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Paclobutrazol Drench Activity in Coir- and Peat-based Root Substrates

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SUMMARY. Paclobutrazol drench applications of 0, 2, and 4 mg a.i./pot were applied to 'Pacino' potted sunflowers (*Helianthus annuus* L.) and 'Red Pigmy' tuberous rooted dahlias (*Dahlia variabilis* Willd.) grown in substrates containing 50%, 60%, 70%, or 80% (by volume) sphagnum peat or coir, with the remainder being perlite, to study the efficacy of paclobutrazol (Bonzi). Potted sunflower plant height differed significantly for peat- and coir-based substrates, with greater plant height being observed in coir-based substrates. Plant diameter was significantly greater at higher percentages of peat or coir in the substrate at 2 and 4

mg of paclobutrazol. Inflorescence diameter also was significantly decreased as paclobutrazol concentration increased. When the percent of height control from the untreated plants for potted sunflower was compared between coir and peat-based substrates, the percent height reduction was similar for peat- and coir-based substrates at 2 mg of paclobutrazol and height control was greater at 4 mg of paclobutrazol in coir-based substrates. The differences in plant growth observed in peat- and coir-based substrates can be attributed to differences in physical properties of these substrates. Dahlia plant height, diameter, and number of days until anthesis were not influenced by substrate type or percentage. However, dahlia growth was significantly reduced as paclobutrazol concentration increased. Coir-based substrates did not reduce the activity of paclobutrazol drenches compared to peat-based substrates, although to compensate for the greater amount of plant growth in coir-based substrates, paclobutrazol concentrations may need to be increased slightly to achieve a similar plant height as with peat-based substrates.

Plant growth retardants (PGRs) are commonly applied to containerized greenhouse crops when plants would normally be disproportionately large relative to the container (Barrett and Nell, 1994). PGRs may be applied as foliar sprays or substrate drenches. Drenches are preferred because they allow for a more precise application, their effectiveness is influenced less by environmental factors than foliar sprays, and there is no risk from spray drift (Quinlan, 1982). However, a major problem with substrate-applied chemi-

cals is that soil organic matter can markedly influence treatment effectiveness (Walker, 1983). Of all organic components used in substrates, pine bark has the greatest impact on PGR efficacy. Tschabold et al. (1975) compared the movement of ancymidol through columns of soil and sand or pine bark and sand mixtures and concluded that ancymidol binds to the bark, which decreased its activity as a PGR. Burchill et al. (1981) further demonstrated that binding of organic compounds such as PGRs by the substrate particles resulted from a hydrophobic attraction between the nonpolar portions of the PGRs molecule and the bark.

Paclobutrazol ((2R, 3R+2S, 3S)-1-(4-Chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)pentan-3-ol) (Bonzi; Uniroyal Chemical Co., Inc., Middlebury, Conn.) effectively controls height on many floricultural crops (Barrett and Nell, 1989). It is active when applied as a substrate drench and taken up by the plant through the roots (Barrett and Bartuska, 1982; Davis et al., 1988), but efficacy has been shown to be influenced by substrate composition. Quarrels and Newman (1994) found a significant reduction in the efficacy of paclobutrazol drenches applied to poinsettia (*Euphorbia pulcherrima* Willd.) grown in a pine bark-based substrate compared to its efficacy on plants grown in a peat-based substrate. Similar results of paclobutrazol activity inhibition were reported by Barrett (1982) for chrysanthemum (*Dendranthema grandiflora* Tzuelev.) grown in a pine bark-based substrate.

Coconut coir dust (coir) is an agricultural waste product derived from the mesocarp or husk of the coconut fruit and is being marketed as an alternative substrate component. In plant production trials, coir has been demonstrated to be a suitable substrate component for greenhouse crop production, promoting shoot and root growth in certain species (Evans and Stamps, 1996; Lokeshia et al., 1988; Talukdar and Barooah, 1987). However, limited information is available regarding the activity of paclobutrazol drenches in coir-based substrates. Therefore, the objective of this study was to determine if the efficacy of paclobutrazol substrate drenches differed when applied to peat- and coir-based substrates.

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Materials and methods

Substrates were formulated to contain 50%, 60%, 70%, or 80% (by volume) sphagnum peat (peat) or coir, with the remainder being perlite. Before formulation of the substrates, dolomitic limestone was added ($4.15 \text{ kg} \cdot \text{m}^{-3}$) to the peat and pasteurized at 60°C for 30 min. The peat was allowed to incubate at 20°C for 10 d before use, at which time the pH was 5.1. Coir was also pasteurized and allowed to incubate for 10 d, after which time the pH was 5.2.

EXPERIMENT 1. On 22 Mar. 1996, dormant tuberous roots of 'Red Pigmy' dahlias were potted into 1.5-L (15-cm-diameter) round plastic containers filled with the experimental substrates listed above. Plants were fertilized at each watering with Excel 15-5-15 Cal-Mag (Scotts, Marysville, Ohio) to provide 155N-21P-127K ($\text{mg} \cdot \text{L}^{-1}$). Greenhouse day/night setpoints were $24/18^\circ\text{C}$, and the plants were grown under naturally occurring day lengths. Twelve days after potting, 133 mL of solution containing 0, 2, or 4 mg (a.i.) paclobutrazol was applied per container. The experimental design was a completely randomized design of six single-plant replications of the eight substrates $\times$ three PGR treatment combinations. At the first sign of anthesis, the number of days from potting until anthesis, leaf canopy height measured from the pot rim to the top of the foliage, inflorescence height above the foliage, total plant height, and plant diameter (measured at the widest dimension, turned 90° , and averaged) were recorded. Data were subjected to analysis of variance by the general linear model procedures and regression analysis (SAS Institute, Cary, N.C.). Means were separated by least significant differences at $P \leq 0.05$.

EXPERIMENT 2. Except where indicated, procedures for Expt. 2 were as described for Expt. 1. On 18 Dec. 1996, 'Pacino' sunflower seeds were sown into cell packs ($8 \times 4 \times 5.5$ -cm cells). On 6 Jan. 1997, the seedlings were transplanted into 1-L (15-cm-diameter) round plastic containers filled with the experimental substrates. Drench treatments of paclobutrazol were applied 15 d after potting at the same doses as in Expt. 1. Inflorescence diameter (measured at the widest dimension, turned 90° , and averaged) was also recorded at anthesis. A com-

pletely randomized design of eight single-plant replications of the eight substrates $\times$ three PGR treatments combinations was used.

Results and discussion

PLANT HEIGHT. Sunflower height was not significantly influenced by substrate percentage for peat- or coir-based substrates; therefore, plant height data were pooled and analyzed by substrate type (peat or coir) and paclobutrazol dose. There was a significant quadratic relationship between sunflower height and paclobutrazol concentration for peat- and coir-based substrates, with a shorter plant height occurring as the concentration of paclobutrazol increased (Fig. 1). Plant height was reduced by 27% and 29% from the untreated control for the 2 and 4 mg of paclobutrazol, respectively, when applied to the peat-based substrates. Plant height was reduced by 28% and 33% from the untreated control for 2 and 4 mg of paclobutrazol, respectively, when applied to the coir-based substrates. There was no significant difference in percent plant height reduction from the untreated control at 2 mg of paclobutrazol for peat- and coir-based substrates. However the percent plant height reduction from the untreated control was significantly greater ($P \leq 0.01$) at 4 mg of paclobutrazol in coir- than in peat-based substrates.

Dahlia height was not significantly different by substrate type (peat or coir) or percentage; therefore, the data was combined and analyzed by paclobutrazol dose. Plant height for dahlias had a significant linear trend for paclobutrazol concentration, with shorter plants as the paclobutrazol concentration increased (Fig. 2). Plants treated with 2 and 4 mg paclobutrazol were 13% and 21% shorter, respectively, than the untreated control.

For all PGR concentrations, sunflower height was greater in coir- than peat-based substrates. This difference in plant height of sunflower may be attributed to the differences in physical or chemical properties between coir and peat. Evans

and Stamps (1996) compared the growth and development of bedding plants produced in sphagnum peat and coir-based substrates and found a higher root fresh mass for geraniums (*Pelargonium $\times$ hortorum* L.H. Bailey), a higher shoot fresh mass for marigolds (*Tagetes patula* L.) and petunias (*Petunia $\times$ hybrida* Vilm-Andr.), and an increase in overall height of petunias grown in coir-based substrates. Evans and Stamps (1996) speculated that the increased growth observed in coir-based substrates may have been a function of the higher water-holding capacity of coir. Evans et al. (1996) examined the physical properties of coir and found that the water-holding capacity of coir ranged from 750% to 1100% of dry weight, while peat held 400% to 800% of its dry weight in water. Therefore, increased height of sunflower in coir-based substrates may have been a function of the properties of coir and not an inactivation of the PGR. This conclusion is further supported by the fact that, at 2 mg of paclobutrazol, the percent height reduction in coir and peat were similar,

Fig. 1. Plant height (cm) of potted sunflowers as influenced by root substrate (peat or coir) and paclobutrazol (mg a.i./pot) drenches. Data were pooled over substrate percentage ($n = 32$). The adjusted r^2 for peat and coir based substrate was 0.713 and 0.864, respectively.

ns,***Nonsignificant or significant at $P \leq 0.001$, respectively; L = linear, Q = quadratic.

Fig. 2. Plant height (cm) of potted dahlias influenced by paclobutrazol (mg a.i./pot) drenches. Data were pooled over the substrate type and percentage (n = 48). The adjusted r^2 for plant height was 0.2546.
^{ns,***}Nonsignificant or significant at $P \leq 0.001$, respectively; L = linear, Q = quadratic.

and at 4 mg the percent height reduction was greater in coir than in peat. Thus, efficacy of the PGR on sunflower was not reduced in coir-based substrates. Our results were similar to those reported by Million et al. (1997) on the similar bioassay activity of paclobutrazol drenches observed in peat- and coir-based substrates.

Increased efficacy of paclobutrazol at 4 mg may be an artifact, as no such results were observed for dahlia. However, it is possible that coir's increased water-holding capacity may have allowed the substrate to retain more of the PGR solution for a longer period of time as compared to the peat. Although root growth was not measured in this study, coir has been shown to promote root growth in several species. If root growth is promoted, uptake of the PGR may be more efficient, and thus the PGR will be more effective. Finally, although statistically significant, the difference in efficacy between the 2- and 4-mg treatment in sunflower would not be practically significant in a commercial production situation.

PLANT DIAMETER. Sunflower plant diameter was not significantly influenced by substrate type; therefore, plant

diameter data were pooled and analyzed by percentage of peat or coir and paclobutrazol dose. There was a significant linear relationship for sunflower plant diameter between the percentage of peat or coir in the substrate and the paclobutrazol concentration. Plant diameter was greater as the percentage of peat or coir increased for 2 and 4 mg of paclobutrazol (data not shown). The increase in percentage of peat or coir was accompanied by a decrease in percentage of perlite in the substrate.

The observed increase in plant diameter as the percentage of perlite decreased from 50% to 20% suggests a decrease in paclobutrazol activity as the percentage of perlite decreased. These results were similar to those of Million et al. (1997), who found that paclobutrazol activity was enhanced in vermiculite and perlite compared with peat.

Dahlia plant diameter was not affected by substrate type or percentage. Data for plant diameter pooled for substrate type and percentage showed a significant linear relationship between plant diameter and paclobutrazol concentration (data not shown). As the paclobutrazol concentration increased from 0 to 4 mg, plant diameter decreased by 6%.

INFLORESCENCE DIAMETER. Data for inflorescence diameter were pooled for all substrate treatments since substrate type or percentage had no effect on sunflower inflorescence diameter. Inflorescence diameter for sunflower decreased linearly with increasing paclobutrazol drench concentration. Inflorescence diameter decreased by 8% as the paclobutrazol concentration increased from 0 to 4 mg (data not shown). Although inflorescence diameter was statistically significant at first pollen, the differences noted would not be a detrimental reduction to commercial growers.

DAYS TO ANTHESIS. There was no significant influence of PGR concentration, substrate type or substrate percentage on the number of days to anthesis for either sunflower or dahlias. The mean number of days to anthesis was 75 for 'Pacino' sunflowers

and 57 for 'Red Pigmy' dahlias (data not shown), and results were similar to those reported earlier for the same crops (Dasoju and Whipker, 1997; Whipker and Hammer, 1997).

Conclusions

The activity of paclobutrazol drenches in coir and peat-based substrates was similar at 2 mg in this study for potted sunflower. Greater percent of plant height reduction in coir-based substrate at 4 mg of paclobutrazol for sunflower suggests an increase in paclobutrazol activity at higher concentrations in coir-based substrates. Increased sunflower plant growth in coir- versus peat-based substrates may be attributed to greater water retention capacity of coir and not a decreased efficacy of paclobutrazol in coir-based substrates. Similar drench concentrations of paclobutrazol as recommended for peat-based substrates could be used for coir-based substrates to control plant size of potted floricultural crops, although, to compensate for the greater amount of plant growth in coir-based substrates, paclobutrazol concentrations may need to be increased slightly to achieve a similar plant height as with peat-based substrates. Growth responses to PGRs and substrates may vary for different crops, which has to be considered in deciding on a working concentration of a PGR for different substrates.

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Using Isothiazolone Biocides to Control Microbial and Fungal Contaminants in Plant Tissue Cultures

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ADDITIONAL INDEX WORDS. bacteria, antibiotics, sterilization, antimicrobial

SUMMARY. Controlling bacterial and fungal contamination in plant tissue cultures is a serious problem. Antibiotics are currently used but are not always effective, can alter plant growth, and are costly, and resistant strains can result with extensive use. Plant preservative mixture (PPM) contains a mixture of two isothiazolones—methylchloroisothiazolinone and methylisothiazolinone, which are a class of broad-spectrum, widely used industrial biocides. The isothiazolones used in PPM are reported by the manufacturer to be nonphytotoxic at concentrations suitable for the prophylactic control of microbial contaminants in plant tissue cultures. Our results indicate that PPM can be routinely added to tissue culture medium to control air- and waterborne bacterial and fungal contaminants effectively.

Microbial and fungal contamination is one of the most persistent problems in plant cell and tissue culture. Contaminants enter the culture environment via

incompletely disinfested explants, explants harboring organisms internally where surface disinfection was unsuitable for their removal, or through a breakdown in the aseptic barriers (e.g., inadequate sterilization of culture medium, defective air flow filters, or poor aseptic techniques in manipulating the plant material).

Surface removal of contaminating organisms from plant tissue being prepared for culture is generally by treatment with oxidizing hypochlorite solutions, ethanol, mercuric chloride, or a combination of these or other treatments (George, 1993; Herman, 1996). The control of persistent or externally introduced contaminants from in vitro plant tissues has generally relied on antibiotics or fungicides (George, 1993; Herman, 1996). The routine use of antibiotics is not recommended for practical and ethical reasons, such as the expense, problems with phytotoxicity, and selection for resistant strains.

The use of industrial biocides has had limited use in plant tissue culture, and almost no applications exist for their use as media addenda for routine contamination control. These compounds include a wide range of materials used as preservatives, bactericides, fungicides, algacides, and slimicides (Lewis et al., 1978). Biocides are commonly used in water-cooling systems, swimming pools and spas, food and feed, paint, medical antiseptics, metalworking fluids, paper pulp processes, cosmetics, soaps, detergents, fabric, and petroleum products (Rossmore, 1995; Singer, 1976). Unlike antibiotics, biocides have a broad spectrum of activity against bacteria, fungi, algae, and slime molds (Rossmore, 1995). Activity against nematodes, mites, and insects has also been demonstrated (Lewis et al., 1978). Biocides generally cause extreme physical or chemical damage to cells (Chapman and Diehl, 1995), suggesting they would be extremely toxic to plant cells and tissues. For this reason they probably have not received consideration for use in plant tissue culture media.

Plant preservative mixture (PPM) is a mixture (patent no. 5,750,402; Plant Cell Technology, Washington, D.C.) of two isothiazolones—methylchloroisothiazolinone and methylisothiazolinone—used to prevent the growth of contaminating organisms in plant tissue culture media. PPM is reported by the manufacturer to be nonphytotoxic when used at 0.5 to 1.0

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