Fertilization Affects Constitutive and Wound-Induced Chemical Defenses in *Gerbera jamesonii*[1]

James D. Spiers[2,6], Fred T. Davies, Jr.[3], Chuanjiu He[3], Terri W. Starman[3], Scott A. Finlayson[4], Scott A. Senseman[4], and Kevin M. Heinz[5]
Department of Horticulture, Auburn University
Auburn, AL 36849

Abstract

The total phenolic content and jasmonic acid (JA) content of foliage from gerbera (*Gerbera jamesonii* 'Festival Salmon') subjected to low and high fertilization rates were measured to determine effects of fertilization on phytochemicals implicated in defense of insect pests. Gerbera seedlings were fertilized with low (only supplied with initial fertilizer charge present in professional growing media) or high (200 mg·liter^{-1} N; recommended rate) fertilization rates. Since JA is induced in response to wounding, treatments consisted of ± mechanical wounding with a hemostat to one physiologically mature leaf and the subsequent harvest of that leaf at specified time intervals (0, 0.5, 1, 3, and 10 h) for JA quantification. Total phenolic content was measured in mature and newly formed leaves. Plants receiving low fertility had reduced aboveground dry mass, were deficient in nitrogen and phosphorous, and had approximately a 9-fold higher concentration of total phenolics in mature leaf tissue compared to high fertility plants. Newly formed leaves had greater concentrations of phenolics than mature leaves in low fertility plants. In response to wounding, JA accumulation was more rapid and higher concentrations of JA were sustained longer in low fertility plants than in high fertility plants. These results show that altering fertilization does affect phytochemicals implicated in defense against insect pests, and these chemical defenses are increased in the foliage of low fertility gerberas.

Index words: jasmonic acid, phenolics, host plant resistance, integrated pest management, wound response, nutrient availability.

Species used in this study: *Gerbera jamesonii* 'Festival Salmon'.

Significance to the Nursery Industry

Many studies have shown that altering host plant fertilization affects insect feeding and fecundity (7, 8, 12, 18, 32), however reason(s) for this is poorly understood. Phenolic compounds have been identified as a group of chemicals closely associated with insect-plant interactions (16, 19, 34, 41), and a higher phenolic content typically exhibits a negative effect on insect feeding. Additionally, accumulation of jasmonic acid and the subsequent effect as a deterrent to insect feeding is well documented (5, 9, 30). This research shows that total phenolic and jasmonic acid content of foliage is affected by fertilization, and low fertility gerbera plants have greater concentrations of these chemical defenses. A basic understanding of effects of cultural practices such as fertilization on general foliar chemistry of plants will help provide explanations for insect pest outbreaks, and potentially provide mechanisms for enhancing host plant resistance and reducing insecticide applications. This is the first study to evaluate relationships between fertilization and chemical defenses in an ornamental greenhouse crop, and future research is warranted to determine whether other horticultural crops respond similarly.

Introduction

Gerbera (*Gerbera jamesonii*) is an economically important ornamental crop sold as a bedding plant, cut flower, and/or flowering potted plant. Gerberas, like many herbaceous floriculture crops, are highly susceptible to insect herbivores. There is much evidence suggesting that reducing fertility may increase host plant resistance to insect pests. Nutrition of host plants has been shown to have a direct effect on the fecundity of numerous insect pests, including western flower thrips [(WFT) *Frankliniella occidentalis*], which are a very problematic insect pest in greenhouse gerbera production. Reduced fertilization decreased WFT abundance in chrysanthemum (7, 8, 12, 32) and tomato (35), and this effect was attributed to reduced availability of essential nutrients for WFT, and possibly increased secondary metabolite levels. However, effects of plant nutrition on constitutive defenses (e.g. secondary metabolites) and induced defenses (e.g. JA accumulation) were not measured in any of these studies.

Plants use constitutive and induced defenses to protect themselves from phytophagous insect attack. Constitutive defenses include stored allelochemicals that can reduce attractiveness of plants to herbivores. Many of these plant secondary metabolites can decrease tissue digestibility and/or increase toxicity to pests, which would influence insect pest feeding, oviposition, fecundity, growth and development (39). Phenolics are the dominant allelochemicals in many plants and are known to decrease insect growth, development and survival (16, 19, 34, 41). When present in high concentrations, these toxic compounds poison generalist insect pests, whereas, specialist insect pests are forced to invest resources in detoxification mechanisms that in turn incur growth and development costs (21). Many natural secondary metabolites adversely affect insect pest colonization (3, 15) and/or attract natural enemies of insect pests (10, 13, 27, 38). These two broad functions have been categorized as direct and indirect resistance mechanisms, respectively.

Many past studies have shown that altering fertilization affects constitutive plant defenses. The quantity and quality of nitrogen (N) available to plants was shown to influence constitutive levels of many types of secondary metabolites,

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[2]Department of Horticulture, Auburn University, Auburn, AL 36849.

[3]Department of Horticultural Sciences, Texas A&M University, College Station, TX 77843.

[4]Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474.

[5]Department of Entomology, Texas A&M University, College Station, TX 77843.

[6]To whom reprint requests should be addressed; jds0017@auburn.edu.

including glucosinolates (17), cardenolides (17), phenolics (18, 36, 41), alkaloids (2), and furanocoumarins (42). Increases in defensive compounds (peroxidase and total phenolics) due to N deficiency was shown to negatively affect feeding and oviposition of various insect pests in tomato (*Lycopersicon esculentum*) (18). In a separate study, tomato plants treated with low N had twice the total phenolic content as compared to high N plants, with highest levels of phenolics in young leaves (36). Similarly, increasing nutrient availability frequently decreases secondary metabolite concentrations in plants (6, 22, 23, 25, 40).

The effect of nutrient availability on induced plant defenses is not well established. With jasmonates established as key regulators of plant responses to insect herbivores (5), jasmonic acid (JA) serves as a useful marker to determine interactions between nutrients and induced defenses. In maize (*Zea mays*), direct positive relationships have been established between beet armyworm (*Spodoptera exigua*)-induced JA accumulation in leaves and subsequent induced volatile emission (30). Low N availability resulted in higher levels of sustained JA accumulation and induced volatile emission, following treatment with insect-derived elicitors, than identically treated plants grown under medium N availability (31). Similarly, low N input resulted in higher increases in JA and volatiles in cotton (*Gossypium hirsutum*) than higher N treatments (9). However, both constitutive and induced JA levels were lower in low N tobacco (*Nicotiana attenuata*) plants compared to high N plants (24). Also contrary to maize and cotton, N supply did not influence elicited release of volatiles in tobacco. Volatile compounds were measured because they are known to attract a parasitoid that parasitizes the beet armyworm (i.e. indirect defense) (1). Based on this documented variability, responses of any given plant species cannot yet be readily predicted. By determining effects of nutrient availability on JA accumulation in other plant species, we may ascertain whether altering fertilization is a possible mechanism for regulating induced and/or constitutive plant defenses.

Despite some advances in agronomic crops (9, 24, 31), no studies to our knowledge have considered interactions between fertilization, JA, and total phenolic content in ornamental crops. The objective of this research was to determine possible effects of fertilization on constitutive defense (i.e. total phenolic content) and induced defense (i.e. JA) mechanisms present in the economically important greenhouse floriculture crop, *Gerbera jamesonii*.

Materials and Methods

Plant cultural conditions. For the first experiment, 24 gerbera 'Festival Salmon' seedlings (Knox Nursery Inc., Winter Garden, FL) were transplanted into 6-inch standard pots (15.5 × 11.5 cm; 2050 cm^3) on June 10, 2005, containing Sunshine Mix #1 (Sun Gro Horticulture Inc., Pine Bluff, AK) and watered with de-ionized water. Plants were grown in growth chambers at 75F day/68F night temperatures, 60% RH, and under a 14:10 (L:D) photoperiod. Light intensity was approximately 300 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Plants were supplied with either low fertility [only supplied with initial fertilizer charge present in the professional growing medium — which was a 5N-2.6P-10K (5-6-12) fertilizer incorporated at 1.6 $kg \cdot m^{-3}$ (2.7 $lbs \cdot yd^{-3}$) of mix], or the recommended fertilizer rate (high fertility). For the high fertility rate, plants received the initial fertilizer charge and were fertilized with a 200 mL (6.76 fl oz) solution of Peters Professional Peat-lite special 15-16-17 (elemental analysis: 15N-7P-14.2K; Scotts-Sierra Horticultural Products Co., Marysville, OH) at 200 ppm N at each watering.

The first study was terminated 29 d after transplanting on July 8, 2005, and total phenolic accumulation was determined. Sixty-four plants were used for the second study that was initiated on September 9, 2006, to ascertain effects on jasmonic acid (JA) accumulation. Plant cultural conditions and methods were the same as above. The second study was terminated 35 d (October 18, 2006) after transplanting, for JA quantification.

Plant growth. To characterize plant growth, dry mass (DM) of shoot tissue was measured at termination of the studies. Plants were cut at the soil line, placed in paper bags and dried in a convection oven at 160F for 120 h. Resultant dry tissue was weighed to determine total above ground DM.

Macro- and micro-nutrient analysis. In both studies, additional plants at low and high fertility were grown to collect leaf tissue for nutrient analysis. Mature leaves were harvested from each treatment at termination of the experiment (n = 4). Mineral status of plants was then determined on a DM basis. Tissue samples were dried in a convection oven at 160F for 120 h, and then were ground in a Wiley mill. Tissue samples were sent to a commercial laboratory for nutrient analysis (J.R. Peters/Scott's Testing Laboratory, Allentown, PA). Nitrogen content was determined using the Kjeldahl procedure (28). Remaining samples were digested in wet acid (20) and macro and micro-element determinations were assessed using an inductively coupled plasma atomic emission spectrophotometer (3510 ICP) based on procedures of Munter and Grande (26).

Total phenolic content quantification. Newly formed leaves [two per plant (rep)] and physiologically mature leaves [one per plant (rep)] were harvested for total phenolic measurements (n = 12). Total phenolic content of leaf tissue was evaluated based on a method adapted from Swain and Hillis (37) that describes the Folin-Ciocalteau reagent assay utilizing chlorogenic acid for a standard curve. In brief, fresh leaf tissue was weighed and ground with mortar and pestle in 80% methanol (6 mL; 0.2 fl oz). Extracts were centrifuged at 14,000 rpm for 15 min and kept in a freezer (–112F) until analysis. The reaction mixture consisted of mixing 30 µL of extract with 90 µL of Na_2CO_3 and 150 µL of Folin-Ciocalteau reagent in a 96-well microplate. After 30 min, absorbance was measured at 725 nm using a KC-4 spectrophotometer (Biotek® Instruments, Inc. Winooski, VT). Results were expressed as milligrams of chlorogenic acid equivalents per gram of fresh weight tissue.

Jasmonic acid quantification. All treatments (n = 4) were initiated (9:00 am) and then harvested on day 35. Treatments consisted of ± mechanical wounding with a hemostat to one physiologically mature leaf from each plant, and subsequent harvest of that leaf at specified time intervals for JA quantification. The JA content from wounded leaves was quantified 0, 0.5, 1, 3, and 10 h after wounding. To determine constitutive levels of JA in unwounded leaves, JA content was measured at 0, 3, and 10 h time intervals in non-wounded leaves. Each mechanical wounding consisted of clamping (mashing) down

with the hemostat to a set point, damaging approximately 90 mm^2 (0.14 in^2) of leaf area. The sampled leaf was wounded twice with the hemostat, once on each side of the midrib in the middle of the leaf, between veins. Tissue samples for JA were frozen in liquid N_2 and stored at −112F before analysis. JA was measured using isotope dilution based gas chromatography-mass spectrometry.

Extraction procedure. Frozen tissue samples (n = 4) were ground to a fine powder in liquid nitrogen with mortar and pestle. A mastermix was prepared that contained 5 mL (0.17 fl oz) of methanol and 50 ng of 1,3-[^{13}C]-JA internal standard (11) for each sample. The extraction mastermix was divided into 15 mL extraction tubes, warmed to 122F, and kept in a beaker of warm water. Frozen tissue samples were weighed and placed in extraction tubes, quickly mixed, and returned to the 122F water bath. Tubes were periodically shaken and returned to the water bath for 15 min. Tubes were then centrifuged for 5 min at 4000 rpm. Supernatant was carefully removed and dried. The extraction process was repeated until supernatants and plant material were colorless.

Sample purification. Samples (n = 4) were initially purified by C_{18} solid phase extraction [C_{18} Bakerbond SPE™ 3 mL extraction columns; packed with reversed phase octadecylsilane (500 mg); J.T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ]. Columns were prepared by adding 3 mL (0.1 fl oz) of 80% methanol, and then preconditioned with 6 mL (0.2 fl oz) of 0.4% acetic acid. Samples were re-suspended in 2 mL (0.068 fl oz) of 0.4% acetic acid and poured through the column three times (0.2 fl oz per sample). Columns were triple-rinsed (0.2 fl oz) with 0.4% acetic acid, and then samples were eluted with 5 mL (0.17 fl oz) of 80% methanol and dried.

Dried samples were re-suspended in 220 µL of filtered MeOH:HOAc (0.1 N) 35:65. Extracts were filtered through 0.2-µM syringe filters and 190 µL was applied to a C_{18} HPLC column (Alltech® alphaBond C_{18}, 300 × 3.9 mm, 125Å, 10µ). Constituents were separated by HPLC on a linear gradient from 35 to 85% methanol in 0.1 N acetic acid, at 0.8 mL per minute. Fractions were collected based on elution times previously determined with authentic JA standards (23.2 to 24.8 min) (11), and then dried.

Gas chromatography-mass spectrometry. Dried fractions were re-dissolved in 100 µL of MeOH, transferred to labeled reacti-vials, and dried under N_2. Samples were then methylated by adding 50 µL of ethereal diazomethane, sealing reacti-vials tightly with caps, and vortexing. Vials were placed under a fume hood for 20 min. After 20 min, caps were removed and diazomethane was allowed to evaporate under the fume hood until dry. The methylation procedure was then repeated. JA concentrations were determined by injecting samples into a Varian 3400 gas chromatograph equipped with a Saturn® 3 mass spectrometer. The sample was suspended in 15 µL of ethyl acetate, and 5 µL was injected with a septum programmable injector (SPI) set at 428F. Column used was a DB-5 (30 m × 0.25 mm × 0.25 µm) with a temperature program of 140 to 482F at a rate of 59F·min^{-1} (12.66 min), with helium as the carrier gas (constant flow rate 1 ml·min^{-1}). Analytes were ionized by electron impact. JA detections were confirmed by matching retention time (R.T. ~ 10.02 min) and mass spectrum of JA standards to unconfirmed analyte. JA levels were determined by comparing ^{12}C JA peak areas (*m/z* 224) to ^{13}C JA internal standard peak areas (*m/z* 226).

Experimental design and statistical data analysis. To determine total phenolic content of gerbera foliage, the first study was arranged as a 2 (fertility) × 2 (young and mature leaves) factorial. There were twelve single-plant replications (n = 12) arranged in a completely randomized design. Data were analyzed using PROC ANOVA (29). The second study was arranged as a 2 (fertility) × 2 (± wounding) × 5 (time period) factorial to determine JA accumulation (n = 4). There were four single-plant replications (n = 4) arranged in a completely randomized design. Data were analyzed using PROC GLM (29), with fertility, wounding, and time period as main effects. When appropriate, mean separations were performed using Fisher's protected least significant difference test to determine treatment differences ($P \leq 0.05$). For nutrient analysis, the study was designed to test the two fertility levels (n = 4) using PROC ANOVA (29).

Results and Discussion

Plant growth and leaf elemental analysis. As expected, fertility had a substantial effect on shoot dry mass and leaf elemental analysis. Shoot dry mass of gerberas receiving high fertility (2.8 g; 0.1 oz) was approximately 2.2-fold that of plants receiving low fertility (1.2 g; 0.04 oz). Plants receiving low fertility had substantially lower N, phosphorus (P), potassium (K), and zinc (Zn) compared to high fertility plants (Table 1). Based on general recommendations for high-quality gerbera tissue nutrient levels, low fertility plants were sufficient for K and Zn, but deficient in N and P (14).

Effects of fertilization on total phenolics. Fertilization had a substantial effect on total phenolic concentration in gerbera leaf tissue (Fig. 1). Physiologically mature leaves of low fertility plants had approximately a 9-fold higher concentration of total phenolics, when compared to high fertility plants. Increase in total phenolic content in low fertility gerbera plants demonstrates that allocation to secondary metabolism, in contrast to primary metabolism (e.g. growth), increases when nutrients are limiting. Similar results were reported in several studies that altered N availability in tomato plants. N stress increased expression of several genes involved in phenolic metabolism (4) and low N resulted in higher levels of phenolics in tomato (36, 41). In our study, young leaves had higher concentration of phenolics compared to older leaves in low fertility plants (Fig. 1), which parallels results reported in nitrogen-deficient tomato plants (36, 41). Though not measured in the present study, low N tomato plants were also reported to have higher constitutive levels of polyphenol oxidase (36), an enzyme that decreases the nutritive value of plants (21). Interestingly, polyphenol oxidase is a N-containing molecule, indicating that the increase in allocation to defense overrides constraints on physiological processes (36). Similar to our findings in gerbera, Inbar et al. (18) reported a negative association between tomato plant growth and chemical defense. Plants subjected to nutrient stress had higher levels of total phenolics and peroxidase, which negatively affected prolificacy of both chewing and cell content-feeding insect pests (18).

Effects of fertilization on jasmonic acid accumulation. Wound-induced JA accumulation was significantly affected

Table 1. Effect of fertilization application on leaf macro- and micro-nutrient content of *Gerbera jamesonii* 'Festival Salmon'. Plants were supplied with either low fertility [only supplied with initial fertilizer charge present in the professional growing medium — which was a 5N-2.6P-10K fertilizer incorporated at 2.7 lbs·yd^{-3} (1.6 kg·m^{-3}) of mix], or the recommended fertilizer rate (high fertility). For the high fertility rate, plants were fertilized with a 200 ml solution of Peters Professional Peat-lite Special 15-16-17 (elemental analysis: 15N-7P-14.2K; Scotts-Sierra Horticultural Products Co., Marysville, OH) at 200 mg·liter^{-1} N at each watering.

	Macro-nutrients					Micro-nutrients				
Fertilizer	N %	P %	K %	Ca %	Mg %	B µg·g^{-1}	Fe µg·g^{-1}	Mn µg·g^{-1}	Cu µg·g^{-1}	Zn µg·g^{-1}
0×	1.1 ± 0.05[z]	0.2 ± 0.01	3.8 ± 0.08	1.8 ± 0.06	0.7 ± 0.03	63.9 ± 4.4	55.1 ± 0.6	128 ± 7.5	8.3 ± 0.87	42.0 ± 3.9
1×	4.1 ± 0.06	1.1 ± 0.04	4.3 ± 0.09	1.8 ± 0.04	0.6 ± 0.02	70.2 ± 2.6	76.1 ± 9.3	110 ± 4.8	5.2 ± 0.25	80.1 ± 1.9
Recommended[y]	2.7 – 4.1	0.3 – 0.7	3.1 – 3.9	0.4 – 4.2	0.3 – 2.8	19 – 50	60 – 130	30 – 260	2 – 10	19 – 80
Fertilizer	< 0.0001[x]	< 0.0001	0.0099	NS	NS	NS	NS	NS	0.0272	0.001

[z]Values are ± SE, n = 4.

[y]Recommended leaf elemental levels for greenhouse gerbera production (14).

[x]Significance ($P \leq F$).

[NS]Nonsignificant at $P \leq 0.05$.

by fertilization (Fig. 2). In response to mechanical damage, JA accumulation increased more rapidly and accumulation was sustained longer in foliage of low fertility plants when compared to high fertility plants. In low fertility plants, JA levels peaked 0.5 h after wounding, and JA accumulation was higher at 0.5 h and 3 h after wounding, as compared to high fertility plants (Fig. 2). JA accumulation peaked 1 hr after wounding in high fertility plants, but this level was not higher than in low fertility plants. Ten hours after wounding, JA concentrations were not different among low and high fertility plants, though levels were still higher than constitutive levels. There were no differences in constitutive levels of JA between low and high fertility plants, and constitutive levels did not differ based on the time samples were taken.

As previously noted, N deficiency enhanced JA accumulation in maize (31) and cotton (9), but JA accumulation was reduced in low N tobacco (24). In the present study, foliage of low fertility gerbera plants accumulated JA more rapidly and sustained higher levels of JA for a longer period of time than high fertility plants — similar to responses found in low N maize and cotton (9, 31). Previous studies have consistently demonstrated the role of JA as a positive regulator of herbivore-induced defenses (5, 9, 31). Hence, enhanced wound-induced JA accumulation observed in low fertility gerbera plants indicates that these plants would be better prepared for defense against phytophagous insects than high fertility gerbera plants.

In summary, constitutive (i.e. total phenolic content) and wound-induced (i.e. JA) chemical defenses were increased in low fertility gerbera plants compared to high fertility plants. Obviously, drastically reducing fertilization would not be a feasible option in greenhouse floriculture crop production, but moderately reducing fertilization or altering fertilization during certain stages of production may be viable options that enhance host plant resistance without reducing marketability. Future research is needed to determine whether optimizing fertilization can be a useful tool in an integrated pest management (IPM) system. If fertilization is reduced to a level that increases host plant resistance while producing marketable crops, then fertilizer run-off, pesticide usage, and associated chemical phytotoxicity (33) could be reduced in production systems.

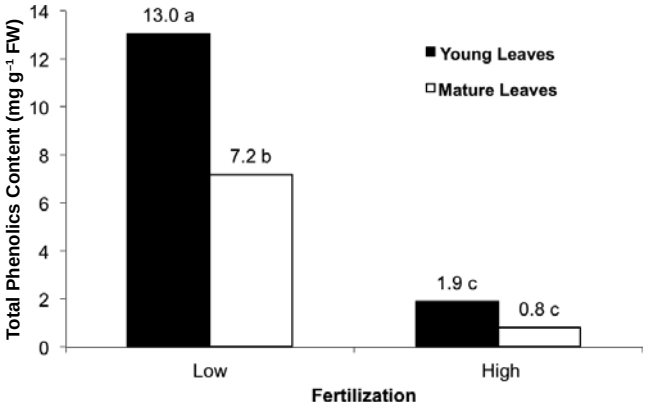

Fig. 1. Total soluble phenolic concentration in young and mature leaf tissue from *Gerbera jamesonii* 'Festival Salmon' fertilized with low and high fertility rates. The interaction between fertilization and leaf age ($P \leq 0.0089$) were significant. Means with different letters are significantly different ($P \leq 0.05$); n = 12.

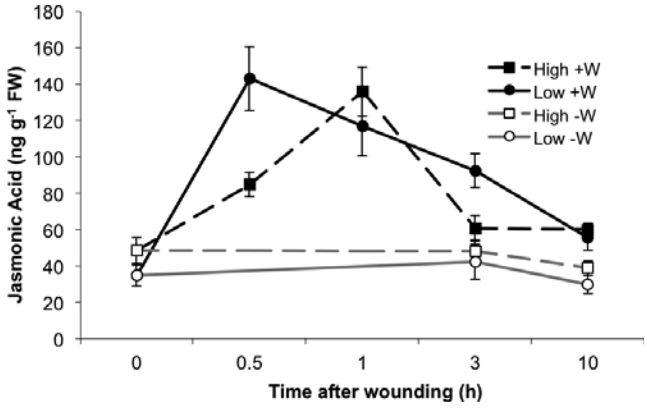

Fig. 2. Jasmonic acid (JA) accumulation in physiologically mature leaves from *Gerbera jamesonii* 'Festival Salmon' plants fertilized with low and high fertilization rates and ± mechanical wounding (W). The interactions of time after wounding, fertilization and ± wounding ($P \leq 0.0067$) were significant effect. Bars ± SE, n = 4.

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