Species of *Pythium* associated with root-rot of vegetables in South Africa

W.J. Botha* and R.L.J. Coetzter

*Agicultural Research Council, Plant Protection Research Institute, Private Bag X134, Pretoria, 0001 Republic of South Africa
Agrometrics Institute, Private Bag X60, Pretoria, 0001 Republic of South Africa

Received 9 November 1995; Revised 8 May 1996

Different species of *Pythium* associated with root-rot of five vegetables were recorded as new records from South Africa. Mature plants and seedlings were affected. Eight species of *Pythium*, namely, *P. dissotocum* Drechsler, *P. sylvaticum* Campbell & Hendrix, *P. spinosum* Sawada, *affin. P. irregulare* Bubman, *P. aphanidermatum* (Edson) Fitzpatrick, *P. polymastum* Drechsler, *P. myriotylum* Drechsler, *P. mamillatum* Meurs and two heterothallic groups, *T*-group and *F*-group were isolated from rotted roots. Characteristic morphological features were described and the optimum growth temperature was determined.

Keywords: Mature plants, morphological features, *Pythium* spp., root-rot, seedlings, vegetables.

*To whom correspondence should be addressed.

Introduction

Numerous reports of *Pythium* spp., associated with or causing root-rot of cultivated plants in South Africa have been published (Wager 1931, 1932, 1933, 1940; Doidge 1950; Doidge et al. 1953; Götter 1977, 1981, 1982). Denman and Knox-Davies (1992) reviewed in detail diseases caused by *Pythium* spp. in South Africa between 1926 and 1989. Other minor reports include McMartin (1973), Loest (1948), Dyer (1951), Malan (1954), Roth (1965), Verbeek (1975), Darvas (1979), Maas & Kotze (1981) and Lamprecht et al. (1988). Although Wager (1931, 1940) attempted to describe the essential morphological features of some isolated *Pythium* spp., his descriptions were incomplete. Other reports lacked detailed morphological descriptions of the isolates examined. Consequently, this study was undertaken to expand the knowledge with regard to the morphology, temperature growth optima and occurrence of several *Pythium* spp. in South Africa obtained from the rotted roots of vegetables.

Methods

Isolation

Diseased and healthy roots (10 sections of 1 cm each), were washed with distilled water and blotted dry on paper towels. Sections in the transition zone between healthy and diseased tissues were taken and plated on a modified selective medium (Masago et al. 1977) consisting of tolclofos methyl 20 mg, quinotone 50 mg, benomyl 10 mg, nystatin 25 mg, ampicillin 50 mg and rifampicin 20 mg per liter distilled water.

Bacteria-contaminated colonies were purified on 2% water agar with ampicillin 50 mg and rifampicin 20 mg per liter distilled water. Hyphal tips were then transferred to corn-meal agar (CMA) containing 20 mg β-sitosterol and incubated at 25°C in the dark. Cultures were deposited at the National Collection of Fungi, Plant Protection Research Institute (PPIR), Private Bag X134, Pretoria, 0001 South Africa.

Morphological studies

Cultures were maintained on clarified V8-juice agar slopes (Ribeiro 1978) immersed under mineral oil, and in sterile distilled water with colonized wheat blades and hemp seeds (Cannabis sativa L.) at 25°C. The keys compiled by Dick (1990) based on oogonial criteria and the revised key of Plaatse-Niterink (Dick 1990) were used to identify the isolates. Sporangia were produced by placing 5-mm-diam CMA plugs in non-sterile soil extract and incubating under near-UV light at 25°C for 24 h. Zoospores were released after cultures were incubated at 4°C for 4 h and returned to room temperature (Ribeiro 1978). Asexual and sexual morphological structures were studied using a compound microscope and the aplerotic, wall and ooplastic indices were calculated (Dick 1990).

Temperature growth studies

The temperature growth response was determined on CMA plates (9 cm diam) from 5–45°C at 5°C intervals with five replicates at each temperature. Cultures on CMA were incubated at 25°C for 7 days. Agar plugs were cut from the growing edge of the colonies with a 5-mm-diam cork borer and placed in the centre of fresh CMA plates. Radial growth of mycelium was marked on two radial axes on each plate after 24 h and 48 h.

Statistical analysis

The function describing the optimum curve was fitted to the observed growth rates at the different temperatures for each isolate separately. The mean of the replicates was taken as the observed growth rates at the different temperatures to be fitted. The PROC NLIN procedure of the SAS System (1989), using the Gaussian Newton method, was used to fit the function of Keen & Smits (1989). The function describing the optimum curve is:

\[ f(x) = Y_{\text{max}} \sin^2(\pi x); \text{if } 0 \leq x \leq 1; \]

\[ 0; \text{elsewhere} \]

where \( x = \frac{T - T_{\text{min}}}{T_{\text{max}} - T_{\text{min}}} \); \( T \) = temperature; \( u = a + bx + cx^2 \)

The function can be expressed in six parameters: minimum temperature \( T_{\text{min}} \), maximum temperature \( T_{\text{max}} \), optimum temperature for growth \( T_{\text{opt}} \), the maximum growth rate \( Y_{\text{max}} \) and \( p \) and \( c \) which are shape parameters. The first shape parameter \( p \) controls the width of the function and shape of the peak of the curve, while \( c \) controls the width of the curve at low growth rates.

Fitting the curve

Function (1) with \( T_{\text{min}} = 4°C \), \( T_{\text{max}} = 45°C \) and \( c = 2 \) were fitted to the data of most of the isolates. Exceptions included *P. mamillatum* where \( T_{\text{max}} \) was set at 1°C; for *P. spinosum*, \( T_{\text{min}} = 2°C \) and for *P. aphanidermatum*, we specified \( T_{\text{min}} = 50°C \) and \( T_{\text{max}} = 4°C \). Estimates for the three free parameters \( T_{\text{opt}} \), \( Y_{\text{max}} \) and \( p \) were named \( T_{\text{opt}} \), \( Y_{\text{max}} \) and \( p \). Specifying these values for \( T_{\text{min}} \), \( T_{\text{max}} \) and fixing \( c \) gave us convergence for each isolate. Gilbert et al. (1995) allowed \( c \) to vary before they achieved an acceptable fit for most of their data.
Goodness of fit
To determine the goodness of fit of the data to function (1), the residual sum of squares and the coefficient of determination ($R^2$) were determined.

Results
Temperature growth studies
Table 3 depicts the parameter estimates obtained by fitting function (1), with $T_{max}$, $T_{min}$ and $c$ fixed, to the data of each isolate separately. The residual sum of squares was low for all the isolates, indicating that the function fitted the data well. Furthermore, as $R^2$ was close to 1 (100% fit), it was a further indication that the function fitted the data adequately for all the isolates. To examine the goodness of fit visually, the data and fitted curves were plotted against temperature (Figure 14a–m) for all the isolates separately. The Figure indicates that the fitted curves followed the data well. As $c$ was fixed for all the isolates, the curves initially climbed steeply in all cases, except for $P. applanidentum$ (PRRI 5257 and PRRI 5256; Figure 14i, k). The different values of $p$ were reflected in the variable shapes (width and peak) of the fitted curves. The optimum growth temperatures for the two isolates of $P. spinosum$, $P. irregulare$ and $P. applanidentum$ were each similar, but the growth rate (mm h$^{-1}$) differed.

Morphological studies
The $Pythium$ spp. isolated from rotted roots of vegetables, as well as the host plants, are listed in Table 1. Additional hosts are listed in Plants-Niterink (1981). $Pythium$ isolates could not be obtained from pieces of healthy root tissue placed on selective medium. Oospores were produced by isolates on CMA with β-sitosterol, and sporangia were formed in non-sterile soil extract under UV light (Figures 1–13). Measurements of oospores, oogonia, ooplasms, wall thickness of oospores and indices of oogonial criteria are listed in Table 2. Measurements and indices of $P. spinosum$, $P. irregulare$ and $P. applanidentum$ are the means of two isolates for each species.

Table 1 List of $Pythium$ spp. associated with root-rot of vegetables in South Africa

<table>
<thead>
<tr>
<th>PPRI no.</th>
<th>Species</th>
<th>Host</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5354</td>
<td>$P. dissotocum$</td>
<td>Pea</td>
<td>George, E. Cape</td>
</tr>
<tr>
<td>5258</td>
<td>$P. sylvaticum$</td>
<td>Lettuce</td>
<td>Western Cape</td>
</tr>
<tr>
<td>5260</td>
<td>$P. spinosum$</td>
<td>Cucumber</td>
<td>Muldersdrift, Gauteng</td>
</tr>
<tr>
<td>5614</td>
<td>$P. spinosum$</td>
<td>Cucumber</td>
<td>Muldersdrift, Gauteng</td>
</tr>
<tr>
<td>5393</td>
<td>$P. mamillatum$</td>
<td>Pea</td>
<td>George, E. Cape</td>
</tr>
<tr>
<td>5259</td>
<td>$P. irregulare$</td>
<td>Cucumber</td>
<td>Muldersdrift, Gauteng</td>
</tr>
<tr>
<td>5616</td>
<td>$P. irregulare$</td>
<td>Pea</td>
<td>George, E. Cape</td>
</tr>
<tr>
<td>5256</td>
<td>$P. applanidentum$</td>
<td>Cucumber</td>
<td>Muldersdrift, Gauteng</td>
</tr>
<tr>
<td>5257</td>
<td>$P. applanidentum$</td>
<td>Phaseolus vulgaris</td>
<td>Pietersburg, N. Prov.</td>
</tr>
<tr>
<td>5359</td>
<td>$P. polymastum$</td>
<td>Cabbage</td>
<td>Brits, N. West</td>
</tr>
<tr>
<td>5848</td>
<td>$P. myriotylum$</td>
<td>Cabbage</td>
<td>Brits, N. West</td>
</tr>
<tr>
<td>5612</td>
<td>$P. myriotylum$</td>
<td>Cucumber</td>
<td>Rustenburg, N. West</td>
</tr>
<tr>
<td>5358</td>
<td>$P. myriotylum$</td>
<td>Arum esculentum</td>
<td>Rustenburg, N. West</td>
</tr>
</tbody>
</table>

$Pythium$ dissotocum Drechsler
Colonies on potato–carrot agar with no special pattern, with downy, low aerial mycelium; on corn-meal agar no aerial mycelium, coarsely radiate pattern; main hyphal diameter 6 μm; sporangia filamentous, slightly inflated, branched, long-digitate, dendroid, oospores discharged through long evacuation tubes, encysted zoospores 10-11 μm diam; oogonia subglobose, terminal, intercalary or lateral, including a small part of the supporting hypha, 21–28 (av. 24.5 μm) diam; antheridia 1–2 per oogonium, monoclinous or diclinous, originating from immediately below the oogonium, crooked-necked and inflated, attached broadly apical to the oogonium, 6–8 × 12–15 μm; oospores aplerotic, 18.5–25 (av. 21.7 μm) diam, abortive oospores numerous; wall

Table 2 Oogonial criteria of $Pythium$ spp. associated with root-rot of vegetables

| Criteria: | Oospore Oogonium Wall Ooplast |
|-----------|-----------------------------|----------------------|------------------------|
|           | data           | data               | data               |
| $P. dissotocum$ | 18.5–25 | 21–28 | 2.0–3.0 | 8–11 |
| $P. sylvaticum$ | 14.5–22 | 15–25.5 | 1.8–2.0 | 6–9 |
| $P. spinosum$ | 17–22.5 | 18.5–23 | 0.9–1.2 | 9–12 |
| $P. irregulare$ | 20–42 | 24–49 | 0.8–1.0 | 12–20 |
| $P. mamillatum$ | 40–59 | 48–65 | 2.0 | 20–28 |
| $P. applanidentum$ | 35–45.5 | 39.5–53 | 1.2–3.0 | 18–27 |

Criteria: $^*$range in measurements (μm); $^\dagger$(x) = mean (μm); % aplerotic index; % wall index; % ooplast index;
$^\ddagger$P. mamillatum with ornamented oogonia;
$^\S$P. mamillatum with smooth oogonia;
up to 3.0 μm thick; ooplast 9–11 (av. 10.1 μm) diam; cardinal temperatures: minimum 5°C, optimum 26.3°C, maximum 40°C; daily growth on corn-meal agar at 26.3°C, 12.3 mm.

**Pythium sylvaticum** Campbell & Hendrix

Colonies on corn-meal agar mainly submerged with no special pattern; on potato–carrot agar appressed with thin, cottony aerial mycelium, no special pattern; main hyphae up to 10 μm wide; sporangia and zoospores not observed; hyphal bodies numerous, globose to ovoid, intercalary and terminal, 11–40 μm; oogonia were smooth, (sub)spherical, produced in single cultures, terminal and intercalary, 15–25.5 (av. 20.2 μm) diam; antheridia 1–3 per oogonium, diclinous with branched stalks, bifurcated near the oogonium, narrow apical attachment, 5–7 × 11–21 μm;

Oospores aplerotic, 14.5–22 (av. 18.2 μm) diam, wall up to 2.0 μm thick; ooplast, 6–9 (av. 7.5 μm) diam; cardinal temperatures: minimum <10°C, optimum 22°C, maximum >30°C; daily growth on corn-meal agar at 22°C, 9.1 mm.

**Pythium spinosum** Sawada

Colonies on corn-meal agar coarsely radiate with floccose aerial mycelium, aerial mycelium dense on potato–carrot agar; main hyphal diameter up to 10 μm; sporangia spherical, 9–31 μm with short discharge tubes, 5–7 × 11–18 μm; encysted zoospores, 6–9 μm diam; hyphal bodies numerous, terminal and intercalary, spherical or limoniform, up to 31 μm; vegetative hyphae with distinct spine-like projections; antheridia 1–3 per oogonium, mainly monolocinous, 6–7 × 10–12 μm, not inflated; oogonia terminal and intercalary, spherical or fusiform, 18.5–23 (av. 20.7 μm) diam; oogonial ornamentations long digitate, evenly spread, 1.5–2 × 6–12 μm; oospores mostly aplerotic, 17–22.5 (av. 19.8 μm) diam, wall 0.9–1.2 (av. 1.0 μm); ooplast, 9–12 (av. 10.6 μm) diam; cardinal temperatures: minimum 5°C, optimum 23°C, maximum 35°C; daily growth on corn-meal agar at 23°C, 19.6 mm.

'afin. **Pythium irregulare**' Buisman

Colonies on corn-meal agar cottony with aerial mycelium at the margin, with dense aerial mycelium on potato–carrot agar; main hyphal diameter up to 7 μm wide; sporangia globose, terminal and intercalary, 10–19 μm diam; encysted zoospores, 8–10 μm diam; hyphal bodies numerous, mainly intercalary, globose, ellipsoid, limoniform and of irregular shape; oogonia globose to irregular, mainly intercalary, 24–49 (av. 36.5 μm) diam with blunt conical projections of variable length and number on some oogonia; oospores aplerotic, 20–42 (av. 31 μm) diam, wall mostly 1.0 μm thick; ooplast, 12–20 (av. 16 μm) diam; cardinal temperatures: minimum <10°C, optimum 22.6°C, maximum >35°C; daily growth on corn-meal agar at 22.6°C, 13.1 mm.

**Pythium aphanidermatum** (Edson) Fitzp.

Colonies on corn-meal agar submerged with a fine distinct radiate pattern; on PCA the aerial mycelium is floccose; main hyphae up to 10 μm wide; sporangia forming complexes of lobulate, inflated elements; encysted zoospores 10–12 μm diam; oogonia terminal, globose, 48–65 (av. 56.5 μm) diam; antheridia monolocinous or diclinous, mostly intercalary, sac-shaped, 1–2 per oogonium, attached broadly apical, mostly 10 × 20 (–25) μm; oospores aplerotic, 40–59 (av. 49.5 μm) diam; ooplast, 20–28 (av. 24 μm) diam; cardinal temperatures: minimum 10°C, optimum 34.1°C, maximum >40°C; daily growth on corn-meal agar at 34.1°C, 26.0 mm.

Figure 14  Temperature growth response of various Pythium spp. based on observed data (+) and fitted curves (0) using the function of Keen & Smits (1989). (a) Pythium T-group; (b) Pythium F-group; (c) P. irregulare; (d) P. sylvaticum; (e) P. spongiosum; (f) P. distotocum; (g) P. mamillatum; (h) P. spinosum; (i) P. aphanidermatum; (j) P. irregulare; (k) P. aphanidermatum; (l) P. myriotylum; (m) P. polymastum.
Pythium polymastum Drechsler
Colonies on corn-meal agar no special pattern with no aerial mycelium; similar appearance on PCA; main hyphae 7 μm wide. Sporangia mostly subglobose to ellipsoid, some empty sporangial envelopes with an undulate contour, 7–15 × 9–16 μm diam; encysted zoospores up to 16 μm diam; oogonia terminal, subglobose, 39.5–53 (av. 46.2 μm) diam, ornamented with numerous conical to mammiform protuberances, 4–6 μm wide at the base, 1 μm wide at the tip, up to 9 μm long; antheridia 1–3 per oogonium, diclinous, broadly apical attached of irregular shape with hyphal projections, often one antheridium entangling the stalk and base of the oogonium, 11–15 × 15–18 μm; zoospores aplerotic, 35–45.5 (av. 40.2 μm) diam, wall up to 3.0 μm thick; oospore 18–27 (av. 22.5 μm) diam; cardinal temperatures: minimum <10°C, optimum 22.3°C, maximum >35°C; daily growth on corn-meal agar at 22.3°C, 16.0 mm.

Pythium myriotylum Drechsler
Colonies on corn-meal agar no special pattern with loose, fluffy aerial mycelium; on PCA no special pattern with floccose aerial mycelium; main hyphal diameter up to 7–9 μm wide; aposporia in clusters, clavate to sickle-shaped, clearly visible through bottom of Petri-dish; sporangia filamentous, inflated, consisting of lobulate to digitate elements with long discharge tubes; encysted zoospores, 6–9 μm diam; oogonia terminal or intercalary, (sub)globose, 27–38 (av. 32.5 μm) diam; antheridia 3–6 per oogonium, mostly diclinous, stalks branched, enveloping oogonium, antheridial cells crook-necked making narrow apical contact with oogonium; oospores aplerotic, 22.5–29 (av. 25.7 μm); wall 1.2–3.0 μm wide; oospore 10.5–16 (av. 13.2 μm) diam; cardinal temperatures: minimum <10°C, optimum 29.3°C, maximum >40°C; daily growth on corn-meal agar at 29.3°C, 13.4 mm.

Table 3 Parameter estimates obtained by fitting function (1), with \( T_{min} \), \( T_{max} \), and \( c \) fixed, to the data for each isolate separately

<table>
<thead>
<tr>
<th>Isolate</th>
<th>( \hat{\eta}_{opt} )</th>
<th>( \hat{\phi}_{max} )</th>
<th>( \hat{\beta} )</th>
<th>( RSS )</th>
<th>( R^2 )</th>
<th>Time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. dissotocum</td>
<td>26.309</td>
<td>0.513</td>
<td>1.1399</td>
<td>0.019</td>
<td>0.93</td>
<td>83.0</td>
</tr>
<tr>
<td>P. sylvaticum</td>
<td>22.129</td>
<td>0.380</td>
<td>3.617</td>
<td>0.018</td>
<td>0.90</td>
<td>112.0</td>
</tr>
<tr>
<td>P. spinosum</td>
<td>22.863</td>
<td>0.671</td>
<td>3.811</td>
<td>0.071</td>
<td>0.88</td>
<td>63.0</td>
</tr>
<tr>
<td>P. spinosum</td>
<td>23.066</td>
<td>0.961</td>
<td>3.400</td>
<td>0.092</td>
<td>0.90</td>
<td>44.0</td>
</tr>
<tr>
<td>P. irregularare</td>
<td>23.715</td>
<td>0.578</td>
<td>2.177</td>
<td>0.013</td>
<td>0.97</td>
<td>74.0</td>
</tr>
<tr>
<td>P. irregularare</td>
<td>21.507</td>
<td>0.514</td>
<td>2.170</td>
<td>0.035</td>
<td>0.89</td>
<td>83.0</td>
</tr>
<tr>
<td>P. ophanidermatum</td>
<td>34.477</td>
<td>0.957</td>
<td>2.498</td>
<td>0.189</td>
<td>0.83</td>
<td>44.0</td>
</tr>
<tr>
<td>P. ophanidermatum</td>
<td>33.808</td>
<td>1.211</td>
<td>3.907</td>
<td>0.117</td>
<td>0.92</td>
<td>35.0</td>
</tr>
<tr>
<td>P. polymastum</td>
<td>22.291</td>
<td>0.668</td>
<td>4.773</td>
<td>0.013</td>
<td>0.97</td>
<td>64.0</td>
</tr>
<tr>
<td>Pythium F-group</td>
<td>24.104</td>
<td>0.536</td>
<td>1.793</td>
<td>0.010</td>
<td>0.97</td>
<td>80.0</td>
</tr>
<tr>
<td>Pythium T-group</td>
<td>30.547</td>
<td>0.611</td>
<td>1.546</td>
<td>0.013</td>
<td>0.96</td>
<td>70.0</td>
</tr>
<tr>
<td>P. irregulare</td>
<td>29.292</td>
<td>0.561</td>
<td>2.221</td>
<td>0.012</td>
<td>0.97</td>
<td>76.0</td>
</tr>
<tr>
<td>P. mamillatum</td>
<td>21.675</td>
<td>0.619</td>
<td>3.199</td>
<td>0.038</td>
<td>0.91</td>
<td>69.0</td>
</tr>
</tbody>
</table>

\( T_{opt} \) = estimate of optimum temperature; \( \hat{\gamma}_{max} \) = maximum growth rate (mm h\(^{-1}\)) of mycelium at optimum temperature; \( \hat{\beta} \) = estimate of shape parameter; \( R^2 \) = coefficient of determination; \( RSS \) = residual sum of squares; time (h) for colony to reach margin of dish.
Pythium mamillatum Meurs

Colonies on corn-meal agar with a coarsely radiate pattern and some loose aerial mycelium at the margin; on PCA a dense, fluffy aerial mycelium; main hyphae up to 8 μm wide; sporangia spherical to ovoid, intercalary or terminal with short discharge tubes, 14–17 x 16–22 μm diam; encysted zoospores 9–10 μm diam; oogonia terminal or intercalary, 14–23.5 (av. 18.8 μm) diam, ornamented with conical protuberances, frequently curved, of variable length, up to 8 μm long and 2–3 μm at the base; smooth oogonia observed, mostly terminal, 17–24.5 (av. 20.7 μm) diam; antheridia 1–2 per oogonium, monolocular, cells clavate making narrow apical content with oogonium; oospores plectotic for both ornamented and smooth oogonia, 13–20 (av. 16.5 μm) diam and 14–21 (av. 17.5 μm) diam respectively; wall thickness, 0.7–1.0 μm and 0.5–1.0 μm respectively; ooplaasts of ornamented and smooth oogonia, 8–10 (av. 9.0 μm) diam and 4–6 (av. 5.0 μm) diam respectively; cardinal temperatures: minimum 5°C, optimum 21.6°C, maximum > 35°C; daily growth on corn-meal agar at 21.6°C, 14.8 mm.

Pythium F-group

Colonies on corn-meal agar with a fine radiate pattern, no aerial mycelium; on PCA no special pattern with a thin aerial mycelium; main hyphal diameter 4–5 μm; sporangia filamentous, slightly inflated, branched, dendroid, with long-digitate elements up to 8 μm wide; discharge tubes long, encysted zoospores 7–9 μm diam; no oogonia produced in single cultures; cardinal temperatures: minimum <10°C, optimum 24.1°C, maximum > 40°C; daily growth on corn-meal agar at 24.1°C, 12.8 mm.

Pythium T-group

Colonies on corn-meal agar with a distinct radiate pattern, no aerial mycelium; on PCA a mixed rosette, indistinct chrysanthemum pattern; main hyphae up to 5 μm wide; sporangia consisting of lobulate, inflated elements; encysted zoospores up to 9 μm diam; no oogonia produced in single cultures; cardinal temperatures: minimum <10°C, optimum 30.5°C, maximum >40°C; daily growth on corn-meal agar at 30.5°C, 14.6 mm.

Discussion

Characteristic of P. dissotocum were: (i) the oogonia which included a small part of the subtending hypha, (ii) sessile, sac-like antheridia immediately below the oogonium, and (iii) slightly inflated, dendroid sporangia. The mean diameter of sexual organs of isolate PPRI 5394 was slightly larger than the mean diameters recorded by Drechsler (1940) and Plaat-Niterink (1981). Mostly two antheridia per oogonium were observed, not three as originally described (Drechsler 1940), but this trait could be variable.

Typical for P. sylvaticum were the branched antheridal stalks which bifurcate near the base of the oogonium. Hendrix and Campbell (1974) and Kageyama et al. (1991) reported much larger dimensions for sexual organs. However, mean diameters of the sexual organs recorded by Plaat-Niterink (1981) were closer to the diameters observed for isolate PPRI 5258.

Characteristic of the South African isolates of P. spinosum was the distinct cylindrical, blunt, digitate oogonial projections of the oogonial wall, as well as similar spine-like projections on some vegetative hyphae. Numerous terminal and intercalary hyphal bodies were also present. No smooth-walled oogonia were observed. Dimensions of sexual organs were in the size range as recorded by Plaat-Niterink (1981), except for the presence of sporangia and zoospores observed in South African isolates (Botha 1993). P. spinosum has also been reported in South Africa from Medicago spp. (medics) (Lamprecht et al. 1988) and papaya (Wagger 1931) and P. irregularare from lucerne (Scott 1987), peach (Verbeek 1975) and wheat (Scott 1987), to name but a few hosts.

South African isolates of P. mamillatum were characterized by the distinctly conical, frequently curved oogonial ornamentations, which differed from the well-defined cylindrical projections of P. spinosum. No projections occurred on the vegetative hyphae. In older cultures of P. mamillatum, some smooth-walled, smaller oogonia were observed, which was also recorded by Plaat-Niterink (1981). Dimensions of sexual organs of isolate PPRI 5393 were smaller than the dimensions recorded by Meurs (1929), but similar to those recorded by Plaat-Niterink (1981).

Hendrix and Papa (1974) regarded P. irregularare, P. spinosum, P. acanthicum and P. mamillatum as a species complex which they considered as closely related or even conspecific. This was proved for a few isolates of P. spinosum and P. irregularare by Chen et al. (1992), using molecular methods. However, many isolates of P. spinosum and P. mamillatum from many sources worldwide need to be investigated and compared at a molecular level, using appropriate techniques and applicable statistical analysis methods, before the two species can be retained as separate species or considered as conspecific.

Typical of the Pythium irregularare-like isolates were the highly variable size and shape of the oogonia as well as the number of oogonial projections (Biesbrock & Hendrix 1967, Plaat-Niterink 1981). Ornamented oogonia were only produced in water cultures with soil extract, which was also observed by Plaat-Niterink (1981). Much larger oogonial and oospore diameters were observed for isolate PPRI 5259 than described by Siders (1932) and Plaat-Niterink (1981). The uncertain taxonomic status of P. irregularare, as an acknowledged species, was confirmed by Hendrix & Papa (1974) with its inclusion in a P. irregularare species complex. In addition, Chen et al. (1992) have shown that P. spinosum and P. irregularare were closely related, based on restriction analysis of transcribed ribosomal DNA. However, before the taxonomic status of all described species of the genus Pythium can be clarified, many isolates from many sources and regions need to be investigated with the aid of appropriate molecular methods.

Isolates of Pythium aphanidermatum were characterized by the presence of intercalary, inflated antheridia and inflated filamentous sporangia. Thermophilic characteristics were revealed in optimum growth at 34°C and a high daily growth rate of 26 mm day⁻¹ at 34°C. Much larger oogonia and oospores were observed for isolates PPRI 5267 and 5257 than the diameters recorded by Plaat-Niterink (1981), although the antheridal sizes were similar. The isolates examined were distinguished from P. deliense by the oogonial stalks which did not curve towards the antheridia and by larger antheridia (Plaat-Niterink 1981).

Typical of P. polyblastum was the presence of large oogonia, aplerotic oospores and 1–4 antheridia per oogonium. Some of the empty sporangial envelopes showed an undulate contour, a feature also observed by Drechsler (1939). A closely related species, P. mastophorom, differed from P. polyblastum by the presence of smaller oogonia and the nearly aplerotic oospores with a single antheridium (Plaat-Niterink 1981). Dimensions of sexual organs were almost identical to the dimensions reported by Drechsler (1939).

The main distinction between isolates in groups F & T was the structure of the filamentous sporangia. Isolate PPRI 5848 in Group F produced filamentous sporangia which were only slightly inflated, with branched long-digitate elements. Isolate PPRI 5612 in Group T produced inflated filamentous sporangia with lobulate elements. Catenulate hyphal bodies and oogonia were absent, indicating that this isolate could not be P. catenulatum Matthews.
Characteristic features of *P. myriotylium* were the presence of clusters of elavate, knob-like appressoria, visible through the bottom of the Petri-dish, the filamentous, inflated sporangia and the antheridia with branched antheridial stalks, enveloping the oogonium. Dimensions of sexual organs fell within the range recorded by Plaat-Niterink (1981), but were slightly larger than the diameters reported by Drechsler (1943) and Meurs (1934) for this species. The presence of smooth oogonia in older cultures was also observed by Plaat-Niterink (1981).

The *Pythium*-host associations reported in this investigation are new records for South Africa. Apart from isolates PPRI 5359 and 5848 from cabbage seedlings, all isolates were isolated from infected roots of mature plants. The prevalence of *Pythium* root-rot on mature vegetable plants should be stressed. Isolates examined in this report were acquired over a period of less than a year and caused substantial losses for producers (A. Thompson, pers. commun.). Wagner (1940) previously reported other hosts infected with *Pythium* spp., namely *Carica papaya* L., *Cucurbita pepo* L., *Nicotiana tabacum* L., to name but a few. *P. aphanidermatum*, *P. spinosum*, *P. irregulare* and *P. myriotylium* were isolated from some of these hosts. *P. myriotylium* was isolated from soil (Darvas et al. 1978; Scott 1987).

Acknowledgements

The authors thank M.J.J. Jansen van Rensburg for assistance with the statistical analyses and Alistair Thompson of the Vegetable and Ornamental Plant Institute, ARC, for the provision of most of the isolates examined in this study.

References


DICK, M.W. 1990. Keys to *Pythium*. Published by the author, Reading, UK.


