THE ROLE OF ALTERNATIVE HOSTS IN SURVIVAL OF *PHYTOPHTHORA DRECHSLERI*

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Abstract

Chlamydospores of *P. drechsleri* Tucker were observed in the roots of six genera of artificially inoculated plants that are common weed species in eastern and southern Australia. Zoospore attraction to the roots of three plant species and subsequent sporulation were demonstrated. The activity of this fungus in agricultural soils is discussed.

I. INTRODUCTION

The acreage sown to safflower (*Carthamus tinctorius* L.) in Australia has doubled since 1968–69 when declining wool prices and the operation of wheat delivery quotas prompted dryland farmers to cultivate alternative crops.

Unsuitable varieties and disease problems have prevented Australia from achieving self-sufficiency in safflower production. Yields in New South Wales compare unfavourably with those overseas, due in part to the widespread incidence of a root rot in both dryland and irrigated crops.

In New South Wales, Kochman and Evans (1969) attributed the cause of root rot of irrigated safflower in the Namoi Valley to *Pythium ultimum* Trow and *P. debaryanum* Hesse. After more appropriate pathogenicity tests, Stovold (1973) concluded that *Phytophthora drechsleri* Tucker was the most important incitant of safflower root rot in New South Wales and, although several *Pythium* spp. could cause a root rot under flooded soil conditions, these fungi were not as virulent as *Phytophthora drechsleri.* Stovold isolated *P. drechsleri* from soils in all safflower-growing areas of New South Wales and from the roots of saffron thistle (*Carthamus lanatus* L.), a common weed in these areas. Root rot has occurred in the first crops sown in virgin soil and the pathogen has been isolated from eucalypt forest soils in Tasmania, the south-west of Western Australia, and the eastern coast of Australia (Pratt and Heather, unpublished data).

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Much is known of the survival and reproduction of many species of *Phytophthora* [see reviews of Hickman (1958) and Zentmyer and Erwin (1970)]. In contrast, very little is known about the behaviour of *P. drechsleri* in the absence of safflower and of its activity in soil. No mention is made in the literature on survival structures of *P. drechsleri* or on its ability to reproduce in the presence or absence of a host. Klisiewicz (1970) studied cultural conditions affecting the production and germination of oospores and reported their formation in paired cultures on autoclaved safflower stems. Klisiewicz claimed that his data support the view that *P. drechsleri* could reproduce and survive in the soil through sexual reproduction if compatible strains were present. No experiments, however, were reported to justify this statement.

The experiments reported here were undertaken to investigate the factors involved in survival of *P. drechsleri* in soil and the possible role of alternative hosts in the propagation of the fungus.

II. Materials and Methods

The isolate of *P. drechsleri* used in these studies was obtained from the roots of diseased safflower plants from Cowra, N.S.W., and the identification was subsequently verified by the Commonwealth Mycological Institute (IMI 164185). Stock cultures were maintained successively on oatmeal agar [20 g oatmeal (boiled and filtered) and 25 g agar per litre] and V8 agar [100 ml V8 juice, 2 g calcium carbonate (filtered), and 25 g agar per litre]. The pathogenicity of the fungus was maintained by periodically inoculating the roots of 3-week-old safflower seedlings (cv. Gila) with a 5-mm agar plug cut from the periphery of a 7-day-old culture on oatmeal agar. The plants were flooded with water for 4 days. The fungus was reisolated 6 days after inoculation by plating roots with lesions onto the surface of antibiotic cornmeal agar (vancomycin 200 p.p.m., pimaricin 8 p.p.m., and pentachloronitrobenzene 100 p.p.m.; 17 g Oxoid cornmeal agar per litre; after Tsao and Ocana 1969). The fungus could then be subcultured to oatmeal agar.

Seedlings and young plants of camel melon [*Citrullus lanatus* (Thunb.) Matsumura & Nakai], Bathurst burr (*Xanthium spinosum* L.), saffron thistle, crowsfoot [*Erodium botrys* (Cav.) Bertol.], Paterson's curse (*Echium plantagineum* L.), and skeleton weed (*Chondrilla juncea* L.) were grown and inoculated as described elsewhere (Cother and Griffin 1973). Control and inoculated plants were flooded for 4 days.

As it can be argued that the use of mycelial inoculum does not approach a natural situation, some infection studies were made using zoospores as inoculum. The isolate of *P. drechsleri* used in these studies, and two other isolates from Batemans Bay and Glen Innes, produced very low numbers of sporangia in Petri's solution, soil extract solution, or pond water. Mehrotra (1970) achieved zoospore concentrations of 30,000–40,000 per millilitre of solution, whereas the highest concentrations achieved in these experiments were 150–200 zoospores/ml.

The susceptibility of roots to zoospore infection was tested on seedlings of Bathurst burr, camel melon, and lucerne (*Medicago sativa* L.). These plants were grown in 1 : 1 sand–soil mixture for 4 weeks, after which time they were removed from the pots and adhering soil and sand was washed from the roots. The plants were suspended by means of cork floats in aerated Hoagland's solution in 1·5-litre glass jars. The sides and tops of each jar were covered with black polythene sheeting to exclude the light. After 7 days in the nutrient solution the plants were inoculated as follows: mycelial mats of *P. drechsleri* were grown in 25 ml of V8 juice broth (100 ml V8 juice, 2 g calcium carbonate, filtered, per litre) in 100-ml flasks for 3 days at 25°C. The mats were washed with water and placed for 36 hr in Petri dishes containing filtered pond water. The mats were then removed, washed in water, and placed in 1·5-litre glass jars containing water at 8°C to initiate zoospore release. When the temperature of the water reached 15°C, seedlings were transferred from the nutrient solution to the inoculum jars for 24 hr at room temperature, then returned to the nutrient solution and examined 36 hr later. Control plants were subjected to the same treatment but no zoospores were added.

III. Results

The reaction of plants to infection by *P. drechsleri* is presented in Table 1. The melon and burr seedlings collapsed 24 hr after the pots were drained and brown watery lesions extended from the roots into the lower stem. Control plants were unaffected. Saffron thistle seedlings did not collapse but dark-brown lesions in the form of dense longitudinal streaks extended from about 5 mm above ground level to well into the root system. Some shrivelling of this area was also evident.

Table 1

Reaction of plants to infection by *P. drechsleri*

Plant	Reaction to infection			Chlamydospores in root tissue	Pathogen reisolated
	Necrosis	Premature senescence	Rapid death		
Bathurst burr	+	−	+	+	+
Camel melon	+	−	+	+	+
Crowsfoot	+	−	+	+	+
Paterson's curse	+	+	−	+	+
Saffron thistle	+	−	−	+	+
Skeleton weed	+	+	−	+	+

Examination of inoculated thistle plants after 4 weeks of apparently normal growth revealed the presence of numerous thick-walled spherical structures of fungal origin in the necrotic cortical cells of stems and roots. These structures have been identified as chlamydospores of *P. drechsleri* (Cother and Griffin 1973). A similar examination was carried out on camel melon and Bathurst burr roots 2 weeks after inoculation. Chlamydospores were found in both cortical and vascular tissue near the point of inoculation in each plant.

All the crowsfoot plants commenced to wilt 1 day after draining and were dead 6 days later. The Paterson's curse and skeleton weed flowered and set seed prematurely compared with the control plants. Deep cavities had formed in the roots of these plants at the points of inoculation and transverse sections of these areas revealed abundant chlamydospores in the necrotic cells of the outer cortex and immediately adjacent cells. There were fewer structures noticed in skeleton weed roots than in the other species.

Segments of roots of all inoculated plants were washed in running tap water, dried on tissue paper, and placed onto antibiotic cornmeal agar in the dark at 25°C. *P. drechsleri* mycelium was subcultured from most plates within 36 hr but faster growing fungi, especially *Mortierella* and *Pythium*, often overgrew the *P. drechsleri* cultures, making isolation difficult. The pathogen was isolated from about 70% of inoculated plants by using a lupin-baiting technique (Chee and Newhook 1965).

There was a similar reaction by all species to zoospore infection. The majority of root tips were dark brown and shrivelled for 2–3 mm from the apex and some

necrotic flecking extended back into the root system. This was particularly evident on lucerne roots.

Microscopic examination of the necrotic root tips showed hyphal penetration of the cortex from encysted zoospores, and sporangia were produced on many of the necrotic areas. When these infected roots were buried for 3 weeks in moist non-sterile field soil, chlamydospores were formed in the necrotic cortical cells.

There was little decline in the viability of chlamydospores with increasing time of burial in soil. Germination in excess of 60% on water agar was achieved with chlamydospores buried for 1–4 months; the germination rate fell to 26% after 10 months in soil but ungerminated spores stained as deeply as younger spores in lactophenol cotton blue. It is likely that the viability of the chlamydospores had not decreased appreciably but that the germination rate had dropped due to the development of constitutional dormancy.

IV. Discussion

P. drechsleri has been isolated from naturally infected saffron thistle (Stovold 1973), but this pathogen has not yet been isolated from field-infected specimens of any of the other weed species used in these experiments. Until this occurs it would be incorrect to claim lucerne, camel melon, Bathurst burr, skeleton weed, Paterson's curse, and crowsfoot as hosts of *P. drechsleri*, although failure to isolate the fungus is not indicative of the absence of the pathogen.

It has now been shown, however, that these plants, which are common throughout eastern Australia, are susceptible under laboratory conditions to infection by *P. drechsleri*. The fungus is capable of reproduction within the invaded tissue and this enables the pathogen to produce resting structures without being subjected to antagonism from other soil microorganisms. Possession of a dormant structure would enable the fungus to persist in soil for many months in the absence of its normally accepted host, safflower. Indeed, it is probably incorrect to visualize this recently introduced plant as anything other than an incidental host with less resistance to infection than the more established naturalized flora.

A hypersensitive reaction developed in inoculated saffron thistle, skeleton weed, and Paterson's curse. Rapid death of plant cells invaded by, or in contact with, the fungus confined the pathogen to the outer cortical tissue. The necrotic streaks seen in these plants are similar to the flecking developed by resistant safflower varieties inoculated with *P. drechsleri* (Klisiewicz and Johnson 1968). These workers reasoned that dead tissue of infected safflower should not hinder saprophytic growth of *P. drechsleri* unless toxic substances were released. Johnson (1970) and Thomas and Allen (1970) suggested that polyacetylenes may have a basic role in disease resistance. This may explain why there was no continued growth of the fungus in saffron thistle, and why the fungus produced resting propagules. The hypersensitivity appears to limit the spread of the fungus and has no doubt played a role in establishing a balance between the pathogen and the naturalized flora. It is envisaged that this pathogen exists in the rhizosphere of many plant species and its low level of activity is usually tolerated by the supporting plant. Only when the balance is upset by extremes in the environment does the flora exhibit signs of infection.

Wilhelm (1956) reported that the typical fungus flora of hairy nightshade roots (*Solanum sarachoides* Sendt) included nine economically important plant pathogens.

Katan (1971) found that some weed species could act as symptomless carriers of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyd. & Hans. and argued that parasitism and pathogenicity were not necessarily related. It was shown by Ham (1962) that the vesicular–arbuscular mycorrhizal condition is one of controlled parasitism. She found that *Pythium ultimum* and an unidentified species of *Pythium* caused damping-off of young onion and lettuce seedlings but both species could induce the development of a mycorrhizal condition in older established seedlings. The same fungus may kill the host or it may enter into a balanced relationship with the host without apparently causing it any harm. A number of genetic and environmental factors determine the behaviour of both host and parasite.

Rotations on agricultural land with "non-susceptible" crops would be of little value in reducing population density of *Phytophthora drechsleri*, or in avoiding root rot in future safflower crops, as it would be virtually impossible to rid a paddock and its surroundings of the ubiquitous saffron thistle and of other possible weed hosts.

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