SCEPTREPLUS

Development of an artificial inoculation method for cavity spot in pot-grown carrots in the glasshouse: update May 2021

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Objectives

1) To develop and refine artificial inoculation methods for cavity spot of carrot caused by *Pythium violae* in glasshouse pot tests
2) To determine if these methods can be used to identify resistant carrot accessions and effective crop protection products for control of cavity spot

Overview

Two experiments were set up to evaluate i) a millet seed *P. violae* inoculum and ii) a compost/carrot *P. violae* inoculum for their ability to induce carrot cavity spot in glasshouse pot trials. Each experiment tested one of the inoculum types at two concentrations and also incorporated the testing of four carrot cultivars and a metalaxyl treatment. This therefore allowed the utility of these artificial inoculation approaches to be assessed for identifying both cavity spot resistance and efficacy of crop protection products in future experiments.

Materials and Methods

Preparation of *P. violae* inoculum

Millet seed inoculum: A millet seed substrate was prepared by mixing 70 g white millet seed with water in 500 ml conical flasks to give a final moisture content of 62% w/w. Flasks were autoclaved three times at 121°C for 30 min with 24 h in-between each cycle. The sterile millet substrate was then inoculated with 14 agar plugs from actively growing cultures of *P. violae* isolate HL path 19 and incubated at 20°C in the dark for three weeks.

Carrot / compost inoculum: A compost / carrot substrate was prepared by mixing 120 g Levington F1 compost (sieved to 4 mm) with water in 500 ml conical flasks to give a final moisture content of 80% w/w. Finely grated carrot (50 g) was then added before the flasks were autoclaved twice at 121°C for 15 min with 24 h in-

between each cycle. The sterile carrot / compost substrate was then inoculated with 14 agar plugs actively growing cultures of *P. violae* isolate HL path 19 and incubated at 20°C in the dark for three weeks.

Carrot cultivars and treatment structure

Two experiments were set up using either millet seed or carrot / compost inoculum with the same structure. Each was set up with four carrot cultivars; the industry standard cv. Nairobi, cv. Eskimo (reported resistance), cv. Criolla (potential high susceptibility) and an additional (coded) susceptible control variety provided by a commercial seed company. Millet or carrot / compost *P. violae* inoculum concentrations of 10 mg and 30 mg g^{-1} growing medium were tested for each cultivar and in addition, the effect of the fungicide metalaxyl was also assessed across all carrot cultivar / inoculum concentration treatment combinations and compared with an untreated control. The experiment comprised a total of 16 treatments with 8 replicate pots of 6 carrots (N=48). Untreated control treatments consisted of four replicate pots with 10 mg and 30 mg g^{-1} dead (autoclaved) inoculum for each carrot variety.

Inoculation of growing medium, experimental set-up and maintenance

A 50:50 v/v mix of sieved compost (John Innes No. 3, Erin, UK) and a horticultural grade sharp sand (Westland, UK) was used as the growing medium in both experiments. Appropriate amounts of each *P. violae* inoculum type were mixed with the compost/sand growing medium in a cement mixer to obtain concentrations of 10 and 30 mg g^{-1} growing medium. Tall plastic pots (5.5 L capacity) were filled with infested growing medium (5.65 kg per pot) at each inoculum concentration. Control pots received a mixture of 50:50 v/v compost/sand and autoclaved (dead) inoculum at the same concentrations of 10 and 30 mg g^{-1} growing medium. Pots were placed in deep saucers in a randomised block design within a 20 m^2 glasshouse compartment. (max 18°C, min 16°C). Supplementary lighting was used from 5:00 to 20:00 h to extend day length and pots watered to ensure a high moisture content before sowing with untreated carrot seed (25 seeds per pot). Spray applications of SL567A (metalaxyl) were made at a rate of 1.3 L/ha in 1000 L water to the soil surface just after sowing and again 3 weeks post-sowing. The growing medium was kept damp by gentle overhead watering with additional watering into the saucers as carrots developed. After 6 weeks, seedlings were thinned out to six per pot.

Assessment of carrot plant growth, cavity spot symptoms and infection by *P. violae*

Carrot seedling emergence and any damping off symptoms were recorded weekly for the first 5 weeks. Mature plants were harvested at 18 - 20 weeks and carrot foliage weight and root weight for each pot determined. Each carrot was assessed for presence / absence of cavity spot (incidence) and also the number of lesions present per carrot (severity).

Results

Effect of *P. violae* inoculum: Overall, the millet seed *P. violae* inoculum generally resulted in more cavity spot disease for the no metalaxyl treatments compared with the compost / carrot inoculum, particularly for the susceptible cultivars (Fig. 1; Fig. 2). For cv. Criolla where the highest levels of cavity spot were observed, disease incidence was 84% carrots affected for the millet grain (Fig. 1a) and 56% for the carrot / compost inoculum (Fig. 1b) at 30 mg g^{-1} concentrations. As expected, the lower concentration of both inoculum types resulted in decreased levels of cavity spot; for instance, for cv. Criolla, disease incidence was reduced to 27% (Fig. 1a) at 10 mg g^{-1}. The same trends were also evident for cavity spot severity which reached a maximum of 4.5 lesions per carrot for cv. Criolla inoculated with 30 mg g^{-1} millet grain inoculum (Fig. 1c). No cavity spot disease was observed for any uninoculated control treatments (data not shown).

Effect of carrot cultivar: High cavity spot levels were observed in the susceptible control carrot variety across both inoculum types and concentrations (Fig. 1, Fig. 2) for no metalaxyl treatments with disease incidence of 78% for the millet grain inoculum and 56% for the carrot / compost inoculum at the 30 mg g^{-1} concentrations. Disease levels for cv. Criolla were also high with 84% and 27% carrots affected respectively for the corresponding inoculum types at the 30 mg g^{-1} concentrations (Fig. 1ab, Fig. 2). While cv. Nairobi resulted in a lower disease incidence than the susceptible control variety and cv. Criolla for the millet grain inoculum at 30 mg g^{-1} (47% carrots affected; Fig. 1a), it exhibited a higher level of cavity spot than cv. Criolla for the carrot / compost inoculum (38% carrots affected, Fig. 1b). The resistant carrot cv. Eskimo consistently resulted in much lower levels of cavity spot across both inoculum types and concentrations ranging from 4.2% for the carrot / compost inoculum to 21% for the millet inoculum for the 30 mg g^{-1} concentrations (Fig. 1ab, Fig. 2).

Effect of metalaxyl treatment: Application of metalaxyl consistently reduced cavity spot incidence and severity across the different carrot cultivars, inoculum types and concentrations (Fig. 1). For instance, when metalaxyl was applied, disease incidence was reduced from 84% in cv. Criolla and 78% in the susceptible control variety to 17% and 29% carrots affected respectively for the millet grain inoculum at 30 mg g^{-1} (Fig. 1a). Similarly, for the carrot / compost inoculum, metalaxyl reduced disease incidence from 27% in cv. Criolla and 56% in the susceptible control variety to 10% and 2% carrots affected respectively at the 30 mg g^{-1} concentration (Fig. 1b).

Conclusions

- Artificial inoculation of carrots with *P. violae* millet grain or carrot / compost inocula at 30 mg g^{-1} growing medium results in high levels of cavity spot
- The susceptible control carrot variety and cv. Criolla resulted in consistently higher levels of cavity spot than the resistant cv. Eskimo.
- Metalaxyl treatment consistently reduced cavity spot disease across different carrot cultivars, inoculum types and concentrations.

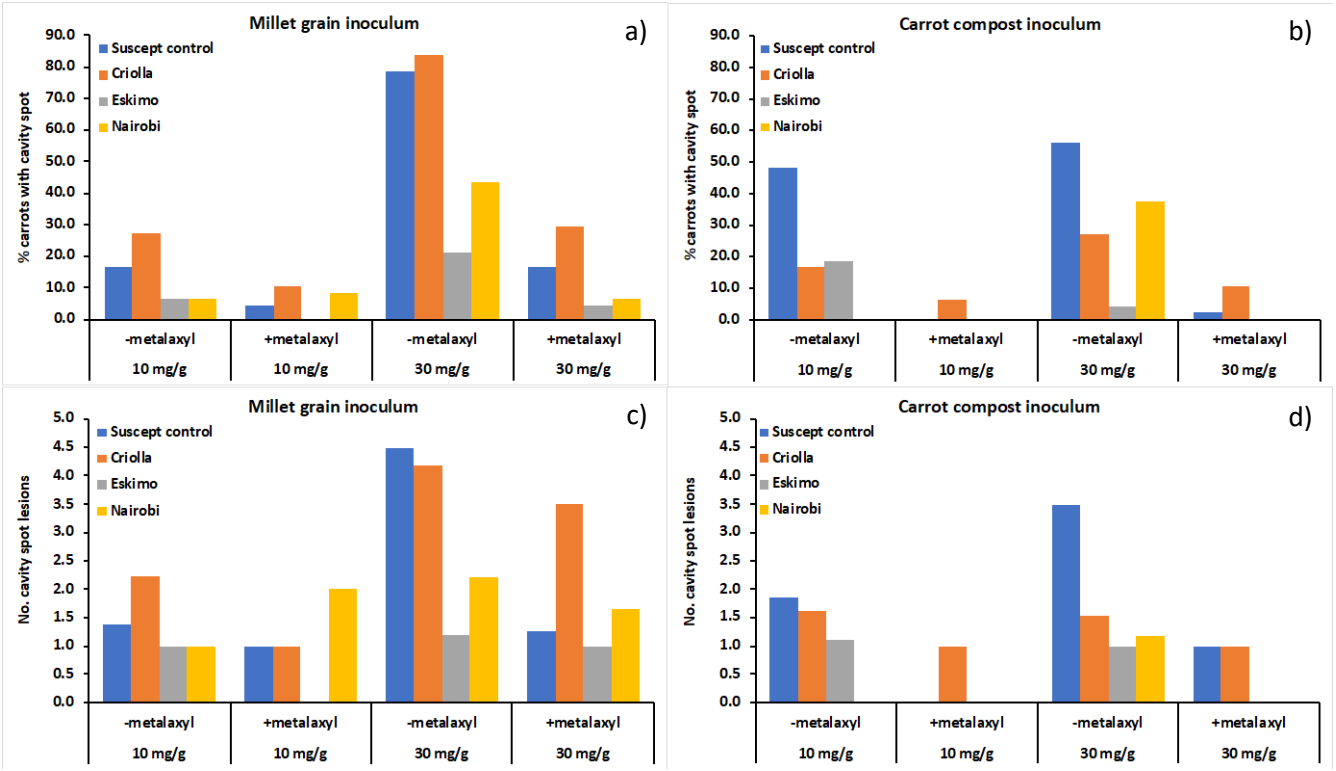

Figure 1. Effect of *P. violae* millet grain inoculum (a & c) and carrot / compost inoculum (b & d), concentration (10 mg-[1], 30 mg^{-1} growing medium), carrot cultivar and metalaxyl treatment on cavity spot incidence (a & b) and severity (c & d) in glasshouse pot experiments.

Figure 2. Photographs showing severe cavity spot lesions in control susceptible (a) and Criolla (b) cultivars and less severe disease symptoms in Nairobi (c) and Eskimo (d) cultivars with *P. violae* millet seed inoculum applied at 30 mg g^{-1} soil (no metalaxyl treatment).