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Whole-plant gas exchange, not individual-leaf measurements, accurately assesses azalea response to insecticides

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Abstract

A comparison of whole-plant and individual-leaf gas exchange measurements was undertaken among azaleas, *Rhododendron* spp., treated with insecticides used to control the azalea lace bug, *Stephanitis pyrioides* (Scott). Azaleas in individual-leaf trials received 3 insecticide treatments during three 21-day trials. The whole-plant study included 2 insecticide treatments and was conducted during a 21-day trial. Individual-leaf studies indicated that insecticidal soap and acephate application at higher than recommended rates caused short-term reductions in gas exchange. However, variability among individual-leaf measurements prevented consistent conclusions. Whole-plant gas exchange measurements provided consistent results and confirmed that insectidical soap caused short-term reductions in P_{net} and R_{dark}. Additionally, whole-plant gas exchange measurements enabled growth analyses and demonstrated a reduction in carbon use efficiency attributed to insecticidal soap treatments. Neither acephate nor horticultural oil significantly affected whole-plant gas exchange. Gas exchange parameters for treatments in all trials were not significantly different from the controls by Day 21.

Keywords: Insecticides; *Rhododendron* spp.; Whole-plant gas exchange

1. Introduction

Studies reporting plant gas exchange responses to pesticides traditionally rely upon measurements taken either on single leaves or on a portion of a single leaf. Reliance on individual-leaf measurements is mostly attributed to technological constraints, because the standard equipment for gas exchange measurements is not large enough to allow measurements to be taken on an entire leaf or whole plant. A literature review by Evans (1993) indicated that investigations relying upon individual leaf measurements do not accurately represent whole plant responses. In many cases, poor correlations in dry matter production and yield are found in conjunction with individual-leaf photosynthesis measurements (Evans, 1993). The poor association has been attributed to: (1) measurements taken from a section of leaf, which is not representative of the entire leaf, (2) selection of a leaf that does not reflect the gas exchange level of the canopy, (3) an inability to account for diurnal changes in the rate of photosynthesis and respiration, and (4) alterations in CO_2 exchange that occur as leaves mature (van Iersel and Bugbee, 2000). Furthermore, reliance upon leaf gas exchange for estimating dry matter production and yield, does not account for the CO_2 exchange that occurs in plant roots and shoots. As a result, many inconsistencies in plant responses to treatments including pesticide applications may be due to inadequate techniques. However, recent research has investigated whole-plant gas exchange analyses in response to fungicide applications using novel technology (van Iersel and Bugbee, 1996; 1997a, b; 2000).

A system, which is capable of taking whole-plant gas exchange measurements among multiple chambers, has been developed (Bugbee, 1992; van Iersel and Bugbee, 2000). Data collection in growth chambers allows the plants to be maintained under controlled environmental conditions. This system directly measures net photosynthesis (P_{net}) and dark respiration (R_{dark}) in each of 10 separate chambers, sequentially. Data can be used to

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calculate gross photosynthesis (P_{gross}), growth or daily carbon gain (DCG), and carbon use efficiency (CUE). This technology will provide a more accurate and meaningful analysis of plant responses to treatments including pesticide applications.

Advocates of integrated pest management programs and non-chemical pest control have urged the use of insecticidal soaps and horticultural oils for managing many pest problems. These chemicals are viewed as safe and environmentally short-lived alternatives to traditional chemical options (Davidson et al., 1990; Miller, 1989). However, soap and oil have been implicated in plant gas exchange reductions (Anderson et al., 1986; Ayres and Barden, 1975; Helson and Minshall, 1962; Schrader and Kammereck, 1996; Wedding et al., 1951). Phytotoxicity to insecticidal soap and horticultural oils has also been reported for sensitive plant taxa (Baxendale and Johnson, 1988; Davidson et al., 1990; Hansen et al., 1992; Johnson, 1985; Olson and Ascerno, 1991; Puritch and Brooks, 1981). Investigations into the morphological and physiological effects of pesticides on azaleas are relatively limited and often do not include insecticidal soaps or horticultural oils (Heungens et al., 1991, 1992; Moore, 1980).

Soap phytotoxicity has been demonstrated on plants but has generally been limited to sensitive plant varieties or may occur in response to applications of higher proportions of active ingredient (Olson and Ascerno, 1985; Puritch and Brooks, 1981). Phytotoxic effects to plants are variable, ranging from chlorosis and mild leaf cupping to necrotic spots and lesions. Further, in greenhouse studies, temperatures often in excess of 80°F may confound phytotoxic responses (Olson and Ascerno, 1985).

Acute plant phytotoxicity to oils, which may be paraffinic or naphthenic by composition, is most likely to occur at temperatures exceeding 35°C and is seen within 24–48 h (Furness and Maelzer, 1981). Plant tissues exposed to aromatic ring structures in unsaturated oils quickly become necrotic. Chronic toxicity, which may result from delayed absorption of the oil film by the plant tissues, is a physical response by the plant that includes chlorosis of tissues, leaf and fruit abscission, reduction in fruit quality and yield, and inhibition of fruit ripening (Furness and Maelzer, 1981). Johnson (1985) outlined several professional conclusions concerning factors likely to cause a phytotoxic response to horticultural oil. The principal contributors included the use of high rates of oil and inappropriate timing of application: during bud break and shoot elongation, during periods of water stress, or when relative humidity is expected to exceed 90% for 48 h or more. Mistaken dormancy, premature fall applications of oils, and genetic variability may also contribute to plant sensitivity to paraffinic oils (Johnson, 1985). A comparison of naphthenic and paraffinic oils of equal weight and viscosity found no immediate differences in the reductions of photosynthesis of Eureka lemon trees (Reihl and Wedding, 1959). However, the photosynthetic rate of naphthenic oil-treated plants recovered more rapidly. While rate of photosynthetic recovery has not been adequately assessed among taxa, Wedding et al. (1951) revealed that respiration and net photosynthesis rates remained depressed for as long as 59 days following treatment of *Citrus limon* (L.) Burm. f. 'Eureka' lemon trees and *Citrus sinensis* (L.) Osbeck. 'Washington' navel orange trees with a 2% solution of an emulsive-type spray oil.

Nursery producers and landscape management professionals commonly use acephate to control azalea lace bug populations (W.E.K., personal observation). Acephate is a water-soluble insecticide that is systemic in leaf tissues. The water solubility of acephate allows it to be transported upward through xylem tissues in plants (Werner, 1972). Acephate residues that are not translocated into plant tissues are short-lived in the environment and may volatilize within 48-h (Bull, 1979). Acephate and its hydrolysis product, methamidophos, have been implicated in reducing the gas exchange parameters of many sensitive plants (Chase and Poole, 1984; Heungens et al., 1991; Oetting et al., 1980), including azaleas and rhododendrons (Heungens et al., 1991, 1992). However, plant phytotoxic responses to pesticides are not consistent among taxa (Davidson et al., 1990).

Using insecticide-treated azaleas, we compared whole-plant gas exchange measurements to the existing technological standard, which is individual-leaf gas exchange measurements. Treatments used to control azalea lace bugs focussed on insecticidal soap, acephate, and horticultural oil. This investigation was intended to accurately quantify the immediate and short-term effects of the applications of these insecticides on azalea CO_2 exchange and to evaluate growth, and carbon-use efficiency variables made available by the whole-plant gas exchange technology.

2. Materials and methods

A series of individual-leaf gas exchange studies were undertaken in greenhouses in July and August 1996 in Georgia. The azaleas used in each trial were obtained from a commercial grower and were selected to have an approximately uniform 30 cm height and width.

Trial 1: Gas exchange responses of *R. indica* var. *alba* 'Delaware Valley White' azaleas treated with 8 insecticides.

In Trial 1, 45 'Delaware Valley White' azaleas were arranged on 38 cm centers into an RCB design with 5 replicates per treatment. Treatments were 8 insecticides, representing a range of chemical classes, and were applied 3 times during 21 d (Table 1). Spray treatments were applied at high volume to 'runoff' on azaleas using

Table 1
Rates of pesticides applied to azaleas during individual-leaf (Trials 1, 2, and 3) and whole-plant (Trial 4) studies of plant physiological responses

Pesticide	Application rate
Trial 1	
Acephate (Orthene T T & O®)	480.04 g a.i./100 l
Azadirachtin (Azatin®)	2.80 ml a.i./100 l
Carbaryl (Sevin®)	101.56 ml a.i./100 l
Chlorpyrifos (Hi Yield Dursban®)	213.90 ml a.i./100 l
Cyfluthrin (Tempo®)	1.90 g a.i./100 l
Imidacloprid (Merit®)	2.77 g a.i./100 l
Horticultural oil (Volck Supreme®)	1.89 l a.i./100 l
Insecticidal soap (M-Pede®)	957.00 ml a.i./100 l
Trial 2	
Acephate (Orthene T T & O®)	60.05 g a.i./100 l
Insecticidal soap (M-Pede®)	957.00 ml a.i./100 l
Trial 3	
Acephate (Orthene T T & O®)	60.05 g a.i./100 l
Acephate (Orthene T T & O®)	120.10 g a.i./100 l
Acephate (Orthene T T & O®)	480.04 g a.i./100 l
Trial 4	
Acephate (Orthene T T & O®)	24.57 g a.i./100 l
Horticultural oil (Volck Supreme®)	1.89 l a.i./100 l
Insecticidal soap (M-Pede®)	957.00 ml a.i./100 l

a hand-held compressed air sprayer with a hollow Conejet nozzle, which delivers 0.38 l/min^{-1} at 2.76 bar (Spraying Systems Co., Wheaton, IL). Treatments were made between 0800 and 1000 h on days 1, 8, and 15 of each study. In the greenhouse, plants were maintained under ambient lighting, which was a 14 h photoperiod. Azaleas were watered prior to sampling at 0800 h. A Li-Cor 6400 portable closed-gas exchange system (Li-Cor, Lincoln, NE) was used to record CO_2 assimilation, transpiration, and leaf stomatal conductance data. The Li-Cor 6400 sample chamber was modified to permit sampling of a 2.25 cm^2 section of the small azalea leaves. Leaf gas exchange in response to azalea lace bug feeding injury was investigated in Trial 1. For comparison, gas exchange measurements were taken between 0800 and 1530 h on one azalea lace bug feeding-injured and one uninjured leaf per shrub on days 0, 2, 4, 9, 11, 14, 16, 18, and 21.

Trial 2: Gas exchange responses of 5 azalea cultivars treated with acephate and insecticidal soap.

In Trial 2, gas exchange was measured among 5 evergreen azalea cultivars representing 4 hybrid lineages. These included *R. indica* var. *alba* 'Delaware Valley White' azaleas, the Indica hybrid 'G.G. Gerbing', the Satsuki hybrid 'Wakaebisu', and the Kurume hybrids 'Mother's Day' and 'Hershey Red' azaleas. Cultivars were chosen that were readily available in the nursery trade and that are commonly found in southeastern landscapes (Galle, 1987). Plants were arranged in a split-plot design with 6 replicates per treatment. Treatments of acephate and insecticidal soap were applied and compared to a water control (Table 1). Azaleas were arranged on 30.5 cm centers. Supplemental lighting from 0730 to 1800 h provided consistent photosynthetic photon flux (PPF) readings of 450–500 μm m^{-2} s^{-1} in the greenhouse for the duration of the study. All plants were watered prior to sampling. Gas exchange measurements were taken on uninjured leaves on days 0, 2, 4, 7, 9, 11, 14, 16, 18, and 21 following Trial 1 sampling procedures.

Trial 3: Gas exchange responses of *R. indica* var. *alba* 'Delaware Valley White' azaleas treated with 3 rates of acephate.

In Trial 3, a split-plot design was used to investigate 'Delaware Valley White' azalea gas exchange in response to acephate treatments at 3 rates (Table 1). Trials 2 and 3 were run concurrently to facilitate data collection. Sampling and pesticide application methodology for Trial 3 was identical to Trial 2. In Trial 3, only undamaged leaves were sampled.

Trial 4: Whole-plant gas exchange measurements

Rooted cuttings of 'Pleasant White' Girard hybrid and *Rhododendron indicum* var. *alba* (L.) Sweet 'Delaware Valley White' azaleas were obtained from a commercial grower. Upon receipt, azaleas were potted into 10-cm square pots (Kord Corp., Lugoff, SC) using Metro Mix 300 potting media (Scotts-Sierra Horticultural Co., Marysville, OH). Leaves with necrotic tissue, damaged during shipping, were removed. Each azalea cultivar was randomly blocked into 6 replicates with 4 treatment groups containing 6 plants. During a 1 week acclimation period, plants were exposed to greenhouse temperatures ranging from 30 ± 3°C (85 ± 5°F) during the day and 21 ± 3°C (70 ± 5°F) at night. Photosynthetic photon flux levels (PPF) were measured using a LI-189 quantum meter (Li-Cor, Lincoln, NE). At the height of azaleas, PPF levels on sunny days measured 675 μmol m^{-2} s^{-1} at 1200 h. Azaleas were watered daily or as the surface of the media became dry.

2.1. Growth chamber design

Throughout the study, growth chambers maintained a CO_2 concentration of 374 ± 11 μmol mol^{-1}. Relative humidity (RH) and temperature were held constant at 85 ± 5% RH and 25 ± 1°C (77 ± 2°F), respectively, under a 16 h photoperiod. Photosynthetic photon flux levels were maintained at approximately 480 μmol m^{-2} s^{-1} at the top of the plant canopy in the chambers by adjusting the height of the growth chamber light source.

2.2. Whole-plant gas exchange measurements and pesticide application

At 0700 h, on the day of measurement, a single replicate was watered and placed in the growth chambers. Chamber assignments for cultivar and treatment combinations were randomized daily. An initial measurement of gas exchange among untreated azaleas was made for 2 h on Day 1. After these baseline measurements were completed, azaleas were removed from the chambers and placed outdoors. Pesticide applications were made simultaneously to both cultivars, using a pressurized CO_2 backpack sprayer with a Conejet T® nozzle (Spraying Systems Co., Wheaton, IL). Cultivar-combined treatment groups of 12 plants received applications of acephate, insecticidal soap, or horticultural oil (Table 1). Azalea responses to insecticide treatments were compared to a control spray of tap water. Azaleas were air dried for approximately 45 min before being placed back in the growth chambers. P_{net} and R_{dark} measurements were taken for the remainder of the 24 h period. Upon completion, azaleas were returned to the greenhouse and maintained as previously described.

A second application of insecticides was made on Day 7 using the same procedures outlined above, except that 3 h of baseline gas exchange measurements were taken prior to applying the second insecticide treatment. Final gas exchange measurements were taken on Day 14.

2.3. Destructive plant sampling

At the completion of Day 14, azaleas were moved to the greenhouse and destructive measurements were initiated. A count of the number of leaves exhibiting chlorosis was made per plant by cultivar and treatment combinations. Afterwards, 50 leaves were randomly collected from each treatment by cultivar. Individual leaf areas were measured using a LI-3100 leaf area meter (Li-Cor, Lincoln, NE). Mean leaf areas were calculated for each treatment-by-cultivar combination and within each replicate. All remaining leaves were removed for dry mass measurements. Stems and roots were separated, washed free of media, and dried to provide dry mass measurements of stems and roots. To provide an estimate of total leaf area, the ratio of summed leaf area for 50 leaves to the dry mass of 50 leaves was determined and multiplied by the total leaf dry mass of the plants in each treatment.

2.4. Calculation of growth, carbon use efficiency and gross photosynthesis variables

P_{net} and R_{dark} CO_2 exchange rates were measured using the whole-plant gas exchange system described by van Iersel and Bugbee (2000). Data collected within 45 min of placing plants in the gas exchange chambers were not used for analysis in order to give the azaleas time to acclimate to chamber conditions. Daily mean values of $P_{net,avg}$ and $R_{dark,avg}$, before and after insecticide applications were calculated from the remaining data.

Mean gross photosynthesis ($P_{gross,avg}$), which is an estimate of the daily rate of CO_2 fixation, was calculated by adding daily mean values of $R_{dark,avg}$ and daily mean $P_{net,avg}$ values:

$$P_{gross,avg} = P_{net,avg} + R_{dark,avg} \quad (1)$$

Rates of respiration in the P_{gross} calculation are assumed to be equivalent in the light and in the dark.

Growth, or daily carbon gain (DCG) mmol d^{-1}, was also calculated. Growth measurements report the net amount of CO_2 fixed by the plants during a 24-h period and accounts for both photosynthesis and the amount of CO_2 lost during nighttime respiration:

$$DCG = [(P_{net,avg} \times t_{light} \times 3600\ s) - (R_{dark,avg} \times t_{dark} \times 3600\ s)]/1000 \quad (2)$$

In our study, t_{light} was 16 h and t_{dark} was 8 h. Finally, carbon-use efficiency (CUE), the ratio of carbon stored as dry mass to the total amount of carbon fixed by photosynthesis (Amthor, 1989) was calculated

$$CUE = [(t_{light} \times P_{net,avg}) - (t_{dark} \times R_{dark,avg})]/(t_{light} \times P_{gross,avg}) \quad (3)$$

To account for differences which may have existed in either canopy area or amount of root and stem tissues of the plants, $P_{net,avg}$, $R_{dark,avg}$, and $P_{gross,avg}$ were adjusted using dry mass measurements of roots and stems, dry mass of canopy leaves, and both dry mass and leaf areas of 50 randomly selected leaves per treatment group. Data presented, therefore, reflect $P_{net,avg}$ expressed per unit leaf area (μmol m^{-2} s^{-1}) and $R_{dark,avg}$ expressed per unit of dry mass (μmol kg^{-1} s^{-1}).

2.5. Statistical analyses

Measurements of gas exchange variables in Trials 1 and 3 were analyzed by general linear model analysis for a randomized complete block design (SAS Institute, 1985). The same variables were analyzed for a split-plot design using the block and treatment interaction as the specified error in general linear model tests for significance in Trial 2. Treatment means in all trials were separated using Fisher's protected least-significant difference procedure (SAS Institute, 1985). Net leaf photosynthetic rate and conductance were regressed on treatments using a linear regression model (SAS Institute, 1985). In Trial 4, measurements were conducted using a 2-cultivar by 4-treatment factorial design with 6 replicates of treatment groups. Each treatment group had 6 azaleas. Because only 8 experimental units could be measured simultaneously, the experiment was replicated in time. Calculated mean average leaf areas were analyzed 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). To establish if the azalea cultivars responded in similar ways to the insecticide treatments, cultivar by treatment interactions were also calculated using the PROC GLM procedure in SAS (SAS Institute, 1985). Where significant differences were detected, means of variables were separated using Fisher's protected least-significant difference test (SAS Institute, 1985).

3. Results

In Trial 1, separate analyses of azalea lace bug-injured and uninjured leaves did not reveal significant differences in photosynthetic rate ($F = 0.54$–1.95; df = 8; $P < 0.81$–0.084), or conductance ($F = 0.38$–1.67; df = 8; $P < 0.92$–0.14). Because no significant differences were detected, data were combined and analyzed together within each sampling date. Photosynthetic rates of insecticide treatments differed significantly from the controls on day 9 ($F = 2.49$; df = 8; $P = 0.03$), day 11 ($F = 5.37$; df = 8; $P < 0.0001$), day 14 ($F = 3.32$; df = 8; $P = 0.01$), and day 18 ($F = 5.92$; df = 8; $P < 0.0001$) (Fig. 1, top). On day 9, insecticidal soap and acephate-treated plants had significantly lower photosynthesis than control plants (Fig. 1, top). Insecticidal soap and acephate photosynthetic rates were still significantly lower than controls on day 11 (Fig. 1, top). Additionally, on day 11, conductance levels for acephate- and soap-treated plants were significantly lower than controls ($F = 3.32$; df = 8; $P = 0.008$) (Fig. 1, bottom). On day 16, photosynthesis and conductance of acephate and soap treatments were not significantly different from the control plants. Rates of photosynthesis in insecticidal soap treatments were significantly lower than control plants on days 14 and 18 (Fig. 1, top). No significant differences in conductance ($F = 1.25$; df = 8; $P = 0.303$) (Fig. 1, bottom) or photosynthesis ($F = 1.57$; df = 8; $P = 0.17$) (Fig. 1, top) were noted on day 21. Other insecticides did not affect leaf gas exchange except on day 14 (data not shown). On day 14, significantly higher photosynthetic rates ($F = 3.45$; df = 8; $P < 0.002$) were recorded for cyfluthrin and azadirachtin treatments compared to controls. Significantly higher rates of conductance ($F = 4.46$; df = 8; $P < 0.0002$) were recorded for cyfluthrin and imidacloprid treatments compared to controls.

In Trial 2, an analysis of cultivars-by-treatment interactions revealed that azaleas responded to insecticides in similar ways, regardless of cultivar. No significant cultivar differences were detected for photosynthesis ($F = 0.11$–1.22; df = 8; $P = 0.99$–0.26) or conductance ($F = 0.19$–1.10; df = 8; $P = 0.92$–0.35). Treatment responses were pooled and analyzed for all cultivars combined.

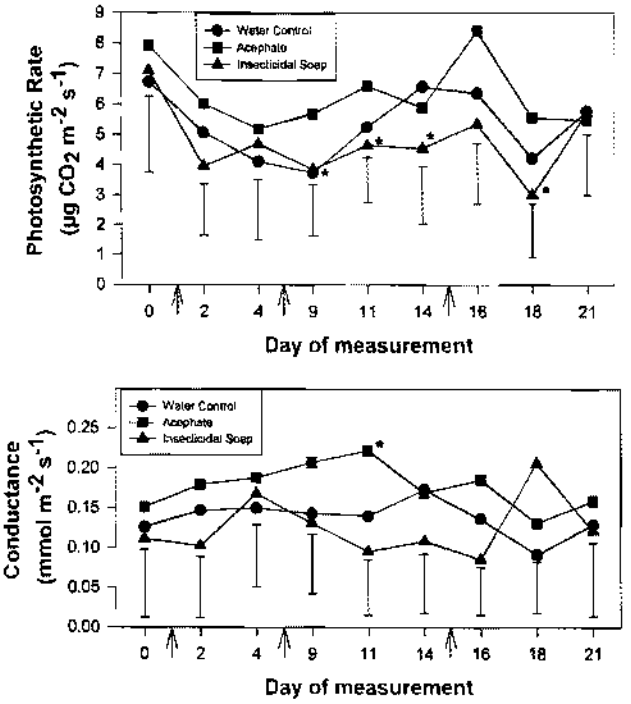

Fig. 1. Single-leaf determinations of photosynthetic rates (top) and conductance (bottom) of 'Delaware Valley White' azaleas treated with pesticides (Trial 1). Results are presented for photosynthetic rates of acephate and insecticidal soap treatments. Arrows indicate applications of pesticide treatments. Error bars represent the least-significant differences among means separated using Fisher's protected LSD test ($\alpha = 0.05$).

Applications of insecticidal soap reduced photosynthetic rates to levels significantly lower than controls on day 9 (LSD = 0.659) (Fig. 2, top). Conductance of soap-treated plants was significantly lower than controls on day 9 (LSD = 0.023) and day 16 (LSD = 0.0287) (Fig. 2, bottom). By day 21, no significant differences among treatments were detected for photosynthesis ($F = 0.04$; df = 2; $P = 0.96$) (Fig. 2, top) or conductance ($F = 0.18$; df = 2; $P = 0.83$) (Fig. 2, bottom).

In Trial 3, Photosynthesis of 'Delaware Valley White' azaleas was not significantly affected by acephate treatments at any of the rates tested on any date ($F = 0.1$–3.04; df = 3; $P = 0.96$–0.07) (Data not shown). Conductance values were not affected by acephate treatments on any date ($F = 0.18$–1.24; df = 3; $P = 0.91$–0.28) except on day 14 where the high rate treatment significantly reduced conductance compared with the controls ($F = 4.83$; df = 3; $P < 0.02$).

Trial 4 showed that within cultivars, neither 'Pleasant White' ($F = 0.73$; df = 3; $P > 0.05$) nor 'Delaware Valley White' azaleas ($F = 2.50$; df = 3; $P > 0.05$) had significant differences in total canopy leaf area among treatments. However, 'Delaware Valley White' plants generally had lower rates of P_{net} and respired more than 'Pleasant White' azaleas, both initially, and after pesticide applications. Mean individual leaf area of 'Delaware Valley

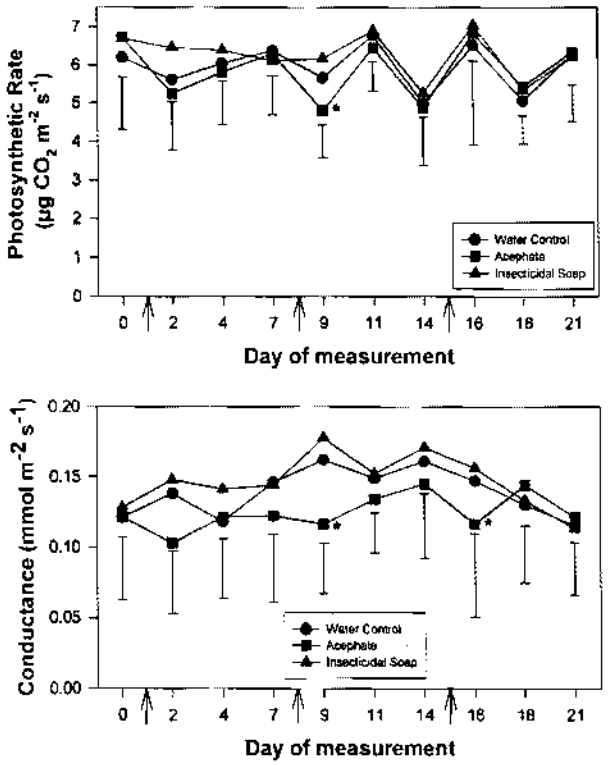

Fig. 2. Single-leaf determinations of mean photosynthetic rates (top) and mean conductance (bottom) of combined azalea cultivars treated with insecticidal soap and acephate (Trial 2). Arrows indicate applications of pesticide treatments. Error bars represent the least-significant differences among means separated using Fisher's protected LSD test ($\alpha = 0.05$).

White' leaves (3.17 ± 0.31 cm^2) was significantly larger than 'Pleasant White' azalea leaves (2.04 ± 0.20 cm^2) ($F = 362.91$; df = 1; $P < 0.0001$). Cultivar P_{net} and R_{dark} differences might also be attributed to dry mass of the plants, which demonstrated significant differences between cultivars. Leaf dry mass, representing the canopy area available for photosynthesis, was significantly greater in 'Pleasant White' at 16.87 ± 2.28 g versus 12.34 ± 2.81 g in 'Delaware Valley White' azaleas ($F = 53.48$, df = 1, $P < 0.0001$). The cumulative dry mass of roots and stems was also significantly greater for 'Pleasant White' at 37.98 ± 8.03 g versus 30.14 ± 7.3 g for 'Delaware Valley White' azaleas ($F = 12.57$, df = 1, $P < 0.0011$). This indicates a greater tissue area available for respiration. Despite these differences, both 'Delaware Valley White' and 'Pleasant White' azalea plants responded similarly to insecticide treatments. No significant effects were detected for cultivar-by-treatment interactions of measured variables ($F = 0.4$–2.09, df = 3, $P < 0.96$–0.06). For this reason, the responses in gas exchange and growth parameters of both cultivars are combined.

As described, P_{net} and R_{dark} values were adjusted to reflect both the cultivar-specific differences in canopy size and stem and root masses, and size differences among treatment groups. These values reveal decreased P_{net} rates following insecticidal soap, and horticultural oil applications. No significant differences were detected for P_{net} rates of the treatments prior to pesticide applications ($F = 0.65$, df = 3, $P > 0.05$). However, reductions in P_{net} are apparent directly following insecticidal soap, and horticultural oil treatments on days 1 and 7 (Fig. 3, top). The sharp decline in P_{net} after insecticidal soap treatments resulted in a significantly lower P_{net} than the control ($F = 14.48$, df = 3, $P < 0.0001$). By the morning of day 7, prior to the second treatment applications, P_{net} levels were returning to their initial rates and were not significantly different from the controls ($F = 0.75$, df = 3, $P > 0.05$). Following the second insecticide application, P_{net} rates of both insecticidal soap and horticultural oil treatments were significantly lower than the controls and significantly different from each other ($F = 18.74$, df = 3, $P < 0.0001$) (Fig. 3, top). Although P_{net} appeared to be returning to normal by Day 14,

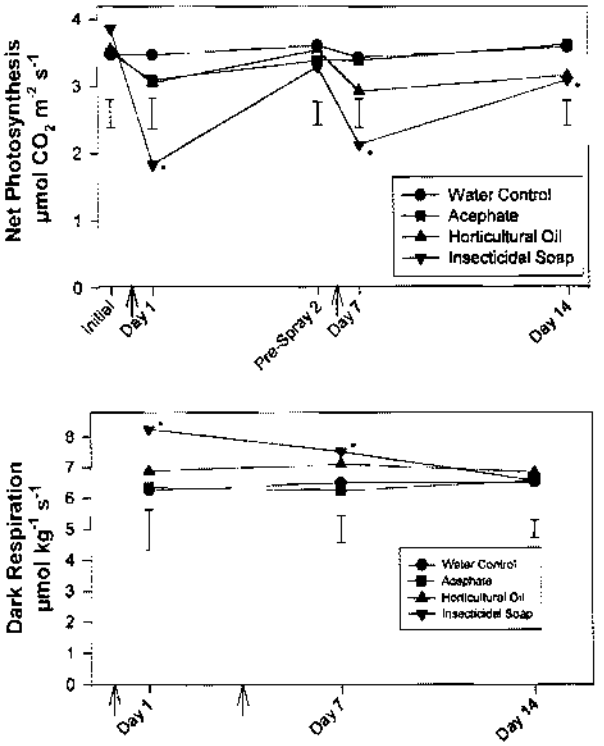

Fig. 3. (Top) Whole-plant net photosynthetic rates of pesticide-treated azaleas. Photosynthesis is expressed per unit leaf area and is averaged for both taxa. Arrows indicate applications of pesticide treatments; (Bottom) The effect of pesticides on the whole-plant nighttime respiration of azaleas. Data were expressed per unit of dry mass and averaged for both taxa. Days 1 and 7 values reflect measurements taken for 21 h following pesticide applications. Day 14 values represent measurements taken for 24 h, 6 days after the application of the second pesticide treatment. Pesticide applications are indicated with arrows. Error bars represent the least-significant differences among means separated using Fisher's protected LSD test ($\alpha = 0.05$).

soap-treated plants still had a significantly lower P_{net} than the controls ($F = 3.63$, df = 3, $P < 0.05$) (Fig. 3, top).

R_{dark} rates of azaleas treated with soap were significantly higher than the controls on Day 1 ($F = 4.20$, df = 3, $P < 0.05$) and day 7 ($F = 6.25$, df = 3, $P < 0.01$). By day 14, no significant differences in R_{dark} were detected among treatments ($F = 0.27$, df = 3, $P > 0.05$) and insecticidal soap treatments approximated the control R_{dark} rates (Fig. 3, bottom).

The efficiency of carbon use, which is the ratio of carbon fixed into dry mass to the total amount of carbon fixed in the photosynthetic process, was significantly lower than controls following the application of insecticidal soap and horticultural oil on day 1 ($F = 54.46$, df = 3, $P < 0.0001$), day 7 ($F = 74.69$, df = 3, $P < 0.0001$) (Fig. 4, top), and on day 14 ($F = 13.92$, df = 3, $P < 0.0001$), which is 6 days after the last treatments were made (Fig. 4, top).

Growth, which represents the carbon fixed by P_{net} less the carbon lost during nighttime respiration, also declined in the soap treatment following pesticide applications (Fig. 4, bottom). Growth among insecticidal soap treatments was significantly lower than controls on day 1 ($F = 10.93$, df = 3, $P < 0.0001$) and day 7 ($F = 9.71$, df = 3, $P < 0.0001$). By day 14, no significant differences were evident among treatments ($F = 2.15$, df = 3, $P > 0.05$). However, a reduced growth trend was evident for horticultural oil treatments throughout the study (Fig. 4, bottom).

Prior to destructive plant sampling, a count of the numbers of leaves exhibiting chlorosis and premature senescence on the plants revealed a significantly greater decline in leaf quality among azaleas receiving insecticidal soap treatments ($F = 4.53$, df = 3, $P < 0.001$). Acephate-treated plants, which had similar CO_2 exchange rates, also had similar mean numbers of chlorotic leaves as the controls (Fig. 5).

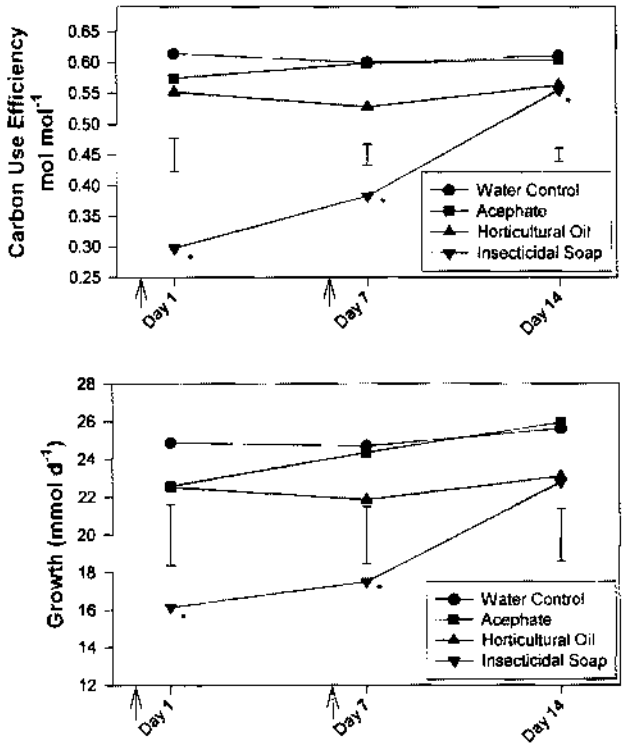

Fig. 4. (Top) Whole-plant determination of the efficiency of carbon use of pesticide-treated azaleas averaged for both taxa. Carbon use efficiencies were calculated as the ratio of carbon fixed into dry mass to the total carbon assimilated during the light period. Days 1 and 7 values reflect measurements taken for 21 h following pesticide applications. Day 14 values represent measurements taken for 24 h, 6 days after the application of the second pesticide treatment; (Bottom) Whole-plant determination of daily carbon gain, or growth, among treatments for azaleas following pesticide applications. Data represent total carbon accumulations that occurred among 6 plants during a 24 h period and are averaged for both taxa. Arrows indicate applications of pesticide treatments. Error bars represent the least-significant differences among means separated using Fisher's protected LSD test ($\alpha = 0.05$).

4. Discussion

Whole-plant measurements were more efficient than individual-leaf measurements at detecting changes in gas exchange following insecticide applications. Gas exchange recorded from individual-leaves, particularly in Trials 2 and 3, presented a high degree of variability. Reliable conclusions about plant responses to treatments

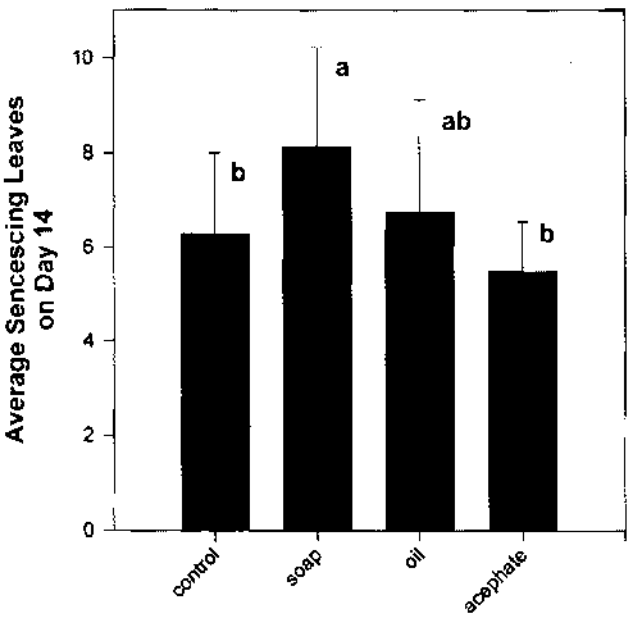

Fig. 5. Whole-plant determinations of the number of chlorotic and senescing azalea leaves following pesticide applications. Data are averaged among both taxa and represent the number of chlorotic leaves per plant on Day 14, 6 days after the application of the second pesticide treatment. Means separations are provided using Fisher's protected least-significant differences test ($\alpha = 0.05$).

would be difficult to make. This is most clearly evident in the photosynthesis and conductance values of individual leaves seen in Trial 3. Also, the results of day 14 gas exchange measurements for Trial 1, which included significantly higher photosynthetic rates among cyfluthrin and azadirachtin treatments and higher rates of conductance among cyfluthrin and imidacloprid treatments, cannot be readily explained. Significant differences were not observed on any other day of measurement, nor were trends apparent for increased photosynthesis or conductance due to these treatments. Gas exchange parameters for these treatments were not significantly different from controls two days later and throughout the remainder of the study.

In our whole-plant study, reduced photosynthetic rates of oil-treated azaleas showed the ability to quickly return to control levels after the initial application. Recovery after the second oil treatment occurred more slowly. No significant differences in gas exchange recorded using individual-leaf measurements were seen among azaleas treated with horticultural oil in Trial 1. Horticultural oil treatments made at the recommended label rate may temporarily reduce plant gas exchange but are unlikely to cause plant phytotoxicity. Our findings support previous research conclusions that the selection of the proper oil and application of an appropriate rate of oil are necessary to provide effective control of arthropod pests while minimizing potential phytotoxic responses (Lawson and Weires, 1991; Schrader and Kammereck, 1996).

In the whole-plant study, treatments of a 2% formulation of M-Pede insecticidal soap resulted in immediate reductions in plant gas exchange. Both P_{net} and R_{dark} levels recovered after 7 days. Gas exchange was also significantly reduced among insecticidal soap treatments on some dates following insecticide applications in the individual leaf studies of Trials 1 and 2. Soap treatments appear to be most appropriate at the lowest proportion of active ingredient that gives effective pest control. Multiple applications, or strict reliance upon insecticidal soaps, might result in reductions in plant growth or a decline in plant quality due to leaf chlorosis. These studies have also shown that acephate applications made at the recommended rate do not cause phytotoxicity or reduce plant gas exchange.

In conclusion, gas exchange studies using individual leaf measurements provided some results similar to those from the whole-plant gas exchange investigation. However, the high degree of variability seen in the individual-leaf studies makes it difficult to draw reliable conclusions. Furthermore, the ability of the whole-plant gas exchange system to independently measure nighttime respiration provides a valuable tool for generating calculations of plant growth and carbon-use efficiency. These measurements are not readily or easily acquired using traditional individual-leaf measurements.

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References

Amthor, J.S., 1989. Respiration and crop productivity. Springer, New York, 215 pp.

Anderson, P.C., Mizell, R.F., French, W.J., Aldrich, J.H., 1986. Effect of multiple applications of pesticides on leaf gas exchange of peach. HortScience 21, 508–510.

Ayres, J.C., Barden, J.A., 1975. Net photosynthesis and dark respiration of apple leaves as effected by pesticides. J. Am. Soc. Hort. Sci. 100, 24–28.

Baxendale, R.W., Johnson, W.T., 1988. Evaluation of summer oil spray on amenity plants. J. Arboric. 14, 220–225.

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

Bull, D.L., 1979. Fate and efficacy of acephate after application to plants and insects. J. Agric. Food Chem. 27, 268–272.

Chase, A.R., Poole, R.T., 1984. Severity of acephate phytotoxicity on *Spathyphyllum* Schott. cv. 'Clevelandii' as influenced by host nutrition and temperature. J. Am. Soc. Hort. Sci. 109, 168–172.

Davidson, J.S., Gill, S.A., Raupp, M.J., 1990. Foliar and growth effects of repetitive summer horticultural oil sprays on trees and shrubs under drought stress. J. Arboric. 16, 77–81.

Evans, L.T., 1993. Crop Evaluation, Adaptation and Yield. Cambridge University Press, Cambridge, UK, 500 pp.

Furness, G.O., Maelzer, D.A., 1981. The phytotoxicity of narrow distillation range petroleum spraying oils to Valencia orange trees in South Australia. Part I: the influence of distillation temperature and spray timing on yield and alternate cropping. Pest. Sci. 12, 593–602.

Galle, F.C., 1987. Azaleas. Timber Press, Portland, OR, 519 pp.

Hansen, J.D., Hara, A.H., Tenbrink, V.L., 1992. Insecticidal dips for disinfecting commercial tropical cut flowers and foliage. Proc. Fla. State Hort. Soc. 104, 61–63.

Helson, V.A., Minshall, W.H., 1962. Effects of petroleum oils on the carbon dioxide uptake in the apparent photosynthesis of parsnip and mustard. Can. J. Bot. 40, 887–896.

Heungens, A., DeClerq, R., Dejonckheere, W., 1991. Fytotoxiciteitsproeven met insecticiden en nematiciden op warmekas-planten geteeld op vloeigoten. Med. Fac. Landbouww. Rijksuniv. Gent. 56, 1343–1363.

Heungens, A., DeClerq, R., Dejonckheere, W., 1992. Fytotoxiciteitsproeven met insecticiden en nematiciden op *Rhododendron simsii* 'Inga' geteeld op vloeigoten. Med. Fac. Landbouww. Rijksuniv. Gent. 57, 1289–1302.

Johnson, W.T., 1985. Horticultural oils. J. Environ. Hort. 3, 188–191.

Lawson, D.S., Weires, R.W., 1991. Management of European red mite (Acari: Tetranychidae) and several aphid species on apple with petroleum oils and an insecticidal soap. J. Econ. Entomol. 84, 1550–1557.

Miller, F.D., 1989. The use of horticultural oils and insecticidal soaps for control of insect pests of amenity plants. J. Arboric. 15, 257–262.

Moore, R.E.B., 1980. Azalea phytotoxicity test, 1979. Insecticide Acaricide Tests 5, 165.

Oetting, R.D., Morishita, F.S., Helkamp, A.L., Bowen, W.R., 1980. Phytotoxicity of eight insecticides to some nursery-grown ornamentals. J. Econ. Entomol. 73, 29–31.

Olson, N., Ascerno, M.E., 1985. Phytotoxicity evaluation of insecticidal soap on bedding plants. Minn. St. Florist's Bull. 34, 12–15.

Puritch, G.S., Brooks, B.C., 1981. Effect of insecticidal soap used on the gypsy moth control program in Kitsilano on insects and vegetation. Can. For. Serv./Pacif. For. Res. Centre, Victoria, B.C., BC-X-218, 21 pp.

Reihl, L.A., Wedding, R.T., 1959. Effects of napthenic and paraffinic petroleum composition at a comparable molecular weight or viscosity on photosynthesis of Eureka lemon leaves. J. Econ. Entomol. 52, 883–884.

SAS Institute, 1985. SAS User's Guide: Statistics. SAS Institute, Cary, NC.

Schrader, L., Kammereck, R., 1996. Effect of horticultural oil on apple/pear physiology. Good Fruit Grower 47, 36–39.

van Iersel, M.W., Bugbee, B., 1996. Phytotoxic effects of benzimidazole fungicides on bedding plants. J. Am. Soc. Hort. Sci. 121, 1095–1102.

van Iersel, M.W., Bugbee, B., 1997a. Dibutylurea reduces photosynthesis and growth of petunia and impatiens. J. Am. Soc. Hort. Sci. 122, 536–541.

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

van Iersel, M.W., Bugbee, B., 2000. A semi-continuous, multi-chamber crop CO_2-exchange system: design, calibration, and data interpretation. J. Am. Soc. Hort. Sci. 125, 86–92.

Wedding, R.T., Riehl, L.A., Rhoads, W.A., 1951. Effect of petroleum oil spray on photosynthesis and respiration in citrus leaves. Plant Physiol. 28, 269–278.

Werner, R.A., 1972. Systemic insecticidal action of disulfoton and Monitor® in loblolly pine seedlings. J. Georgia Entomol. Soc. 7, 67–73.