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Suitability of ten plant baits for the rapid detection of pathogenic *Pythium* species in hydroponic crops

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Abstract

Ten types of plant baits were tested in the laboratory to assess their capacity to detect pathogenic *Pythium* species. These were orange tree leaves, tomato leaves, pepper leaves, geranium leaves, Bermuda grass leaves, pine needles, immature carnation petals, hemp-seed cotyledons, pepper and cucumber fruits. The *Pythium* spp. tested were *P. aphanidermatum*, *P. irregulare* and *Pythium* 'group F' from hydroponic market garden crops in the Poniente region of Almería (south-east Spain). The test consisted of observing the velocity at which five baits were colonized and the day of colonization of the first bait. Results indicated that the slowest baits to be infected were immature carnation petals and pine needles. These two, together with Bermuda grass leaves, were also the baits infected in lowest number, such that practically no further infection was produced in the baits after the fifth day of contact with the inoculated water. The other plant baits tested were equally suitable for detection of *Pythium* spp. over the first two days, although only orange leaves and hemp-seed cotyledons were infected on the first day.

Abbreviations: APH – *Pythium aphanidermatum*; BGL – Bermuda grass leaves; C – control; CF – cucumber (fruit); CP – immature carnation petals; F – *Pythium* 'group F'; GL – geranium leaf; HSC – hemp-seed cotyledons; IRR – *Pythium irregulare*; NFT – nutrient film technique; OTL – orange tree leaf; PCA – potato carrot agar; PF – pepper (fruit); PL – pepper leaf; PN – pine needles; SE – standard error; SDW – sterile distilled water; TL – tomato plant leaf.

Introduction

The expansion of soilless cultivation of market garden crops has created an upsurge in interest in the pathogenic species of *Pythium* found in irrigation water. In addition to being the traditional agents in the damping-off of nursery seedlings (Hendrix and Campbell, 1973), *Pythium* spp. have also been the cause for the increase in the incidence of various root diseases in adult plants grown in greenhouses. *Pythium* can cause loss of rootstock, root rot, reduced crop yield, wilting of aerial parts and can even cause plant death (Blancard et al., 1992; Moulin et al., 1994; Menzies et al., 1996).

The detection of propagules of *Pythium* spp. in the irrigation water of hydroponic crops is therefore of considerable interest in the control of these diseases. As a consequence, more and more precise methods (selective media, filtration, ELISA techniques) are being assessed as means of detecting *Pythium* in irrigation water (Pittis and Colhoun, 1984; Ali-Shtayeh et al., 1991; Wakeham et al., 1997; Sánchez, 1998). However, these methods are all tedious or excessively expensive.

An alternative simple method uses plant baits. However, the possibilities of using plant baits as a rapid means of detecting *Pythium* have not been determined. The bait chosen must be appropriate for the species of

Pythium to be detected (Hendrix and Campbell, 1973). This is because each bait releases a certain quantity of nutrients to the water which influences the behaviour of the fungus (Emerson, 1958), for example, in the production of zoospores and oospores (Shahzad and Dick, 1990).

The present article presents a study of the speed and efficacy of ten plant baits in detecting important species of pathogenic *Pythium* spp., which have been found in hydroponic greenhouse crops in Almería province (an important European centre for the production of market garden crops).

Materials and methods

Plant material

Plant material for use as bait was collected from the University of Almería, from the nearby La Cañada de San Urbano and from the Poniente region of Almería. This plant material comprised (Figure 1): orange tree leaves (OTL) (*Citrus aurantium* L.), tomato leaves (TL) (*Lycopersicon esculentum* Mill.), geranium leaves (GL) (*Pelargonium zonale* (L.) Aiton), pepper leaves (PL) (*Capsicum annuum* L.), pine needles (PN) (*Pinus halepensis* Miller), immature carnation petals (CP) (*Dianthus caryophyllus* L.), Bermuda grass leaves (BGL) (*Cynodon dactylon* (L.) Pers.), hemp-seed cotyledons (HSC) (*Cannabis sativa* L.), pepper fruits (PF) (*Capsicum annuum* L.) and cucumber fruits (CF) (*Cucumis sativus* L.).

Preparation of baits

After collection, the plant material was washed in abundant soap and tap water and left to dry at laboratory temperature on filter paper. The hemp-seeds were disinfected by immersing them for two days in a mixture of water and 20% ethanol. To prepare the plant baits, pieces of 0.7 cm in diameter, were taken from the leaves of the geranium, orange, pepper and tomato, as well as from the CP. The PF and CF were cut using the same leaf punch and the skin of the fruits was eliminated. The PN and BGL were cut into approximately 1.5 cm lengths. Lastly, the hemp-seeds were opened with forceps and the cotyledons extracted. The baits were submerged in ethanol for 3 s, washed copiously with sterile distilled water (SDW) and dried on sterile paper.

Isolates of Pythium *spp.*

The isolates came from the Centro de Investigación y Formación Hortícola 'La Mojonera' (Almería), kindly donated by J. Gómez. These isolates were obtained

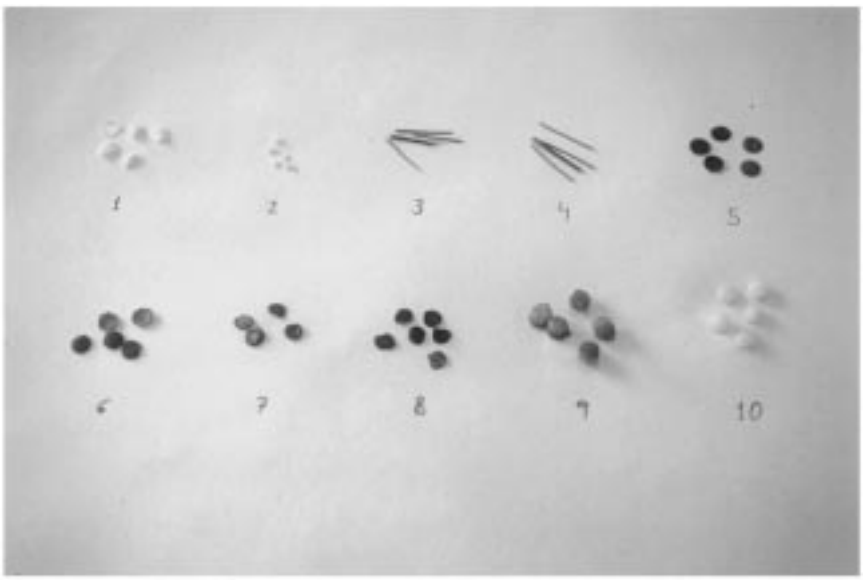

Figure 1. Plant baits tested (1. CP, 2. HSC, 3. BGL, 4. PN, 5. OTL, 6. GL, 7. TL, 8. PL, 9. PF, 10. CF).

Table 1. *Pythium* spp. isolates utilized

Fungal bank code	Species
PY11	*P. aphanidermatum*
PY13	*P. aphanidermatum*
PY17	*P. aphanidermatum*
PY1	*P. irregulare*
PY14	*P. irregulare*
PY20	*P. irregulare*
PY145	*P. irregulare*
PY147	*P. irregulare*
PY12	*Pythium* 'group F'
PY18	*Pythium* 'group F'

from roots and stems of diseased horticultural plants which had been grown in hydroponic or soilless culture in south-east Spain (Gómez, pers. comm.). Table 1 gives the isolate codes, their radial growth rates on PCA at 25 °C and the *Pythium* spp. to which they belong.

In vitro *detection of* Pythium *spp. in SDW using plant baits*

To detect the presence of *Pythium* propagules, the plant baits were placed in contact with water contaminated with *Pythium* spp. The *Pythium* spp. were seeded on a Petri dish (9 cm diameter) with 15 ml potato carrot agar (PCA). They were incubated in darkness at 25 °C until, after two days, they reached the edge of the Petri dish. 10 ml of SDW was placed in each of the test Petri dishes. A 9 mm diameter piece of the colony on agar medium of the corresponding isolate was transferred to each Petri dish. The control samples received only the agar medium maintained under the same conditions. Five plant baits of the same type were placed in each Petri dish. The dishes were sealed with Parafilm® and incubated in darkness at 20 °C. Three duplicates were made for each isolate and each bait. Ten isolates and ten different plant baits and controls were tested. Colonization of the baits was detected with a stereoscopic hand lens (Olympus® SZ60-Japan) which allows the whitish mycelium of the *Pythium* genus to be discerned on the bait, especially around its edges (Figure 2). The number of colonized baits was counted over the next 20 days.

Two new concepts are introduced in this paper as indicators of the character of the baits:

(1) *Baiting period*: the time needed for at least one bait to be infected. This was measured in days.

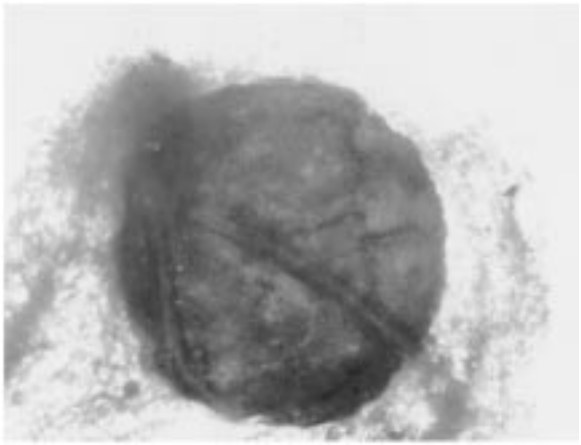

Figure 2. Details of a plant bait infected by *Pythium* spp. A fungal colony developing in the water can also be seen.

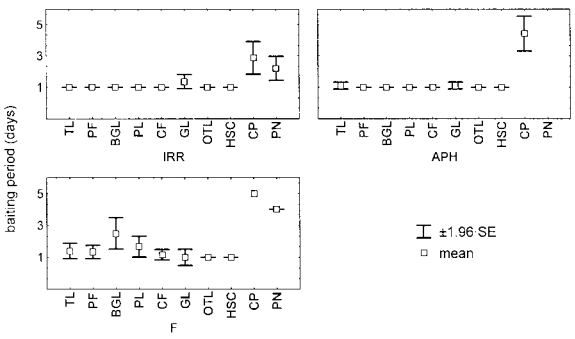

Figure 3. Baiting period of *Pythium* spp.

(2) *Baiting intensity*: measured as the % of baits colonized during a set time. In this study, the period chosen was one day, since the factor of interest is the speed of detection of the isolates.

Results

Baiting period

For the majority of plant baits employed, the mean baiting period (Figure 3) was approximately one day, and always shorter than two days, although three types of baits (BGL, CP and PN) required a longer period in some cases. On BGL, an average baiting period of 2.5 days was required for *Pythium* 'group F' (F) isolates. For the other two species of *Pythium* considered, the average time was one day.

PN were totally unattractive to the *P. aphanidermatum* (APH) isolates, which did not invade even a single bait over the entire 20-day test period. PN were effective for *P. irregulare* (IRR) and F. Nevertheless, the mean baiting period exceeded two days for IRR and four days for F isolates.

CP were the slowest to show the *Pythium* spp. (excluding consideration of PN to detect APH). The mean baiting period required was almost three days for IRR, rising to four or five days for APH and F.

Baiting intensity

The mean baiting intensity (mean percentage of baits invaded by *Pythium* spp. during the first day of contact with contaminated water) varied considerably according to the species in question (Figure 4), although for the majority of baits the behaviour was similar. Thus, IRR isolates were generally the most aggressive, closely followed by APH, whilst those of F were the least aggressive. However, all three were equally aggressive on OTL and HSC, which were always invaded at close to 100% intensity. On the other hand, CP and PN exhibited a low percentage infection rate by IRR whilst the infection rate for APH and F was practically nil.

A comparison of different baits, for each species (Figure 5) shows that for IRR, maximum values of close to 100% were achieved on TL, PF, PL, CF, OTL and HSC baits. PN showed the lowest values, at around 20%. BGL, GL and CP exhibited intermediate values.

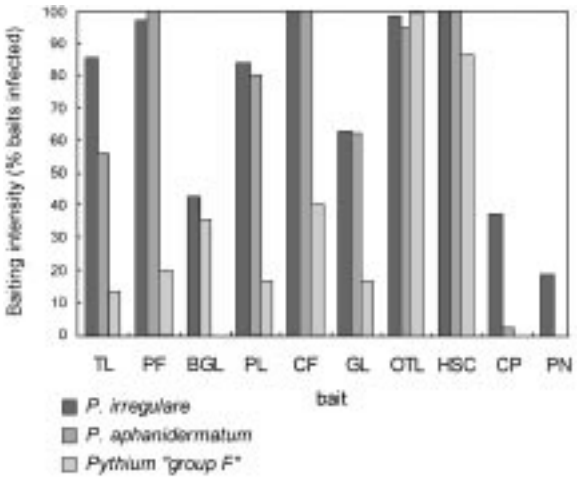

Figure 4. Mean baiting intensity, of *Pythium* spp.

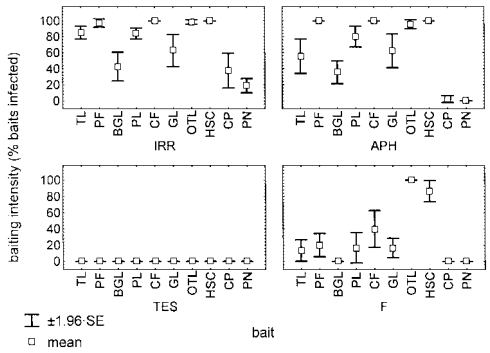

Figure 5. Baiting intensity, of *Pythium* spp.

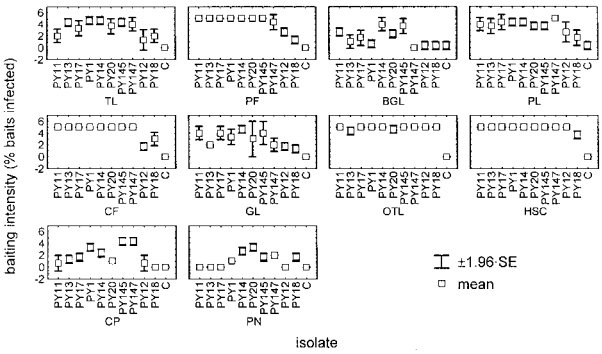

Figure 6. Baiting intensity of the isolates of *Pythium* spp. and the baits (PY11, PY13, PY17: *P. aphanidermatum*; PY1, PY14, PY20, PY145, PY147: *P. irregulare*; PY12, PY18: *Pythium* 'group F'; C: control).

For APH, the maximum values of close to 100% were reached on PF, CF, OTL and HSC baits. CP and PN registered nil or practically nil invasion. Other baits were intermediate to these with values of about 80% for PL and OTL, somewhat lower for TL and GL, and around 40% for BGL. For F, only OTL and HSC achieved close to 100% invasion. Baits of TL, PL and GL registered low values; consistently nil results were recorded for BGL, CP and PN, whilst CF and PF exhibited intermediate values.

It is clear that not all the baits are infected to the same degree by the same fungus; in terms of baiting intensity, three factors are relevant: the type of bait, the species of *Pythium* and the interaction between these two (Figure 6). This phenomenon was confirmed by 2-factor ANOVA for the isolates and baits used under experimental conditions (Table 2).

Table 2. Results of 2-factor ANOVA for the baits and species used under experimental conditions

Effect	df effect	MS effect	df error	MS error	*F*	*p*-level
Baits	9	25.867	290	0.798	32.419	0.000**
Species	3	147.524	290	0.798	184.892	0.000**
Interaction	27	4.569	290	0.798	5.727	0.000**

df: degree of freedom; MS: mean of squares; *F*: statistic *F*; *p*-level: level of significance; ** $p < 0.01$.

Discussion

For the species of *Pythium* in this study, the plant baits behaved differently with respect to the period or intensity of baiting, i.e. with respect to when and how many baits are invaded. The detection of *Pythium* spp. occured during the first 24 h, or within two days at the outside. To utilize this method of rapid detection, the OTL and HSC baits are recommended.

Some plant baits detect certain *Pythium* spp. better. Accordingly, the BGL baits detected only IRR and APH; baits of CP and PN practically detected only IRR; baits of TL, PF, PL, CF and GL detected IRR and APH better than F. Only OTL and HSC were effective in the equal detection of all three species of *Pythium*. These results suggest that certain baits can be targeted to detect certain species of *Pythium.*

Thus, the plant baiting method has been shown to be valid for the rapid detection of *Pythium* in water used for irrigating hydroponic crops. However, various problems exist in the practical application of this method in the field that cannot be solved in an *in vitro* test, and for which a subsequent adjustment is required. Firstly, these plant baits detect other fungal species (e.g. *Phytophthora* spp.) present in the water (Ponchet et al., 1972). So, if the result of the test is positive, confirmation in the laboratory by a qualified technician would be required. Secondly, the volume of water necessary to obtain a *Pythium* propagule could be too large for the technique to be useful. However, if the aim is to avoid phytosanitary problems by *Pythium* spp. in hydroponic crops, then large volumes of water would not need to be sampled. Further, other authors have noted that if propagules are not detectable in 45.5 ml water, then no loss of crop occurs (nor are there any symptoms of the disease) in cucumber on NFT by APH (Menzies et al., 1996). However, the opposite has also been cited: 240 ml was insufficient in an ebb-and-flow recirculating subirrigation system (Sanogo and Moorman, 1993). Thirdly, the quantity of baits employed could be insufficient to detect the presence of a single *Pythium* propagule in the volume of water under consideration. The number of baits used in this study appears to be adequate (five baits in 10 ml), based on the fact that two pieces of either grass or hemp in 150 ml have been cited as being sufficient (Emerson, 1958).

A further problem for detection in the field could be contamination of the water by other organisms. These could influence the results of the test, for example, by retarding the invasion by *Pythium* spp. A possible solution might be to add fungicides and antibiotics to the water. In this respect, many authors have studied the effect of fungicides and antibiotics on the development of different *Pythium* spp. (Tsao, 1970; Ponchet et al., 1972; Jeffers and Martin, 1986).

Unfortunately, the presence of *Pythium* in the nutrient solution evaluated by the bait procedure does not necessarily mean that there is a real pathological risk for the crop. Indeed, some isolates may not be pathogenic and furthermore the severity of root rot caused by *Pythium* spp. is closely related to the susceptibility of the crop, depending on the environment (Lemanceau, pers. comm.). For instance, intraspecific variation in virulence has been reported for APH on 12 different plant species (McCarter and Littrell, 1970) or tomato (Grover and Dutt, 1973), for IRR on parsley (Cother and Gilbert, 1993), and for irrigation water isolates of *P. catenulatum* from the same region of Almería on cucumber (Sánchez, 1998).

On the other hand, many factors can have a significant influence on the expression of disease caused by *Pythium* spp.: soil moisture, soil temperature, soil pH and presence of specific soil minerals. These environmental parameters can influence fungal growth, development competing or antagonistic micoorganisms, host susceptibility and symptom expression (Martin and Loper, 1999).

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