum production cycle because of favorable greenhouse growing environments and the prolific reproduction of aphids, which are both viviparous and parthenogenic. Chrysanthemum cultivars differ in susceptibility to aphids (Bethke et al., 1998; Guldmond et al., 1995). For example, reproduction of the cotton aphid (*A. gossypii*) was affected by cultivars of chrysanthemums, but not by irrigation or fertilizer treatments (Bethke et al., 1998). The morphology and physiological status of the host crop determines aphid susceptibility (Kennedy, 1958; Slosser et al., 1989). The greenhouse environment and cultural conditions practiced contributes to host–plant resistance or susceptibility (Warring and Cobb, 1992). Greenhouse photoperiod, light irradiance (Wyatt and Brown, 1977), temperature (Kocourek et al., 1994; Wyatt and Brown, 1977), nitrogen fertility (Pettit et al., 1994) and host plant water status (Morris, 1992) affect aphid reproduction.

Fertilizers used in nursery–greenhouse industries are an important source of essential elements for both chrysanthemums and

aphids. Optimal nitrogen fertility is important for plant growth and development. Nitrogen fertility can indirectly influence uptake of other ions, chlorophyll synthesis, photosynthesis and photosynthates. Some photosynthates are allocated to secondary metabolism (i.e., phenolics) that can increase the plant’s resistance to insect pests (herbivores) (Bryant et al., 1987; Larsson et al., 1986). High nitrogen fertility can increase insect populations and decrease total phenolics, with minimal effect on cinnamic acids, benzoic acids or flavonoids (Muzika and Pregitzer, 1992). However, the chemical resistance of host plants may not be the primary factor of plant tolerance to herbivores. Rather, herbivore tolerance may be partially or fully compensated for by increased plant growth in response to herbivore pressures (Herms and Mattson, 1992; McNaughton, 1983). Increased growth may help plants cope with or recover faster from herbivore damage, such as from aphids. During commercial production, chrysanthemums are fertilized at high fertility, except for the last several weeks of production when fertility is discontinued to enhance postharvest keeping quality of flower blooms (Yoder Brothers Inc., 2003). However, unlike agronomic or other field crops, there is near zero tolerance for any aphids during greenhouse crop production of chrysanthemum. In short, strategies for optimizing greenhouse chrysanthemum nitrogen fertilization and controlling aphid population development have led to mixed results (Bethke et al., 1998).

As part of best management practices (BMP), reduction of fertilizer usage and subsequent reduction in nitrogen run-off has caused changes in nitrogen usage for a number of ornamental crops (Cabrera et al., 1993; Yeager et al., 1997). The Environmental Protection Agency (EPA) is enforcing the 1972 Federal Clean

Received for publication 3 June 2003. Accepted for publication 9 Dec. 2003. We gratefully appreciate the technical assistance of Ross Hanson, Brad Seever and Lucila Carpio. We thank Peter C. Andersen for critical review of the manuscript. We also thank Yoder Brothers, Inc. for donation of chrysanthemums. Financial support was provided in part by the USDA-National Research Initiative for Floriculture and Nursery Research, grant 58-6204-0.106, the American Floral Endowment, and Texas Agricultural Experiment Station.

Water Act that all states implement a total maximum daily load program for all watersheds (Lea-Cox, 2001). Knowing the influence of nitrogen fertility on host plant quality and pest populations may lead to precision based fertilization that maintain high crop quality, but minimize insect damage. The ultimate result would be to reduce the usage of pesticides and associated chemical phytotoxicity. Greater precision chemical usage can also potentially reduce pesticide runoff and contamination of surface and ground water (Yaeger et al., 1997). Hence this study tested the hypothesis that moderate levels of nitrogen would better control cotton aphids on chrysanthemum. While aphids are known to reduce plant quality, there have been few comprehensive studies on plant response to aphid population dynamics—analyzing changes in plant growth and development, gas exchange and ethylene evolution.

Materials and Methods

PLANT CULTURAL CONDITIONS. One hundred and twenty rooted cuttings of chrysanthemum (*Dendranthema grandiflora* 'Charm') were transplanted into thirty 6-inch standard pots (15.5 × 14.5 cm) on 13 Dec. 2001. Sunshine Mix #1 (Sun Gro Horticulture Inc., Pine Bluff, Arizona) was used for potting media. Four rooted cuttings, which is the industry standard, were evenly spaced within each pot. The plants were kept in growth chambers at 20 °C night/24 °C day, 75% RH and initially placed under long-day conditions: 16L:8D photoperiod. The recommended range of nitrogen for potted chrysanthemum is around 375 ppm N for pulse feeding (Scotts-Sierra Horticultural Products Co., Marysville, Ohio). Plants were fertilized twice a week and watered as needed between fertilizations. Plants were fertilized twice weekly with 200 mL of Peters Professional Peat-lite special 15N-16P-17K (Scotts-Sierra Horticultural Products Company, Marysville, Ohio) at 0, 19, 38, 75 or 375 ppm N, which is respectively, 0%, 5%, 10%, 20%, or 100% of the recommended rate.

When there was 2 to 2.5 cm of new growth, each plant was soft pinched to about seven laterals (18 d after transplanting). After another 7 d of long-day conditions, when there was 1.3 to 2.5 cm of new growth, plants were put under short-day conditions (11L13D) to initiate flower primordia. Seven days after switching to short-days, the growth retardant, B-9, (Uniroyal Chemical Company Inc., Middlebury, Conn) was applied to all pots at 3500 ppm to the point of run-off to reduce internode elongation. The experiment was terminated after 57 d on 8 Feb. 2002.

APHID CULTURE AND INOCULATION. Five apterous early adults of *A. gossypii* (6 to 7 d old) were transferred to each pot with a camel's hair brush the day after B-9 application. Only green morphs (spring morphs) were used for the experiment. The aphids were placed on the apical region of the plants. The plants were ≈5 weeks old at the time of aphid transfer. A ring of Tangle-Trap (1.3 cm wide) (Tanglefoot Company, Grand Rapids, Mich.) was applied to the outside of each pot, nine cm below the rim to control possible aphid movement. No aphids were found in the Tangle-Trap at the end of the experiment, which suggests that aphids do not crawl or migrate down the walls of pots. Cotton aphids used in this experiment were from a colony established with individuals collected from naturally infested chrysanthemums ('Miramar') in the greenhouse and maintained in the laboratory at 26 ± 1 °C, 46% ± 5% RH and 11L: 13D photoperiod on chrysanthemums. The average photosynthetic photon flux at plant canopy in the growth chambers was 650 μmol·m⁻²·s⁻¹.

LEAF DEVELOPMENTAL STAGES. For determining plant

development and aphid populations—nutritional analysis of nitrogen (N) and carbon (C), determination of photosynthesis, ethylene evolution and leaf chlorophyll—leaves were divided into three strata (regions): 1) two to three newly unfurled young leaves from the apical region, 2) physiologically mature leaves, and 3) basal, postphysiologically mature leaves from the oldest remaining one to three laterals just above the soil line (Fig. 1).

APHID DETERMINATION. The number of aphids on each pot was counted weekly until apterous aphids or alates started to leave the pots. Aphids were quantified in the three leaf stages (strata). Aphid counts were terminated when ≥10 alates were found in any given pot (n = 3).

PLANT GROWTH MEASUREMENTS. Leaf area, plant height, flower bud number, specific leaf area [(SLA) cm²·g⁻¹ dry mass (DM) of leaves] and DM of leaves, shoots, flower buds, stems and roots were measured at the termination of the experiment (day 57) to characterize plant growth; three pots per treatment each containing four established, clonally-rooted cuttings were evaluated (n = 3).

TOTAL CARBON/ NITROGEN (C/N) ANALYSIS. At the termination of the experiment, leaves of aphid inoculated (AI) plants were lightly washed and rinsed with deionized water to remove residual honeydew and aphids. Leaves were subsequently dried with paper towels and leaf area taken with a leaf area meter (model 3000; LI-COR Inc., Lincoln, Neb.). All leaves were dried at 70 °C for 96 h and ground with a Wig-L-bug microgrinder (Dentsply Rinn Corp., Elgin, Ill.). Leaves were later analyzed for carbon and nitrogen content using the micro-Dumas combustion assay (Kirsten, 1983) at the Analytical Chemistry Lab., Institute of Ecology, University of Georgia, Athens, Georgia. (See <http://www.uga.edu/~sisbl/udumas.html> for overview of elemental analysis by Micro-Dumas Combustion). Plants from the *Non-AI*

Fig. 1. Schematic of chrysanthemum plant with leaf location used in analysis of aphids, ethylene, gas exchange, carbon and nitrogen levels. The three regions were 1) young leaves—two to three newly unfurled leaves from the apical region, 2) physiologically mature leaves and 3) older leaves—basal, postphysiologically mature leaves from the oldest one to three laterals above the soil surface. Note that there were four, clonally rooted cuttings per pot, not one, which is depicted for schematic purposes in the figure.

group were harvested in the same way as *AI* plants. To determine if there were any leaching effects from washing leaves, half the *Non-AI* leaves were not washed. There were no statistical differences between washed and unwashed leaves using the *t* test (Data not presented).

MACRO- AND MICRONUTRIENT ANALYSIS. Leaf tissue of physiologically mature leaves was harvested at the termination of the experiment using leaves from individual pots per treatment ($n = 3$). The mineral status of plants was then determined on a dry mass basis. Dry leaves were ground in a Wiley mill and part of the leaves used for N analysis and the remainder for other macro- and microelemental analysis. Nitrogen content was determined using the Kjeldahl procedure (Rund, 1984). The remaining samples were digested in wet acid (Jones et al., 1991), and macro- and microelemental determination was made on an inductively coupled plasma atomic emission spectrophotometer (3510 ICP) at a commercial lab (J.R. Peters/Scott's Testing Laboratory, Allentown, Pa.) using the procedures of Munter and Grande, 1981.

CHLOROPHYLL DETERMINATION. Leaf chlorophyll was determined with a SPAD-502 portable chlorophyll meter (Minolta Camera Co., LTD, Japan). The SPAD-502 meter readings were correlated with a chlorophyll content prediction equation: $y = -0.001x^3 + 0.104x^2 - 1.730x + 11.702$, where y = chlorophyll content ($\mu\text{g}\cdot\text{cm}^{-2}$), x = meter reading ($r^2 = 0.98$). This equation was obtained by running a linear regression analysis between the SPAD-502 readings obtained from physiologically mature leaves from two to three pots of each fertility level, and the total chlorophyll content of the same pair of leaves. Leaf chlorophyll was extracted with aqueous acetone (80%) and the total content was determined by optical density of filtered aqueous acetone supernatant, which was measured at 647 nm and 664 nm with a spectrophotometer (Yadava, 1986). During the experiment, two leaves from three pots per treatment were randomly selected from each of the three leaf regions per pot and weekly measured with a SPAD-502 ($n = 6$). Only the final data is reported.

NET PHOTOSYNTHESIS. Net photosynthesis (P_n) and stomatal conductance (g_s) measurements of individual leaves were taken between 8:00 and 11:00 using a portable photosynthesis system (LI-6400; LI-COR Inc., Lincoln, Neb). The LI-6400 was programmed with a constant leaf chamber block temperature at 25 °C; a fixed substrate level of 360 $\mu\text{L}\cdot\text{L}^{-1}$ CO_2 was provided with a 12-gram cartridge, and the light source was an LED 6400 R/B at 300 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Each leaf was a single replication, and there were three replications per treatment ($n = 3$). Only the final measurements are reported which were taken 2 d before harvesting the experiment. There were no treatment effects on g_s , so only P_n is reported.

ETHYLENE DETERMINATION. Buds, young leaves, physiologically mature leaves and old leaves (Fig. 1) were excised at the time the experiment was terminated. These samples were immediately transferred to 10-mL glass vials and sealed with a rubber serum stopper. After 10 min of incubation, a 1-mL volume of gas was withdrawn with a needle and syringe and immediately injected into PhotoVax 10 plus digital gas chromatograph (PhotoVac Inc., Waltham, Mass.) with a photo detector; compressed air (ultra zero grade, Praxair Inc., Danbury, Conn.)

was the carrier gas. Ethylene concentration was determined by a standard ethylene curve programmed in the GC.

EXPERIMENTAL DESIGN AND STATISTICAL DATA ANALYSIS. For plant growth data and leaf macro- and microelements, the experiment was arranged in a 5 fertility $\times$ 2 $\pm$ aphid factorial. Each pot with four established-rooted cuttings was a single replication. There were three replications arranged in a completely randomized design. Data from all variables were analyzed by using analysis of variance (ANOVA) (SAS Institute Inc., 2000), with aphid inoculation and fertility concentration as main effects. For analysis of total leaf carbon and nitrogen ($n = 3$), net photosynthesis ($n = 3$), leaf chlorophyll ($n = 6$), the experiment was arranged in a 5 fertility $\times$ 2 $\pm$ aphid $\times$ 3 leaf positions (young, physiologically mature, old) factorial using ANOVA. For aphid populations ($n = 3$), the experiment was arranged in a 5 fertility $\times$ 3 leaf position factorial using ANOVA. None of the control (not inoculated with aphids) became infested with aphids during the experiment. Aphid inoculated and noninoculated plants were grown in two separate chambers to avoid contamination. The experiment was repeated and chambers switched among aphid treatments to avoid possible chamber effects; there were none. Since the experiment was repeated twice, only the second run is presented which mirrored the results of the first run.

Results

APHID ABUNDANCE. Aphid abundance was greatest at high fertility ($P \leq 0.0001$) (Fig. 2). A greater population of aphids were observed in physiologically mature and older leaves at low fertility, whereas at high fertility young leaves had 33% more aphids than older, basal leaves.

PLANT GROWTH. Aphid inoculated (*AI*) plants had reduced individual flower bud DM from 0 to 75 ppm N (Fig. 3, Table 1). At the highest fertility level (375 ppm N), *AI* plants had lower total flower bud, leaf and total top DM and reduced flower bud

Fig. 2. Effect of fertility and leaf development on aphid populations in chrysanthemum at termination of experiment; $n = 3$; bars indicate the standard error of the mean, when absent it falls under the symbol; treatment effects of fertility (F), leaf position (L) and $F \times L$ were highly significant ($P \leq 0.0001$).

Fig. 3. Effect of aphids and fertility on (A) individual flower bud dry mass (DM), (B) total flower bud DM, (C) leaf DM, (D) total top DM (flower bud, leaf and shoot), (E) shoot DM, and (F) total plant DM; $n = 3$; bars indicate the standard error of the mean, when absent it falls under the symbol; see Table 1.

number than aphid free (*Non-AI*) plants (Figs. 3 and 4, Table 1). However aphids had no effect on plant height or shoot and total plant DM (total top DM and root DM). *AI* plants at high fertility had increased specific leaf area [(SLA), i.e., thinner leaves] and greater leaf area than *Non-AI* plants (Fig. 4, Table 1). Fertility affected all growth parameters (Figs. 3 and 4, Table 1). At highest fertility, greatest growth increases occurred: individual flower bud DM, total flower bud, leaf, shoot and total above ground plant DM, and plant height, flower bud number and highest SLA (Figs. 3 and 4, Table 1). Nutritional deficiency led to reduced growth of low fertility plants.

LEAF MACRO- AND MICRONUTRIENT ANALYSIS, AND TOTAL CARBON/TOTAL NITROGEN (C/N). In physiologically mature leaves, *AI* plants had decreased leaf nitrogen (N) at all fertility levels compared to *Non-AI* treatments (Table 2). *AI* plants also had decreased leaf phosphorus (P) from 19 to 75 ppm N compared to *Non-AI*

treatments (Table 2). Boron (B) tended to be lower and zinc (Zn) higher in *AI* plants. There was not a consistent *AI* response in potassium (K) and calcium (Ca). Leaf N, P, iron (Fe), B, manganese (Mn) and molybdenum (Mo) increased at higher fertility (Table 2). Among the three types of leaves, total Leaf N was highest in young and physiologically mature leaves compared to old, basal leaves (Fig. 5). Treatment effects of aphids (A), fertilization (F) and leaf type or location (L) on total leaf N were significant with values, respectively, of $P \leq 0.04$, $P \leq 0.0001$, and $P \leq 0.01$; there was a significant interaction between $F \times A$, $P \leq 0.01$. At highest fertility, young leaves of *AI* plants had lower total leaf N than *NonAI* treatments (Fig. 5). From 38 to 375 ppm N, physiologically mature leaves of *AI* plants had lower leaf N than *NonAI* treatments (Fig. 5). The total percent leaf carbon was unaffected by aphids or fertility treatments (data not presented).

CHLOROPHYLL AND NET PHOTOSYNTHESIS. Chlorophyll and

Table 1. ANOVA *P* values of growth parameters of chrysanthemum-aphid study with aphids (A) and fertility (F) as main treatments.^z

Individual flower bud DM ^y		Flower bud DM		Leaf DM		Total top DM		Shoot DM	
Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>
A	<0.0001	A	0.0256	A	NS	A	NS	A	NS
F	<0.0001	F	<0.0001	F	<0.0001	F	<0.0001	F	<0.0001
A × F	NS	A × F	<0.0001	A × F	0.0200	A × F	<0.0001	A × F	NS

Total plant DM		Plant ht		Leaf area		Flower bud no.		Specific leaf area	
Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>	Source	<i>P</i>
A	NS	A	NS	A	0.0025	A	<0.0001	A	0.0020
F	<0.0001	F	<0.0001	F	<0.0001	F	<0.0001	F	<0.0001
A × F	NS	A × F	NS	A × F	0.0004	A × F	<0.0001	A × F	<0.0001

^zSee Figs. 3 and 4.^yDM = dry mass.^{ns}Nonsignificant.

photosynthetic rates increased at higher fertility (Fig. 6). Treatment effects of aphids (A), fertilization (F) and leaf location (L) on leaf chlorophyll were, respectively, non-significant, $P \leq 0.0001$ and $P \leq 0.0001$. Hence, aphids had no significant effect on leaf chlorophyll. Aphids reduced photosynthesis in young leaves of high fertility plants, whereas physiologically mature and older leaves were unaffected [treatment effects of aphids (A), leaf position (L) and $A \times L$ were significant with values, respectively of, $P \leq 0.0001$, $P \leq 0.0207$, and $P \leq 0.0531$] (Fig. 6). There were

no aphid or fertility effects on stomatal conductance (data not presented).

ETHYLENE. Aphids caused greater ethylene production in reproductive buds and young leaves of high fertility plants, but had no effect on ethylene evolution in physiologically mature or older basal leaves (Fig. 7).

Discussion

This is one of the first reports on the susceptibility of chrysanthemum to cotton aphids (*Aphis gossypii*)—detailing the influence

Fig. 4. Effect of aphids and fertility on (A) plant height, (B) leaf area, (C) flower bud number, and (D) specific leaf area ($\text{cm}^2 \cdot \text{g}^{-1}$) of chrysanthemum; $n = 3$; bars indicate the standard error of the mean, when absent it falls under the symbol; see Table 1.

Table 2. Effect of fertility (fert) and aphids on leaf macro- and microelements from physiologically mature leaves of chrysanthemum.

Fertilizer	Aphids	N (g·kg ⁻¹)	P (g·kg ⁻¹)	K (g·kg ⁻¹)	Ca (g·kg ⁻¹)	Mg (g·kg ⁻¹)	B (μg·g ⁻¹)	Fe (μg·g ⁻¹)	Mn (μg·g ⁻¹)	Cu (μg·g ⁻¹)	Zn (μg·g ⁻¹)	Mo (μg·g ⁻¹)
0	No	10.3 ± 0.4 ^a	4.3 ± 0.1	24.2 ± 2.9	8.3 ± 0.4	3.1 ± 0.1	71.5 ± 6.3	20.2 ± 1.0	19.2 ± 4.9	5.0 ± 0.7	55.3 ± 6.9	1 ± 0.11
	Yes	9.3 ± 0.1	3.9 ± 0.4	20.5 ± 0.8	8.6 ± 0.2	3.0 ± 0.1	65.8 ± 0.9	19.8 ± 2.1	20.0 ± 1.4	5.0 ± 0.4	62.1 ± 10.6	0.9 ± 0.3
19	No	12.7 ± 0.1	5.0 ± 0.1	24.7 ± 1.0	7.0 ± 0.1	2.8 ± 0.1	83.3 ± 0.8	23.1 ± 1.4	30.4 ± 1.0	10.1 ± 8.5	47.7 ± 3.7	1 ± 0.1
	Yes	11.2 ± 0.1	4.1 ± 0.1	23.7 ± 0.3	7.4 ± 0.3	2.6 ± 0.1	72.0 ± 1.7	21.5 ± 0.4	21.8 ± 1.7	5.0 ± 0.3	57.8 ± 6.9	0.9 ± 0.1
38	No	14.1 ± 0.1	5.2 ± 0.1	25.2 ± 1.0	7.2 ± 0.3	2.8 ± 0.1	77.3 ± 2.2	46.4 ± 2.5	30.9 ± 2.7	5.5 ± 0.8	44.5 ± 4.4	1.1 ± 0
	Yes	12.5 ± 0.1	4.5 ± 0.2	24.1 ± 0.5	6.8 ± 0.1	2.4 ± 0.2	72.6 ± 1.5	24.7 ± 4.8	21.6 ± 2.0	5.1 ± 1.0	54.6 ± 4.2	0.8 ± 0.0
75	No	18.0 ± 0.1	6.1 ± 0.3	27.6 ± 0.9	6.9 ± 0.4	2.7 ± 0.2	77.4 ± 2.4	33.1 ± 4.1	27.2 ± 5.7	6.0 ± 0.5	42.1 ± 4.7	1.4 ± 0.2
	Yes	16.0 ± 0.1	5.1 ± 0.4	27.3 ± 0.9	7.8 ± 0.9	2.6 ± 0.5	75.9 ± 3.9	29.6 ± 0.3	33.5 ± 14.	5.9 ± 0.4	50.8 ± 5.0	1.4 ± 0.3
375	No	24.3 ± 0.1	7.1 ± 0.4	26.6 ± 1.5	7.2 ± 0.7	2.6 ± 0.3	95.8 ± 4.9	56.2 ± 12.7	36.2 ± 4.0	6.5 ± 0.8	25.6 ± 3.0	4.6 ± 0.5
	Yes	21.3 ± 0.1	7.0 ± 0.4	25.0 ± 1.6	8.1 ± 0.6	3.1 ± 0.1	90.6 ± 4.0	87.7 ± 20.3	40.1 ± 3.2	6.0 ± 0.5	33.5 ± 2.9	4.3 ± 0.1
Significance												
Fertilizer (F)		<0.0001	<0.0001	<0.0001	0.007	0.006	<0.0001	<0.0001	<0.0001	NS	<0.0001	<0.0001
Aphids (A)		<0.0001	<0.0001	0.0047	0.02	NS	0.0002	NS	NS	NS	0.0002	NS
F × A		NS	NS	NS	NS	0.005	NS	0.001	0.05	NS	NS	NS

^aValues are means ± SE; (n = 3).

of fertility on herbivore abundance, plant growth, photosynthesis and ethylene evolution. We hypothesized that moderate levels of N would better control aphids on chrysanthemum. In fact, aphid levels were reduced by 75% or more at moderate to low fertility levels. The greater aphid abundance at the highest fertility level of 375 ppm N led to the sharpest reduction in plant performance.

A basic question of this research was how does aphid feeding influence plant growth, and is this altered by plant fertility at different N levels? Aphids significantly influenced carbohydrate partitioning of chrysanthemum. While aphids did not alter total plant biomass, they did reduce reproductive growth (lower flower bud number and total flower bud dm) and decreased leaf biomass.

Phenotypic plasticity is the ability of a plant to modify its physiology and/or morphology in response to biotic and abiotic stimuli. The biotic influence of aphids and abiotic response to high fertility triggered morphological changes in leaf development by increasing the specific leaf area (SLA) {cm²·g⁻¹ leaf mass}, i.e., thinner leaves and increasing the leaf area. In another study, we observed increased leaf area in *AI* plants at high fertility (Davies et al., 2003). A higher SLA indicates that fewer leaf mesophyll cells develop and that biomass is reduced per unit leaf area (Hunt and Lloyd, 1987; McDonald, 1990). Differences in SLA can also be caused by increased light, as well as increases in water content and cell size, or altered thickness of leaves and veins (Dijkstra, 1990). Chrysanthemum is a fast-growing, nutritionally high-demanding plant. As such, fast-growing plants could benefit from a high SLA if leaves increased photosynthetic levels under certain environmental conditions (Van der Werf, et al., 1993). In a study on volatile communication (aerial allelopathy) between barley cultivars, plants exposed to volatiles from another cultivar had a higher SLA, which enabled them to maintain the same relative growth rate (RGR) (Ninkovic, 2003). In our study with chrysanthemum, the greater SLA with *AI*-plants at high fertility may be due to aphid-induced volatiles (including ethylene) and as compensation for reduced photosynthesis (Pn)—due in part to aphid induced leaf sooty mold, which reduced light penetration at the leaf surface. Furthermore, the greater leaf area of *AI*-plants would increase the potential surface area for capturing sunlight for photosynthesis.

Aphids are phloem feeders and cause minimal tissue damage, relative to leaf miners which attack leaf mesophyll tissue or thrips which target individual cells. As such, plants perceive aphids as pathogens and activate the salicylic acid-dependent and jasmonic

acid/ethylene dependent signalling pathways (Walling, 2000). These aphid-triggered physiological responses may also cause changes in leaf SLA.

AI chrysanthemum had reduced total N at high fertility in both young and physiologically mature leaves, but not in older leaves compared to *Non-AI* plants (Fig. 5). Physiologically mature leaves of *AI* plants from 0 to 375 ppm N, had lower leaf N than *Non-AI* plants (Table 2). Leaf N is a crude index of nitrogen available in the phloem (Dixon, 1970). However, dietary nitrogen, principally in the form of amino acids in the phloem, is an important factor influencing aphid development (Douglas, 1993). Hence aphid consumption of soluble nitrogen via amino acids is likely contributing to the decline in total N. Aphids are known to concentrate more in newly developing leaves than older leaves, in part because of greater amino acid concentration in the phloem of the former (Douglas, 1993).

While increasing N fertilization can increase plant growth (biomass), decreases occur in carbon-based defensive chemicals, such as phenolics, tannins and terpenes (Muzika and Pfregritzer, 1992). Phenolics represent a large and structurally diverse class of compounds, second only to carbohydrates in their abundance in higher plants. Phenolics are ubiquitous and have antibiotic and antimicrobial activity (Harborne 1985); consequently many phenolics have some level of phytotoxicity which may play a role in plant defense. Hence, reduced levels of allelochemicals at high N may also be contributing to increased aphid populations. It should also be noted that aphids do not always respond favorably to N-fertilized plants. Too high a fertility level and supraoptimal N in chrysanthemum can reduce aphids, in part due to high salinity mediated through the plant root system and reduced soluble proteins in the host plant (Bethke, et al., 1998).

The percent leaf carbon (C) was similar among all treatments, consequently any difference in C/N ratios are attributed to differences in N. The carbon analysis combined both structural and soluble carbohydrates, so we were unable to determine if soluble carbohydrates differed among treatments. The principal carbon source utilized by most aphids is sugar, particularly sucrose (Douglas, 1993). Insects such as beetles will consume 5-fold more leaf tissue in plants low in carbon and rich in nitrogen (Larsson et al., 1986).

Leaf P was lower in *AI* plants from 19 to 75 ppm N, however the tissue P levels were in the sufficiency range among all treatments (Jones, et al., 1991). There were differences in B and Zn in *AI* plants, however, both microelements were considered in the

sufficiency range for all treatments (Jones, et al., 1991). Among treatments, Ca, K and Mn were considered in the deficiency range, while Mg, Fe (at high fertility) and Cu were considered in the sufficiency range (Jones, et al., 1991).

At higher fertility, chlorophyll and net photosynthesis (P_n) increased. Leaf chlorophyll may vary according to light conditions (Pospisilova et al. 1997) and other factors such as mineral status of the plants, in which N, Mg, Cu, and Fe have important roles (Taiz and Zeiger 1998). Leaf N was higher in *Non-AI* plants. While aphids did not statistically alter leaf chlorophyll, there was a reduction in P_n at high fertility (375 ppm N) in *AI* young leaves, but not physiologically mature or older leaves. Lower N in *AI* plants may have indirectly reduced P_n . The reduced P_n in young leaves of *AI* plants may also be due to physical stress of phloem-feeding aphids, i.e., causing higher ethylene evolution or other signalling compounds. Aphids excrete residue on the leaf surface which can lead to a build-up of sooty mold fungi, reducing light irradiance which can decrease P_n , as previously discussed. Changes in carbon partitioning with reduced leaf mass and higher SLA (fewer leaf mesophyll cells) may also have reduced P_n .

Aphids caused greater ethylene production in reproductive buds and young leaves of high fertility plants, but had no effect on physiologically mature or older leaves. Ethylene is a phytohormone that can occur with plant injury, i.e., aphid stylets penetrate leaf mesophyll cells in locating phloem tissue. Genes activated on herbivore attack are strongly correlated with the mode of herbivore feeding and the degree of tissue damage at the feeding site (Walling, 2000). Phloem feeding aphids (and white flies) produce marginal injury to plant foliage and are instead perceived as pathogens and can activate ethylene-dependent signaling pathways (Walling, 2000). This may in part explain the higher ethylene in flower buds and young leaves of *AI* plants. Ethylene can adversely effect plant growth, and with other plant hormones—controls such important physiological processes as leaf and flower senescence and abscission. Ethylene is also associated with leaf chlorophyll degradation and senescence (Abeles et al. 1992). High rates of ethylene production are normally associated with shoot apical areas, presumably because of higher auxin content in meristematic tissue. But in our study with chrysanthemum, no differences in ethylene occurred between young vs. physiologically mature leaves (except with the presence of aphids). Aphids caused ethylene to increase more than 2-fold in *AI* young leaves and buds vs. *Non-AI* plants. Autocatalytic ethylene production occurs in floral senescence (Abeles et al., 1992), and possibly with *AI* plants. In general, ethylene does not have a direct effect on photosynthe-

sis (Abeles et al. 1992). In a nonaphid study subjecting plants to environmental stress, there was a negative linear correlation between increasing ethylene concentrations and decreasing leaf chlorophyll (He et al., 2003).

Fig. 5. Effect of aphids and fertility on leaf elemental nitrogen (N) in (A) young, (B) physiologically mature, and (C) old leaves of chrysanthemum; $n = 3$; bars indicate the standard error of the mean, when absent it falls under the symbol. Treatment effects of aphids (A), fertilization (F) and leaf type or location (L) on total leaf N were significant with values, respectively, of $P \leq 0.04$, $P \leq 0.0001$, $P \leq 0.01$; there was a significant interaction between $F \times A$, $P \leq 0.01$.

Fig. 6. Effect of leaf stage, fertility and aphids on (A) leaf chlorophyll [treatment effects of aphids (A), fertilization (F) and leaf location (L) on leaf chlorophyll were, respectively, nonsignificant, $P \leq 0.0001$ and $P \leq 0.0001$; $F \times A$, $F \times L$, $A \times L$ and $F \times A \times L$ were, respectively, $P \leq 0.02$, $P \leq 0.0001$, nonsignificant and $P \leq 0.0001$]; (B) net photosynthesis (Pn). Treatment effects of leaf position, (L), fertility (F), and $L \times F$ were highly significant ($P \leq 0.0001$); and (C) effect of aphid and leaf stage on Pn of chrysanthemum at 375 ppm N. Treatment effects of aphids (A), leaf position (L), and $A \times L$ were significant with values, respectively of, $P \leq 0.0001$, $P \leq 0.0207$, and $P \leq 0.0531$; $n = 6$ for leaf chlorophyll and $n = 3$ for gas exchange; bars indicate the standard error of the mean, when absent it falls under the symbol.

While low to moderate N fertility greatly reduced aphid populations in our study, the chrysanthemums were nutritionally deficient and not of marketable quality. Aphids respond favorably to N fertility of plants (Herms, 1989; Pettit et al., 1994). Soluble nitrogen has been positively correlated to increased aphid levels (Van Emden and Bashford, 1969). In several greenhouse studies (data not reported), differences between 75 and 375 ppm N were much less dramatic than in our growth chamber studies. In part this was because of different environmental conditions and that no pesticides were used in the growth chambers. Greenhouse production requires the use of pesticides to control aphid outbreaks

at higher fertility. This can potentially lead to phytotoxic effects and a reduction in plant quality.

The increased aphid populations in younger than older leaves at high fertility in our study may be due to higher amino acid levels (which we did not measure); leaf N was higher in younger leaves. Soluble amino acids levels fluctuate in response to plant stress (Bethke et al., 1998; Jansson et al., 1987). Water stress can increase host plant susceptibility to aphids due to higher soluble N in the vascular system (Kennedy, 1958; Kennedy and Booth, 1959; Mattson, 1980). However, it is soluble amino acid levels per se that affects aphid populations, not just plant fertility or leaf N (Bethke et al., 1998). Douglas (1993) reported that amino acid content of phloem exudates from leaves and of aphid honey dew was greater in summer than fall, and higher in rapidly developing leaves in a growth flush than mature leaves. Hence, higher fertility likely contributed to subsequent increases in soluble nitrogen, amino acids and potentially lowered levels of carbon-based phenolic allelochemicals (Herms, 1989)—enabling greater aphid populations in young chrysanthemum leaves.

Besides N, aphids also require carbohydrates (sucrose), which would be high in the metabolic sink area of new developing vegetative and reproductive meristems and young, developing leaves. In our study, aphids were not confined to a particular leaf and had the opportunity to move and locate optimal feeding sites. At low fertility, the higher aphid populations in physiologically mature and older leaves may have been due to their larger canopy (platform on which aphids could congregate compared to the limited surface area of stunted, younger leaves). Relative to young leaves, there maybe some microhabitat environmental advantages of aphids congregating in older leaves of low N plants, such as closer proximity to the high relative humidity of the moist media (soil mix). Also the relative sink strength of N between young and older leaves decreased under low fertility. At low fertility, plant growth rates were very slow, so there were potentially higher levels of soluble carbohydrates and comparably low nitrogen levels among the three leaf types. In another study, differences in chrysanthemum cultivars and desirability of aphid populations to occur in newly developing leaves and shoot apices (in N sufficient plants) affected the vertical distribution of aphids (Wyatt, 1969). More aphid resistant cultivars tended to have fewer aphids colonizing on physiologically mature leaves, compared to lower basal leaves and apices. However, with increasing population pressure and the development of alates, aphids will move throughout the plant canopy (Wyatt, 1969).

In summary, commercial production of chrysanthemum entails a rapid crop cycle where nutrients and water are applied in abundance. A basic tenant of BMP and integrated pest management (IPM) systems is the reduction of quantities of fertilizers and pesticides through more judicious use and integration of pest control methods (Holtzer et al. 1996). IPM is highly applicable to a single plant-pest system—hence the potential with the chrysanthemum–aphid system. The morphology and physiological status of chrysanthemum determines its susceptibility to aphids. This paper demonstrates the influence of aphids on increasing ethylene, decreasing Pn, decreasing carbon allocation to leaves and reproductive structures. Selecting chrysanthemum cultivars with greater resistance to aphids is one approach to IPM. With other crops such as potatoes, cultural practices are used to control aphid populations (Harrewijn, 1983; Jansson and Smilowitz, 1986). Controlling fertility and irrigation practices to reduce and manage aphids in chrysanthemum are other options. Fine-tuning fertility to slow aphid growth may reduce the number of pesticide applications

Fig. 7. Effect of aphids and organs of chrysanthemum on ethylene production at 375 ppm N; n = 3; bars indicate the standard error of the mean, when absent it falls under the symbol; treatment effects of aphids (A), leaf position (L), and A $\times$ L were highly significant ($P \leq 0.0001$).

and associated chemical phytotoxicity. Furthermore, how aphids influence plant growth and development could also be used for establishing aphid density thresholds for spraying. While growing plants under deficient fertility levels is not a satisfactory strategy for reducing insect pests, more precise chemical practices that reduce fertility and pesticide levels that produce healthier, less stress susceptible plants are feasible.

Literature Cited

- Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in Plant Biology. Academic Press, Inc., New York.
- Bethke, J.A., R.A. Redak, and U.K. Schuch. 1998. Melon aphid performance on chrysanthemum as mediated by cultivar, and differential levels of fertilization and irrigation. Entomol. Expt. Appl. 88: 41–47.
- Blackman, R.T. and V.F. Eastop. 2000. Aphids on the world's crops: an identification and information guide. 2nd ed. Wiley, New York.
- Bryant, J.P., T.P. Clausen, P.B. Reichardt, M.C. McCarthy, and R.A. Werner. 1987. Effect of nitrogen fertilization upon the secondary chemistry and nutritional value of quaking aspen (*Populus tremuloides* Michx.) leaves for the large aspen tortrix (*Choristoneura conflictana* [Walker]). Oecologia 73:513–517.
- Cabrera, R.I., R.Y. Evans and J.L. Paul. 1993. Leaching losses of N from container-grown roses. Scientia Hort. 53:333–345.
- Davies, F.T. Jr., K.M. Heinz, A. Chau, C. He, and A.D. Cartmill. 2003. Manipulating fertility of potted chrysanthemum influences cotton aphid (*Aphis gossypii*) populations. Proc. S. Nurs. Assn. Res. Conf. (in press).
- Dijkstra, P. 1990. Cause and effect of differences in specific leaf area, p. 125–140. In: Lambers, H., M.L. Cambridge, H. Konings, and T.L. Pons (eds.). Causes and consequences of variation in growth rate and productivity of higher plants. SPB Acad. Publ., The Hague, Netherlands.
- Dixon, A.F.G. 1970. Quality and availability of food for a sycamore aphid population, p. 271–288. In: A. Watson (ed.). Animal populations in relation to their food resources. Symp. Brit. Ecol. Soc. 10, Blackwell Scientific, Oxford, U.K.

- Douglas, A.E. 1993. The nutritional quality of phloem sap utilized by natural aphid populations. Ecol. Entomol. 18:31–38.
- Guldemon, A.J., W.T. Tiggs, and P.W.F. de Vrijer. 1995. Host races of *Aphis gossypii* (Homoptera: Aphididae) on cucumber and chrysanthemum. Environ. Entomol. 23:1240–1245.
- Harborne J. 1985. Phenolics and plant defense. In: C.F. Van Sumere and P.J. Lea (eds.). The biochemistry of plant phenolics. Clarendon Press, Oxford, U.K.
- Harrewijn, P. 1983. The effect of cultural measures on behavior and population development of potato aphids and transmission of viruses. Med. Fac. Landbouww. Rijksuniv. Gentetics 48(3):791–799.
- He, C.J., F.T. Davies, Jr., R.E. Lacey, M.C. Drew, and D.L. Brown. 2003. Effect of hypobaric conditions on ethylene evolution and growth of lettuce and wheat. J. Plant Physiol. 160:1341–1350.
- Hermes, D.A. 1989. Plants vs. herbivores: Nature's arms race. Amer. Nurs. (Sept. 1):65–81.
- Hermes, D.A. and W.J. Mattson. 1992. The dilemma of plants: To grow or to defend. Quart. Rev. Biol. 67:283–335.
- Holtzer, T.O., R.L. Anderson, M.P. McMullen and F.B. Peairs. 1996. Integrated pest management of insects, plant pathogens and weeds in dry land cropping systems of the great plains. J. Prod. Agr. 9:200–208.
- Hunt, R. and P.S. Lloyd. 1987. Growth and partitioning. New Phytol. 106:235–249.
- Jansson, R.K., G.C. Elliott, Z. Smilowitz and R.H. Cole. 1987. Influence of cultivar maturity time and foliar nitrogen on population growth of *Myzus persicae* on potato. Entomol. Experiment. Applicat. 43:297–300.
- Jansson, R.K. and Z. Smilowitz. 1986. Influence of nitrogen on population parameters of potato insects: abundance population growth and within plant distribution of the green peach aphid *Myzus persicae* (Homoptera: Aphididae). Environ. Entomol. 15:979–992.
- Jones, J.B., Jr., B. Wolf and H.A. Mills. 1991. Plant analysis handbook. Micro-Macro Publ., Inc., Athens, Ga.
- Kennedy, J.S. 1958. Physiological condition of the host plants and susceptibility to aphid attack. Entomol. Expt. Appl. 1:50–65.
- Kennedy, J.S. and C.O. Booth. 1959. Host alternation in *Aphis fabae* Scop. 1. Feeding preferences and fecundity in relation to the age and kind of leaves. Ann. Appl. Biol. 38:25–64.
- Kirsten, W. 1983. Organic elemental analysis: Ultramicro, micro, and trace methods. Academic Press/Harcourt Brace Jovanovich, New York.
- Kocourek, F., J. Havelka, J. Berankova, and V. Jaroski. 1994. Effect of temperature on developmental rate and intrinsic rate of *Aphis gossypii* reared on greenhouse cucumbers. Entomol. Expt. Appl. 71:59–64.
- Larsson, S., A. Wirén, L. Lundgren, and T. Ericsson. 1986. Effect of light and nutrient stress on phenolic chemistry in *Salix dasyclados* and susceptibility to *Galeurucella lineola* (Coleoptera). Oikos 47:205–210.
- Lea-Cox, J.D. 2001. Water and nutrient planning-monitoring of irrigation and fertilization practices. Comb. Procd. Intl. Plant Prop. Soc. 51:575–580.
- Mattson, Jr., W.J. 1980. Herbivory in relation to plant nitrogen content. Annu. Rev. Ecol. Syst. 11:19–161.
- McDonald, A.J.S. 1990. Phenotypic variation in growth rate as affected by N-supply: Its effects on net assimilation rate (NAR), leaf weight ratio (LWR) and specific leaf area (SLA). In: H. Lambers, M.L. Cambridge, H. Konings, and T.L. Pons (eds.). Causes and consequences of variation in growth rate and productivity of higher plants. SPB Publ.
- McNaughton, S.J. 1983. Compensatory plant growth as a response to herbivory. Oikos 40:329–336.
- Morris, W.F. 1992. The effects of natural enemies, competition, and host plant water availability on an aphid population. Oecologia 90: 359–365.
- Munter, R.C. and R.A. Grande. 1981. Plant tissue and soil extract

- analysis by ICP-atomic emission spectrometry, p. 653–672. In: R.M. Barnes (ed.). *Developments in atomic plasma spectrometry*. Heyden, London.
- Muzika, R.M. and K.S. Pregitzer. 1992. Effect of nitrogen fertilization on leaf phenolic production of grand fir seedlings. *Trees*. 6:241–244.
- Ninkovic, V. 2003. Volatile communication between barley plants affects biomass allocation. *J. Expt. Bot.* 54(389):1931–1939.
- Pettitt, F.L., C.A. Loader, and M.K. Schon. 1994. Reduction of nitrogen concentration in the hydroponic solution on population growth rate of the aphids (Homoptera: Aphididae) *Aphis gossypii* on cucumber and *Myzus persicae* on pepper. *Environ. Entomol.* 23:930–936.
- Pospisilova J., J. Catsky, and Z. Sestak. 1997. Photosynthesis in Plants Cultivated in vitro, p. 525–540. In: M. Pessarakli (ed.). *Handbook of photosynthesis*. Marcel Dekker, New York.
- Rund, R.C. 1984. Fertilizers, p. 8–37. In: S. Williams (ed.). *Official methods of analysis of the Association of Official Analytic Chemists*. 14th ed. AAOAC, Arlington, Va.
- SAS Institute, Inc. 2000. SAS/stats user's guide. Version 8. SAS Inst. Inc., Cary, N.C.
- Slosser, J.E., W.E. Pinchak, and D.R. Rummel. 1989. A review of known and potential factors affecting the population dynamics of the cotton aphid. *S.W. Entomol.* 14:302–313.
- Storer, J.R. and H.F. Van Emden. 1995. Antibiosis and antixenosis of chrysanthemum cultivars to the aphid *Aphis gossypii*. *Entomol. Expt. Appl.* 77:307–314.
- Taiz, L. and E. Zeiger. 1998. *Plant physiology*. 2nd ed. Sinauer Assoc. Inc. Publ., Sunderland, Mass.
- Van der Werf, A. A.J. Vissar, F. Schieving, and H. Lambers. 1993. Evidence for optimal partitioning of biomass and nitrogen at a range of nitrogen availabilities for a fast- and slow-growing species. *Funct. Ecol.* 7:63–74.
- Van Emden, H.F. and M.A. Bashford. 1969. A comparison of the reproduction of *Brevicoryne brassicae* and *Myzus persicae* in relation to soluble nitrogen concentration and leaf age (leaf position) in the Brussels sprout plant. *Entomol. Expt. Appl.* 12:351–364.
- Walling, L.L. 2000. The myriad plant responses to herbivores. *J. Plant Growth Regul.* 19:195–216.
- Waring, G.L. and N.S. Cobb. 1992. The impact of plant stress on herbivore population dynamics, p. 167–226. In: E. Bernays (ed.). *Insect plant interactions*. vol. 4. CRC Press, Boca Raton, Fla.
- Wyatt, I.J. 1969. Factors effecting aphid infestation of chrysanthemums. *Ann. Appl. Biol.* 63:331–337.
- Wyatt, I.J. and S.J. Brown. 1977. The influence of light intensity, daylength and temperature on increase rates of four glasshouse aphids. *J. Appl. Ecol.* 14:391–399.
- Yadava, U.L. 1986. A Rapid and nondestructive method to determine chlorophyll in intact leaves. *HortScience* 21:1449–1450.
- Yeager, T., D. Fare, C. Gilliam, A. Niemiera, T. Bilderback, and K. Tilt. 1997. Best management practices—Guide for producing container-grown plants. S. Nurs. Assn., Atlanta, Ga.
- Yoder Brothers Inc. 2003. Pot mums: Cultural tips. <http://www.yoder.com/grower/pot/tips/cultural.asp>. Barberton, Ohio.