Short Note

The effect on spore viability of storing the nematode parasite *Pasteuria penetrans* in either dried root material or water suspension

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Pasteuria penetrans is a mycelial and endospore forming bacterium parasitising root-knot nematodes (*Meloidogyne* spp.), with potential as a biocontrol agent (Chen & Dickson, 1998). The mass production of *P. penetrans* is based on a method described by Stirling and Wachtel (1980). A host plant for a *Meloidogyne* species is inoculated with second-stage juveniles (J2) encumbered with spores of *P. penetrans* and after an appropriate period, depending on the conditions of plant growth, the plant is uprooted and soil is washed from the root, which is then dried thoroughly, so that egg masses of uninfected females die; the spores of the parasite inside the infected females remain viable for long periods. A spore suspension of *P. penetrans* is released from this stock material by thoroughly grinding the dried roots with a pestle and mortar, suspending the ground material in distilled water, and sieving it through a 38 µm sieve to remove coarse root material; the spore density is then estimated using a haemocytometer.

Previous studies showed that a storage period of *P. penetrans* in the form of dried root material for 6 or 11 years did not reduce the ability of spores to attach to juveniles but significantly decreased the infection of females (Espanol *et al.*, 1997; Giannakou *et al.*, 1997). Similarly, Nasiou *et al.* (2020) reported that spores of *P. penetrans* derived from dried roots that had been stored for 24 years, attached readily to J2 but their ability to germinate and infect might have been lost, since 50 days after J2 inoculation mature spores were not detected in females without egg masses in roots.

In order to find out if the way that spores are stored influences their attachment and infection potential, a study was conducted to compare spores stored as a spore suspension (in distilled water), with spores derived from infected *Meloidogyne* females in dried roots. The spore suspensions used by Nasiou *et al.* (2020) were prepared in 2019, from dried roots stored for 24 years and were kept stored in glass bottles in a domestic refrigerator at temperature c. 4°C. The isolates tested were *P. penetrans* 3 (*Pp*3) and *P. penetrans* blend (*Pp*blend; a blend of six isolates). In 1995, when the dried root material had been produced, spore suspensions were also prepared and stored in a domestic refrigerator for 24 years. In 2020, a test of spore attachment and infectivity was conducted using a population of *M. javanica* from Crete. Four spore suspensions were used: *Pp*3 old and *Pp*blend old (prepared in 1995 from the freshly dried root material), *Pp*3 new and *Pp*blend new (prepared in 2019 from the same root material after 24 years of storage). The *Pp* old suspensions were sieved through a 38-µm sieve to remove algae and debris accumulated during this long storage period. Eggs of *M. javanica* were collected from roots of tomatoes grown in pots (Hussey & Barker, 1973) and incubated in an extraction dish for 3 days. Hatched J2 were transferred to 5.5-cm diam. Petri dishes (*ca* 200 per dish) containing suspensions of 20,000 spores with a final volume of 8 ml. After 48 h of incubation in the spore suspension at 25-28°C, the number of spores that had attached to the cuticle of 30 randomly selected J2 per dish was examined with an inverted

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microscope at ×200. The J2 encumbered with spores were used to inoculate tomato plants ('Ace') grown in 250-ml plastic cups filled with a commercial soil substrate. Plants were kept in a growth room at 25-32°C and 14 h photoperiod for 60 days. After that period, they were uprooted, the roots were washed thoroughly and 15-20 females without egg masses were selected and dissected from the root using a dissecting microscope. The females were put in drops of water on glass slides, crushed with cover slips and examined at ×400 for the presence of mature spores of *P. penetrans*.

In the case of *Pp* new treatments, a few females without oocytes or eggs in their ovaries were found but none of them contained mature spores. By contrast, in the case of *Pp* old treatments, 5-10 females with mature spores were found out of the 15-20 examined per root. The cover slips were removed, and the pieces of the squashed females stuck on glass slides and coverslips were washed in a glass beaker and vortexed so as to disperse the spores. These fresh suspensions of *Pp*3 and *Pp*blend derived from *M. javanica* were kept stored in a domestic fridge for 2 months to ensure full hydration of spores. Afterwards the ability of spores to attach and infect nematodes were tested on *M. javanica*, *M. incognita*, *M. hapla* and *M. luci* as previously described. Spores attached readily on J2 of all species and J2 encumbered with spores inoculated on tomato plants; infected females containing mature spores were found in tomato roots as described before, for the four tested nematode species.

This study strongly indicates that *P. penetrans* spores stored in the form of dried root for a 24-year period attached to juveniles but did not cause infection, whereas when stored as water suspensions for the same period, they retained their ability to attach to juveniles and subsequently infected the females.

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