Environmental Factors Influencing Safflower Screening for Resistance to *Phytophthora cryptogea*

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ABSTRACT

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Sand texture influenced infection of safflower seedlings by zoospores of *Phytophthora cryptogea*. In fine sand, an inoculum of 10^7 zoospores was required to uniformly infect trays of safflower seedlings, but when coarser sand was used, 2×10^6 zoospores were sufficient. Withholding water from seedlings to induce wilt symptoms before inoculation improved the reliability of glasshouse screening. Breeding lines resistant to infection as 10-day-old seedlings also expressed resistance when tested after 90 days in pot experiments. Selected breeding lines were screened in a field disease nursery. When sown on ridges, some safflower lines reacted differently from those sown on the flat for border check irrigation. Ponding of water in border check irrigation for as little as 2 hr led to successful infection provided air temperatures exceeded 35 C. Some breeding lines expressed field resistance but failed to show resistance under glasshouse conditions. One breeding line showed little or no infection in both field and glasshouse screenings.

Root-rot diseases in safflower that are attributed to Phythiaceous fungi have been reported in Australia (17,20) and in other countries, including the United States (15,24), Canada (4), and Argentina (11).

Although species of both *Pythium* and *Phytophthora* have been implicated in safflower root rot, *Phytophthora cryptogea* Pethybr. & Laff., which we believe is synonymous with *P. drechsleri* Tucker (2), is reported to be the major cause of root rots in Australia (20) and the United States (15). Disease symptoms appear as vascular wilting followed by rapid desiccation of the entire plant. Crop losses can be high, particularly in seasons of high rainfall or when irrigation is applied to crops growing in soils with poor surface drainage.

In addition to high soil moisture, soil temperatures of 25–30 C favor infection by *P. cryptogea* (10). The susceptibility to Phytophthora root disease of commercial cultivars presently available in Australia is a major obstacle to the expansion of safflower within the Australian oilseeds industry. In the absence of economic chemical control measures, a plant breeding program has been initiated to develop root-rot-resistant cultivars.

A glasshouse/controlled-environment procedure to screen for root-rot resistance is described. Some environmental and cultural conditions such as temperature (9,14,22), light intensity and inoculum density (14), soil texture and water potential (5), plant water potential (6), and plant age (21) are critical for safflower infection by *P. cryptogea*. An examination of some of these factors was undertaken to develop a screening procedure that would provide conditions favorable to both fungal infection and host expression of disease resistance.

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MATERIALS AND METHODS

Safflower germ plasm. Safflower accessions tested in these experiments formed part of a world collection held at the CSIRO, Centre for Irrigation Research at Griffith, New South Wales (12). The origins of accessions examined in this study are shown in Table 1.

Gila, a widely grown commercial cultivar with broad adaptability to the Australian climate, is susceptible to *P. cryptogea*, particularly at the hypocotyl or lower stem. It was used as a susceptible check cultivar in all screening tests performed.

Inoculum. A field isolate of *P. cryptogea* from safflower was used in all experiments. The inoculum used in disease-resistance tests consisted of standardized suspensions of zoospores produced axenically by a method described by Chen and Zentmyer (3).

Controlled-environment techniques. Safflower seedlings were grown in plastic seedling trays (280 × 340 × 50 mm) containing river sand that had been treated with aerated steam at 80 C for 30 min to destroy soilborne pathogens. The effect of sand texture (fine, 0.25–0.50 mm, and coarse, 0.70–1.5 mm) on disease screening were compared. When screening breeding lines for root-rot resistance, 11 rows of 10 seeds were planted, with Gila sown in alternate rows. Ten days after sowing, the sand was saturated with water at 25 C and 200 ml of zoospore suspension in water was distributed over the sand surface between the rows of seedlings. Zoospore densities of 10^5–10^8 in 200 ml of water were used for screening. Seedling trays were then held at 25 C in a glasshouse or controlled-environment cabinet for 7 days before disease rating and repotting. Severe wilting symptoms resulting in plant death developed during this time in susceptible plants. Disease assessment was based on percentage of plant death.

Single safflower plants growing in U.C. potting mix (1) in 150-mm-diameter plastic nursery pots were inoculated at floral initiation after the soil was water-saturated. An inoculum of 2×10^5 zoospores in 50 ml of water was used to inoculate each pot in four 5-mm-diameter holes made in the soil, 15–20 mm from the root. Percentage of plant death was recorded at 2 wk.

Field disease nursery screening. A root-rot disease nursery was established in a 0.5-ha field at the Centre in 1975. In subsequent years, this field was sown to safflower and irrigated by border check to incite root-rot infection. Cucurbits, also a host for *P. cryptogea* (7,8), have

Table 1. Reference numbers and country of origin of safflower accessions

Accession no.[a]	Other references	Country of origin
A461	PI 273874[b]	Ethiopia
A504	PI 237538[b]	Turkey
A566	PI 250192[b]	Pakistan
A783	PI 279345[b]	Japan
A949	PI 311738[b]	Poland
A1090	CPI 81609[c] (USB)[d]	United States
A1091	CPI 81610[c] (VFR-1)[d]	United States
A1092	CPI 81611[c] (VF stp-1)[d]	United States
A1108	CPI 169056[c]	Iran
A1110	CPI V50/243[c]	Iran
A1111	CPI V51/49[c]	Iran
A1112	CPI 83387[c] (14-5)[d]	United States

[a] Held at CSIRO, Centre for Irrigation Research.
[b] Plant Introduction number of Western Regional Plant Introduction Station, Pullman, WA.
[c] Commonwealth Plant Introduction Number of CSIRO, Division of Plant and Industry, Canberra City, ACT, Australia.
[d] USDA registered name.

been sown in recent years to sustain the field inoculum during the summer season.

Safflower breeding lines were screened in the disease nursery for resistance to Phytophthora root rot. Lines were sown on the flat in a randomized block design. Within each block, smaller blocks were hilled up for border check irrigation, which enabled water to be ponded to a depth of 150 mm. Ponding within border checks was maintained for 2 or 6 hr. Ponding treatments were applied at floral initiation and when air temperatures exceeded 35 C. Soil temperature was measured at various depths using dial-gauge thermometers.

The susceptible cultivar Gila was included in each irrigation subblock as a control. Breeding lines were sown in rows 5–7 m long at a density of about 20 plants per meter. Percentage of plant death was measured 7–14 days after ponding.

Table 2. Percentage death of susceptible and resistant safflower plants inoculated with different densities of *Phytophthora cryptogea* zoospores

Zoospore density (no./200 ml)	Percentage death of seedlings[a]	
	Gila	A1110
10^5	10.3 (3.8)	0.0 (0)
10^6	67.6 (3.4)	0.0 (0)
10^7	95.0 (1.8)	4.0 (3.3)
10^8	100.0 (0.0)	...

[a] Means of four replicated trays; standard deviation of mean is shown in parentheses.

Table 3. Effect of sand texture on safflower infection by *Phytophthora cryptogea* zoospore

Susceptible accession	Percentage death of seedlings[a]	
	Fine sand	Coarse sand
Gila	91.5 (21.2)	94.6 (11.8)
US10	48.0 (18.2)	87.0 (9.1)
A1112	95.0 (12.8)	97.5 (5.0)

[a] Means of four replicated trays; standard deviation of mean is shown in parentheses.

Table 4. Effect of plant maturity on safflower susceptibility to Phytophthora root rot

Safflower accession	Percentage death of plants	
	Seedlings[a]	Mature plants[b]
Gila	94.6 (5.8)	100
A504	2.6 (4.8)	0
A461	10.4 (5.5)	33

[a] Means of four replicated trays; standard deviation of mean is shown in parentheses.
[b] Means of six replicated pots.

Table 5. Effect of irrigation method on Phytophthora root rot of safflower in a field nursery

Safflower accession	Percentage death of plants[a]	
	Furrow irrigation	Border check irrigation
Gila	57 (6.4)	98 (1.2)
Biggs	18 (2.7)	60 (4.8)
A567	98 (2.3)	100 (0)
A1110	26 (2.8)	28 (2.9)

[a] Means of four replicated plots; standard deviation of mean is shown in parentheses.

RESULTS AND DISCUSSION

Controlled-environment studies. *Inoculum density.* To investigate the minimum inoculum density of *P. cryptogea* required to ensure both complete loss of the susceptible control (Gila) and reliable survival of resistant breeding material, different densities (10^5–10^8 zoospores per flat) were used.

Percentage of death of seedlings of the susceptible variety Gila and a resistant breeding line (A1110) inoculated with different zoospore densities is shown in Table 2.

An inoculum density of 10^7 zoospores in 200 ml of water was the optimum level for use in this screening technique. This inoculum level was ideal when breeding material was grown in fine river sand, but in further investigations using coarse sand and prior water stress, a reduced level (2×10^6/200 ml) proved more satisfactory.

Soil texture. Large variability in results of seedling deaths had been recorded in early screening for resistance using zoospore inoculations. We investigated sand texture used to grow seedlings and found that coarse rather than fine sand was a more effective medium for testing resistance of safflower seedlings to root rot (Table 3). Facilitated movement of zoospores toward roots through the coarse sand is a probable explanation for this phenomenon. Laboratory observations of restricted motility of *P. cryptogea* zoospores in fine sand have been reported (5,18). Coarse sand was subsequently used in the screening program to enhance infection of safflower seedlings.

Soil moisture regime. Withholding water from safflower seedlings for 2 days before inoculation resulted in visible symptoms of moisture stress. At the time of inoculation, sand in the seedling trays of both stressed and unstressed treatments was saturated to facilitate zoospore movement toward the hypocotyls and roots.

Symptom development was increased from 88% in unstressed to 96% in stressed treatments. Increased susceptibility of safflower to Phytophthora root rot caused by water stress before inoculation has been demonstrated previously by Duniway (6) using 3- to 5-wk-old plants. Our results show a predisposing effect of water stress on Phytophthora infection in young 10-day-old safflower seedlings. This practice was adopted in the screening technique to ensure infection conditions.

Infection of mature plants. Phytophthora root rot in mature 90-day-old safflower plants showed a pattern similar to root rot observed in 10-day-old seedlings. Examples of a typical reaction are shown in Table 4. Gila remained fully susceptible to hypocotyl infection during both early and later stages of growth. Two breeding lines (A504 and A461), which showed resistance to root rot as young seedlings, maintained this resistance after 90 days (at floral initiation).

The results obtained at these two stages of safflower growth indicate that the more rapid seedling screening technique provides a reliable assessment of the resistance of maturing plants.

Field disease nursery studies. *Furrow and border check irrigation.* Safflower lines differed in their responses to root infection in the disease nursery (Table 5). The percentage of diseased plants of cultivars Gila and Biggs was lower in furrow-irrigated than border check treatments. Border check irrigation exposes both roots and hypocotyls to infection, whereas hypocotyls remain dry

Table 6. Percentage death of safflower lines after ponding treatments in a field disease nursery compared with glasshouse tests

Line	Percentage death of plants		
	Duration of ponding (hr)		
	2[a]	6[b]	Glasshouse screening
Gila	100 (0.9)[c]	100 (0)	100 (0.9)
A566	50 (3.3)	100 (0)	100 (0)
A783	0 (0)	0 (0)	85 (8.2)
A949	50 (4.1)	75 (3.6)	25 (1.6)
A1090 (USB)	25 (2.8)	0 (0)	92 (2.8)
A1091 (VFR-1)	25 (3.2)	75 (2.2)	97 (1.4)
A1092 (VF stp-1)	0 (0)	0 (0)	97 (0.9)
A1108	0 (0)	0 (0)	68 (3.2)
A1110	0 (0)	0 (0)	4 (1.6)
A1111	0 (0)	0 (0)	62 (2.2)
A1112	0 (0)	0 (0)	100 (0)

[a] Air temperature 35 C; soil temperature 35 C (1 cm deep), 35 C (5 cm deep), and 25 C (15 cm deep).
[b] Air temperature 42 C; soil temperature 50 C (1 cm deep), 50 C (5 cm deep), and 30 C (15 cm deep).
[c] Four replicated plots and trays in field and glasshouse tests, respectively; standard deviation of mean is shown in parentheses.

when plants are furrow irrigated.

Moderate root-rot resistance was expressed by Gila when grown under furrow irrigation. This confirms descriptions of hypocotyl susceptibility combined with moderate root-rot resistance in Gila (16,19,23). The susceptibility of Biggs to infection in border check treatments was unexpected, given reports of high levels of hypocotyl resistance (23). However, Biggs' hypocotyls have previously succumbed to infection by highly virulent strains of *P. cryptogea* in the United States (16), indicating that similarly virulent strains or races may also exist in Australia.

Poor survival of safflower accession A567 in both irrigation treatments indicates that it is both stem- and root-rot susceptible. Accession A1110 showed satisfactory resistance to field infection in both treatments, indicating both stem- and root-rot resistance.

Ponding time. Breeding lines differed in their responses to flood irrigation (Table 6). Gila was fully susceptible in both ponding trials, confirming results obtained in the glasshouse. Five breeding lines examined (A783, A1108, A1110, A1111, and A1112) showed excellent resistance to both 2 and 6 hr of ponding; however, these lines varied in resistance to *Phytophthora* in the glasshouse (Table 5). In fact, although A1112 was fully resistant to Phytophthora infection under ponding, it succumbed to infection even faster than Gila in the glasshouse.

Selections from accession A1110 have proved consistently resistant in both field and glasshouse screening, with only 26–28% loss of plants under field conditions (Table 5). This line is being considered for interstate evaluation as a root-rot-resistant cultivar for use in irrigated, fine-textured soils (12).

Accession A504, which expressed superior resistance in controlled-environment screening (Table 4), was not tested in the disease nursery because of lack of seed.

Accessions A1091 and A1092 from the United States were included because of their resistance to *Verticillium* and the possession of the favorable striped hull character. A1092 performed well in the field nursery but both lines were susceptible in the glasshouse. Responses of A1090 (USB) in the glasshouse were unexpected because of its registration as a root- and stem-resistant breeding line. Accessions A566 and A949 performed poorly in both field and glasshouse screening, but because of their superior resistance to frost and leaf blight (*Alternaria carthami*), respectively, they were included in these trials.

Similar results were obtained for 2- and 6-hr ponding treatments in the field trial in 1980. These results prompted us to extend ponding times to 12, 24, and 48 hr in the 1981 season. Ponding times of 24 hr are not uncommon in commercial operations. Ponding for 48 hr was applied as an extreme treatment to establish whether any of the breeding lines could withstand this period of flooding. Unfortunately, cool air temperatures (25–30 C) were experienced throughout the later stages of growth in 1981 and the results from the disease nursery were inconclusive because of variable response of the susceptible controls. Depleted oxygen, which is associated with flooding at elevated soil temperature, appears to be a critical factor in root-rot development (13).

Staggered sowing dates could provide a solution to the climatic variability at the early bud stage of the crop. Small seed quantities of the breeding lines being tested and the limited area of the disease nursery makes this difficult to achieve.

Glasshouse screening for Phytophthora root-rot resistance proved more severe than field testing. However, 5.5% of the safflower lines screened in the glasshouse were resistant and have subsequently performed consistently well under field conditions in the disease nursery.

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