Whole-Plant CO_2 Exchange Measurements on Azaleas Injured by Azalea Lace Bug (Heteroptea: Tingidae) Feeding

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ABSTRACT Whole-plant gas exchange was measured continuously for 24 h on rooted cuttings of Girard's 'Pleasant White' azaleas. Azalea treatments were azalea lace bug, *Stephanitis pyrioides* (Scott), feeding injury levels that averaged 6, 13, or 31% leaf-area injury throughout the plant canopies. Gas exchange parameters, including net photosynthesis, dark respiration, carbon use efficiency, and growth, were compared with undamaged control plants. Responses of Girard's 'Pleasant White' azaleas suggested that azaleas were tolerant of lace bug feeding injury levels above the aesthetic threshold. Azalea tolerance can be incorporated into an integrated management plan to reduce chemical inputs into the urban landscape.

KEY WORDS *Stephanitis pyrioides*, azalea lace bug, *Rhododendron*, photosynthesis, respiration, carbon use efficiency

INJURY TO RHODODENDRONS and azaleas occurs when feeding by azalea lace bugs, *Stephanitis pyrioides* (Scott), removes cell contents in the leaves resulting in chlorotic stipples (Mead 1967, Johnson and Lyon 1991). Both nymphal and adult azalea lace bugs feed by inserting mouthpart stylets into stomata on the abaxial surface of leaves (Ishihara and Kawai 1981). Stylets penetrate cells within the palisade parenchyma of the leaf mesophyll and remove chloroplasts and other cellular contents (Ishihara and Kawai 1981, Buntin et al. 1996). Typically, feeding by single adult females was more injurious than individual adult males or nymphs (Neal and Douglass 1988, Buntin et al. 1996). In addition to the direct removal of chloroplasts, Buntin et al. (1996) found that photosynthesis in the remaining cells and leaf respiration were reduced. The authors attributed these effects to stomatal closure in injured leaves (Buntin et al. 1996).

The azalea lace bug studies, like the majority of arthropod–plant gas exchange investigations, have relied primarily upon single-leaf measurements of gas exchange. Evans (1993) cited several factors that may result in a poor correlation between leaf photosynthesis measurements and both dry matter production and yield. Leaves selected for measurement vary with age or condition. Analysis of a particular stage of leaf maturity is not necessarily representative of the overall photosynthetic rate of a plant canopy. Variability may also exist within a single leaf. The section of leaf chosen for measurement may not be typical of the whole. Collection of the many samples needed for statistical accuracy virtually ensures that leaf samples will be conducted over a long period. Variation, caused by diurnal changes in photosynthetic rate, is likely to confound conclusions drawn from research results. Most importantly, root and shoot respiration are not measured. Growth and carbon use efficiency variables are not adequately quantified and cannot be reliably interpreted. To avoid the shortcomings of gas exchange analysis made on individual leaves, whole-crop CO_2 exchange systems have been developed (Dutton et al. 1988, Bugbee 1992, van Iersel and Bugbee 1997). A system capable of taking whole-plant gas exchange measurements in multiple chambers has been developed, which incorporates growth chambers to maintain controlled environmental conditions (Bugbee 1992, van Iersel and Bugbee 1997). The system directly measures net photosynthesis (P_{net}) and nighttime respiration (R_{dark}), in each of 10 separate chambers, sequentially. Data can be used to calculate gross photosynthesis (P_{gross}), the daily increase in photosynthetically fixed carbon or daily carbon gain, and carbon use efficiency, the ratio of carbon fixed by photosynthesis less the carbon lost during respiration (see *Materials and Methods*) (Fig. 1).

An integrated management plan for azalea lace bugs in landscape plantings might incorporate plant tolerance of feeding injury into a holistic control strategy. Our knowledge of the physiological effects of *S. pyrioides* feeding injury would be greatly enhanced by studies that measure gas exchange among several levels of damage. Some researchers have indicated that plant vigor is often reduced among feeding injured azaleas, and that premature leaf abscission or plant death might occur (Bailey 1951, Mead 1967, Nalepa and Baker 1994). However, whole-plant physiological response to lace bug feeding injury has not been quantified. To better understand the relationship of azalea lace bug feeding injury to gas exchange rate we undertook a whole-plant analysis of gas exchange in Girard's 'Pleasant White' azalea hybrids. We also examined the possible fate of photosynthetic assimilate

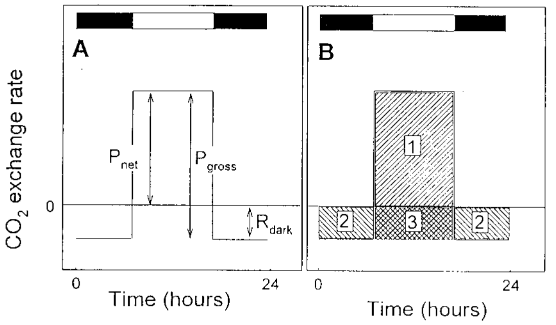

Fig. 1. A schematic representation of CO_2 exchange of whole plants during a 24-h period. Net photosynthesis and dark respiration rate measurements were used to estimate gross photosynthetic rate (A). Daily carbon accumulation of plants (area 1–area 2) and total gross photosynthesis during the light period (area 1 + area 3) were calculated (B). These values were then used to estimate carbon use efficiency (area 1–area 2)/(area 1 + area 3). The bars in the top of the diagrams indicate periods of light (open bar) and dark (closed bar).

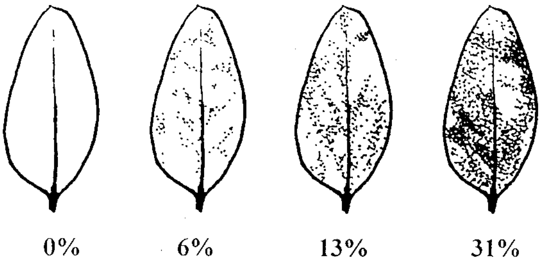

Fig. 2. Negative images of 'Girard's Rose' hybrid azalea leaves are approximations of azalea lace bug feeding injury levels used in this study. Images are representative of treatment group damage levels that were inflicted throughout the foliage of the rooted plants.

in damaged azaleas. To maximize the usefulness of our findings for urban pest management, we created leaf area injury levels similar to those commonly encountered in the landscape.

Materials and Methods

Preparation of Girard's 'Pleasant White' Azaleas. Rooted cuttings of Girard's 'Pleasant White' azaleas, a cultivar of uncertain parentage, were obtained from a single wholesale nursery to ensure uniformity of type and size. Upon receipt, azalea cuttings were potted into 10-cm pots (Kord, Lugoff, SC) using Metro Mix 300 potting media (Scotts-Sierra Horticultural, Marysville, OH). Azaleas were acclimated to November and December greenhouse conditions over a 3-wk period. Plants were maintained at 20 ± 1°C during the daytime 17 ± 1°C at night under a photoperiod of 10:14 (L:D) h. To maintain the 42 undamaged control plants in a lace bug-free condition during the study, a single acephate application of 0.296 g (AI)/liter, made from the Orthene T T & O (wettable powder) formulation, was made 3 wk before measurements were initiated. Previous studies revealed no reductions in plant gas exchange when acephate was used at the recommended rate (Klingeman 1998).

Azalea Lace Bug Colony. A colony of azalea lace bugs, housed in 1.0-m^3 screen cages in the entomology insect rearing facility at Griffin, GA, was used to create experimental injury levels. Colonies were established and periodically replenished with adult azalea lace bugs collected from natural populations found near Griffin. Colonies were reared on several varieties of evergreen azaleas at 27°C and a photoperiod of 14:10 (L:D) h.

Imposing and Assessing Azalea Lace Bug Injury Levels. Low, medium, and high leaf area injury treatments were selected to represent a range of damage commonly seen in the landscape (Fig. 2). Infestation of plants was undertaken between 3 November and 17 November 1997 in the 1.0-m^3 screen cages. Adult lace bugs were shaken onto foliage and allowed to feed until an approximation of the targeted percentage of leaf area injury was reached. Azaleas representing low injury treatments required between 2 and 4 d of feeding pressure, whereas plants in the high injury treatments were infested for 10–14 d. After injury infliction, lace bugs were shaken from the foliage into the rearing chambers. Azaleas were then inspected visually and remaining lace bugs were removed by hand. Inspections of plants made before gas measurements revealed no additional lace bugs.

To quantify the percentage of leaf area injured within treatments, plants were halved by temporarily inserting an index card at random into the plant canopy. All of the leaves on each rooted azalea half, which averaged 55 ± 18 leaves per plant, were compared with an array of 24 images of injured leaves. Leaf images in the array were analyzed using Mocha software: a computer-assisted measurement program that used contrasting color overlays to quantify both total leaf area and injured leaf area (Jandel Scientific 1993). Images on the array represented lace bug injury ranging from 0.5 to 82%. A record of the estimated percent injury on each leaf was quantified and reported as actual injury for each treatment group, which included 7 plants. After the initial assessment, plants that required additional injury to meet targeted percentage levels were reintroduced to the lace bug colony. Plants were remeasured after the 2nd infestation was completed. Once damage assessments were concluded, azaleas were arranged in a randomized complete block design with 6 replicates, of 7 plants per replicate, for each of 4 treatments. Final injury levels among replicated treatment groups averaged 6% of the canopy for the low level, 13% for the medium level, and 31% of the treatment canopy for the high level of injury. Thus, results are presented for 42 plants in each treatment level for an experimental total of 168 rooted azaleas. Plants were reacclimated in the greenhouse from 22 November to 30 November 1997 before gas exchange measurements were initiated.

Whole Plant Gas Exchange Measurements. A multichamber whole plant CO_2-exchange system (Bednarz and van Iersel 1998) was used to measure net photosynthesis (P_{net}) and dark respiration (R_{dark}) for treatment groups during a 24-h period. Plants were watered before being placed in the chambers to alleviate potential water stress. In the chambers, treatment groups received a 14-h photoperiod. Fluorescent and incandescent lights provided average photosynthetic photon flux levels of 600 μmol m^{-2} s^{-1} at canopy height. Diurnal conditions in the growth chambers were 23 ± 1°C and 50% RH, whereas nocturnal conditions were 19 ± 1°C and 70% RH.

P_{net} and R_{dark} of each treatment group of plants were measured once every 10 min. Ambient air was blown into the acrylic chambers and airflow was measured with mass flow meters (GFM37–32, Aalborg Instruments and Controls, Monsey, NY). The difference in the CO_2 concentrations of the air entering and exiting the chamber was measured with an infrared gas analyzer (LI-6251, Li-Cor, Lincoln, NE). Whole chamber CO_2 exchange (μmol s^{-1}) was calculated as the product of mass flow (mol s^{-1}) and the difference in CO_2 concentration (μmol mol^{-1}). Both photosynthesis and respiration data are reported as positive quantities. Carbon use efficiency (CUE), the ratio between carbon incorporated in plant dry mass and the total amount of carbon fixed in photosynthesis (Amthor 1989), was calculated as follows:

$$CUE = [(P_{net} * t_{light}) - (R_{dark} * t_{dark})] / (P_{gross} * t_{light}), \quad [1]$$

where P_{net} = average net photosynthesis during the light period, t_{light} = length of the light period (seconds), t_{dark} = length of the dark period (seconds), R_{dark} = average respiration during the dark period, and P_{gross} = average gross photosynthesis during the light period (estimated as the sum of P_{net} and R_{dark} assuming equal respiration rates in the light and dark periods) (Fig. 1).

Daily carbon gain (DCG) may be calculated as the amount of CO_2 fixed by the canopy less the amount of CO_2 lost by respiration:

$$DCG = (P_{net} * t_{light}) - (R_{dark} * t_{dark}). \quad [2]$$

Daily carbon gain is similar to crop growth rate. Long-term experiments using summed daily carbon gains to provide a cumulative carbon gain have found a close correlation (r^2 = 0.998) between cumulative carbon gain and plant dry mass (van Iersel and Bugbee 1997).

Values reported for each variable represent measurements made among 7 plants in each treatment group. Beginning 1 December 1997, treatment groups were analyzed in 3 separate runs lasting 24 h each. Each run consisted of measurements taken on 2 complete replicates, each with the four 7-plant treatments, as well as measurements taken within 2 empty acrylic chambers, which provided a baseline for comparison. Baseline measurement of slight changes within the empty chambers enabled us to correct for error potentially introduced by zero-drift of the infrared gas analyzer as it measures CO_2 concentrations.

Destructive Sampling Measurements. Once measurements of gas exchange were concluded, 25 fully expanded leaves from each treatment group were randomly selected and removed. Leaf area of the 25 leaves was determined using a LI-3100 leaf area meter (Li-Cor, Lincoln, NE), and a dry mass of the 25 leaves was taken. All remaining leaves were removed for dry mass measurements. Stems and roots were separated by treatments, washed free of media, and dried to provide dry mass measurements of stems and roots. To provide an estimate of the total treatment canopy area, the ratio of the summed leaf areas of the 25 sampled leaves to the sample dry mass was multiplied by the total dry mass of leaves in each treatment.

Statistical Analysis. Analysis of variance (ANOVA) analyses were used to calculate mean average leaf areas using the PROC GLM procedure in SAS (SAS Institute 1985). Cultivar gas exchange parameters and dry mass variables among treatments were compared using PROC GLM in SAS (SAS Institute 1985). Where significant differences were detected, means of variables were separated using Fisher protected least significant difference (LSD) test (LSD, P = 0.05) (SAS Institute 1985).

Results

Leaf Area Injury Levels. Artificial infestation of Girard's 'Pleasant White' azaleas, using azalea lace bugs to inflict feeding injury, yielded 6% actual leaf area injury throughout the canopy of the low level treatment. Previous work on azaleas has indicated that leaf damage becomes readily apparent when 2% or more of its area is injured by feeding azalea lace bugs (Klingeman 1998). Moreover, in a recent survey, consumers were highly discriminatory and refused to accept azaleas that had >10% proportional injury (Klingeman 1998). We used this 2% threshold to calculate a mean proportional injury level for the 7 azalea plants comprising each treatment group. Leaves with <2% injury among the leaf area were not counted as injured for the assessment of proportional injury. As a result, 6% actual leaf injury corresponded to 42% proportional injury based on the 2% threshold. The medium treatment had 13% actual leaf area injury with 61% proportional injury, and the high treatment had 31% actual leaf area injury with 76% proportional injury throughout the canopy.

Whole Plant CO_2 Exchange Measurements. Plant gas exchange responses to treatments were not explained by size differences in tissues of the treatment plants. No significant size differences were detected for individual leaf area (F = 1.59; df = 3, 15; P < 0.24), canopy leaf area (F = 2.59; df = 3, 15; P < 0.10), or combined root and stem dry masses (F = 1.71; df = 3, 15; P < 0.21) among the 7-plant treatment groups. Significant differences among azalea lace bug feeding injury treatments were evident for net photosynthesis (F = 9.35; df = 3, 15; P < 0.001), dark respiration (F = 5.79; df = 3, 15; P < 0.01), carbon use efficiency (F = 29.66; df = 3, 15; P < 0.0001), and growth (F = 10.14; df = 3, 15; P = 0.0007) values. Mean separation for P_{net} indicated that the high-injury treatment group, with 31% of the canopy leaf area showing azalea lace bug

Table 1. Mean ± SD net photosynthesis (P_{net}), dark respiration (R_{dark}), carbon use efficiency (CUE), and daily carbon gain (DCG) in azaleas with varying levels of azalea lace bug feeding injury

Injury level	Actual leaf area injured %	Proportional injury %	P_{net} (μmol s^{-1})	R_{dark} (μmol s^{-1})	CUE (mol mol^{-1})	DCG (mmol d^{-1})
None	0	0	1.02 ± 0.15a	0.25 ± 0.02a	0.66 ± 0.03c	42.57 ± 7.09a
Low	6	42	1.15 ± 0.05a	0.26 ± 0.02a	0.69 ± 0.02b	48.48 ± 2.42a
Medium	13	61	1.01 ± 0.17a	0.21 ± 0.03b	0.72 ± 0.03a	43.77 ± 7.21a
High	31	76	0.78 ± 0.06b	0.21 ± 0.02b	0.63 ± 0.02d	31.52 ± 2.84b

Values followed by the same letter within column are not statistically different (Fisher LSD test, $P < 0.05$).

injury, had significantly lower net photosynthesis than undamaged, low, or medium injury treatments. Mean separation of R_{dark} results revealed that both the medium (13% actual leaf area injury) and high (31% leaf area injury) levels were significantly lower than respiratory rates demonstrated by undamaged and low (6% leaf area injury) treatments (Table 1).

Carbon use efficiency is calculated as the proportion of all carbon fixed by photosynthesis that may be recovered as dry mass. Mean separation of carbon use efficiency among treatments revealed that all injury levels had significantly different efficiencies of carbon use (Table 1). The treatment having the highest level of injury demonstrated the least efficiency in carbon use: only 63.1% of the total carbon fixed by photosynthesis was attributed to dry mass production, the rest was lost in the respiratory process. The trend described by growth, or daily carbon gain values (Table 1) closely paralleled that of net photosynthetic rate (Table 1). Mean separation by LSD analysis indicated that only plants averaging 31% actual leaf area injury had significant reductions in growth (Table 1).

Discussion

The gas exchange measurement system used in this study enabled continuous data recording during a 24-h period and supported current concerns, that root and stem respiration are important contributors to the accuracy of measured plant gas exchange parameters that are often overlooked (Evans 1993). Dark respiration data were adjusted for root and stem dry mass and analyzed in conjunction with the net photosynthetic rate, which was adjusted for the dry mass of the canopy leaf area. The results revealed net photosynthetic rates comparable to the uninjured controls with feeding injury as high as 13% of the canopy leaf area and included injury levels that may have been much higher on individual leaves. Dark respiration also was used to provide measures of carbon use efficiency and growth among treatments. Growth measurements are most descriptive in long-term studies using cumulative carbon gain values. In our short-term study, growth reductions were not apparent until feeding lace bugs had inflicted 31% canopy leaf area injury. The close parallel trends demonstrated for P_{net} and growth (daily carbon gain) values were expected because of the small losses in CO_2 attributed to dark respiration relative to the higher rates of CO_2 fixed by net photosynthesis.

Carbon use efficiency levels for undamaged, low, and medium injury levels exhibited a trend suggesting that carbon use efficiency increased with azalea lace bug feeding injury. This phenomenon cannot be readily explained by the physiological apportionment of sucrose, which is the end product of photosynthesis. However, efficient carbon use, defined as a high ratio of carbon fixation in dry mass to a low rate of carbon lost to the respiratory process, is greatest when photosynthetic rate is high relative to dark respiration within a plant. For example, carbon use efficiency among plants in the medium treatment having a moderate rate of net photosynthesis was reduced by the low rate of dark respiration. Thus, 28.3% of carbon photosynthetically fixed by plants in the medium feeding injury treatment was lost in dark respiration and plants were 71.7% efficient at retaining carbon for dry mass production. This phenomenon is consistent with our adjusted P_{net} and R_{dark} values, which suggest that physiological resilience may be demonstrated for azaleas under feeding pressure from azalea lace bugs. In our study, azaleas that had up to 13% canopy leaf area injury caused by *S. pyrioides* feeding continued to photosynthesize at levels comparable to the uninjured controls.

Azalea lace bug feeding reduced net photosynthesis and dark respiration in comparison to undamaged controls. These findings are consistent with the reported effects, summarized by Welter (1989), of mesophyll-feeding arthropods including spider mites, scales, and leafhoppers that are gas exchange reducers. Reduced photosynthesis, conductance, and transpiration in response to arthropod feeding injury, has been attributed to the loss of chlorophyll or a reduced capacity of remaining chlorophylls (Walstad et al. 1973, Cockfield and Potter 1986). In addition, stomatal guard cells may be functionally impaired or closed in response to water loss (Sances et al. 1979, 1981; DeAngelis et al. 1982; Mizell et al. 1986; Anderson and Mizell 1987). Azalea tolerance to injury demonstrated in this study might be explained by the behavior of azalea lace bugs, which feed in the palisade parenchymal cells leaving the photosynthetic cells of the spongy mesophyll largely intact (Ishihara and Kawai 1981, Buntin et al. 1996).

The amount of feeding injury inflicted by lace bugs throughout the plant canopies of each treatment was assessed using a 2% injury-recognition threshold (Klingeman 1998) to tally presence or absence of damage to individual leaves. These assessments established that all of the treatments chosen would be considered aesthetically damaged in the landscape. Whole-plant gas exchange measurements revealed that neither net photosynthesis, carbon use efficiency, nor growth are significantly reduced, in comparison to undamaged controls, among azaleas with lace bug feeding injury less than or equal to 13% of the plant canopy. Dark respiration was not significantly different from the controls with injury to 6% of the plant canopy. Importantly, these findings highlight the phenomenon that highly discriminatory consumers act on aesthetic injury levels for azaleas that are well below the level of injury required to cause physiological damage to plants.

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References Cited

Anderson, P. C., and R. F. Mizell III. 1987. Impact of peach silver mite, *Aculus cornutus* (Acari: Eriophyidae), on leaf gas exchange of 'Flordaking' and 'June Gold' peach trees. Environ. Entomol. 16: 660–663.

Amthor, J. S. 1989. Respiration and crop productivity. Springer, New York.

Bailey, N. S. 1951. The Tingoidea of New England and their biology. Entomol. Am. 31: 1–140.

Bednarz, C. W., and M. W. van Iersel. 1998. Semi-continuous carbon dioxide exchange rates in cotton treated with commercially available plant growth regulators. J. Cotton Sci. 2: 136–142.

Bugbee, B. 1992. Steady-state canopy gas exchange: system design and operation. HortScience 28: 41–45.

Buntin, G. D., S. K. Braman, D. A. Gilbertz, and D. V. Phillips. 1996. Chlorosis, photosynthesis, and transpiration of azalea leaves after azalea lace bug (Heteroptera: Tingidae) feeding injury. J. Econ. Entomol. 89: 990–995.

Cockfield, S. D., and D. A. Potter. 1986. Predatory insects from suburban lawns in Lexington, Kentucky. Gt. Lakes Entomol. 17: 179–184.

DeAngelis, J., K. C. Larson, R. E. Berry, and G. W. Krantz. 1982. Effect of spider mite injury on transpiration and leaf water status in peppermint. Environ. Entomol. 11: 975–978.

Dutton, R. G., J. Jiao, M. J. Tsujita, and B. Grodzinski. 1988. Whole plant CO_2 exchange measurements for nondestructive estimation of growth. Plant Physiol. 86: 355–358.

Evans, L. T. 1993. Crop evaluation, adaptation and yield. Cambridge University Press, Cambridge.

Ishihara, R., and S. Kawai. 1981. Feeding habits of the azalea lace bug, *Stephanitis pyrioides* Scott (Hemiptera: Tingidae). Jpn. J. Appl. Entomol. Zool. 25: 200–202.

Jandel Scientific. 1993. User's manual, version 1.2.10. Jandel Scientific, San Rafael, CA.

Johnson, W. T., and H. H. Lyon. 1991. Insects that feed on trees and shrubs. Cornell University Press, Ithaca, NY.

Klingeman, W. E. 1998. Developing decision-making guidelines for controlling the azalea lace bug, *Stephanitis pyrioides* (Scott) (Heteroptera: Tingidae). Ph.D. dissertation, University of Georgia, Athens.

Mead. F. W. 1967. *Stephanitis* lace bugs of the United States (Hemiptera: Tingidae). Fla. Dep. Agric. Div. Plant Ind. Entomol. Circ. 62.

Mizell, R. F., P. C. Anderson, and D. E. Schiffhauer. 1986. Impact of twospotted spider mite on some physiological processes of 'Flordaking' peach. J. Agric. Entomol. 3: 143–151.

Nalepa, C. A., and J. R. Baker. 1994. Winter oviposition of the azalea lace bug (Hemiptera: Tingidae) in North Carolina. J. Entomol. Sci. 29: 482–489.

Neal, J. W., Jr., and L. W. Douglass. 1988. Development, oviposition rate, longevity, and voltinism of *Stephanitis pyrioides* (Heteroptera: Tingidae), an adventive pest of azalea, at three temperatures. Environ. Entomol. 17: 827–831.

Sances, F. V., J. A. Wyman, and I. P. Ting. 1979. Physiological responses to spider mite infestation on strawberries. Environ. Entomol. 8: 711–714.

Sances, F. V., J. A. Wyman, I. P. Ting, R. A. van Steenwyk, and E. R. Oatman. 1981. Spider mite interactions with photosynthesis, transpiration, and productivity of strawberry. Environ. Entomol. 10: 442–448.

SAS Institute. 1985. SAS user's guide: statistics, 5th ed. SAS Institute, Cary, NC.

van Iersel, M. W., and B. Bugbee. 1997. Increased organic matter in the growing medium decreases Benlate DF phytotoxicity. Plant Dis. 81: 743–748.

Walstad, J. D., D. G. Neilsen, and N. E. Johnson. 1973. Effect of pine needle scale on photosynthesis of Scots pine. For. Sci. 19: 108–111.

Welter, S. C. 1989. Arthropod impact on plant gas exchange, pp. 135–150. *In* E. A. Bernays [ed.], Insect–plant interactions, vol. 1. CRC, Boca Raton, FL.

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