Influence of Surflan (Oryzalin) Concentrations in Irrigation Water on Growth and Physiological Processes of *Gardenia jasminoides radicans* and *Pennisetum rupelli*[1]

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Abstract

This study investigated the effect of oryzalin concentrations on growth, CO_2 assimilation, stomatal conductance, transpiration and ethylene synthesis in dwarf gardenia and fountain grass (tolerant and sensitive species). The plant species were subjected to irrigation water containing 10 µg/liter, 100 µg/liter and 1000 µg/liter oryzalin. CO_2 assimilation, stomatal conductance and transpiration were measured 2 and 4 weeks after treatment initiation, and ethylene evolution was determined at the end of the study. Oryzalin did not affect net CO_2 assimilation, stomatal conductance, transpiration or ethylene evolution for dwarf gardenia or fountain grass. Dwarf gardenia shoot and root weights were not affected by oryzalin, but shoot and root weights of fountain grass were reduced by the highest concentration.

Index words: carbon dioxide assimilation, container plants, herbicide residues, irrigation water, photosynthesis, stomatal conductance, transpiration.

Species used in this study: dwarf gardenia (*Gardenia jasminoides radicans* Thunb.); fountain grass (*Pennisetum rupelli* Steud.).

Herbicide used in this study: Surflan (oryzalin) 3,5-dinitro-*N*4,*N*4-dipropylsulfanilamide.

Significance to the Nursery Industry

The detection of herbicide residues in containment ponds of water recycling systems is a major concern to nursery growers. However, in this study oryzalin concentrations comparable to previously reported oryzalin concentrations in nursery runoff water did not reduce growth or influence physiological processes of a sensitive and tolerant plant species. Oryzalin appears to be safe for weed control in conjunction with water recycling systems in container plant production. If oryzalin concentrations reach 1000 µg/liter (1 ppm) in irrigation water for a sustained period, the growth of sensitive species such as fountain grass could be reduced.

Introduction

In response to water conservation regulations and measures, water recycling systems are being developed to capture and reuse runoff as irrigation water. An inherent drawback of the system is the application of herbicide residues to the growing crop in the irrigation water. Preemergence herbicides are broadcast applied or sprayed over the top of containers. As much as 80% of the applied herbicide may settle in spaces between containers (9), and is available for move-

[1]Received for publication September 16, 1996; in revised form July 28, 1997. Technical contribution no. 4322 from South Carolina Agricultural Experiment Station.

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The *Journal of Environmental Horticulture* (ISSN 0738-2898) is published quarterly in March, June, September, and December by the Horticultural Research Institute. Subscription rate is $65.00 per year for educators and scientists; $85.00 per year for others; add $25.00 for international orders. Periodical postage paid at Washington, D.C. and at additional mailing office. POSTMASTER: Send address changes to HRI, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005.

ment in runoff water generated by overhead irrigation or rainfall (17).

Oryzalin is an active ingredient in commercial herbicide formulations [Surflan (DowElanco, Indianapolis, IN), Rout (O.M. Scotts, Marysville, OH)], which commonly are used for nursery weed management. Oryzalin was detected in runoff water from a container nursery on the day of application (12), and in nursery containment pond water and sediments (6). The highest concentration in the containment pond water was 0.146 mg/liter immediately after herbicide application. Concentrations declined to 0.001 mg/liter 14 days after application and were undetectable one month after treatment. However, materials adsorbed to pond sediment particles are released over time resulting in the detection of herbicides in containment pond water throughout the year (6). Low levels of herbicides in irrigation water have caused plant injury in agronomic crops. Cotton and soybeans were injured by low concentrations of picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) in irrigation water (5), and low levels (0.05-0.08 µg/ml) of atrazine [6-chloro-*N*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] injured seedling and mature creeping bentgrass (16). The possibility of crop damage by herbicide residues in irrigation water is a concern of nursery growers.

Applications of oryzalin for weed control may injure both herbaceous and woody nursery crops. Oryzalin injured pampas grass and daylilies (10) and caused root growth reduction of *Rhododendron* 'Stewartsonian' and *Ilex crenata* Thunb. 'Helleri' (18). Root inhibition due to oryzalin application can be expected, as the primary effect of oryzalin is to cause gross cell morphological abnormalities in root tips. In our earlier study, most woody species, including dwarf gardenia, were tolerant to low levels of oryzalin (1.0 and 10.0 µg/ml), but shoot growth and root growth of fountain grass were reduced (4).

Photosynthesis, stomatal conductance (g_s), transpiration (E) and ethylene production are interrelated physiological processes that may reflect herbicide induced stress. Carbon dioxide assimilation (A) reveals herbicide susceptibility prior to visual injury (11). G_s partially regulates the movement of CO_2, affecting A, and water vapor, affecting E, between internal leaf tissue and surrounding atmosphere and is involved in regulation of photosynthetic capacity (7). Herbicide induced changes in g_s are well documented (21), and herbicide tolerance has been related to g_s (11). The production of ethylene due to water stress has been reported (2). Inhibition of root growth by oryzalin may result in similar water stress conditions leading to increased ethylene production.

The objective of this study was to evaluate the influence of residual concentrations of oryzalin on growth and physiological processes of fountain grass (sensitive species) and dwarf gardenia (tolerant species) (4).

Materials and Methods

One-year-old dwarf gardenia liners, rooted in the spring of 1993, were procured from a commercial nursery, and fountain grass seeds were sown in pure fine pine bark in February 1994. Dwarf gardenia liners were transplanted into 3.1 liter (#1) plastic containers in late February, and one-month-old fountain grass seedlings were transplanted in mid March 1994. Unamended, pure fine pine bark was used as the growing medium for both species. Plants were fertilized within one week after transplanting with 4.5 g nitrogen (50% urea, 50% urea formaldehyde), 1.6 g phosphorus and 2.7 g potassium (16–4–8 N–P–K). Fertilizer applications at similar rates were repeated for both species one week prior to treatment. Foam plates were placed beneath each container to collect leachates and resupply the plant by capillary uptake. The experiment was conducted in a glass greenhouse to avoid any external source of water. Average light intensity in the greenhouse on a clear cloudless day was 1685 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. Average minimum and maximum temperatures were 19C (66F) and 31C (88F), respectively.

Oryzalin treatments were initiated in the third week of April and terminated the first week of June. Treatments were 10, 100 and 1000 µg/liter oryzalin in irrigation water, water control with 1% acetone, and water only. Oryzalin from the commercial formulation Surflan was dissolved in acetone prior to mixing with water. The concentration of acetone was maintained at 1% by volume. Each plant received 240 mls of irrigation water as needed, applied to the medium with no foliar contact. Total oryzalin applied to fountain grass was 3.36 mg for the 1000 µg/liter (1 ppm) treatment, 0.34 mg for the 100 µg/liter (0.1 ppm) treatment, and 0.03 mg for the 10 µg/liter (0.01 ppm) treatment. Total oryzalin applied to dwarf gardenia was 4.32, 0.43 and 0.04 mg for the same three treatments.

The experimental design was a randomized complete block with four single plant replications. The experiment was repeated under similar conditions. Physiological parameters measured to evaluate stress caused by oryzalin treatments included A, g_s, E and ethylene production.

CO_2 assimilation. CO_2 assimilation was determined using an ADC Model LCA-2 portable infrared gas analyzer equipped with a Parkinson broad leaf chamber (Analytical Development Co., Hoddesdon, England). The first fully expanded leaves of dwarf gardenia and the third or fourth leaf of fountain grass, depending on development and maturity, were analyzed. Two leaves per plant were measured twice during the study, at two and four weeks after treatment initiation. The differential CO_2 concentration between the leaf chamber and ambient air was recorded directly from the analyzer between 1100 and 1350 hours on a cloudless day with average light levels of 1685 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. Leaves of dwarf gardenia used to obtain A were traced on paper to determine leaf area by LI-3100 Area meter (LI-COR, Inc., Lincoln, NE). The average of the two widths of leaf in the leaf chamber were measured and total leaf area was calculated by multiplying average width by length. Data was converted into A ($\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$) for statistical analysis.

Transpiration and stomatal conductance. The first fully expanded leaves of dwarf gardenia and third or fourth leaf of fountain grass, depending on development and leaf maturity, were selected to measure g_s and E. Stomatal conductance and E were measured directly using a LI-COR steady state porometer model 1600 (LI-COR, Lincoln, NE) two and four weeks after treatment initiation. All measurements of g_s and E were recorded one day after irrigation, between 1100 and 1350 hours, on cloudless days.

Ethylene production. After final irrigation, plants were transferred into a growth chamber with 12 hour light period, 19–21C (66–70F) temperature and light intensity at 1300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. A randomized complete block design was utilized and plants were acclimatized for two days to the growth chamber environment before determination of ethylene production following the method of Feng and Baker (8). Whole plant shoots were harvested and weighed and the cut ends were placed in tap water. The plant shoots were placed in 1-liter (34-oz) mason jars with lids equipped with a serum cap for collection of air samples. The air in the mason jar was mixed by retracting and advancing the plunger of a plastic syringe 10 times before collecting air samples. Four air samples of 1 cc per jar were collected and ethylene content was determined within six hours of sampling using a Shimadzu gas chromatograph (Model GC-9A) equipped with a phenyl isocyanate/porosil C column and a flame ionization detector. Column temperature was 30C (86F) and injection temperature was at 140C (284F). Nitrogen was used as the carrier gas and 11 ppm ethylene was used as a standard. Ethylene content of the samples were calculated by comparing sample peak to that of standard peak. Data was converted into ethylene produced per gram of fresh shoot for statistical analysis.

Plants were harvested at the termination of the study and root and shoot fresh weights were taken.

Statistical analysis. Data were subjected to ANOVA and treatment means were separated using least significant difference (LSD) at $P = 0.05$. No differences were observed between water with acetone control and water alone. Therefore, water with 1% acetone was used as control, as all treatments contained 1% acetone. Due to differences in time of measurement, variations in microclimates around the plants led to differences in data between the repeated experiments, and hence, data were not pooled for A, g_s, E. However, treatment results were similar between the two experiments and only the data from experiment 1 is presented. Data for ethylene were pooled due to lack of differences in conditions and values between the two repeated experiments.

Results and Discussion

Only the highest rate of oryzalin in irrigation water 1000 µg/ml (1 ppm) reduced shoot and root fresh weights of fountain grass (Fig. 1). Oryzalin treatments did not reduce shoot and root fresh weights of dwarf gardenia, and an increase in shoot fresh weights occurred with 100 µg/liter (0.1 ppm) of oryzalin.

Grasses are sensitive to dinitroaniline herbicides. Oryzalin caused reduction in root growth of pampas grass (10), and fountain grass was sensitive to low concentrations of oryzalin in irrigation water (4). Mode of action of oryzalin involves inhibition of primary and lateral roots (13). Reduction of root growth may have resulted in reduced shoot weight in this study. Fountain grass was only affected at the highest rate of oryzalin [1000 µg/liter (1 ppm)], which is approximately ten times the highest amounts detected in irrigation water (6).

CO_2 assimilation. Herbicide irrigation treatments had little effect on A for either species at either measurement time. The highest rate of oryzalin reduced A in fountain grass two weeks after treatment in the first experiment (Fig. 2). None of the treatments reduced A in fountain grass at four weeks after treatment in both experiments. No reduction in A of dwarf gardenia was observed in either experiment at two and four weeks after treatment. CO_2 assimilation increased in dwarf gardenia with 10 µg/liter oryzalin in irrigation water

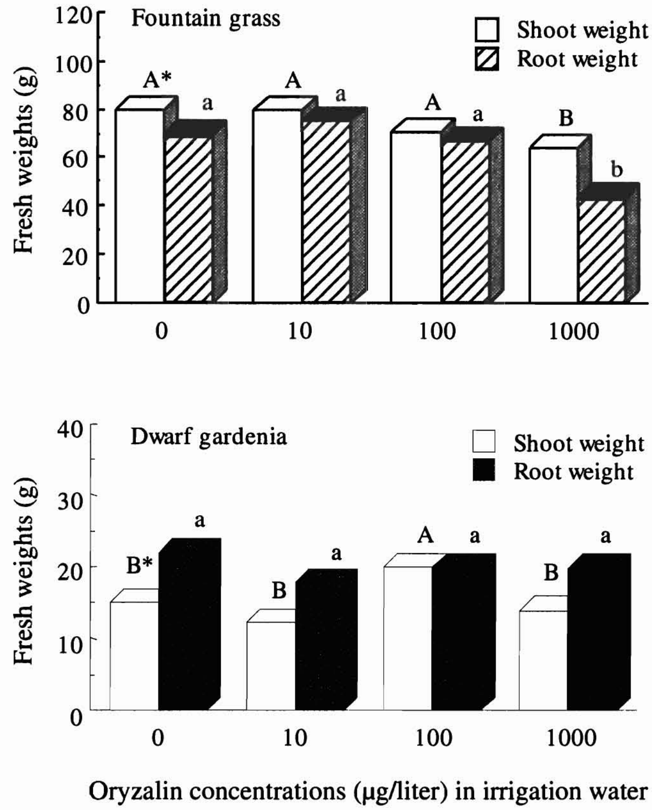

Fig. 1. **Fresh shoot and root weights (grams) of fountain grass and dwarf gardenia treated with oryzalin in irrigation water (experiment 1).**
***Values followed by the same capital and lower case letter within species are not different according to LSD at $P = 0.05$. Coefficient of variation for shoot and root weights of fountain grass are 7.71% and 12.0%, respectively. Coefficient of variation for shoot and root weights of dwarf gardenia are 15.3% and 18.5%, respectively.**

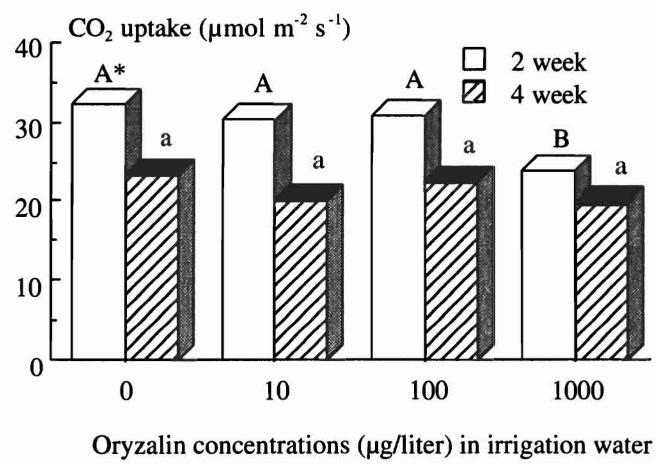

Fig. 2. **CO_2 assimilation (µmol·m^{-2}·s^{-1}) in fountain grass irrigated with oryzalin in irrigation water (experiment 1).**
***Values followed by the same capital and lower case letter within species are not different according to LSD at $P = 0.05$. Coefficient of variation for 2 and 4 weeks after treatment in fountain grass are 11.2% and 17.3%, respectively.**

two weeks after treatment (data not shown) but no other differences occurred.

Mode of action of oryzalin does not involve direct inhibition of A (19). Oryzalin inhibited oxidative photophosphorylation in isolated chloroplasts and mitochondria (14), and partially reduced A (measured as oxygen evolution) in excised spinach foliar tissue (15). Similar reductions in A could occur if the compound successfully penetrates cells and partitions into cell organelles, which is unlikely due to the limited root to shoot translocation of dinitroaniline herbicides. Several triazine herbicides are known to increase A at low rates but no such increase attributable to oryzalin has been reported. The temporary increase in A in dwarf gardenia at 10 ng/liter in the second experiment cannot be explained.

Stomatal conductance and transpiration. Reduction in root growth could influence root water uptake and result in water stressed conditions which may alter leaf water potential and turgor potential. Several species have shown a gradual reduction in g_s in response to a more negative water potential (1, 20). Simazine in irrigation water and oxyfluorfen spray affected g_s in our preliminary experiments (unpublished data). However, stomatal conductance and E were not affected in either species by oryzalin treatments (data not shown). Reduction in the root growth of fountain grass at the highest oryzalin rate was not accompanied by a reduction in g_s or E at two and four weeks after treatment.

Ethylene evolution. No treatment differences in ethylene production in fountain grass or dwarf gardenia were observed (data not shown). Water stress causes an initial rise in ethylene evolution followed by a rapid decline (3). Root fresh weight reduction caused by the highest oryzalin rate may cause water stress. However, the lack of differences in stem ethylene evolution suggests that oryzalin residues in irrigation water may not result in stress induced ethylene. Measurement of ethylene production in roots may be a better indication of stress due to oryzalin in irrigation water.

The results of this study indicate that only when residues of oryzalin in irrigation water reach relative concentrations above 1000 µg/liter (1 ppm) for a sustained period, is the growth of a sensitive plant species affected. The root exposure of tolerant plant species to similar levels of oryzalin in irrigation water for up to four weeks has no influence on plant growth or key physiological processes such as CO_2 assimilation, g_s, E and ethylene evolution.

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