Proc. VII. Int. Symp. Biol. Contr. Weeds, 6-11 March 1988, Rome, Italy.
Delfosse, E.S. (ed.). Ist. Sper. Patol. Veg. (MAF), pp. 531 - 7 (1989).

Biological Control of *Parthenium* Weed Using Two Rust Fungi

A. Parker

CAB International Institute of Biological Control (CIBC), Silwood Park, Ascot, Berks. SL5 7TA UK [1]

Abstract

Parthenium hysterophorus **is a native of Central America which has become a problem weed of Australian rangeland since its accidental introduction in 1958. Investigations of potential pathogens for classical introduction were begun in 1983 in order to supplement insect biological control. An evaluation has been made of the biology and host range of two autoecious rust fungi, *Puccinia melampodii* and *P. abrupta* var. *partheniicola* which were collected in north-east Mexico. Preliminary studies have shown that *P. melampodii* has an unacceptably wide host range and hence this rust has been rejected as a biological control agent. *P. abrupta* var. *partheniicola* has a narrow host range and causes severe damage to Parthenium weed. Infection with this rust hastens leaf senescence and significantly reduces the life cycle and dry weight of plants. Also the production of mature, seed-bearing flowers is reduced 10-fold on diseased plants. Thus *P. abrupta* var. *partheniicola* appears to be a promising biological control agent for Parthenium weed.**

Introduction

Parthenium hysterophorus L. (false ragweed; Asteraceae: Heliantheae) is a widespread noxious weed of many sub-tropical and tropical countries. It was introduced into Australia from Texas in 1958 with a consignment of pasture seed, and its rapid spread in Queensland since the 1970s is seriously curtailing the grazing acreage (Haseler 1976). In addition, Parthenium weed is reported to cause allergic responses, such as respiratory malfunction and dermatitis, in susceptible humans (Evans 1987). As chemical treatment of the weed is uneconomic, the search for biological control agents began in 1978.

The centre of origin of *P. hysterophorus* is believed to be Central America and surveys for predators and pathogens have been performed in Mexico. Six insect species have since been introduced into Australia but only one, the moth *Epiblema strenuana* (Walker) (Lepidoptera: Tortricidae) causes significant damage to Parthenium (McClay 1987). Also, this stem borer is only damaging in the wetter areas and hence the investigations have turned to pathogens to provide additional injury to the weed. Two rust fungi were chosen from the records of pathogens of Parthenium held at the CAB International Mycological Institute (Evans 1987*a*). These fungi are obligate parasites and hence are more likely to be host specific than facultative parasites. Both rusts were collected in Mexico in 1986: *Puccinia melampodii* Diet. & Holw. (Uredinales) from the humid lowland areas; *P. abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parm. from upland semi-arid areas. They were brought to the quarantine facilities at CIBC (UK) for assessment. Initial investigations to determine the life-cycles of the rusts showed that both are autoecious. All five spore stages of *P. abrupta* var. *partheniicola* have been produced on Parthenium plants (Evans 1987*b*) whereas *P. melampodii* only exists in the telial stage (Parmelee 1967) and is presumed to have lost the other stages.

This paper describes recent work on the biology of the weed-fungal interactions and on the host range of the two rusts prior to considering them for introduction into Australia.

---

[1] Present address: Department of Horticulture, University of Reading, Earley Gate, Reading RG6 2AU UK

Methods and Materials

Rust and Plant Materials

Uredia and telia of *P. abrupta* var. *partheniicola* and telia of *P. melampodii* were collected on plant material from different areas of Mexico. Seed of *P. hysterophorus*, *P. confertum* L. and *P. argentatum* L. were also collected. Additional samples of *P. abrupta* var. *partheniicola* uredia and seed of *P. hysterophorus* were made in Kenya, a new locality record for this rust species on the recently introduced and spreading weed. Seed of *P. hysterophorus* and those species to be included in the host range tests were sent from Australia. The selection of native and crop plants for testing was made by the Queensland Department of Primary Industry and was based on the mainly centrifugal procedure of Wapshere (1974). Plants were grown in sterilised soil (50:50 John Innes No. 3 compost: peat) in 5" pots and maintained in a greenhouse (14 h light at 25°C and 40 to 50% RH, 10 h dark at 15°C and 90 to 100% RH) held under negative pressure conditions to satisfy UK quarantine requirements.

Inoculation Procedures

Uredospores from 3-wk-old pustules of *P. abrupta* var. *partheniicola* were knocked from a leaf into a sterile Petri dish and mixed with a sterile solution of 0.01% Tween 20 in distilled water. The suspension was then painted onto both surfaces of young leaves of the test plants and Parthenium before being placed in a chamber in which free water was maintained on the leaves by a Defensor 505 humidifier for 16 h overnight. Telia of *P. melampodii* were cut into small pieces which were then pasted onto leaves with sterile distilled water and humidified for 48 h. Host range tests were performed at least twice using four plants of each species.

Disease Assessments

Pustule development was assessed macroscopically and the number counted per leaf. Leaves were also sampled 2, 4, 6 or 8 d after inoculation and stained and cleared using the method of Bruzzese and Hasan (1983). The extent of fungal growth on and in the leaves was then assigned to categories of development on a quantitative assessment of spores. The effect of disease on the weed was determined by comparison of ten uninoculated plants with ten plants which were inoculated with uredospores of *P. abrupta* var. *partheniicola* every 3-4 d from 13-wks-old during the growing season. Various parameters of host vigour were assessed including leaf senescence, leaf and lateral shoot production, plant height and seed production.

Results

Biotypes

Two distinct biotypes of *P. hysterophorus* grew from seed collected in Mexico in both 1984 and 1986. Seed from Saltillo (Coahuila) produces a plant with a rosette of leaves which does not elongate until flowering. Plants grown from seed collected in Ciudad de Maiz (San Luis Potosi) and La Ascension (Nuevo Leon) have no rosette stage and their leaves are more hirsute than the Saltillo biotype. Both produce white flowers and hence the South American biotype of *P. hysterophorus* (Dale 1981) is not involved. Plants grown from Australian and Kenyan seed are like the Saltillo biotype.

Isolates

Assessment of pustule formation and sporulation produced by the one Kenyan and five Mexican isolates of *P. abrupta* var. *partheniicola* on Australian Parthenium plants indicated that no one isolate was consistently more virulant than the others. However one of the isolates collected from Saltillo (isolate 3) produced the most vigorous infection during the

summer (Table 1) and hence this isolate was chosen for all subsequent host range tests. This choice of isolate was further supported by its having been found on the same plant biotype as Australian Parthenium.

Table 1. Number of pustules produced per leaf after inoculation with six isolates of *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parm. (mean of 14 replicates).

Isolate	Source	Altitude (m.a.s.l.)	Mean number of pustules/leaf
Mexican			
1	La Esperanza (Nuevo Leon)	1015	7.8
2	Ciudad de Maiz (San Luis Potosi)	1390	9.5
3	Saltillo (Coahuila)	1540	10.5
4	Saltillo (Coahuila)	1700	1.3
5	La Ascension (Nuevo Leon)	1750	3.4
Kenyan	Kahawa-Ruiru (Thika)	1490	0.9
	LSD ($P = 0.05$):		2.56

Host Range

Telia of *P. melampodii* were produced on leaves of *P. hysterophorus*, *P. confertum*, *P. argentatum* and on distorted flowering shoots of *Calendula officinalis* L. (Asteraceae: Calenduleae). As this fungus can sporulate on species from different tribes in the Asteraceae, *P. melampodii* could no longer be considered as a potential biological control agent and no further host range tests were performed using this rust.

Uredia of *P. abrupta* var. *partheniicola* have only been observed on plants of *P. hysterophorus* and *P. confertum*. The degree of infection produced in leaves of other plant species tested is shown in Table 2. Uredospores germinated to various extents on all of the test plants inoculated to date. On Parthenium leaves, spore germination was 85-95% whereas on leaves of other plants, such as *P. confertum*, germination was as low as 48%. Germ tubes of *P. abrupta* var. *partheniicola* also formed appressoria over stomata on all of the tested plants although the proportion of germ tubes forming appressoria was not correlated with the taxonomic closeness of the species to Parthenium. Thereafter the plants varied in their response to the fungus. In some of the Heliantheae, such as the sunflower cultivars and non-host members of the Ambrosiinae, the fungal growth was limited by a hypersensitive response in which the substomatal vesicles and short internal hyphae were surrounded by necrotic mesophyll cells. In others, the fungus produced longer, branched hyphae and haustoria but did not develop sufficiently to produce chlorotic spots or pustules on the leaves. Of the other tribes in the Asteraceae, leaves of the two representatives of the Lactuceae tested produced the necrotic hypersensitive response whereas those of *Aster* sp. (Asteraceae) and *C. officinalis* allowed long intercellular hyphae to grow. However, members of the Anthemideae

and Cynareae limited the growth of the short internal hyphae without a hypersensitive response.

Effect of P. abrupta *var.* partheniicola *on Weed Vigour*

Although uredia were produced on all the green parts of diseased Parthenium plants, repeated inoculation failed to reduce significantly their height, leaf production and lateral shoot production (Table 3). However infected leaves senesced significantly quicker than non-infected leaves. Also the length of flowering shoots and dry weight produced by diseased plants were significantly less than produced by healthy Parthenium plants. There were highly significant differences between the life expectancies and production of mature, seed-bearing flowers in diseased and healthy plants; the life expectancy was reduced by 40% and the seed production by 90% when infected with uredia of *P. abrupta* var. *partheniicola.*

Discussion

In Mexico, Parthenium weed is seriously damaged by infection from *P. abrupta* var. *partheniicola* in the upland arid areas. This damage has been repeated in the CIBC greenhouse in similar environmental conditions which also resemble those found in the Parthenium-infested areas of Australia (Dale 1981, Tomley, A., pers. comm., 1987). It is presumed that the hastened senescence of rust-infected leaves reduces the amount of photosynthate produced which in turn reduces the length of flowering shoots and the number of seed-bearing flowers. As the weed is an annual with no perennating organs, the highly significant reduction in seed production caused by rust infection is likely to seriously curtail its spread. Also a rust-infected plant is more stressed than a healthy one for additional reasons such as increased transpiration which should reduce its competitiveness with the native flora and rangeland grasses. Observations in Australia have shown that after field release of *Puccinia chondrillina* Bubak & Syd., the rust became widespread within a short time and caused a remarkable reduction in the population of skeleton weed (Cullen *et al.* 1973, Cullen 1978). Of the two candidate rusts, *P. abrupta* var. *partheniicola* is better suited to be a biological control agent as it produces large numbers of powdery uredospores which are wind dispersed. In contrast, the telia of *P. melampodii* are borne on the undersurface of Parthenium leaves and the teliospores remain attached. Also uredinia of *P. abrupta* var. *partheniicola* occur on all green plant parts of the weed and thus this rust is more damaging to seed production than *P. melampodii* which is limited to the leaves.

From inoculations performed in the greenhouse, the host ranges of *P. abrupta* var. *partheniicola* and *P. melampodii* correspond with those published by Parmelee (1967) and Cummins (1978) respectively. The restricted host range of the former rust indicates that *P. hysterophorus* and *P. confertum* are more closely related to each other than to *P. argentatum.* This relationship is also implied from the morphological similarities of the first two species. McClay (1987) also found that the moth, *E. strenuana* fed and oviposited on *P. hysterophorus* and *P. confertum* but not on *P. argentatum* although it could use additional plant species as host. The resistance of *P. argentatum* to infection by *P. abrupta* var. *partheniicola* is auspicious as this plant has a latex which may be developed as a commercial source of rubber.

The ability of *P. abrupta* var. *partheniicola* to establish haustoria in several non-host plants indicates that the fungus is able to overcome some of the resistance mechanisms of the leaves. The formation of unnecrosed haustoria contrasts with the usual degree of infection produced by other rust fungi in their respective non-host plants (Heath, 1977). Due to the lack of sporulation on these plants, the reaction to *P. abrupta* var. *partheniicola* is considered as immune (Kochman, J.K., pers. comm., 1987). A similar degree of disease development which followed inoculations of *Rubus* spp. (Rosaceae) with *Phragmidium violaceum* (Schultz) Wint. (Teliomycetes: Uredinales) was considered as highly resistant by Bruzzese and Hasan (1986). Additional plant species are still be screened for their reaction to *P. abrupta* var. *partheniicola,* but as they are further removed taxonomically from Parthenium than those already tested, it is expected that these plants will also show immune responses.

Table 2. Infection development from inoculations of species of Asteraceae with uredospores of *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parm.

Host	Tribe	Sub-tribe	Host / Symptom seen [1]							
			1	2	3	4	5	6	7	8
Parthenium hysterophorus L.	Heliantheae	Ambrosiinae	+	+	+	+	-	+	+	+
P. confertum L.	"	"	+	+	+	+	-	+	+	+
P. argentatum L.	"	"	+	+	+	+	+	-	-	-
Ambrosia artemisiifolia L.	"	"	+	+	+	+	+	-	-	-
Xanthium pungens Wallr.	"	"	+	+	+	+	+	-	-	-
Helianthus annuus L. cv. Flora	"		+	+	+	+	+	-	-	-
H. annuus cv. Hysun 22	"		+	+	+	+	+	-	-	-
H. annuus cv. Dekalb 500	"		+	+	+	+	-	-	-	-
H. annuus cv. Dynamite	"		+	+	+	+	+	-	-	-
Cosmos bipinnatus Cav.	"		+	+	+	+	-	+	+	-
Dahlia cv. Pompone	"		+	+	+	+	-	+	+	-
Dahlia cv. Cinderella	"		+	+	+	+	-	+	+	-
Aster sp.	Asteraeae		+	+	+	+	-	+	+	-
Calendula officinalis L.	Calenduleae		+	+	+	+	-	+	-	-
Cichorium endivia L.	Lactuceae		+	+	+	+	+	-	-	-
C. intybus L.	"		+	+	+	+	-	-	-	-
Chrysanthemum hortorum	Anthemideae		+	+	+	+	-	-	-	-
C. cinerariifolium (Trev.) Vis.	"		+	+	+	-	-	-	-	-
Leucanthemum vulgare Lam.	"		+	+	+	-	-	-	-	-
Cynara scolymus L.	Cynareae		+	+	+	+	-	-	-	-
Carthamus tinctorius L.	"		+	+	+	-	-	-	-	-

[1] Infection symptoms:
microscopic- 1 = spore germination; 2 = appressoria formation over stomata; 3 = substomatal vesicle formation; 4 = short internal hyphae present (< 1 cell long); 5 = necrosis visible around stunted internal hyphae; 6 = longer, branched internal hyphae present; 7 = haustoria seen;
macroscopic- 8 = chlorotic spots or pustules present.

Work is also in progress to determine the reaction of some closely related plants to the rust in a variety of environmental conditions which will simulate the range of environments in which Parthenium grows in Australia, but these are thought unlikely to alter the responses of the plants.

Table 3. Assessments of weed vigour after inoculation with *Puccinia abrupta* Diet. & Holw. var. *partheniicola* (Jackson) Parm.

Parameter	Mean from ten plants Uninoculated	Inoculated
Height to tip of terminal shoot (cm)	97.8	72.7 NS
Leaf production[1]	7.9	8.0 NS
Lateral shoot production[1]	4.6	3.9 NS
Index of leaf senescence[2]	8.8	21.2**
Length of flowering shoots (cm)	180.0	54.9***
Mature flower production	2420.3	212.2***
Life of plants (d)	384.1	225.9***
Plant dry weight (g)	9.32	4.37**

[1] Leaf and shoot production over 4 wks in the middle of the growing period.
[2] Total index of senescence for 10 leaves/plant where 0 = healthy green leaf and 5 = dead.
NS = no significant difference between the populations.
** = significantly different at $P = 0.01$ level.
*** = significantly different at $P = 0.001$ level.

From the seed sent from Australia, the non-apomictic Parthenium appears to be of only one biotype. Hopefully this genetic homogeneity will prevent the problems encountered in the biological control programme for skeleton weed in Australia when two of the three biotypes were resistant to infection by the first rust isolate to be introduced (Burdon *et al.* 1981). Hence the restricted host range of *P. abrupta* var. *partheniicola* and its damaging effect on Parthenium make this fungus a promising candidate for introduction into Australia as a biological control agent of the weed.

Acknowledgements

The author wishes to thank Drs. H.C. Evans and C. Prior for assistance in the preparing of this manuscript. This work formed part of a project (PAR. 475) funded by the Queensland Department of Lands (Land Administration Commission). The U.K. research was carried out under MAFF licence number PHF 873/36.

References

Bruzzese, E. and S. Hasan. 1983. A whole leaf clearing and staining technique for host specificity studies of rust fungi. *Pl. Path.* **32**:335-8.

Bruzzese, E. and S. Hasan. 1986. Host specificity of the rust *Phragmidium violaceum*, a potential biological control agent of European blackberry. *Ann. Appl. Biol.* **108**:585-96.

Burdon, J.J., R.H. Groves and J.M. Cullen. 1981. The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. *J. Appl. Ecol.* **18**:957-66.

Cullen, J.M. 1978. Evaluating the success of the programme for the biological control of *Chondrilla juncea* L. *Proc. IV Int. Symp. Biol. Contr. Weeds*, 30 August - 2 September 1976, Gainesville, Florida, Freeman, T.E. (ed.). Univ. Florida, pp. 117-21.

Cullen, J.M., P.F. Kable and M. Catt. 1973. Epidemic spread of a rust imported for biological control. *Nature* **244**:462-4.

Cummins, G.B. 1978. *Rust Fungi on Legumes and Composites in North America.* University of Arizona Press, Tucson, Arizona, p. 106.

Dale, I.J. 1981. Parthenium weed in the Americas. A report on the ecology of *Parthenium hysterophorus* in South, Central and North America. *Aust. Weeds* Sept.:8-14.

Evans, H.C. 1987*a*. Fungal pathogens of some subtropical and tropical weeds and the possibilities for biological control. *Biocontr. News and Inform.* 8(1):7-30.

Evans, H.C. 1987*b*. The life cycle of *Puccinia abrupta* var. *partheniicola*, a potential biological control agent of *Parthenium hysterophorus*. *Trans. Br. Mycol. Soc.* 88:105-11.

Haseler, W.H. 1976. *Parthenium hysterophorus* L. in Australia. *Pest Articles and News Summaries* 22:515-7.

Heath, M.C. 1977. A comparative study of non-host interactions with rust fungi. *Physiol. Pl. Path.* 10:73-88.

McClay, A.S. 1987. Observations on the biology and host specificity of *Epiblema strenuana* (Lepidoptera, Tortricidae), a potential biocontrol agent for *Parthenium hysterophorus* (Compositae). *Entomophaga* 32:23-34.

Parmelee, J.A. 1967. The autoecious species of Puccinia on Heliantheae in North America. *Can. J. Bot.* 45:2267-339.

Wapshere, A.J. 1974. A strategy for evaluating the safety of organisms for biological weed control. *Ann. Appl. Biol.* 77:201-11.