

Host Range Screening of *Puccinia abrupta* var. *partheniicola* for the Biological Control of *Parthenium hysterophorus* in Queensland

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The host range screening of the rust *Puccinia abrupta* var. *partheniicola* is reported with reference to the range of resistant host reactions observed within the 109 species and varieties tested. Sori development and sporulation were only observed on *Parthenium hysterophorus* and *P. confertum* var. *lyratum*. In most non-host plants tested the fungus produced internal hyphae whose further development was restricted by host cell necrosis or callose deposition. In a few species, including some sunflower varieties, the host response occurred relatively late, after the development of limited haustoria. Additional testing of these species was required under 4 extreme environmental conditions involving combinations of light, temperature and humidity to confirm the inability of the rust to sporulate on them. The final stage of the host range screening involved inoculation with *P. helianthi* onto 2 sunflower varieties, 1 resistant and 1 susceptible to sunflower rust, to establish that prior inoculation did not predispose plants to subsequent infection by *P. abrupta* var. *partheniicola*.

Introduction

Parthenium hysterophorus L. (Parthenium weed, false ragweed; Asteraceae: Heliantheae) is an aggressive annual weed introduced into Australia from Texas in 1958. Its rapid spread since then has led to concern in central Queensland where it competes in both pastures and crops. Despite an intensive programme for containment it has continued to spread. Economic losses as a result of the weed are high with direct costs for control by local and state authorities estimated to be \$A 1.5 million, a figure that would be equaled by private landowners, and estimates for lost production considered to be in excess of \$A 5 million/yr. Chemical treatment of the weed is not economically feasible (Holman and Dale 1981), so a biological control programme was instigated in 1978. Surveys in Mexico, the purported centre of origin of parthenium weed, led to the collection of the rust *Puccinia abrupta* Diet. & Holow. var. *partheniicola* (Jackson)

Parmelee (Uredinales) (Parmelee 1967). Evans (1987) confirmed the rust to be a macrocyclic autoecious species and a promising candidate biological control agent. Parker (1988) reported that infection of the rust-hastened leaf senescence and significantly reduced plant dry weights, with diseased plants showing a 10-fold reduction in the number of seed-bearing flowers.

This paper describes the completion of the host range screening of the rust that included the testing of a number of species under a range of extreme environmental conditions, and the inoculation of *P. abrupta* var. *partheniicola* onto 2 sunflower varieties after prior inoculation with the sunflower rust, *Puccinia helianthi* (Schw.).

Materials and Methods

Study 1. Host Range Screening

The protocol followed throughout the host range tests was that described by Parker (1988). Conditions are given in Table 1.

Table 1. Environmental conditions as part of the host range studies for *Puccinia abrupta* var. *partheniicola*.

Regime	Temperature (°C)		Hrs	Light Intensity	Humidity (%)
	Day	Night			
1	34	22	14	600 $\mu\text{Em}^{-2}\text{sec}^{-1}$	30 ¹
2	34	22	14	600 $\mu\text{Em}^{-2}\text{sec}^{-1}$	85
3	17	9	12	400 $\mu\text{Em}^{-2}\text{sec}^{-1}$	30 ¹
4	17	9	12	400 $\mu\text{Em}^{-2}\text{sec}^{-1}$	85

¹ Humidity increased to 70% during darkness.

Species included in the host range screening list were selected by the Queensland Department of Lands according to the centrifugal phylogenetic testing procedure of Wapshere (1974).

Disease Assessment

In addition to macroscopic assessment of pustule development, inoculated leaves were sampled 10 d after inoculation to be cleared and stained according to the method of Bruzzese and Hasan (1983). Fungal development within the leaf was assessed and defined according to a numerical assessment system (Table 2). In addition macroscopic symptoms were assessed over a 3-wk period, according to the method of Kochman and Goulter (1983), and compared to a system devised by Bruzzese and Hasan (1986) for measuring the susceptibility of blackberry plants tested against *Phragmidium violaceum* (Schultz) Winter (Uredinales).

Study 2. Environmental Regime Testing

Eight species, marked with an asterisk in Table 2, that had appeared to allow growth of internal mycelium by *P. abrupta* var. *partheniicola* during Study 1 were challenged with the rust under 4 extreme environmental regimes to confirm the inability of the rust to form pustules on these species. Plants were inoculated as for Study 1, however, after inoculation, plants were maintained at 1 of 4 regimes listed in Table 1.

Study 3. Inoculation of Sunflowers after Prior Inoculation with *Puccinia helianthi*

On occasions, extensive internal hyphal development by *P. abrupta* var. *partheniicola*

had been observed in some sunflower varieties.

This study was designed to investigate the possibility that infection of sunflowers with sunflower rust *P. helianthi* (races 1 and 3) may predispose plants to infection when subsequently challenged with *P. abrupta* var. *partheniicola*. Two varieties of sunflower, 1 susceptible to the chosen races of *P. helianthi* (*Sunfola 68*) and 1 resistant (*Hysun 33*) were inoculated with *P. helianthi* prior to being challenged with *P. abrupta* var. *partheniicola*. The inoculum was applied to both leaf surfaces in a band 2-3 cm wide, delimited by 4 marks on the edge of each leaf. Inoculated plants were misted overnight (16 h) at 15-18°C, prior to being maintained at 18°C/12 h light. Three plants of each variety were challenged with *P. abrupta* var. *partheniicola* at the same time as the inoculation of *P. helianthi* or 1, 2, 4, 8 and 16 d after the initial inoculation with *P. helianthi*. Urediniospores of *P. abrupta* var. *partheniicola* were applied to both upper and lower leaf surfaces adjacent to the areas previously inoculated with *P. helianthi*.

Results and Discussion

Study 1

There was no obvious association between the host reaction of individual species challenged with *P. abrupta* var. *partheniicola* and their phylogenetic relationship to *P. hysterothorus* (Table 2). Pustules were formed only on *P. hysterothorus* and to a lesser extent on *Parthenium confertum* var. *lyratum*. The assessment systems of Bruzzese and Hasan or Kochman and Goulter classified these species as being susceptible—scoring a rating of 4 or 0 in

Table 2. Assessment of the host range of *Puccinia abrupta* var. *partheniicola* by inoculation of urediniospores onto selected test species.

Test Plant Species	Assessment of Infection ¹								
	1	2	3	4	5	6	7	8	9
Target plant									
<i>Parthenium hysterophorus</i> L.	+	+	+	-	-	+	-	-	+
Plants in sub-tribe Ambrosiinae									
<i>Ambrosia artemisiifolia</i> L.* ²	+	+	+	+	+	-	+	-	-
<i>Iva annua</i> L.*	+	+	+	+	-	-	+	-	-
<i>Parthenium argentatum</i> L.*	+	+	+	+	+	-	-	-	-
<i>Parthenium confertum</i> var. <i>lyratum</i> L.	+	+	+	-	-	+	-	-	+
<i>Xanthium pungens</i> Walfr.*	+	+	+	+	+	-	-	-	-
Major crop plants in Tribe Heliantheae									
<i>Guizotia abyssinica</i> (L.f.) Cass.	+	+	+	+	+	-	+	+	-
<i>Helianthus annuus</i> L. (main commercial cultivars)									
Dekalb 500, Flora, Hysun 22, Hysun 33*, Suncross 40, Sunking, Sunmac 40, SF5215, Lady Dekalb 600, Dynamite	+	+	+	-	+	+	+	-	-
Hysun 32, Sirosun 25H, Sunfolia 68*, Thunder Sunbird 11	+	+	+	+	-	+	+	-	-
Suncross 25	+	+	+	+	-	-	+	-	-
<i>H. agrophyllus</i> Torrey & Gray.	+	+	+	+	-	+	+	-	-
<i>H. debilis</i> var. <i>cucumerfolius</i> Nutt.	+	+	+	+	-	+	+	-	-
<i>H. tuberosus</i> L.	+	+	+	+	-	+	+	-	-
Major crop plants in other Tribes									
<i>Bidens pilosa</i> L.* (Heliantheae)	+	+	+	+	+	+	+	+	-
<i>Carthamus tinctorius</i> L.	+	+	+	+	-	-	-	-	-
<i>Chrysanthemum cinerariifolium</i> (Trev.) Vis.	+	+	+	+	-	-	-	-	-
<i>C. hortorum</i> L.H. Bailey	+	+	+	+	-	-	-	-	-
<i>Cichorium endiva</i> L.	+	+	+	+	-	-	-	-	-
<i>C. intybus</i> L.	+	+	+	+	-	-	-	-	-
<i>Cynara scolymus</i> L.	+	+	+	+	-	-	-	-	-
<i>Lactuca sativa</i> L.	+	+	+	+	-	-	+	+	-
Plants of ornamental importance in Tribe Heliantheae									
<i>Coreopsis lanceolata</i> L.	+	+	+	+	+	+	+	-	-
<i>Cosmos bipinnatus</i> Cav.	+	+	+	+	+	+	+	-	-
<i>Dahlia</i> spp. cv pompone, cv cinderella	+	+	+	+	-	+	-	-	-
<i>Echinacea purpurea</i> Moench	+	+	+	+	+	-	+	+	-
<i>Gaillardia aristata</i> Pursh	+	+	+	+	-	+	+	-	-
<i>Rudbeckia hirta</i> L.	+	+	+	+	+	+	+	-	-
<i>Tithonia diversifolia</i> Gray	+	+	+	+	+	-	+	-	-
<i>Wedelia</i> sp. (Singapore daisy)	+	+	+	+	+	-	-	-	-
<i>Zinnia elegans</i> Jacq.	+	+	+	+	-	+	-	-	-
Ornamentals in other Tribes									
<i>Ageratum houstonianum</i> Mill.	+	+	+	+	-	-	-	-	-
<i>Aster</i> sp.	+	+	+	+	-	+	-	-	-
<i>Calendula officinalis</i> L.	+	+	+	+	+	-	-	-	-

Table 2. Continued.

Test Plant Species	Assessment of Infection ¹									
	1	2	3	4	5	6	7	8	9	
<i>Centaurea cyanus</i> L.	+	+	+	+	+	-	-	-	-	
<i>Gazania x hybrida</i> Hort.	+	+	+	+	+	-	-	-	-	
<i>Gerbera jamesonii</i> H. Bolus.	+	+	+	+	+	-	-	-	-	
<i>Helichrysum subulufolium</i> F. Muell.	+	+	+	+	-	-	-	-	-	
<i>Leucanthemum vulgare</i> Lam.	+	+	+	+	-	-	-	-	-	
<i>Senecio cruentus</i> DC.	+	+	+	+	+	+	-	-	-	
<i>Tagetes erecta</i> L.	+	+	+	+	+	-	-	-	-	
Native plants in Tribe Heliantheae										
<i>Eclipta alba</i> Hassk.	+	+	+	+	+	-	-	+	-	
<i>E. platyglossa</i> F. Muell.	+	+	+	+	+	-	-	+	-	
<i>Enydra luctuans</i> Lour.	+	+	+	+	+	-	-	-	-	
<i>Glossogyne tenuifolia</i> (Labill.) Less.	+	+	+	-	+	-	-	-	-	
<i>Spilanthes grandiflora</i> Turcz.	+	+	+	+	-	-	+	+	-	
<i>Wedelia biflora</i> DC.	+	+	+	+	+	-	-	-	-	
<i>W. spilanthisoides</i> F. Muell.	+	+	+	+	-	-	+	-	-	
Native plants in other Tribes										
<i>Actites megalocarpa</i> (J.D. Hook.) Lander	+	+	-	-	+	-	+	-	-	
<i>Adenostemma lavaenia</i> O. Kuntze	+	+	+	+	-	-	+	-	-	
<i>Ammobium alatum</i> R.Br.	+	+	+	+	+	-	-	-	-	
<i>Calotis cuneata</i> R.Br.	+	+	+	+	-	-	+	-	-	
<i>Brachycombe iberidifolia</i> Benth.	+	+	+	+	-	-	+	-	-	
<i>B. multifida</i> DC.	+	+	+	+	-	-	-	-	-	
<i>Calocephalis brownii</i> (Cass.) F. Muell.	+	+	+	+	-	-	-	-	-	
<i>Cassinia laevis</i> R.Br.	+	+	+	+	-	-	+	-	-	
<i>Centepeda minima</i> L.A. Br. & Ascherson	+	+	+	+	-	-	-	-	-	
<i>Conyza sumatrensis</i> (Retz) E. Walker	+	+	+	+	-	-	-	-	-	
<i>Cotula australis</i> Sprengel JD Hook	+	+	+	+	-	+	-	-	-	
<i>Craspedia chrysantha</i> (Schidl.) Benth.	+	+	+	+	-	-	+	-	-	
<i>Cymbonotus pressianus</i> Steetz.	+	+	+	+	-	-	-	-	-	
<i>Emilia sonchifolia</i> (L.) BDC.	+	+	+	+	-	-	-	-	-	
<i>Epaltes australis</i> Less.	+	+	+	+	+	-	-	-	-	
<i>Flaveria australasica</i> Hooker	+	+	+	+	-	+	+	-	-	
<i>Gnaphalium sphaericum</i> Willd.	+	-	-	-	-	-	-	-	-	
<i>Gynura drymophila</i> (F. Muell.) F. Davies	+	+	+	+	+	-	-	-	-	
<i>Helichrysum bracteatum</i> Andr.	+	+	-	-	-	-	-	-	-	
<i>H. ramossissimum</i> Hook.	+	+	+	+	-	-	-	-	-	
<i>Helipterum albicans</i> (Gunn) DC.	+	+	-	-	-	-	-	-	-	
<i>H. roseum</i> Benth.	+	+	+	+	+	-	-	-	-	
<i>Minuria integerrima</i> DC. Benth.	+	+	+	+	-	-	-	-	-	
<i>Myriocephalus stuartii</i> (Sonder) Benth.*	+	+	+	+	+	+	+	-	-	
<i>Olearia nernstii</i> (F. Mueller)	+	+	+	-	-	-	-	-	-	
<i>Podolepis arachnoidea</i> (Hook.) Druce	+	+	+	+	+	-	-	-	-	
<i>Rhaponticum australe</i> (Gaudich.) Soskov	+	+	+	+	-	-	-	+	-	
<i>Schoenia cassiniana</i> (Gaudich.) Steetz	+	+	+	+	+	-	-	-	-	
<i>Senecio lautus</i> G. Forster ex. Willd s. lat.	+	+	+	+	-	-	+	-	-	
<i>Veronica cinerea</i> L.	+	+	+	+	-	-	-	-	-	
<i>Vittadinia sukata</i> L.	+	+	+	+	-	-	+	-	-	

Table 2. Continued.

Test Plant Species	Assessment of Infection ¹								
	1	2	3	4	5	6	7	8	9
<i>Youngia japonica</i> L.	+	+	+	+	-	-	+	-	-
Important crop plants attacked by other <i>Puccinia</i> spp.									
<i>Arachis hypogea</i> L.	+	+	+	+	-	-	-	-	-
<i>Gossypium hirsutum</i> L.	+	+	+	+	-	-	+	-	-
<i>Medicago sativa</i> L.	+	+	+	+	-	-	-	-	-
<i>Phaseolus vulgaris</i> L.	+	+	+	+	-	-	+	-	-
<i>Saccharum officinarum</i> L.	+	+	-	-	-	-	-	-	-
<i>Sorghum bicolor</i> (L.) Moench. cv. DK 37, DK 46, DK 57	+	+	-	-	+	-	-	-	-
<i>Triticum aestivum</i> L.	+	+	-	-	-	-	-	-	-
<i>Zea mays</i> L.	+	+	-	-	-	-	-	-	-
Representatives from families in the Order Campanulales									
<i>Isotoma anethifolia</i> Summerh.	+	+	+	+	+	-	-	-	-
<i>Pratia purpurescens</i> (R. Brown) F. Wimmer	+	+	+	+	+	-	-	-	-
<i>Wahlenbergia</i> sp.	+	+	+	+	+	-	-	-	-
<i>Scaevola humilis</i> R. Brown	+	+	+	+	-	-	-	-	-
<i>Stylidium graminifolium</i> Swartz	+	+	-	-	+	-	-	-	-

¹ Codes: 1. Spore germination; 2. appressoria formation over stomata; 3. substomatal vesicle formation; 4. short internal hyphae present; 5. necrosis around point of infection and internal hyphae; 6. short or longer internal hyphae with haustoria; 7. callose formation around haustoria or on host cell walls around infection; 8. host cell granulation; 9. sorus formation.

² Species included in additional host range tests.

the respective systems. All of the other species tested were considered either as immune or highly resistant in both systems. For all species, the rate of host response was modified by the age of the inoculated leaves, and older tissues were more resistant to penetration by the rust.

Necrosis of cells into which internal hyphae had developed was the most common host reaction as exemplified in *Parthenium argentatum* L., *Centaurea cyanus* L. and *Eclipta alba* Hassk. In species such as *Ageratum houstonianum* Mill. and *Chrysanthemum cinerariifolium* (Trev.) Vis., necrosis was more restricted and occurred only around the guard cells beneath the appressorium. For most of the sunflower varieties, both callose and cell necrosis restricted hyphal growth. This was also observed in a number of other species, including *Ambrosia artemisiifolia* L. however, callose alone appeared to be the only defence response in a few species such as *Wedelia spilanthis* F. Muell. and *Adenostemma lavaenia* O. Kuntze. The deposition of callose appeared to be associated with host cell

granulation in *Lactuca sativa* L., but generally granulation was a less commonly observed defence response, although in *Rhaphanum australe* (Gaudich.) Soskov it appeared to be the only form of reaction. In a few species such as *Stylidium graminifolium* Swartz and some members of the Gramineae, the rust did not develop beyond the formation of an appressorium although no host response was apparent. Necrosis of cells under the appressorium prevented penetration of the *Sorghum bicolor* (L.) Moench. varieties tested. Frequently, as for *Bidens pilosa* L., the challenged plant responded to infection with a variety of defence mechanisms rather than just one.

Study 2

The inoculation of selected test species with *P. abrupta* var. *parthenicola* under 4 extreme environmental regimes did not generally modify the host response observed in the original host range study. In each of the conditions uredinia

were formed only on *P. hysterophorus*, and in none of the test species was extensive internal mycelium with haustoria formed. Fungal development was most rapidly inhibited under conditions of increased light and high humidity and possibly reflected an increase in the metabolic activity of the plant. Short internal mycelium was observed in *A. artemisiifolia*, *P. argentatum*, and *Myriocephalus stuartii* (Sonder) Benth., but in each case the hyphae were constricted. Cell necrosis was observed in a number of species, but in *M. stuartii* it appeared that the rust was unable to obtain sufficient nutrients to sustain its development beyond the substomatal cavity although there was an absence of any discernible host response. In *Iva annua* L. thickening of the guard cell walls acted as an initial barrier to penetration, although, where infection did occur, cell necrosis was observed in cells adjacent to the point of penetration. This had not been seen in *Study 1*, in which fungal development was restricted by deposition of callose onto cell walls. Only in *P. argentatum* did the rust develop to a greater extent than in the original study, although this was probably a consequence of leaf age at the time of inoculation.

Study 3

The prior inoculation of *P. helianthi* onto the 2 sunflower varieties tested did not enhance the level of infection of *P. abrupta* var. *partheniicola*. The rate of the hypersensitive response was not modified by the prior inoculation of *P. helianthi*. In *Sunfolia 68*, chlorotic lesions and cell necrosis in response to infection by *P. abrupta* var. *partheniicola* were observed adjacent to areas colonised by extensive internal mycelium of *P. helianthi*. In no instance did mycelium of the 2 rusts occur in the same cell, and hyphae of *P. abrupta* var. *partheniicola* were always separated from *P. helianthi* by an area of necrosed cells. *P. helianthi* did not develop in *Hysun 33*, and the same strong necrotic reaction was induced by *P. abrupta* var. *partheniicola*.

No evidence was produced from the studies to indicate that *P. abrupta* var. *partheniicola* could develop sufficiently to sporulate on any of the hosts tested except *P. confertum* var.

lyratum on which weak sporulation was observed. A proposal for the importation of the rust was submitted by the Queensland Department of Lands (Tomley 1990) based on the findings of these studies, and in February 1991 permission to import the rust into Australia was granted. The rust was released into the field in June 1991 where its spread and effectiveness are being monitored.

Acknowledgments

This work was funded by the Queensland Department of Lands (Land Administration Commission). We wish to acknowledge the invaluable contribution made by Dr. H.C. Evans through his advice and guidance during this study.

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