Assessment of host specificity among different species of glyphosate synergistic *Pythium*

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A total of 39 *Pythium* isolates representing 14 species of *Pythium* was assessed for host specificity as glyphosate synergists. This was done using three groups of *Pythium* isolates from roots of glyphosate-treated bean (PBI), wheat (PWI), and isolates from various glyphosate-untreated hosts. PBI consisted of 15 isolates and included *P. ultimum, P. sylvaticum, P. coloratum, P. irregularare* and *P. group 'HS'*. PWI consisted of 14 isolates representing the first four of these species. *Pythium* from glyphosate-untreated hosts included single isolates representing *P. aphanidermatum, P. spinosum, P. paroecandrum, P. hypogynum, P. splendens, P. sulcatum, P. vanterpooli, P. acanthicum, P. arhenomanes* and *P. coloratum*. The glyphosate synergistic potential of the *Pythium* isolates was determined by treating 2-week-old seedlings growing in soil infested with individual isolates of *Pythium* with different doses of glyphosate. LD₅₀ values associated with each isolate were estimated by logistic regression analysis of plant mortalities recorded 4 wk after treatment with glyphosate, and compared with LD₅₀ values for plants grown in the absence of *Pythium*. Host specificity was assessed by comparing the glyphosate synergistic potential of PBI on bean and PWI on wheat seedlings, with the potential of these same isolates of PBI on wheat and PWI on bean seedlings. Glyphosate synergistic potential of PBI was also estimated on sunflower and pepper, to test whether PBI were capable of glyphosate synergistic interaction (GSI) on other unrelated dicot yledonous species. *Pythium* isolates from glyphosate-untreated hosts were tested on bean to determine if *Pythium* species not represented in the PBI and PWI groups were capable of GSI.

The glyphosate synergistic potentials of the PBI and PWI on wheat seedlings were low and inconsistent compared to those observed on dicot plants. All PWI and 12 of the 15 PBI were glyphosate synergists on beans, and all the PBI were glyphosate synergistic on sunflower and pepper seedlings. All *Pythium* isolates from glyphosate-untreated sources tested were also glyphosate synergists on bean seedlings. These various tests of glyphosate synergistic potential of *Pythium* isolates from diverse sources on various plant species revealed no evidence of host specificity among the isolates and species tested.

Soilborne species of *Pythium* have been shown to augment the herbicidal activity of glyphosate by colonizing the roots of glyphosate-treated plants. This enhancement of herbicidal efficacy was termed glyphosate synergistic interaction (GSI) by Johal & Rahe (1984). Soilborne *Pythium* spp. were found to be the first and predominant root colonizers of glyphosate treated plants grown in different soils (Lévesque et al., 1993). Several different species of *Pythium* were involved in GSI on bean (Descalzo et al., 1996). A possible indication of host-specificity of some glyphosate synergistic *Pythium* (GSP) was suggested from the result of an experiment by Lévesque, Rahe & Eaves (1992), in which *Pythium* isolates previously collected from the roots of glyphosate-treated apple and bean seedlings were glyphosate synergists only on the plant species from which the fungus originated. No other tests conducted so far substantiate this initial suggestion of host specificity among glyphosate synergistic *Pythium* (GSP). This paper describes the results from research to address the host specificity of GSP. The specific objectives were to determine if: (i) isolates of *Pythium* species from the roots of 2-week-old glyphosate-treated bean and wheat seedlings were selectively synergistic on the host species from which they were isolated; (ii) isolates of *Pythium* species from the roots of glyphosate-treated bean seedlings were capable of GSI on various unrelated dicot yledonous species; (iii) isolates of *Pythium* species from glyphosate-untreated hosts were capable of GSI on bean seedlings; and (iv) the relative pathogenicity of isolates of *Pythium* species differed on glyphosate-untreated hosts.

MATERIALS AND METHODS

Sources of *Pythium* isolates

Isolates of five *Pythium* species previously collected from the roots of glyphosate-treated bean and wheat seedlings grown separately in various soils were used (Lévesque et al., 1993; Descalzo et al., 1996). *Pythium* isolates obtained from glyphosate-treated beans or wheat are referred to hereafter as...
**Glyphosate synergistic Pythium**

**Table 1.** Pythium species and the numbers of genotypes represented among isolates obtained from the roots of glyphosate-treated bean and wheat seedlings

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. ultimum</em> Trow</td>
<td>Bean 3, Wheat 4</td>
</tr>
<tr>
<td><em>P. sylvaticum</em> W. A. Campb. &amp; F. Hendrix</td>
<td>6, 7</td>
</tr>
<tr>
<td><em>P. coloratum</em> Vaartaja</td>
<td>2, 2</td>
</tr>
<tr>
<td><em>P. irregularis</em> Buismann</td>
<td>2, 1</td>
</tr>
<tr>
<td><em>P. group</em> 'HS'</td>
<td>2, 0</td>
</tr>
<tr>
<td>Total</td>
<td>15, 14</td>
</tr>
</tbody>
</table>

* Bean seedlings were grown in soils collected from Summerland and Fraser Valleys, B.C., Canada.
* Wheat seedlings were grown in soils collected from Fraser Valley, B.C., Canada.

**Pythium** bean isolates (PBI) and Pythium wheat isolates (PWI). The Pythium genotypes were defined on the basis of restriction fragment length polymorphisms (RFLP) of the total DNA (Lèvesque et al., 1993; Descalszo et al., 1996). The number of genotypes represented per Pythium species is shown in Table 1. To indicate the original source of the isolates of the same *Pythium* species obtained from glyphosate-treated bean or wheat seedlings in the succeeding Tables and Figures, letters B or W was placed after the RFLP type designation. Except for *P. acanthicum* which was isolated from soil, all other *Pythium* species in these collections were originally isolated from the roots of various host plants that were untreated with glyphosate (Table 2).

**Determination of glyphosate LD₉₀ values for plants grown in soils infested with various Pythium isolates**

The relative efficacy of the different *Pythium* isolates to act as glyphosate synergisers on various plant species was assessed by estimating the glyphosate LD₉₀ values associated with each *Pythium* isolate on each of the host species. LD₉₀ values were estimated using PBI on bean seedlings, *Phaseolus vulgaris* L. cv. Topcrop (Descalszo et al., 1996) and PWI on winter wheat seedlings, *Triticum aestivum* L. cv. Northstar. Reciprocal tests were then conducted using PBI on wheat and PWI on bean seedlings to determine whether the *Pythium* isolates were specific glyphosate synergisers for the host plant species from which they were initially isolated. GSI tests were also done using PBI on two dicot hosts: sunflower, *Helianthus annuus* L. cv. Sunwheat 101 and pepper, *Capsicum frutescens* cv. California Wonder. The final GSI tests were done to determine whether other *Pythium* species from various glyphosate-untreated hosts and from soil were also capable of GSI on bean seedlings.

The method used for all GSI tests consisted of planting surface-sterilized seeds in plastic trays (54 x 28 x 7 cm) filled with 3-5 kg autoclaved clay loam soil (OM = 22%, pH = 5.0). Twenty seeds were planted in each of six equally spaced rows oriented across the width of each tray. The plants were grown in a growth room under a 16:8 h, day:night photoperiod and 25:19'C temperature regimes. The light intensity at plant height was 230 µmol m⁻² s⁻¹. The soil was kept moist by addition of distilled water as needed. The plants were watered with a dilute solution made up of 4 g 20-20-20 fertilizer 1⁻¹ distilled water 1 wk after planting.

Inoculum was made by growing individual *Pythium* isolates in stationary culture in V8-cholesterol broth (Ayers & Lumsden, 1975) for 8 d. The cultures were incubated in the dark at 20-23°C. The mycelium was decanted into a Büchner funnel lined with filter paper and rinsed with sterile distilled water. The mycelial mat was aseptically cut into nominal 1 cm² square pieces with a scalpel. The mycelium pieces were suspended in 0-08% sterile water agar (WA) at 1 g 100 ml⁻¹, and macerated in a Sorvall blender for 20 s. Inoculum was applied 1 d before glyphosate treatment by drenching 25 ml of the mycelial suspension between each row of seedlings when the seedlings had two fully expanded primary leaves (2 wk after seeding).

Glyphosate treatments were made 1 d later as two 1 µl droplets of aqueous dilutions of Roundup® herbicide (360 g a.i. 1⁻¹) applied to the stem at the cotyledonal node for dicot yledonous plants and in the whorl of the wheat plants. Different glyphosate doses were assigned at random to each row of plants, but all plants in a row received the same dose. Doses of 0, 2, 6, 20, 50 and 150 µg glyphosate a.i. per plant were used for the *Pythium* inoculated bean, sunflower and pepper plants. A lower range of doses (0, 0.5, 1.5, 5, 15 and 40 µg glyphosate a.i. per plant) was used for wheat seedlings. To obtain 100% mortality at the highest glyphosate dose, *Pythium* uninoculated plants (controls) had to be treated with glyphosate ranging from 0 to 1200 µg glyphosate a.i. per plant for dicot yledonous seedlings and 0-60 µg glyphosate a.i. per plant for wheat seedlings. The proportions of plants

**Table 2.** Isolates of different *Pythium* species obtained from hosts and soil that were not treated with glyphosate

<table>
<thead>
<tr>
<th>Source</th>
<th>Place of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aphanidermatum</em></td>
<td>English cucumber</td>
</tr>
<tr>
<td>(Edson) Fitzp.</td>
<td>B.C., Canada</td>
</tr>
<tr>
<td><em>P. sulcatum</em></td>
<td>Carrot</td>
</tr>
<tr>
<td>R. C. Pratt &amp; J. E. Mitchell</td>
<td>B.C., Canada</td>
</tr>
<tr>
<td><em>P. hypogynum</em></td>
<td>Lamb's-quarters</td>
</tr>
<tr>
<td>Middleton</td>
<td>Quebec, Canada</td>
</tr>
<tr>
<td><em>P. coloratum</em></td>
<td>Alfalfa</td>
</tr>
<tr>
<td>Vaartaja</td>
<td>Ontario, Canada</td>
</tr>
<tr>
<td><em>P. splendens</em></td>
<td>Blueberry</td>
</tr>
<tr>
<td>Braun</td>
<td>Nova Scotia, Canada</td>
</tr>
<tr>
<td><em>P. acanthicum</em></td>
<td>Soil</td>
</tr>
<tr>
<td>Drechsler</td>
<td>Ontario, Canada</td>
</tr>
<tr>
<td><em>P. arrhenomanes</em></td>
<td>Oat</td>
</tr>
<tr>
<td>Drechsler</td>
<td>Manitoba, Canada</td>
</tr>
<tr>
<td><em>P. spinosum</em></td>
<td>Turfgrass</td>
</tr>
<tr>
<td>Sevada</td>
<td>North Carolina, U.S.A.</td>
</tr>
<tr>
<td><em>P. purpureum</em></td>
<td>Turfgrass</td>
</tr>
<tr>
<td>Drechsler</td>
<td>North Carolina, U.S.A.</td>
</tr>
<tr>
<td><em>P. suteriopollis</em></td>
<td>Turfgrass</td>
</tr>
<tr>
<td>V. Kouyeas &amp; H. Kouyeas</td>
<td>North Carolina, U.S.A.</td>
</tr>
</tbody>
</table>

*Pythium* isolates from Canada were provided by Dr. Donald J. S. Barr of Biosystematic Research Institute, Ottawa, Canada, and isolates from U.S.A. were from Dr. Gloria Abad, North Carolina, State University, U.S.A.
killed by each dose were recorded 4 wk after treatment with
glyphosate. One tray was used for each Pythium isolate per
experiment and each experiment was repeated a minimum of
two times.

Plant mortality data for each experiment were modelled by
standard backward regression method with logistic regression
program (Baker & Nelder, 1987) using the computation
method for LD_{so} and the associated variance described
previously (Rahe, Lévesque & Johal, 1990; Lévesque, Rahe &
Eaves, 1992). Glyphosate LD_{so} values for plants inoculated
with different Pythium isolates were compared with the
control using Bonferroni’s test (Neter, Wasserman & Kutner,
1990).

Pathogenicity of Pythium isolates

The ability of different Pythium isolates to cause infection of
germinating seeds was assessed by growing individual isolates
for 5 d on 16 ml solidified potato dextrose agar (PDA)
contained in 100 x 15 mm Petri dishes. The culture surface
was then covered with sterilized peat-based potting mix
(Metro-mix™, grade 290) to a depth of 5-6 mm. Twenty
surface sterilized bean, wheat, sunflower, or pepper seeds were
put on the surface of the Metro-mix and additional Metro-mix
was added to barely cover them. Approximately 20 ml of
sterilized distilled water was added and the plates were placed
in individual plastic bags and kept for 7 d at the same
conditions used to grow plants, after which the numbers of
ungerminated seeds, and dead and living seedlings in each
Petri dish were recorded. Three to five plates were used for
each Pythium isolate in each replicate experiment. The dead
plants were counted 4 wk after inoculation and ANOVA was
performed on arcsine transformed data using SAS Statistical

RESULTS

There were significant interactions between the replicate
experiments and various Pythium isolates in all GSI tests. It was,
therefore, necessary to analyse each experiment separately.

Effect of PBI on bean and wheat seedlings

The effect of different Pythium isolates on glyphosate LD_{so}
values on beans was described by Descalzo et al. (1996). The
PBI GSI tests on wheat seedlings gave an inconsistent GSI
response in three replicated experiments (Fig. 1). In two out of
three experiments, plants inoculated with P. irregulare RFLP
type B1; P. sylvaticum RFLP types B1 and B2; and P. coloratum
RFLP type B2 had significantly (P ≤ 0.05) lower LD_{so} values
than the control. In one out of three experiments, plants
inoculated with Pythium ultimum RFLP types B1a and B1b; P.
irregulare RFLP type B2; P. ′HS’ RFLP types B1 and B2; P.
sylvaticum RFLP types B3, B4, B5 and B6; and P. coloratum
RFLP type B1 had significantly lower LD_{so} values than the
control. However, it was also observed that plants inoculated
with P. ultimum RFLP type B2; P. irregulare RFLP type B2; P.
coloratum RFLP B1; P. ′HS’ RFLP type B1; and P. sylvaticum
RFLP types B3 and B6; as well as those inoculated with P.
ultimum RFLP type B1b; P. coloratum RFLP type B2 and P.
sylvaticum RFLP type B4 required similar amounts of
glyphosate to kill 50% of the plant population as was needed
for the control in one out of three, and two out of three tests
respectively. It was also noted that in some instances plants
inoculated with P. ultimum RFLP type B1a and B1b; P.
ultimum RFLP type B2; P. irregulare RFLP type B1; P.
sylvaticum RFLP types B1, B2, B4 and B5 had higher LD_{so}
values than the control.

The effects of PWI on wheat and bean seedlings

The effect of different PWI on glyphosate LD_{so} values on
wheat seedlings is shown in Fig. 1. Plants inoculated with P.
ultimum RFLP types W1, W2, W3 and W4; P. coloratum RFLP
types W1 and W2; P. sylvaticum RFLP type W5 significantly
(P ≤ 0.05) enhanced the herbicidal activity of glyphosate on
2 wk old wheat seedlings in each of two tests done at different
times. In one out of two tests, plants inoculated with P.
irregulare RFLP type W1; P. sylvaticum RFLP types W1, W2,
W3, W4, W6 and W7 significantly enhanced the herbicidal
efficacy of glyphosate on wheat seedlings. In one of two tests,
plants inoculated with P. sylvaticum RFLP types W2, W3 and
W7 needed similar amounts of glyphosate to kill 50% of the
plant population as was required in the control treatment, and
in the remaining instances, plants inoculated with P. sylvaticum
RFLP types W1, W4 and W6; and P. irregulare RFLP type W1
had higher LD_{so} than the control treatment.

The effect of PWI on glyphosate LD_{so} values on beans is
shown in Fig. 2. All PWI consistently and significantly
enhanced the herbicidal efficacy of glyphosate on 2-week-old
bean seedlings in two experiments. Mortality of 50% was
observed at doses 1/10 to 1/30 of those required to cause
50% mortality in the absence of Pythium (control).

The effects of PBI on sunflower and pepper

All PBI enhanced the herbicidal activity of glyphosate on
2-week-old sunflower seedlings by 20 to 50 fold (Fig. 3). All
PBI except P. ′HS’ group RFLP type B1 and P. coloratum RFLP
type B2 enhanced herbicidal activity on pepper seedlings by
six to 30-fold. P. ′HS’ group RFLP type B1 and P. coloratum
RFLP type B2 caused smaller but significant reductions of
glyphosate LD_{so} on pepper seedlings (Fig. 3).

GSI of Pythium species obtained from soil and
glyphosate-untreated hosts on beans

The effect of different Pythium species obtained from soil and
from glyphosate-untreated plants on glyphosate LD_{so}
values on beans are shown in Fig. 4. All Pythium species tested
significantly enhanced (P ≤ 0.05) the herbicidal effect of
glyphosate on 2 wk old bean seedlings, and 50% mortality
was observed at doses seven to 80-fold less than of those
required to cause 50% mortality in the absence of Pythium,
Pythium coloratum, P. hypogynum, P. aphanidermatus and
Glyphosate synergistic Pythium

Fig. 1. Effect of isolates of Pythium obtained from the roots of glyphosate-treated bean (top panel) and wheat (bottom panel) seedlings on glyphosate LD<sub>50</sub> on wheat seedlings. Means (vertical bars) and standard errors (horizontal bars) from two or three experiments shown on each line.

P. spinosum were significantly more effective as synergists than were other Pythium species in this group.

Pathogenicity of PBI on germinating seeds

Symptoms of pre-emergence damping-off caused by various species of Pythium were similar in all types of plant tested. Pythium entered the germinating seeds in the soil by infecting the emerging radicle. After the initial infection, the mycelia colonized the growing embryo and cotyledons. Susceptible seeds were eventually killed and were covered with white mycelia 1 wk after infection. Infected seeds seldom emerge from the soil, and those that did emerge from the soil were weak and had obvious stem necrosis immediately above the soil surface. Infected seedlings usually topple down 3–4 d after emergence and showed symptoms of stem girdling.

The 15 PBI varied in their virulence on germinating bean seedlings (Table 3). All isolates of P. ultimum and P. irregular are highly virulent, causing 100% and 88–98% pre-emergence damping-off on beans, respectively. P. sylliciticum RFLP types B2 and B3, and Pythium 'HS' group RFLP types B1 and B2 were moderately virulent, causing 35–52% pre-emergence damping-off. Other isolates of P. sylliciticum and all isolates of P. coloratum were moderately to weakly virulent, causing a range of pre-emergence damping-off from as high as 27% to as low as 9%.
The same isolates were also pathogenic to varying degrees on germinating wheat seeds. *P. ultimum* RFLP type B1a was the most virulent isolate, causing 95% pre-emergence damping-off. *P. ultimum* RFLP types B1b and B2; *P. irregularare* RFLP types B1 and B2; and *Pythium 'HS'* group RFLP type B2 caused pre-emergence damping off ranging from 63% to 93%. *P. sylvaticum* RFLP types B1, B2 and B3 caused 76, 63 and 50% respectively. The least virulent isolates, *P. coloratum* RFLP types B1 and B2; *Pythium 'HS'* group RFLP type B1; and *P. sylvaticum* RFLP types B4, B5 and B6 caused a range of pre-emergence damping-off ranging from 9 to 26%.

The different PBI were pathogenic to varying degrees on germinating pepper seeds (Table 3). All isolates of *P. ultimum, P. irregularare* and *P. coloratum*, as well as *Pythium 'HS'* RFLP type B2 and *P. sylvaticum* RFLP type B1 caused 100% pre-emergence damping-off. *P. sylvaticum* types B2, B3 and B5 caused mortalities ranging from 72 to 90%. *Pythium 'HS'* group RFLP type B1 and *P. sylvaticum* RFLP types B4 and B6 were the least pathogenic, causing 15 to 30% mortality on pepper seedlings.

PBI were generally less pathogenic to germinating sunflower seeds than to bean, wheat and pepper. The most pathogenic isolates were *P. ultimum* RFLP type B2 and *P. irregularare* RFLP type B2, causing 48% and 47% mortalities respectively. *P. ultimum* RFLP types B1a and B1b; *P. irregularare* RFLP type B1; *P. sylvaticum* RFLP type B1, B2 and B3; and *P. coloratum* types B1 and B2 caused mortalities ranging from 10 to 35%. Mortalities caused by the least pathogenic isolates, *Pythium 'HS'* RFLP types B1 and B2, and *P. sylvaticum* RFLP types B4, B5 and B6, ranged from 0 to 5%.

**Pathogenicity of PBI on germinating bean and wheat seeds**

All isolates of PBI were pathogenic to varying degrees on germinating wheat seeds (Table 4). *P. ultimum* RFLP types W1, W2 and W3 were the most pathogenic, causing 95% mortality. *P. coloratum* RFLP type W1 and *P. irregularare* RFLP type W1 were the least pathogenic causing 20% mortality. All the other PWI were intermediate in pathogenicity, causing mortality ranging from 25 to 63%. When the same *Pythium* isolates were tested on beans, all except *P. coloratum* type W2 were pathogenic to varying degrees. *P. ultimum* RFLP types W1, W2, W3 and W4 were the most pathogenic, causing 85% pre-emergence damping-off while *P. coloratum* RFLP type W1, *P. irregularare* RFLP type W1 and *P. sylvaticum* types W2 and W6 were the least pathogenic, causing 8–23% mortality. *P. sylvaticum* types W1, W3, W4, W5 and W7 were intermediate in pathogenicity, causing 25–45% mortality on germinating bean seeds.

**Pathogenicity of Pythium species from soil and various glyphosate-untreated hosts on germinating bean seeds**

The pathogenicities of *Pythium* species obtained from glyphosate-untreated hosts and soil were tested on germinating bean seeds (Table 5). *P. aphanidermatum* caused the highest pre-emergence damping-off (94%), followed by *P. spinosum* (66%) and *P. splendens* (27%), *P. paracaeorum* (19%) and *P. arthemomanes* (5%). *P. coloratum, P. aphanidermatum, P. hypogynum, P. sulcatum* and *P. aphanidermatum* isolates did not cause mortality on germinating bean seeds.

**DISCUSSION**

The significant differences sometimes observed between the results of repeated experiments probably reflects the complexity of interactions between numerous components of the soil ecosystem during GSI. Since it is impossible to completely control all of the variables in the soil ecosystem, conclusions
Glyphosate synergistic *Pythium*

Fig. 3. Effect of isolates of *Pythium* obtained from the roots of glyphosate-treated bean on glyphosate LD_{50} on sunflower (top panel) and pepper (bottom panel) seedlings. Means (vertical bars) and standard errors (horizontal bars) from two experiments shown on each line.

regarding *Pythium*-plant species specificities during GSI must be based on the overall trend of several replicated GSI tests.

In general, the significance of GSI, as evidenced by the magnitude of differences in glyphosate LD_{50} values obtained in the absence and presence of different *Pythium* isolates, was greater on dicotyledons than on monocotyledons test plants. This may be due to the substantial difference in glyphosate sensitivity observed between the two plant groups, as seen in the lower glyphosate dosages needed to cause equivalent plant mortalities on monocotyledons than on dicotyledonous plant species.

The GSI tests of PWI on wheat and reciprocal GSI tests of PBI on wheat were generally not conclusive with regard to the question of host specificity of these isolates as glyphosate synergists. However, it was interesting to observe that seven PWI *Pythium* isolates consistently caused significantly lower glyphosate LD_{50} values on wheat compared with the control. In contrast, no PBI isolates gave any evidence of consistent GSI on wheat.

The lack of specificity among *Pythium* isolates during GSI was clearly evident in the results of the GSI tests done on various herbaceous dicotyledons. All PWI were glyphosate synergistic on bean seedlings, and PBI that were glyphosate synergistic on beans were also synergists on sunflower and
pepper seedlings. Results from these tests showed no strong evidence for host specificity during GSI involving *Pythium* species on herbaceous dicot seedlings.

A differential GSI response was observed on apple seedlings for two isolates of *Pythium* (Lévesque et al., 1992). In their experiment the isolate obtained from bean was *P. ultimum*, but the isolate obtained from apple was not identified. It was possible that the contrasting GSI effect that they observed on apple seedlings was due to different *Pythium* species used. It was also likely that the difference between a woody and an herbaceous dicot yleodinous species might confer specificities not observed among herbaceous species. This possibility notwithstanding, our results clearly support the conclusion that many different isolates and species of *Pythium* are capable of GSI with a general lack of specificity on diverse species of herbaceous dicot seedlings.

The type of *Pythium* species that were isolated from the roots of glyphosate-treated bean and wheat seedlings were generally similar except for the presence of *Pythium 'HS' group* among FBI but not PWI. Thus, the GSI tests that
utilized *Pythium* species not represented in the PBI and PWI groups were needed to confirm whether other *Pythium* species were also capable of GSI. So far, we have tested 14 different *Pythium* species for GSI (five from glyphosate-treated plants, eight from glyphosate-untreated hosts, and one from soil). The finding that at least some isolates of all 14 species are capable of GSI suggests that other species of *Pythium* in nature may also behave as glyphosate synergists on herbicide-treated plants in the field.

The different species of *Pythium* varied in their pathogenicity. This is an expected result since *Pythium* in general causes diseases on diverse plant species, both monocotyledons and dicotyledons ranging from herbaceous to woody (Hendrix & Campbell, 1973). *Pythium* causes different diseases depending on the growth stage of the host. Mature plants are affected by *Pythium* at root tips and root hairs, causing a sublethal overall decline of plant health over time (Mirechet, 1971). Succulent and juvenile stem and root tissues of seedlings and germinating seeds are commonly attacked below the soil line, which results in girdling of the seedling stem and a watery decay of germinating seeds (Singh & Singh, 1984). The most prominent indication of severe *Pythium* attack on seedlings is manifested by damping-off, characterized by toppling-over of affected seedlings on the soil surface. *Pythium* can also be part of a disease complex on mature trees by causing synergistic interaction with other types of soil-borne microorganisms, as in apple replant disease (Braun, 1991), crown rot of apple trees (Jeffers *et al.*, 1982) and root rot of young apple trees (Utkhede & Smith, 1991).

The pathogenicity of the isolates of *Pythium* species from PWI and PBI groups on their respective reciprocal hosts showed indications of host preferences when the most virulent *Pythium* isolates were considered. The most virulent isolate of *P. ultimum* in the PBI group appeared to be more virulent on germinating bean seeds than on wheat. In contrast, the most virulent isolate of *P. ultimum* in the PWI group was less virulent on beans than on wheat. PBI were generally less pathogenic on sunflower than on bean or pepper seeds.

The antifungal metabolites sesquiterpene lactones (Spring, Albert & Hager, 1982) present in sunflower probably have contributed to its natural resistance against *Pythium*.

The relative pathogenicity of a *Pythium* isolate to glyphosate-untreated plants could not be used to predict its glyphosate synergistic potential on the same host. This was apparent from the results of GSI tests conducted using *Pythium* isolates from soil and various glyphosate-untreated plant hosts. *P. corymbiferum* and *P. hypogynum* were non-virulent on germinating bean seedlings; however, their efficiencies as glyphosate synergists were similar to those of *P. aphani-dermatum*, the most virulent isolate. The ability of the non-virulent *Pythium* species to act as glyphosate synergists on bean seedlings clearly suggests that pathogenicity is not the only factor involved in GSI.

The evidence that diverse *Pythium* species are capable of GSI and are generally non-host specific, at least on herbaceous dicot seedlings, and that these same isolates are pathogenic on germinating seeds highlights possible risks that might be associated with recurrent use of glyphosate as a herbicide and crop desiccant. Such risks might also be associated with the use of glyphosate on genetically engineered glyphosate-tolerant crops. There is both direct and indirect evidence that the population dynamics of soil microbes in the soil can be affected for short periods by glyphosate treatment (Blowes, 1987; Lévesque, Rahe & Eaves, 1987; Smiley, Ogg & Cook, 1992). However, the small number of published reports in relation to the extensive use of glyphosate-containing herbicide over the past two decades suggests that noticeable enhancement of root disease potential by glyphosate is not common. *Pythium* infections on mature plants are usually not discernible due to the insidious nature of pathogen development; as a result, significant effects that are reflected in overall yield may also go unnoticed. Research to assess the impact of glyphosate on long-term root rot disease potential of field soils and of sublethal doses on the activity of deleterious root microflora in perennial crops is clearly needed.

We acknowledge the assistance of Dr D. J. S. Barr in the confirmation of *Pythium* identification. This work was supported by the Natural Sciences and Engineering Research Council of Canada.

### REFERENCES


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**Table 5. Mortalities observed in pathogenicity tests on germinating bean seeds using isolates of *Pythium* species obtained from glyphosate-untreated hosts and soil**

<table>
<thead>
<tr>
<th><em>Pythium</em> species</th>
<th>Pre-emergence damping-off mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aphani-dermatum</em></td>
<td>94 a</td>
</tr>
<tr>
<td><em>P. spinosum</em></td>
<td>66 b</td>
</tr>
<tr>
<td><em>P. splendens</em></td>
<td>27 c</td>
</tr>
<tr>
<td><em>P. paracaudatum</em></td>
<td>19 c</td>
</tr>
<tr>
<td><em>P. arrhenomanes</em></td>
<td>5 d</td>
</tr>
<tr>
<td><em>P. corymbiferum</em></td>
<td>0 d</td>
</tr>
<tr>
<td><em>P. suterpetooli</em></td>
<td>0 d</td>
</tr>
<tr>
<td><em>P. hypogynum</em></td>
<td>0 d</td>
</tr>
<tr>
<td><em>P. sulcatum</em></td>
<td>0 d</td>
</tr>
<tr>
<td><em>P. acanthium</em></td>
<td>0 d</td>
</tr>
</tbody>
</table>

* Mean mortalities from three trials, with 3–5 replicate plates for each isolate per trial. Twenty seeds per plate and mortality recorded 2 wk after inoculation. Values within column followed by the same letter(s) are not significantly different from each other (P ≤ 0.05) according to Bonferroni's test.


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