Stimulation of Plant Growth by (3-Methoxyphenyl)acetonitrile Applied as a Foliar Spray In Vivo or as a Medium Amendment In Vitro

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Abstract. Meadowfoam (*Limnanthes alba* Hartweg ex. Benth.) seedmeal, a coproduct of oil extraction from meadowfoam seeds, has been found to increase the growth of greenhouse plants when added to the growing medium. (3-Methoxyphenyl)acetonitrile (3-MPAN) is a biologically active glucosinolate degradation compound previously identified at high levels in meadowfoam seedmeal. 3-MPAN was tested as a foliar spray at several concentrations (0 μ M, 0.18 mM, 0.37 mM, 0.73 mM, 2.2 mM, and 7.3 mM) on lime basil (*Ocimum basilicum* L.), spearmint (*Mentha spicata* L.), cuphea (*Cuphea lanceolata* L.), and French marigold (*Tagetes patula* L.) seedlings grown in the greenhouse. 3-MPAN increased the fresh and dry weights of all four species tested. However, this effect was dose-dependent among species with spearmint growth higher at all 3-MPAN application rates, whereas basil growth was promoted at only the 2.2-mM rate. 3-MPAN increased the tissue concentrations of the secondary compound (–)-carvone at the 7.3-mM application rate. In addition, 3-MPAN added to sterile nutrient media stimulated the growth of spearmint plants in vitro. These results indicate that 3-MPAN may have applicability as a postemergent growth stimulant for a wide variety of plants.

Meadowfoam (*Limnanthes alba* Hartweg ex. Benth.) is a commercial oilseed crop grown for its unusual fatty acid profile, which makes it attractive to the cosmetics industry (Kleiman and Princen, 1991). Meadowfoam seeds contain copious amounts of the secondary metabolite glucolimanthin (3-methoxybenzyl glucosinolate) (Daxenbichler and VanEtten, 1974). Meadowfoam seeds are heated during oil extraction to prevent the enzymatic formation of 3-methoxybenzyl isothiocyanate, which has high mammalian toxicity (Carlson et al., 1998). Although the heating step prevents the formation of 3-methoxybenzyl isothiocyanate, the corresponding nitrile, (3-methoxyphenyl)acetonitrile (3-MPAN), is formed by thermal degradation instead and remains in the seedmeal (Vaughn et al., 1996). Although 3-MPAN is phytotoxic at higher application rates, it has been found to promote growth at

lower rates (Vaughn et al., 1996). Meadowfoam seedmeal contains, on average, 1.5 mg of 3-MPAN/g seedmeal (Vaughn et al., 1996) and has been found to promote or inhibit plant growth, depending on the application rate, when incorporated into soil or potting mixtures (Dueul, 2003; Linderman et al., 2006). Preliminary tests that we conducted indicated that 3-MPAN promoted the growth of greenhouse-grown plants when applied as aqueous foliar sprays. Our principal objective in the present study was to determine the growth effects of various 3-MPAN concentrations on several different plant species grown in vivo in the greenhouse when applied as foliar sprays or when added to liquid growth medium for plants grown in vitro.

Materials and Methods

Greenhouse experiments. Lime basil (*Ocimum basilicum* L.), cuphea (*Cuphea lanceolata* L.), French marigold (*Tagetes patula* L.), and spearmint (*Mentha spicata* L.) seeds were obtained from the U.S. Department of Agriculture, ARS, National Germplasm Repository, Corvallis, OR, and were planted in Cone-tainers (Hummert International, Earth City, MO; 25-mm diameter $\times$ 160-mm length) containing 10 g of a soilless potting medium formulated with 1 peatmoss : 1 vermiculite (by volume) and amended with 10.9 g·kg^{–1} Micromax (Scotts Co., Marysville, OH) and 62.3 g·kg^{–1} Osmocote (14% to 6% to 12% N–P–K; Scotts Co.).

3-MPAN (Aldrich, St. Louis) was formulated as aqueous solutions at rates of 0.0 mM, 0.18 mM, 0.37 mM, 0.73 mM, 2.2 mM, and 7.3 mM. Four-week-old seedlings of each species were sprayed using a 500-mL spray bottle until liquid began to drain off the leaves. Seedlings were watered three times per week without additional fertilizer during the experimental incubation periods. Experiments were repeated twice using 20 replications per treatment. All experiments were conducted in the greenhouse from March through June 2006. The average daily temperature during this period was $\approx$ 25 °C and varied from a low of 20 °C to a high of 29 °C. Illumination during experiments was provided by natural sunlight with an average daily photosynthetic photon flux (PPF) of 650 μ mol·m^{–2}·s^{–1}.

Carvone analysis. Levels of the secondary compound (–)-carvone, the dominant component of spearmint oil, were analyzed in treated spearmint plants. (–)-Carvone concentrations have been previously shown to be influenced by culture conditions (Tisserat and Vaughn, 2004). For analysis, the terminal 4-cm portions of mint plants from individual plants were excised, mixed together, and then sampled. One gram fresh weight samples were extracted with dichloromethane using a Dionex ASE 300 Accelerated Solvent Extractor (Dionex Corp., Sunnyvale, CA). Extracts were analyzed on a Hewlett-Packard (HP, Palo Alto, CA) 5890 Series II gas chromatograph equipped with a flame ionization detector (GC-FID). Mass spectra were produced by a HP 5972 A Series Mass selective detector. The columns used were fused silica HP-5MS capillaries (0.25- μ m film thickness, 30-m length $\times$ 0.25 mm i.d.). The GC-FID operating parameters were as follows: splitless injection mode temperature programmed from 70 to 250 °C at 10 °C/min^{–1}, helium carrier gas flow rate 1.1 mL·min^{–1}, and the injector temperature set at 250 °C.

Plant liquid culture experiments. The response of spearmint plants to 3-MPAN applied as an in vitro additive to culture medium instead of as an in vivo treatment was also examined. Plants were grown in a liquid basal medium (BM) consisting of Murashige and Skoog salts (Murashige and Skoog, 1962) plus (per liter): 30,000 mg sucrose, 0.5 mg thiamine/HCl, and 100 mg myoinositol. The pH was adjusted to 5.0 $\pm$ 0.1 with 0.1 N HCl or NaOH and dispensed in 50-mL aliquots into Magenta polycarbonate culture boxes (Sigma Chemical Co., St. Louis). Culture boxes consisted of a bottom GA-7-3 Magenta box (77 mm $\times$ 77 mm $\times$ 77 mm, length $\times$ width $\times$ height) interlocked with a polypropylene coupler with an aerial GA-7 Magenta box (77 mm $\times$ 77 mm $\times$ 97 mm, length $\times$ width $\times$ height). Within the boxes, a polypropylene platform, consisting of an inverted 25-mm diameter Kim-Kap closure (Kimble, Vineland, NJ) and drilled with a 12-mm hole centrally positioned in a Magenta B-cap (Sigma Chemical Co.), was situated. Plantlets were positioned in the platform and media was accessible through the Kim-Kap hole. Medium was autoclaved

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for 15 min at $1.05 \text{ kg}\cdot\text{cm}^{-2}$ at 121°C . Stocks of shoots of spearmint were maintained on BM before testing with 3-MPAN. Solutions of 3-MPAN in BM were tested at rates of 0.0 mM, 0.037 mM, 0.073 mM, 0.37 mM, 0.73 mM, 2.2 mM, and 7.3 mM. Cultures were grown in a culture room maintained at $25 \pm 1^\circ\text{C}$ and using a photoperiod of 16-h light/8-h dark. Light was supplied by a combination of cool white fluorescent tubes, metal-halide, and incandescent lamps for a total *PPF* density of $80 \mu\text{mole}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the vessel periphery. Ten replicates were planted originally and experiments were repeated at least three times. After 8 weeks of incubation, data on culture fresh weight, leaf number, shoot number, and root number were recorded. Data from all three replicates were pooled

and analyzed with the Student-Newman-Keuls multiple range test.

Results and Discussion

Absolute growth responses differed among the plant species studied, but the trends were similar. Significant growth increases in cuphea shoot fresh weight and dry weight were obtained from foliar spray treatments at the 0.73- and 2.2-mM 3-MPAN levels (Fig. 1). However, above 2.2-mM 3-MPAN treatment levels, growth responses decreased. Highest shoot lengths were obtained from seedlings treated with 2.2 mM 3-MPAN. Spraying cuphea seedlings with 2.2 mM caused shoot length, fresh weight, and dry weight to increase 17.1%, 59.5%,

and 46.7%, respectively, compared with untreated controls (Fig. 1).

French marigold seedlings benefited from 0.37- and 0.73-mM foliar sprays showing a 34.1% and 37.1% increase in fresh and dry weights, respectively, compared with that obtained from untreated controls (Fig. 2). Lime basil shoot height, fresh and dry weight increased from all 3-MPAN spray treatments (Fig. 3). Spearmint cuttings were found to benefit the most from 2.2-mM treatments and exhibited an 80% and 68% increase in fresh and dry weights compared with untreated controls (Fig. 4). Analysis of (–)-carvone concentrations in spearmint cuttings subjected to the various 3-MPAN treatments showed significant increases in carvone at several of the treatment levels, with much

Fig. 1. Cuphea shoot height, fresh and dry weights after treatment with (3-methoxyphenyl)acetonitrile foliar sprays. Data were averaged for 10 replications/treatment. Mean separation by Student-Newman-Keuls multiple range test ($P < 0.1$).

Fig. 2. French marigold shoot height, fresh and dry weights after treatment with (3-methoxyphenyl)acetonitrile foliar sprays. Data were averaged for 10 replications/treatment. Mean separation by Student-Newman-Keuls multiple range test ($P < 0.1$).

Fig. 3. Lime basil shoot height, fresh and dry weights after treatment with (3-methoxyphenyl)acetonitrile foliar sprays. Data were averaged for 10 replications/treatment. Mean separation by Student-Newman-Keuls multiple range test ($P < 0.1$).

higher (–)carvone levels being found in the 7.3-mm treatment (Fig. 4). From these results we can conclude that no detrimental effect on secondary metabolism occurred in spearmint regardless of the concentration of 3-MPAN administered in this study.

Liquid culture experiments. Spearmint plantlets grown in vitro were found to benefit from much lower 3-MPAN concentrations than those administered to plants grown in vivo (Fig. 5). Administration of 3-MPAN concentrations above 0.73 mm were toxic to cultured spearmint plantlets (results not shown). Of course, it should be mentioned that the concentrations of 3-MPAN in vitro were continuous, whereas applications of foliar sprays were singular. Nevertheless, it was found that 3-MPAN tested in vitro caused a typical dosage–growth response relationship to occur. Highest fresh and dry weights and number of roots occurred at the 0.73-mm 3-MPAN treatment, whereas lower growth rates occurred when more or less 3-MPAN was administered (Fig. 5). However, morphogenesis responses did not always follow this trend. For example, the highest number of leaves and shoots occurred from cultures treated with 0.07 and 0.37 mm, and 0.07, 0.37 and 0.73-mm 3-MPAN treatments, respectively. Analysis of carvone concentrations in spearmint plants grown in vitro revealed no change in carvone concentrations (data not shown).

The use of bioregulators to improve growth and yields of greenhouse crops such as cut flowers has been extensively studied (Latimer, 1992; Nickell, 1982). Several other compounds have been shown to promote plant growth at low levels when applied as foliar sprays or soil drenches. Gibberellic acid drenches at 10, 100 or 250 ppm increased stem length, leaf area, and plant dry weight of greenhouse-grown tomato (*Lycopersicon esculentum* Mill.) seedlings (Latimer, 1992). The synthetic tertiary amine DCPTA [2-(3,4-dichlorophenoxy)triethylamine] was found to promote growth and initiate flowering in *Phalaenopsis* orchids and heliconias (*Heliconia stricta* Huber) when applied as a foliar spray (Broschat and Svenson, 1994; Keithly and Yokoyama, 1990). Van Staden et al., (2006) found that 3-methyl-2Hfuro[2,3-c]pyran-2-one, a compound present in smoke produced by burning plants, promoted seedling growth and vigor in the crop plants tomato, okra [*Abelmoschus esculentus* (L.) Moench], green bean (*Phaseolus vulgaris* L.), and maize (*Zea mays* L.). Elsayed (1995) and Belakbir et al. (1998) demonstrated that Biozyme, the proprietary combination of gibberellic acid, indole-3-acetic acid, zeatin, and micronutrients, increased vegetative growth and fruit yields of bell peppers (*Capsicum annuum* L.). Khan et al. (2005) found that spraying solutions of autoclaved fungal materials, including freeze-dried mycelia and spore suspensions, increased the fresh weight and carvone concentrations of greenhouse-grown spearmint plants. The mechanism of action of the fungal materials, however, was not identified.

Fig. 4. Spearmint shoot height, fresh and dry weights, and tissue carvone concentrations after treatment with (3-methoxyphenyl)acetonitrile foliar sprays. Data were averaged for 10 replications/treatment. Mean separation by Student-Newman-Keuls multiple range test ($P < 0.1$).

Fig. 5. Spearmint leaf number, shoot number, and fresh and dry weights when grown in liquid medium containing various concentrations of (3-methoxyphenyl)acetonitrile. Data were averaged for five replications/treatment. Mean separation by Student-Newman-Keuls multiple range test ($P < 0.1$).

Summary

Enhanced growth through 3-MPAN applications is obtainable both in vivo and

in vitro. Our results suggest that growth promotion for a variety of plants is possible using (3-methoxyphenyl)acetonitrile as an aqueous foliar spray. Because very low rates

of 3-MPAN were effective in promoting growth and applications are relatively simple (i.e., the solutions are applied to the point where excess solution drips off the target plant), 3-MPAN appears to offer potential as a commercial plant growth promotion agent for greenhouse crops.

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