Selected Cultural and Environmental Parameters Influence Disease Severity of Dandelion Caused by the Potential Bioherbicidal Fungi, *Phoma herbarum* and *Phoma exigua*

S. M. STEWART-WADE¹ AND G. J. BOLAND²

¹School of Resource Management, The University of Melbourne, Melbourne, Victoria 3010, Australia; ²Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

(Received 25 April 2003; returned 9 June 2003; accepted 3 December 2003)

Selected cultural and environmental variables were investigated for their influence on the efficacy of *Phoma herbarum* and *Phoma exigua* to cause disease on dandelion (*Taraxacum officinale*) under growth room conditions. In both species, mycelial fragments caused significantly greater disease severity on dandelion than spore suspensions. Mycelial age was not an important factor in disease severity caused by *P. herbarum*, with all cultures causing high disease ratings. However, younger cultures of *P. exigua* caused the greatest disease severity on dandelion, but significantly less than that caused by *P. herbarum*. The initial pH of the growth medium (potato dextrose broth) did not affect disease severity caused by either Phoma species. Increasing concentrations of mycelia of *P. herbarum* were applied to dandelions that were then exposed to various leaf wetness durations. Disease severity increased with increasing leaf wetness duration. For dandelions exposed to no leaf wetness duration, the greater the mycelial concentration, the greater the disease rating. However, for dandelions exposed to all leaf wetness durations, all concentrations of mycelia caused similar disease ratings. As *P. herbarum* caused high disease ratings on dandelion, it therefore warrants further investigation as a potential bioherbicide for the control of this weed.

**Keywords:** *Taraxacum officinale*, mycoherbicide, bioherbicide, biological control

**INTRODUCTION**

Dandelion (*Taraxacum officinale* G. H. Weber ex Wiggers) is a broadleaf perennial plant that occurs worldwide (Holm *et al.*, 1997). It is considered a problem weed in turf management in...
golf courses, municipal parks and home gardens (Burpee, 1992), as well as in various agricultural and horticultural crops (Stewart-Wade et al., 2002).

Several fungi have been considered as possible biological control agents for dandelion including Sclerotinia sclerotiorum (Lib.) de Bary, S. minor Jagger (Riddle et al., 1991) and Phoma taraxaci von Hofsten (Von Hofsten, 1954). Recently, two other species of Phoma, namely P. herbarum (Westend.) and P. exigua (Desm.), have been studied as potential biological control agents for dandelion (Neumann Brebaum, 1998; Neumann Brebaum & Boland, 1999). Neumann and Boland (1999) found that disease severity caused by P. herbarum on dandelion could be increased by the addition of selected adjuvants.

Other Phoma species have also been considered for biological control of weeds. Phoma aquilina Sacc. & Penz., one of the causal agents of curl-tip disease, has been studied for the control of bracken, Pteridium aquilinum (L.) Kuhn in the UK (Burge & Irvine, 1985; Burge et al., 1986). Also, Phoma proboscis Heiny was investigated for the control of field bindweed, Convolvulus arvensis L., in the USA and combined with chemical herbicides to reduce the rate of spores required for control (Heiny, 1994).

Optimisation of infection is essential to the successful development of a bioherbicide, and factors to be considered include the inoculum type, concentration and physiological status; and environmental parameters such as the required leaf wetness duration (dew period) (Boyette & Turfitt, 1988). The most suitable infective units of a potential bioherbicide must be determined, and then produced in a timely and cost-effective manner (Boyette et al., 1991). Factors that affect the physiology of the agent, such as inoculum age, and production parameters including pH and nutrition, may affect the efficacy of the bioherbicide (Boyette et al., 1991; Schisler et al., 1991). Finally, one of the most important environmental constraints on a bioherbicide is a period of leaf wetness, usually of at least 6 h, which is required for germination and establishment of the pathogen (Dhingra & Sinclair, 1995; Amsellem et al., 1991).

The purpose of this research was to investigate selected cultural and environmental parameters for their effect on the efficacy of the potential biological control agents, P. herbarum and P. exigua, to cause disease on dandelion.

MATERIALS AND METHODS

Inoculum Production

P. herbarum and P. exigua were originally isolated from dandelion leaf lesions collected in southern Ontario, Canada in 1994 (Neumann Brebaum, 1998; Neumann Brebaum & Boland, 1999). Each Phoma species were centrally inoculated onto a potato dextrose agar (PDA) plate and grown for 5 days at room temperature (~ 22°C) on the laboratory bench. Mycelial inoculum was used in all experiments unless stated otherwise. Mycelial inoculum was produced by inoculating five plugs (5 mm diameter) from the actively growing margin of the PDA culture of each isolate into 100 mL of potato dextrose broth (PDB) in a 250-mL flask. Flasks were shaken at 100 rpm (Lab-Line Orbit Shaker, Lab-Line Instruments Inc., Melrose Park, IL, USA, Model 3520) at room temperature for 4 days. Mycelial biomass was harvested by vacuum filtration using Whatman No. 2 filter paper and blended for 20 s in a blender set on high (Waring Commercial Blender, Waring Products Co., New Hartford, CT, USA, Model 5011).

Dandelion Production

Dandelion seeds were collected from the field and stored at 4°C for at least 1 month prior to planting. Seeds were sown into 5 × 5-cm plastic pots (Kord Products, Burlington, ON, Canada) filled with Promix BX (Les Tourbieres Premier Ltee, Riviere du Loup, PQ, Canada) and covered with a thin layer of Turface (Applied Industrial Materials Corporation, Deerfield, IL, USA). Pots were placed in trays and covered with clear plastic covers for
1 week. Pots were maintained in a growth room at 22°C with a 14:10-h light:dark photoperiod and a light intensity of 200 μE/m² per s. After seedling emergence, plants were thinned to one plant per pot. Plants received experimental treatments at four weeks of age.

**Inoculation and Post-Inoculation**
In all experiments, control plants were treated with PDB only. Treatments were applied to plants until run-off using a handheld plastic sprayer. Following treatments, plants were subjected to various leaf wetness durations. Leaf wetness was achieved and maintained using ultrasonic humidifiers (Super Electronic Co., Montreal, PQ, Canada, Model SH8DH3) that fed humid air into plexi-glass chambers (132 x 51 cm). Plants were returned to the growth room bench and assessed for disease at 3, 7 and 14 days after treatment (DAT) using the Horsfall-Barratt scale (Horsfall & Barratt, 1945; Horsfall & Cowling, 1978; Neumann & Boland, 1999), where 0 = 0% diseased tissue, 1 = 0 < 3% diseased tissue, 2 = 3 < 6% diseased tissue, 3 = 6 < 12% diseased tissue, 4 = 12 < 25% diseased tissue, 5 = 25 < 50% diseased tissue, 6 = 50 < 75% diseased tissue, 7 = 75 < 88% diseased tissue, 8 = 88 < 94% diseased tissue, 9 = 94 < 97% diseased tissue, 10 = 97 < 100% diseased tissue, and 11 = 100% diseased tissue (which equates to plant mortality).

**Inoculum Type**
Filtered spore suspensions, unfiltered spore suspensions (which contained some mycelial fragments) or mycelial suspensions of *P. herbarum* and *P. exigua* were tested for their efficacy to control dandelion. Filtered spore suspensions were prepared by flooding an actively sporulating PDA culture (up to 14 days old) with 10 mL of PDB, rubbing with a spatula to dislodge the spores and filtering the suspension through six layers of cheesecloth (grade 50). Spore suspensions were adjusted to $\times 10^6$ spores/mL with the aid of a haemocytometer. This concentration was chosen based on preliminary experiments (unpublished data, Stewart-Wade) testing a range of concentrations for their efficacy to control dandelion. Unfiltered spore suspensions were prepared as described above except they were not filtered through cheesecloth. Mycelial suspensions were produced as described previously and were made up as a 10% (v/v) mycelia/PDB solution. Treatments were applied to six replicate plants, which were then subjected to 48 h leaf wetness duration (LWD). This experiment was repeated.

**Mycelial Age**
Mycelial suspensions of *P. herbarum* and *P. exigua* were prepared using 4-, 7-, 11-, 14- or 18-day-old PDB cultures. Treatments were applied to 10 replicate plants, which were then subjected to 48 h LWD. This experiment was repeated.

**Initial pH of PDB**
Mycelia of *P. herbarum* and *P. exigua* was grown as described above in PDB with initial pH adjusted to 5, 6, 7, 8 or 9 using HCl and NaOH before autoclaving (pH of unadjusted PDB = 6.2). Treatments were applied to 10 replicate plants, which were then subjected to 48 h LWD. This experiment was repeated using five replicate plants per treatment.

**Mycelial Concentration and Leaf Wetness Duration (LWD)**
Mycelial concentrations of *P. herbarum* in PDB (v/v) were 10, 20, 40, 60, 80 or 100% (i.e., undiluted) (Neumann & Boland, 2002). *P. exigua* was not tested. Treatments were applied to six replicate plants, which were then subjected to the following LWDs: 1 = 0 h; 2 = 6 h; 3 = 6 h wet/18 h dry/6 h wet; 4 = 12 h; 5 = 12 h wet/12 h dry/12 h wet; and 6 = 48 h. The repetitive leaf wetness durations, LWD#3 and LWD#5, were included to simulate field conditions. This experiment was repeated using five replicate plants per treatment.
General
Data from each set of experiments were analysed using the non-parametric Kruskal–Wallis one-way ANOVA. Data were then square root-transformed and analysed using a parametric general two-way ANOVA. Since the same conclusions could be drawn from the statistical analysis of each experimental run, the data were then pooled, reanalysed and means were separated using a one-way ANOVA and an LSD value at $P = 0.05$.

RESULTS
Inoculum Type
Both fungus and inoculum type were significant ($P = 0.05$) factors in disease of dandelion at 3, 7 and 14 DAT. At 3 DAT, the highest mean disease rating was 10.1, caused by *P. herbarum* mycelia. At 14 DAT, unfiltered spores or mycelia of *P. herbarum* caused significantly ($P = 0.05$) greater mean disease ratings on dandelion than inoculum of *P. exigua* (Table 1). For both fungi, mycelial fragments caused significantly ($P = 0.05$) greater disease severity on dandelion than spore suspensions (Table 1). For *P. herbarum*, unfiltered spore suspensions caused significantly ($P = 0.05$) greater mean disease ratings than filtered spore suspensions (Table 1). This was most likely due to the presence of mycelial fragments in the unfiltered suspensions.

Mycelial Age
Both fungus and age were significant ($P = 0.05$) factors in disease of dandelion at 3, 7 and 14 DAT. At 3 DAT, mean disease ratings caused by *P. herbarum* ranged from 7.6 to 9.2, significantly ($P = 0.05$) greater than those of *P. exigua*. At 14 DAT, mycelial age of *P. herbarum* did not significantly ($P = 0.05$) affect the mean disease rating on dandelion, with all ages causing high mean disease ratings (Table 2). In general, the younger the mycelial preparations of *P. exigua*, the greater the mean disease ratings ($P = 0.05$), with 4- and 7-day-old mycelia causing significantly ($P = 0.05$) greater ratings than those of 14- or 18-day-old mycelia (Table 2). However, these ratings were significantly less than those caused by *P. herbarum* ($P = 0.05$) (Table 2).

Initial pH of PDB
Fungus was a significant ($P = 0.05$) factor in disease of dandelion at 3, 7 and 14 DAT (Table 3). *P. herbarum* caused significantly ($P = 0.05$) greater mean disease ratings on dandelion than *P. exigua* at all assessment dates, ranging from 9.4 to 10.2. The initial pH of the PDB, however, did not affect disease severity caused by either pathogen ($P = 0.05$) (Table 3).

Mycelial Concentration and Leaf Wetness Duration (LWD)
Both mycelial concentration and leaf wetness duration were significant ($P = 0.05$) factors in disease of dandelion at 7 and 14 DAT. For dandelions exposed to 0 h of leaf wetness (i.e., LWD #1), the greater the mycelial concentration, the greater the disease rating, at 14 DAT.

TABLE 1. Effect of inoculum type on efficacy of *P. herbarum* and *P. exigua* to cause disease on dandelion, at 14 days after treatment. Plants were subjected to 48 h leaf wetness duration and rated using the Horsfall–Barratt scale. LSD (0.05) = 1.77

<table>
<thead>
<tr>
<th>Inoculum type</th>
<th><em>P. herbarum</em></th>
<th><em>P. exigua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered spores</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Unfiltered spores</td>
<td>5.4</td>
<td>2.8</td>
</tr>
<tr>
<td>Mycelia</td>
<td>10.6</td>
<td>6.9</td>
</tr>
</tbody>
</table>
TABLE 2. Effect of mycelial age on efficacy of *P. herbarum* and *P. exigua* to cause disease on dandelion, at 14 days after treatment. Plants were subjected to 48 h leaf wetness duration and rated using the Horsfall–Barratt scale. LSD (0.05) = 1.49

<table>
<thead>
<tr>
<th>Mycelial age (days)</th>
<th><em>P. herbarum</em></th>
<th><em>P. exigua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>9.7</td>
<td>6.3</td>
</tr>
<tr>
<td>7</td>
<td>9.2</td>
<td>5.5</td>
</tr>
<tr>
<td>11</td>
<td>9.7</td>
<td>5.2</td>
</tr>
<tr>
<td>14</td>
<td>9.4</td>
<td>3.9</td>
</tr>
<tr>
<td>18</td>
<td>8.3</td>
<td>4.1</td>
</tr>
</tbody>
</table>

TABLE 3. Effect of pH of the potato dextrose broth growth medium on efficacy of *P. herbarum* and *P. exigua* to cause disease on dandelion, at 14 days after treatment. Plants were subjected to 48 h leaf wetness duration and rated using the Horsfall–Barratt scale. LSD (0.05) = 1.96

<table>
<thead>
<tr>
<th>pH</th>
<th><em>P. herbarum</em></th>
<th><em>P. exigua</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>9.6</td>
<td>6.9</td>
</tr>
<tr>
<td>6</td>
<td>9.6</td>
<td>6.5</td>
</tr>
<tr>
<td>7</td>
<td>9.6</td>
<td>7.5</td>
</tr>
<tr>
<td>8</td>
<td>9.6</td>
<td>5.9</td>
</tr>
<tr>
<td>9</td>
<td>9.6</td>
<td>7.0</td>
</tr>
</tbody>
</table>

However, with dandelions exposed to all other leaf wetness durations, all concentrations of mycelia caused similar disease ratings (Table 4). Disease severity increased significantly (*P* = 0.05) with increasing LWD. At 14 DAT, inoculated plants subjected to LWD #6 (48 h continuous LWD) scored a mean disease rating of 11 (100% disease), which was significantly (*P* = 0.05) greater than all other treatments. Under LWD #3 (2 × 6 h), disease ratings of all plants were significantly (*P* = 0.05) lower than ratings under LWD #4 (12 h continuous), indicating that continuous leaf wetness duration is more important than total hours of leaf wetness duration. Under LWD #4 (12 h continuous) and #5 (2 × 12 h), mean disease ratings of inoculated plants ranged from 3.9 to 6.1 and were statistically equivalent (*P* = 0.05) for each of the three lowest mycelial concentrations, and only slightly improved for the three

TABLE 4. Effect of mycelial concentration and leaf wetness duration on efficacy of *P. herbarum* to cause disease on dandelion, at 14 days after treatment. Plants were subjected to the following LWDs: #1 = 0 h; #2 = 6 h; #3 = 6 h wet/18 h dry/6 h wet; #4 = 12 h; #5 = 12 h wet/12 h dry/12 h wet; and #6 = 48 h, and rated using the Horsfall–Barratt scale. LSD (0.05) = 1.35

<table>
<thead>
<tr>
<th>Mycelial concentration (%)</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
<th>#5</th>
<th>#6</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1.3</td>
<td>1.0</td>
<td>0.9</td>
<td>4.2</td>
<td>5.5</td>
<td>11.0</td>
</tr>
<tr>
<td>20</td>
<td>0.4</td>
<td>1.3</td>
<td>1.5</td>
<td>4.3</td>
<td>5.4</td>
<td>11.0</td>
</tr>
<tr>
<td>40</td>
<td>1.0</td>
<td>1.5</td>
<td>2.1</td>
<td>4.4</td>
<td>5.7</td>
<td>11.0</td>
</tr>
<tr>
<td>60</td>
<td>1.8</td>
<td>1.4</td>
<td>2.4</td>
<td>3.9</td>
<td>5.6</td>
<td>11.0</td>
</tr>
<tr>
<td>80</td>
<td>2.0</td>
<td>1.9</td>
<td>1.5</td>
<td>3.9</td>
<td>5.4</td>
<td>11.0</td>
</tr>
<tr>
<td>100</td>
<td>2.2</td>
<td>2.2</td>
<td>1.6</td>
<td>4.2</td>
<td>6.1</td>
<td>11.0</td>
</tr>
</tbody>
</table>
highest mycelial concentrations. Again, this emphasises the importance of continuous leaf wetness for successful disease development.

**DISCUSSION**

In this *Phoma*:dandelion system, mycelia of both *Phoma* species caused greater disease ratings on dandelion than spores, whereas spores are usually considered the most infective of fungal propagules (Boyette *et al.*, 1991). In the application of bioherbicides, mycelia are thought to be easily damaged and then desiccate readily on the leaf surface (Lawrie *et al.*, 2001). However, mycelia have been shown to provide equivalent or better control of weeds in several systems, including *Alternaria cassiae* Jurair & Khan/Cassia obtusifolia L. (Stowell *et al.*, 1989) and *Chondrostereum purpureum* (Pers. Ex Fr.) Pouzar/Prunus serotina Erhr. (Grosclaude, 1964; Scheepens & Hoogerbrugge, 1989). There are several possible explanations of poor infection due to spores, including the use of a sub-optimal spore concentration (too low, or too high resulting in spore dormancy due to self-inhibition) (Macko *et al.*, 1976; Heiny & Templeton, 1991; Dhingra & Sinclair, 1995); or reduced infectivity potential due to culture age or a sub-optimal culture environment (Stowell, 1991; Griffin, 1994; Dhingra & Sinclair, 1995). The infectiveness and ease of production of mycelia in liquid culture precluded any further investigation of spores as inoculum in this system.

It was expected that mycelia of different ages would be in different phases of growth and so, would be in different states of physiological fitness to infect a plant (Hall *et al.*, 1994; Dhingra & Sinclair, 1995; Hallsworth & Magan, 1996). This did not seem to be the case for *P. herbarum* that was highly virulent at all ages tested. However, young cultures of *P. exigua* caused greater disease ratings than older cultures and this was expected as young cultures generally have higher infectivity potential (Dhingra & Sinclair, 1995). The ability to use young mycelia of both species has the commercial advantage of minimising the required production time and so, minimising costs and the chance of contamination (Auld, 1992). However, there may also be disadvantages including reduced survival during downstream processing and poor shelf life (Churchill, 1982; Boyette *et al.*, 1991), and these factors would need to be investigated.

Since pH has been recorded as affecting the morphological and cultural characteristics of these two species of *Phoma* (Rajak & Rai, 1984), it was expected that pH may also affect virulence (Hallworth & Magan, 1996). The lack of an effect of pH on disease severity may be due to the fungi altering the pH by metabolising the substrate (Hall, 1981; Griffin, 1994), so that after 4 days incubation, the final pH was similar for all cultures and different from their initial pH. Alteration of pH can be difficult to control as buffers have a limited effective range, and the concentration sufficient to be effective may inhibit growth (Hall, 1981; Griffin, 1994). Also, autoclaving can alter pH (Dhingra & Sinclair, 1995) and so, actual initial pH may have been quite different to the measured initial pH. Also, *Phoma* species may be able to withstand a broad range of pH, which has been reported for many fungi (Smith, 1988; Griffin, 1994). This can be advantageous during industrial production of inocula, since the substrate pH can be adjusted to inhibit the growth of some contaminants and favour the growth of the biological control agent (Hallsworth & Magan, 1996). Scale-up from shake-flask culture to large fermenters will entail numerous challenges, among them, pH, aeration and temperature (Smith, 1988).

Increased inoculum concentrations are often associated with greater initial disease severities, as reported from other plant–pathogen systems (Makowski, 1993; Hong & Fitt, 1995; Vloutoglou & Kalogerakis, 2000), before tapering off over time. In this study, all concentrations of mycelia used generally caused similar disease ratings, and so the ability to use low concentrations, as long as a sufficient LWD is available or can be provided, reduces the cost of production and is commercially desirable (Heiny, 1994). In a previous study, mycelial concentrations of 10–20% incited higher disease severity than greater concentrations under a continuous LWD of 32 h (Neumann & Boland, 2002). However, interrupted
LWDs used in this study, which reflect natural conditions more accurately, showed no such concentration effect. In general, disease severity increased with increasing LWDs, as expected (Makowski, 1993; Hong & Fitt, 1995; Vloutoglou & Kalogerakis, 2000). The requirement for a long continuous LWD for 100% control of dandelion is not ideal (Makowski, 1993; Mortensen, 1998); however, under LWD#5, equivalent to two consecutive nights of 12 h dew, *P. herbarum* provided approximately 50% control of dandelion. This level of control could be improved by formulation and application technologies (Neumann & Boland, 1999), and label specifications as to the most suitable time for application that could optimise the performance of the bioherbicide (Walker & Boyette, 1986; Makowski & Mortensen, 1990).

The use of 4-week-old seedlings of the perennial weed dandelion, whilst not desirable, was necessary for these initial studies. Other researchers studying bioherbicides have also used young perennial plants (ranging from ‘newly emerged’ up to 6 weeks old) to determine basic requirements and give an indication of potential field performance (Burge & Irvine, 1985; Burge et al., 1986; Heiny & Templeton, 1991; Heiny, 1994; Neumann & Boland, 1999). Similarly the use of 48 h LWD in these experiments, whilst being unlikely to occur in the field, provided a standard comparative treatment and ensured that leaf wetness was not limiting while evaluating other parameters. Many other studies have included a 48 h dew period when evaluating potential bioherbicides (Heiny & Templeton, 1991; Makowski, 1993; Ghorbani et al., 2000; Lawrie et al., 2001).

*P. herbarum* mycelia, easily produced in shake-flask liquid culture, consistently caused high disease ratings on dandelion. The main drawback of this plant pathogen as a bioherbicide is its requirement for prolonged leaf wetness periods, which may be reduced through improvements in formulation (Neumann & Boland, 1999) and application technologies.

ACKNOWLEDGEMENTS

The authors would like to thank E. A. Smith for technical assistance.

REFERENCES


