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SHORT NOTE

A method to monitor the activity of *Phytophthora* spp. in the root zone of *Pistacia* spp.

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Summary. A method was developed to monitor the formation of zoospores of *Phytophthora* species in the soil around potted host plants grown under greenhouse conditions. Inoculum of the pathogen was grown in vermiculite amended with hemp seed extract for 4 weeks at room temperature and 10 ml was placed around each plant. Pots were periodically flooded with water overnight with the drainage hole closed. Water was drained and collected next day and baited with citrus leaf discs for 48 h at room temperature and plated on a *Phytophthora* selective antibiotic medium. Baits colonized with *Phytophthora* were good indicators of the pathogen activity in the root zone of susceptible and resistant hosts.

Key words*:* rhizosphere, bait, monitoring, soilborne pathogen.

Soilborne fungal pathogens of plants and the diseases they cause are difficult to study because of the environment in which they are found (Singleton *et al.*, 1992). The inocula of soilborne plant pathogens are of two general types: motile (e.g. zoospores) or non-motiles (e.g. oospores and chlamydospores). Simultaneous study of the plant growth and the activity of a pathogen with non-motile propagules in the soil is difficult. In contrast, the quantitative determination of motile propagules in soil percolate may help the study of the concurrent activity of the pathogen and the pathogen development of the disease it causes.

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The majority of *Phytophthora* species are soilborne micro-organisms causing crown and root rots in important agricultural crops (Erwin and Ribeiro, 1996). *Phytophthora* infections are aggravated when the soil is saturated for prolonged periods (Duniway, 1979). Under waterlogged conditions most *Phytophthora* spp. produce sporangia and release zoospores if other environmental conditions are also favourable. Sporangia are not dispersed from their point of formation within soil, since the sporangia of most *Phytophthora* spp. are not caducous and most natural soils do not contain many pores large enough for the movement of sporangia with water flow (Duniway, 1983). Zoospores are the major infective propagules, which swim actively in the soil but for long distance transport rely on water flow for their dispersal (Carlile, 1983, Duniway, 1983).

Several techniques have been developed to monitor the population of soilborne plant pathogens (Campbell and Madden, 1990) but information concerning the activity of soilborne plant pathogens without disturbing the host is scarce (Mole and Riessen, 1995). The purpose of the present study was to develop a technique to monitor the activity of *Phytophthora* spp. in the root zone of several species of *Pistacia*.

Materials and methods

Three species of *Phytophthora* isolated from pistachio growing areas in the Kerman province in Iran were used (Banihashemi, 1995). *Phytophthora citrophthora*, and *P. drechsleri* were isolated from the roots of pistachio trees and *P. nicotianae* from soil around a pistachio tree. Among the isolates used, *P. citrophthora* was the most aggressive on *Pistacia* spp. (Banihashemi, 1995). All hyphal tip isolates were maintained on corn meal agar (CMA) at 15°C.

Phytophthora isolates were grown on autoclaved vermiculite amended with hemp seed extract in 500 ml flasks (200 ml vermiculite and 120 ml of extract of 60 g hemp seeds in 1 l of distilled water) for four weeks at room temperature (Banihashemi, 1987).

Shelled seeds of *Pistacia vera* L. cv. Momtaz were soaked overnight in water, treated with 0.1% PCNB and germinated between two sheets of moist cheese-cloth at room temperature for 3–5 days. Similarly, shelled seeds of *P. mutica* Fisch. & Mey, *P. khinjuk* Stochs. (local wild cultivars) and *P. atlantica* Deff. (from USA) were removed, soaked overnight, dried at room temperature, treated with 0.5% benomyl (w:w) and incubated in moist sterilized sand at 4–6°C for 1–3 months to resume germination. Plants obtained from germinated seeds were grown in a greenhouse at 22–28°C with supplemental fluorescent illumination.

Nine-month-old seedlings of *P. vera* and 15-month-old seedlings of other *Pistacia* species were inoculated with 10 cc of the inoculum at the base of the seedling close to the root and covered with soil. Controls received only vermiculite supplemented with hemp seed extract. All treatments were arranged in a completely randomized design with 4 replications each containing 4 seedlings. Pots were flooded overnight, with the drainage hole closed with melted paraffin.

The following day, the drainage holes were opened and water was collected from each pot, filtered through cheese cloth, decanted into a disposable plastic container (20×15 cm) to about 2 cm depth, baited with 30–50, 5-mm in diam. fresh citrus leaf disks (usually sweet lime) (Banihashemi *et al.*, 1992) and incubated at room temperature. After 48 h, the baits were collected on the screen, washed on the screen under running tap water on screen, blotted dry with a paper towel and plated on *Phytophthora* selective medium (PARPH) (Kannwischer and Mitchell, 1978). The number of baits colonized with *Phytophthora* spp. was determined with boiled hemp seeds on individual colonies. Four to five boiled hemp seeds were placed overnight on each colony and then transferred to distilled water in Petri dishes and incubated at room temperature under continuous fluorescent illumination. Sporangia formation around the colonized hemp seeds gave a positive indication of *Phytophtora* occurrence at genus level. Water from each pot was sampled for 8 weeks as described above. Between flooding periods, pots were kept saturated by periodical irrigation.

Individual plants in each treatment were examined daily and disease symptoms including foliage discoloration, wilting and death were recorded. At the end of the experiment, the seedlings were

Table 1. Monitoring *Phytophthora* spp. as affected by host, pathogen species and time after inoculation.

Parameter	Colonization (%)
Pistacia spp.	
Pistacia atlantica	1.28 c
Pistacia vera	20.50 b
Pistacia mutica	25.15 a
Pistacia khinjuk	25.40a
Phytophthora spp.	
Phytophthora citrophthora	21.67 a
Phytophthora drechsleri	12.91 b
Phytophthora nicotianae	13.49 b
Weeks after inoculation	
I	20.72 a
II	11.23 d
III	17.02 b
IV	14.97 c

Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.01$).

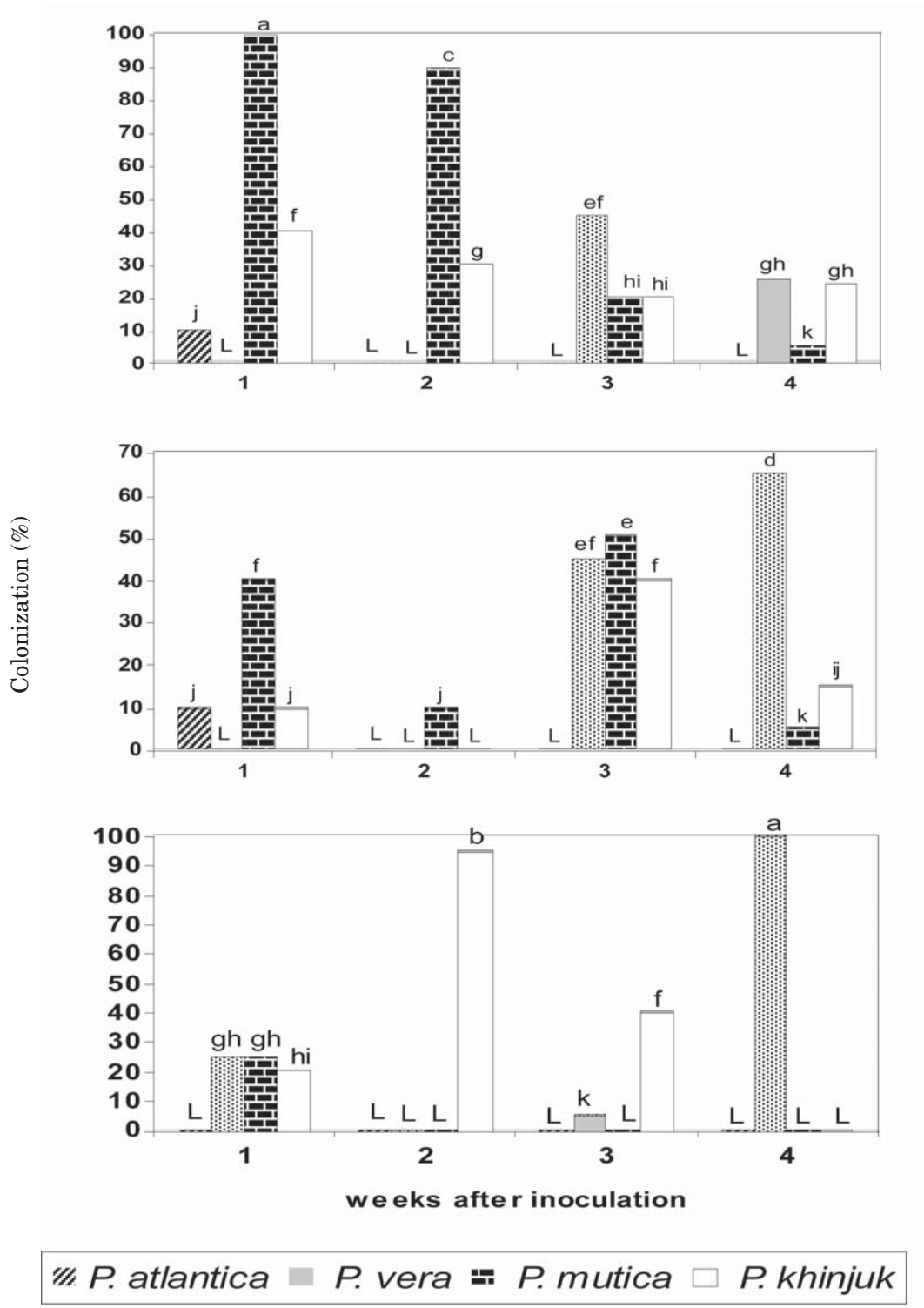

Fig 1. Population changes of *Phytophthora* spp. in the root zone of *Pistacia* spp. during 4 weeks under greenhouse conditions. Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P \leq 0.01$).

removed and isolations of the pathogen from infected tissues were done. Analysis of variance was performed and means were compared using Duncan's multiple range test. All experiments were repeated at least twice.

Results and discussion

Disease symptoms usually appeared as foliage discoloration and wilting of the terminal leaves, followed by the drying and death of individual branches, which eventually resulted in the total collapse of the plants. *P. mutica* and *P. khinjuk* showed disease symptoms within a week of inoculation whereas on *P. vera* symptoms appeared two or more weeks after inoculation. *P. atlantica* did not produce any aerial symptoms throughout the experiment and all seedlings of this species remained healthy even after two years. Reinoculation of *P. atlantica* after the end of the experiment did not lead to disease symptoms. *Phytophthora* spp. were consistently isolated only from infected tissue of diseased plants. *P. citrophthora* was the most aggressive fungus on *Pistacia,* and *P. khinjuk* was the tree most susceptible to *Phytophthora*. *P. nicotianae* was mildly pathogenic.

Phytophthora spp. were present in drained water collected from all treatments (including the control soil without a host plant) the first day after inoculation. This indicated that the pathogen had formed sporangia and released zoospores into the soil. Drained water was collected at weekly intervals and assayed for the pathogen. In *P. vera*, none of the *Phytophthora* spp. was detected seven days after the first flooding. By the 3rd and 4th weeks all the *Phytophthora* spp. were present (Fig. 1). In *P. atlantica* a few baits were colonized with *P. citrophthora* and *P. drechsleri* in the first week but on this species no *Phytophthora* species was detected in subsequent samplings. *P. mutica* and *P. khinjuk* supported all *Phytophthora* species in the first week but the rate of colonization of *Phytophthora citrophthora* progressively decreased. In control soil without a host plant only 10–20% of the baits were colonized by *P. citrophthora* 2 weeks after soil infestation (data not shown).

The effect of the host tree, the *Phytophthora* species and the time after inoculation on bait colonization indicated that there were significant differences between *Pistacia* species. The resistant *P. atlantica* showed the lowest rate of bait colonization, and the susceptible *P. mutica* and *P. khinjuk* the highest. Among the *Phytophthora* species examined, *P. citrophthora* was the most pathogenic and resulted in highest bait colonization, confirming our previous finding (Banihashemi, 1995).

Literature cited

Banihashemi Z., 1989. Study of pistachio gummosis in southern province of Iran. In: *Proceeding of 9th Iranian Plant Protection Congress,* September 1989, Mashhad, Iran, 87.

Banihashemi Z, 1995. Identification of *Phytophthora* species associated with pistachio gummosis in southern Iran. *Acta Horticulturae* 419, 349–352.

Banihashemi Z., J.D. MacDonald and J. Stites, 1992. Combined baiting and ELISA to detect and quantify *Phytophthora* spp. in container media. *Phytopathology* 82, 1161 (abstract).

Campbell L.L. and L.V. Madden, 1990. *Introduction to Plant Disease Epidemiology*. John Wiley & Sons, New York, NY, USA, 532 pp.

Carlile M.J., 1983. Motility, taxis and tropism in *Phytophthora*. In: *Phytophthora*, *Its Biology, Taxonomy, Ecology and Pathology* (D.C. Erwin, S. Bartnicki-Garcia, P.H. Tsao, ed.). The American Phytopathological Society Press, St. Paul, MN, USA, 95–107.

Duniway J.M., 1979. Water relation of water molds. *Annual Review of Phytopathology* 17, 431–460.

Duniway J.M., 1983. Role of physical factors in the development of *Phytophthora* diseases. In: *Phytophthora*, *Its Biology, Taxonomy, Ecology and Pathology* (D.C. Erwin, S. Bartnicki-Garcia, P.H. Tsao, ed.). The American Phytopathological Society Press, St. Paul, MN, USA, 175–178.

Erwin D.C. and O.K. Ribeiro, 1996. *Phytophthora Diseases Worldwide*. The American Phytopathological Society Press, St Paul, MN, USA. 561 pp.

Mole L. and H.W. Van Riessen , 1995. Effect of plant roots on germination of microsclerotia of *Verticillium dahliae*. *European Journal of Plant Pathology* 101, 673–678.

Singleton L.L., D.L. Mihail and C.M. Rush, 1992. *Methods for Research on Soilborne Phytopathogenic Fungi*. APS Press, St. Paul, MN, USA. 265 pp.

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