COLORADO FLOWER GROWERS ASSOCIATION, INC.

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Fusarium Stem Rot of Carnations: Uptake of Benomyl by Mature Plants

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Previous papers in this series (5, 10, 11, 12) have reported efforts to control Fusarium stem rot of carnation in propagative operations. While cuttings and young transplants are highly susceptible (2, 3, 4) and indeed epidemics have occurred in the past, current control procedures have reduced losses almost completely. For instance, in one commercial test of a population of 7316 transplants previously propagated in a clean stock program using recommended procedures, only 0.01% loss occurred over a 6 month period (10).

Subsequently mature flower producing plants may succumb to the branch rot also caused by the same pathogen. A higher incidence of this phase of the disease has been observed with plants growing in gravel culture and irrigated with spray systems containing nutrients. It is likely that the frequent periodic inundations of lower branches with water predisposes the tissue to invasion and infection by *Fusarium roseum*. The conventional preventative measures have not been effective in control of this branch rot of mature plants and losses may be high even when the grower has taken all possible precautions. A systemic fungicide which could enter the plant, be transported to the potential locus of infection, and then act directly on the pathogen appears to be a useful combative procedure in this situation. Furthermore, continuous application of the systemic chemical in small quantities could provide an internal chemical resistance that would reduce the loss to disease at a later date. This paper gives results of basic experiments to determine whether benomyl has appropriate properties for realizing these possibilities.

METHODS

Carnation cv Pink Sim cuttings were used in experiments designed to determine the characteristics related to uptake and systemic activity of benomyl. Rooted cuttings were transplanted into 12 inch plastic azalea pots (four plants/pot). These contained potting media composed of either 1 part sand, 2 parts peat, and 3 parts Fort Collins loam or gravel. Three plants in each pot were pinched and later served as material for the bioassays. A complete nutrient solution was used for irrigation. Plants in soil treatments were watered on demand. Plants grown in gravel were irrigated automatically to slight excess at each watering.

Benomyl as a 50% wettable powder was incorporated into the soil around the root zones of the young carnation plants or applied as an aqueous suspension to plants growing in gravel or soil. In the drench treatments, the medium in each pot was drenched 2 and 8 weeks after transplanting with 200 ml of a 65 ppm active suspension of benomyl. Following the same application schedules, a powder form of benomyl at 65 ppm active (w/w oven dry potting medium) was applied to the immediate soil about the root zones. In total, two separate applications of the fungicides were made representing 130 ppm total of active material. All treatments were randomized in blocks on the greenhouse bench and each treatment contained 6 replications.

To determine the presence and concentration of the fungicide in plant tissue, a quantitative bioassay was employed. Beginning 2 weeks after the first application of benomyl, one cutting from each of the 6

replications per treatment was taken on a weekly basis. After the second applications of benomyl and during the second 6-week sampling period, when plant material was more available, 3 cuttings from each of 6 replications per treatment were taken on a weekly basis. The cuttings were processed into small pieces and inserted into freeze-dryer tubes. The tubes and plant material were then submerged in a container of liquid oxygen for 10 minutes to arrest any possible metabolic breakdown of benomyl. Next, the frozen carnation tissue was placed on a VirTis freeze-dryer for 24 hours. The desiccated plant material was ground with a mortar and pestle and passed through a 40 mesh screen. The resulting powder-like material was weighed into 100 mg. aliquots and placed into individual Falcon Petri dishes (40 mm x 12 mm). Five ml of potato-dextrose agar amended with streptomycin (30 ppm) to suppress bacterial contamination was added to each micro Petri dish. To insure an even suspension of plant material in the agar, each plate was stirred gently with a sterile spatula. Once the agar had solidified, 7 mm plugs were cut with a No. 3 cork borer. The plugs were then lifted and positioned on the plastic test plates previously seeded with *Penicillium expansum*. Two plugs per plate were used and a total of 12 plates were tested per treatment. The test plates were incubated at 25 C for 36 hours. When fungitoxic substances diffused from the agar plugs containing microparticles of plant material treated with benomyl, the *P. expansum* spores did not germinate and a clear zone of inhibition was formed. The average diameters from 2 axes of the zones of inhibition minus the diameter of the agar plug were recorded in mm. The bioassay data reported represents in each case an average of 24 measurements from agar plugs containing plant material treated with benomyl. When zones of inhibition were over 5 mm, usually 95 to 100% of the agar plugs showed measurable zones of inhibition. With average zones of less than 3 mm, frequently less than half of the plugs showed positive zones of inhibition.

The test plates were prepared by rehydrating the ingredients for Sabouraud's agar in 500 ml of distilled water, which was autoclaved for 20 minutes at 15 PSI, and cooled to approximately 50 C. Freshly collected spores of *P. expansum* from 2-week-old slant tube cultures were suspended in sterilized distilled water and incorporated in the cooled but fluid Sabouraud's agar, making a suspension containing approximately 300 spores per ml; 10 ml of this suspension was pipetted into each of the necessary number of Fisherbrand plastic Petri dishes (100 mm), allowed to solidify, and then held at 2 C until used, 12 hours later.

Spore suspensions were prepared from 2-week-old cultures growing on Sabouraud's agar slants held at 24 C. Spores were collected by flooding the slant tube cultures with 5 ml distilled water per tube. The spore suspension was then filtered through a glass wool plug to remove any extraneous mycelial debris. The concentration of spores was estimated with a Spencer Bright Line Hemacytometer by taking the mean of 4 counts of the spores from the third drop from a Pasteur pipette. A sufficient quantity of the spore suspension of *P. expansum* was added to the agar to give a final concentration of approximately 300 spores per ml.

Fusarium roseum, *Verticillium albo-atrum* and *P. expansum* were all tested as bioassay organisms. Since *P. expansum* was the most sensitive to benomyl (growth inhibited at 0.1 ug/ml), this fungus was used to determine the possible toxicity of substances in carnation tissues (7).

RESULTS AND DISCUSSION

According to the quantitative bioassays, the largest amount of the fungitoxicant accumulated in carnation plants grown in soil treated with benomyl drenches or when benomyl was incorporated in the soil (Fig. 1). The least amount of accumulation occurred in plants grown in gravel. The amount of fungitoxin in the plants of the soil drench treatments greatly increased between the second and third weeks and then remained fairly constant until after the second application of benomyl on the sixth week. During the second 6 weeks, both treatments reached the highest concentration for the 12-week period. In soil amended with benomyl, there was a high concentration of fungitoxin during the fifth week, then a rapid decline until after the second application of benomyl at the sixth week. The plants grown in gravel probably had the most rapid uptake of all treatments since the fungitoxin was detected at the first sampling period. However, it was almost nondetectable by the sixth week. During the second 6 weeks, an almost identical pattern of immediate uptake and steady decline was again noted. Thus the planting media and mode of application influenced the total uptake as well as the rate of accumulation of the fungitoxicant.

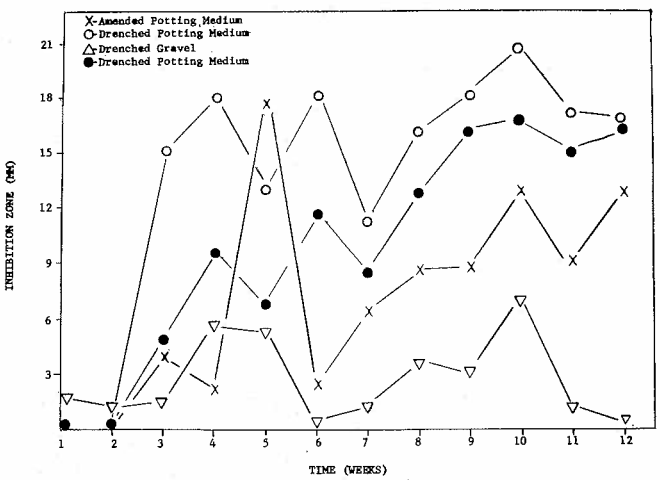

Figure 1. Zones of inhibition from *Penicillium expansum* bioassays of carnation cuttings from 1 to 12 weeks. The first sampling period began 2 weeks after the initial application of benomyl. At the sixth week of sampling another application of benomyl was applied.

As ideal growing media for the utilization of systemic fungicides, inert media are more likely than soil to permit rapid and consistent uptake of benomyl

since they do not vary so widely in composition as soil. Soils containing large amounts of clay or humus material vary more than gravel or sand in base exchange capacity and in the populations of microorganisms they contain. In the gravel treatment, reduced uptake of benomyl was related for the most part to the amount of irrigation needed to sustain an actively growing plant and the subsequent leaching of the fungicide from the substrate. The aggregate size of gravel recommended for use under normal conditions in carnation culture (8) seemingly provided too much porosity for single dosage applications of benomyl to be taken up by the plant in any large amount. The problem was further compounded by more frequent irrigations, up to 4 times daily in the summer, as compared to twice weekly for plants grown in soil.

LEACHING TESTS

To detect the movement of benomyl through the various potting media, leachate samples were taken for the first 8 weeks. Pots in all treatments were slightly elevated above the bench by placing inverted Petri dish covers under the pots to facilitate collection of samples. The treatments having soil media were irrigated to over field capacity and leachates collected. In the gravel treatment the pots were slightly elevated and the resulting run-off trapped. Ten to 20 ml of leachate from each of the 6 pots per treatment was collected and consolidated to represent one individual treatment.

A paper-disc bioassay was performed with the leachate samples. Schleicher and Schuell filter paper discs (12.7 mm)^2 were impregnated with 0.2 ml of the leachate that was to be tested for fungitoxicity. Six discs per treatment were placed on test plates of Sabouraud's agar amended with streptomycin (30 ppm) to suppress bacterial contamination. The test plates previously seeded with *P. expansum*, were incubated at 23 C for 24 hours. A zone of inhibition of the *P. expansum* indicated the presence of a fungitoxic substance. Preparation of the test plates and measurements of zones of inhibition were carried out as previously described.

Leachate samples taken during the first week from gravel exhibited a very high rate of activity when bioassayed against *P. expansum* followed by a rapid decline in activity for the next 5 weeks (Fig. 2). The second application of benomyl after 6 weeks produced a subsequent increase in activity 1 week later, followed by another rapid decline in activity. This would suggest, together with the trends noted in Fig. 1, that most of the fungicide was being leached through the gravel before the roots could absorb it.

In contrast, leachate from soils contained only barely detectable levels of the fungitoxin (Fig. 2).

These results have significance for future considerations in control of Fusarium branch rot of carnations. Systemic uptake of benomyl is rapid in carnations immediately after application to gravel.

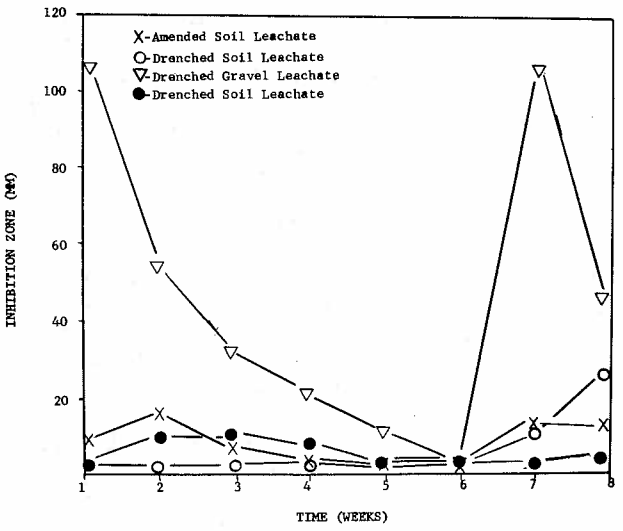

Figure 2. Zones of inhibition from *Penicillium expansum* bioassays of potting media leachates containing varying amounts of benomyl from 1 to 8 weeks. The first sampling period began 2 weeks after the initial application of benomyl. At the sixth week of sampling another application of benomyl was applied.

The frequent watering required in gravel culture, however, leads to rapid leaching of the chemical. Thus, continuous application of the fungitoxin with each watering may be required to maintain an adequate level in the tissue.

Positioning of the very insoluble benomyl near roots is no more efficient in uptake than a drench applied to the soil. Presumably this is due to the conversion of benomyl to the more soluble form of methyl 2-benzimidazole carbamate (MBC) in an aqueous suspension (1, 9, 13, 14, 15). Indeed, Clemons and Sisler (6) observed that aqueous technical samples of benomyl converted to MBC at the rate of about 50 per cent in one hour.

The question now becomes whether systemic uptake of benomyl reaches high enough concentrations to effect invasion of carnation tissue by *F. roseum*. This will be the subject of the next paper in this series.

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