Abstract

Cavity spot is a major disease of carrots in Western Australia, which is the largest exporter of carrots in the southern hemisphere.

In a survey of a 400 ha commercial carrot field conducted to determine the causal agent(s) of cavity spot, 51 out of a total of 235 fungal isolates belonged to *P. coloratum* and 120 to *P. sulcatum*, while the remainder were from other genera. Laboratory pathogenicity tests were conducted by inoculating surface sterilised mature carrots with these fungi as 5 mm diameter colonised agar plugs, and incubating at 28°C in the dark. All 51 isolates of *P. coloratum* produced large brownish-black water soaked and depressed lesions, whilst only 32 isolates of *P. sulcatum* produced lesions which were small. The other fungi isolated did not cause lesions.

A glasshouse inoculum density trial (0, 0.1, 0.3, 0.5, 0.8 or 1 %) showed that *P. coloratum* produced substantial and numerous lesions at an inoculum density of 0.5% and above. In contrast, *P. sulcatum* produced few and small lesions at inoculum densities of 0.8 and 1 % and none at 0.5 %. This is the first record of *P. coloratum* as a causal agent of cavity spot of carrots in Australia or elsewhere.

Three experiments were then conducted with *P. coloratum* to study the relationships between time of infection, host age and development of cavity spot lesions. These showed that: a) *P. coloratum* infected carrot seedlings asymptptomatically 3 weeks after sowing, (b) seedling grown in artificially infested soil from 0-3 weeks of age and subsequently transferred to pathogen-free soil developed cavity spot at harvest (16 weeks) and (c) there was no significant (*P*>0.05) association between carrot age and the ability of *P. coloratum* to infect the roots and to cause cavity spot lesions at harvest.

In Western Australia, the application of lime has been shown to reduce the incidence of cavity spot in the field. Therefore, a glasshouse pot trial using soil artificially infested with *P. coloratum* was conducted to examine the effect of lime or gypsum amendments on the development of cavity spot disease of carrots. Each amendment was applied to field soil at 4000 or 8000 kg ha⁻¹. Lime at both rates of
application (pH 6.8 and 7.1, respectively) significantly ($P<0.05$) reduced the incidence of the disease, with the higher lime rate being more effective. In contrast, both gypsum treatments (pH 5.2) had no effect on the incidence of cavity spot. There was no significant difference ($P>0.05$) in the calcium concentration between carrot roots grown in unamended or lime- or gypsum-amended soil with or without the pathogen. Therefore, calcium did not appear to play a direct role in the reduction of cavity spot. Reduction of the incidence of cavity spot appeared to be related to the increase in soil pH associated with the application of lime.

Microbial biomass in a limed field soil (pH 6.9) known to reduce cavity spot incidence was compared to a conducive non-limed soil (pH 5.1) from an adjacent plot. The limed soil showed significantly ($P<0.01$) high soil microbial activity as measured by the hydrolysis of fluorescein diacetate and by arginine ammonification. It also had significantly higher ($P<0.01$) total numbers of colony forming units (cfu) of aerobic bacteria, fluorescent pseudomonads, Gram negative bacteria, actinomycetes and significantly ($P<0.01$) decreased cfu of filamentous fungi and yeasts compared to non-limed soil. In addition, limed soil had higher cfu of non-streptomycete actinomycetes, rarely reported in similar studies. These non-streptomycete actinomycetes were estimated and isolated using polyvalent *Streptomyces* phages and the dry heat technique to reduce the dominance of streptomycetes on isolation plates. The non-streptomycete actinomycetes isolated included species of *Actinoplanes, Micromonospora, Streptoverticillium, Nocardia, Rhodococcus, Microbispora, Actinomadura, Dactylosporangium* and *Streptosporangium*. Isolates from limed and non-limed soils were screened *in vitro* using a dual culture technique on Hussein's fishmeal extract agar for antagonism against *P. coloratum*. The numbers of actinomycetes antagonistic to *P. coloratum* were higher in the lime amended soil.

Three hundred and fifty two out of 817 actinomycetes isolated from both soils produced compounds inhibitory to *P. coloratum*. Of these, 45 isolates were chosen for further *in vitro* studies since they were the most antagonistic to *P. coloratum*. These belonged to *Streptomyces* spp. (12), *Streptoverticillium* spp. (6), *Actinoplanes*
spp. (8), Micromonospora spp. (5), Actinomadura spp. (9), Microbispora spp. (2), and Streptosporangium spp. (3). They were screened against *P. coloratum* using *in vitro* carrot bioassay, which involved pairing agar plugs colonised by each actinomycete and *P. coloratum* on the carrot surface and incubating the carrots at 28°C in the dark for 4 days. Lesion diameter was used as a criterion for the disease index to compare the efficacy of the different isolates. Out of the 45 isolates screened, the strongest seven isolates which reduced or prevented lesion formation were used for additional *in vitro* and *in vivo* antagonism trials. These seven isolates were identified using cultural, morphological, physiological, biochemical and cell wall characteristics as *Streptomyces janthinus*, *S. cinerochromogenes*, *Streptoverticillium netropsis*, *Actinomadura rubra*, *Actinoplanes philippinensis*, *Micromonospora carbonaceae*, and *Streptosporangium albidum*.

All seven isolates tested produced antifungal non-volatile metabolites on nutrient-rich and impoverished media, but failed to produce volatile antifungal compounds. All these isolates significantly (*P*<0.05) reduced the incidence of cavity spot in soil artificially infested with the pathogen in the glasshouse. *S. janthinus* and *Strepto. albidum* were the most effective. In addition, all the actinomycetes species, except *Ac. rubra* and *M. carbonacea*, in the presence or absence of the pathogen significantly (*P*<0.05) increased mean fresh root weight compared to the treatment which included *P. coloratum* only.

This study shows that these actinomycetes have considerable potential for future use as biocontrol agents of cavity spot in the field. It also highlights the importance of including polyvalent *Streptomyces* phages and the dry heat technique for the isolation of non-streptomycete actinomycetes to be used as potential biological control agents.