EFFECT OF TEMPERATURE AND LEAF WETNESS ON INFECTIVITY OF *PUCCINIA ABRUPTA* VAR. *PARTHENIICOLA*, A POTENTIAL BIOCONTROL AGENT OF PARTHENIUM WEED

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Summary Parthenium weed (*Parthenium hysterophorus* L.) is declared as a noxious weed in Queensland and is uneconomic to control using chemical herbicides. As it is an introduced weed, this led to the exploration of biological control as a long-term option. The rust *Puccinia abrupta* var. *partheniicola* from upland areas of Mexico is a potential biocontrol agent for the weed. Infection by the pathogen causes a significant reduction in vegetative growth of young plants and seed production of older ones. The environmental requirements of the infection of the rust were investigated. The infectivity of the pathogen depended on post-inoculation temperature and leaf wetness. The optimum temperature for infection was 15°C with leaves continuously moist for 12 h. Leaf wetness for 9 h at 15 or 20°C also enabled considerable rust infection and subsequent leaf damage. A 6 h period of leaf wetness was a minimum requirement for the infection when plants were inoculated and held at 10, 15 or 20°C, and subsequently grown on in a controlled temperature, naturally lit glasshouse maintained on a day/night regime of 18/13°C. When plants were grown on at 30/26°C, a wet leaf period of 6 h at 15 or 20°C was sufficient for infection, but 9 h was required at 10°C. No infection occurred when leaves were wet for 3 h or less. Plants exposed to the lower day/night temperatures of 18/13°C had more infection than those exposed to 30/26°C. The optimum temperature for urediniospore germination was 15°C, with a drastic decline at 30°C, and a much smaller decline at 5, 10 and 20°C.

INTRODUCTION

Parthenium weed (*Parthenium hysterophorus* L.), a plant native to tropical America, is an aggressive coloniser of rangeland in central Queensland (Parsons and Cuthbertson 1992). Because of its high competitive ability, the weed becomes a dominant species, and in 1992 it covered an area of 170 000 km^2 (Haseler 1976, McFadyen 1992). The weed is declared noxious in Queensland (Parsons and Cuthbertson 1992). Since the weed infests large areas, control using chemical herbicides is uneconomic (Holman and Dale 1981), biological control is being explored as a long term option. The rust *Puccinia abrupta* var. *partheniicola* from upland areas of Mexico is a potential biocontrol agent to curtail the weed. Infection by the pathogen caused a significant reduction in vegetative growth of young plants and seed production of older ones (Evans 1987). In addition, the rust is very host specific (Parker *et al*. 1994).

Because little is known about the environmental requirements for the infection of the rust on parthenium weed, experiments were undertaken to determine temperature and leaf wetness duration necessary for infection, and temperature required for optimum germination of urediniospores.

MATERIALS AND METHODS

Puccinia abrupta var. *partheniicola* urediniospores collected from upland areas of Mexico by researchers of Alan Fletcher Research Station, Queensland Department of Land were used for these experiments.

Urediniospores of *P. abrupta* var. *partheniicola* were produced by infecting parthenium weed grown in 25 cm diameter plastic pots with the rust spores. Urediniospores were collected by sucking them up from the leaf surface using an 'Air Cadet' Model No. 7530-50 (Cole-Palmer Instrument Co., Chicago). Collected spores were placed in vials and stored at 10°C before used for inoculation.

Plant preparation and inoculation Parthenium seeds were sown in 10 cm pots previously filled with California mix. Each pot was planted with several seeds. At the plant age of two weeks, plants were thinned to only one uniform plant per pot.

Six weeks old plants were inoculated with a solution of urediniospores in sterile distilled water previously mixed with two drops of 0.01% Tween 20 with an approximate concentration of 20 000 spores mL^{-1}. The urediniospores used for inoculation were collected from 5-week-old pustules, and used after storage for four months in liquid nitrogen. The plants were inoculated by spraying them with the solution using a Wattyl Jet-Pack (Wattyl Australia Pty., Canada Bay, New South Wales). The inoculated plants were then individually covered with previously misted plastic bags to provide leaf wetness for the duration of 3, 6, 9 or 12 h and placed in a growth chamber at a temperature of 10, 15 or 20°C. Plastic bags were removed at the end of the dew period and the plants placed on glasshouse benches. The experiment

was arranged in a completely randomized design with six replicates and conducted twice. In the first experiment, inoculated plants were held in a glasshouse with day/night temperatures of 30/26°C and in the second experiment at 18/13°C. The infectivity of the rust was measured by counting the number of pustules per plant at the end of the experiment.

Spore germination Urediniospores used for this experiment were obtained from a vial of spores stored in liquid nitrogen for four months. The urediniospores in sterile distilled water were spread on the surface of water agar in petri dishes. Ten petri dishes were incubated in each section of a thermogradient set at 5, 10, 15, 20, 25 or 30°C in the dark. After 24 h, the percentage of spores germinated was determined on population of at least 100 spores by scoring using a compound microscope. Urediniospores were considered to be germinated if the length of the germ tube was at least equal to the spore diameter. The experiment was arranged in a completely randomized design with 10 replicates and conducted twice.

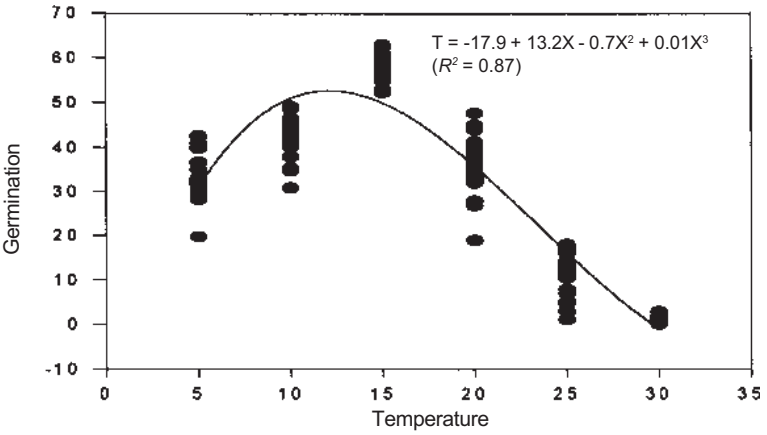

Figure 1. Effect of post-inoculation temperature on urediniospore germination (%) for *P. abrupta* var. *partheniicola*. Data from two experiments were combined.

Statistical analysis Data from the temperature and leaf wetness effects were analysed with analysis of variance procedures to determine the significance of treatment effects and their interactions (Steel and Torrie 1981). A Student-Newman-Keuls (S-N-K) test at the 0.05 level was carried out to compare treatments. Data from the spore germination test were analysed with polynomial regression.

Table 1. Effect of temperature and leaf wetness on infectivity of parthenium weed by *P. abrupta* var. *partheniicola* when the plants were grown on at a glasshouse day/night temperature of 30/26°C and 18/13°C.

Temperature	Pustules per plant[A]			
	Duration of leaf wetness (h)			
	3	6	9	12
		30/26°C		
10	0	0.0 a[B]	0.5 a	11.0 c
15	0	6.5 b	17.0 d	32.2 e
20	0	4.7 b	16.3 d	16.3 d
		18/13°C		
10	0	0.2 a[B]	3.0 a	52.3 e
15	0	10.3 b	30.2 d	83.5 f
20	0	2.3 a	29.3 d	25.5 c

[A] Data are average of six replicates.

[B] Since there was interaction between temperature and leaf wetness, all possible combinations were compared. Values followed by the same letter within each glasshouse temperature are not significantly different at P=0.05 according to S-N-K test.

RESULTS

Infectivity of the rust *P. abrupta* var. *partheniicola* on parthenium weed depended on post-inoculation temperature and leaf wetness duration. The number of pustules per plant was zero when the post-inoculation wetness period was £3 h, regardless of temperature. When the plants were grown on in a controlled temperature, naturally lit glasshouse maintained at a day/night temperature of 30/26°C, the minimum leaf wetness period required for infection was 6 h at a post-inoculation temperature of 15 and 20°C but 9 h at 10°C with the maximum infection occurring at 15°C for 12 h wet (Table 1).

Plants grown on in a controlled temperature, naturally lit glasshouse maintained on a day/night temperature of 18/13°C were infected more severely than those maintained on 30/26°C. The minimum leaf wetness period of 6 h was required for infection, with the maximum infection occurred when the plants were exposed to post-inoculation temperature of 15°C with 12 h leaf wetness (Table 1).

The variance between the two spore germination experiments was not significantly different, hence both experiments were pooled, so that the experiment had 20 replicates. The maximum urediniospore germination of 58% was achieved at incubation temperature of 15°C, with a marked decline at 25 and 30°C (11% and 0.9% of spore germinated respectively). There was a much

smaller decline at 5, 10 and 20°C with spore germination of 34, 42 and 36% respectively (Figure 1). The relationship between temperature and the percentage of urediniospore germinated was described by the polynomial regression $Y = -17.9 + 13.2X - 0.7X^2 + 0.01X^3$ where Y is the percentage of spore germinated and X is the temperature. The R^2 of the equation was 0.87

DISCUSSION

Urediniospores used in these experiments had sufficient viability after being stored in liquid nitrogen for four months, much as reported by Holden (1992). The temperature range for urediniospore germination of *P. abrupta* var. *partheniicola* was quite wide (5–25°C). This feature suggests that *P. abrupta* var. *partheniicola* could be a successful biocontrol agent for parthenium weed. Similarly, urediniospores of *P. chondrillina,* which has been successful in controlling skeleton weed, *Chondrilla juncea* L., in Australia and USA (Hasan and Wasphere 1973, TeBeest 1991) germinated over a wide range of temperature.

Two main environmental conditions affecting the success of biocontrol agents in suppressing weeds are temperature and moisture (as humidity). Each has to be suitable for the growth and development of the pathogens (TeBeest 1991). Infectivity of *P. abrupta* var. *partheniicola* to parthenium weed in our experiments depended on post-inoculation temperature and leaf wetness. The optimum temperature observed for infection of the rust to parthenium weed was 15°C, which is a common night temperature in the growing areas of the weed in central Queensland (Tomley 1990). However, optimal leaf wetness duration for *P. abrupta* var. *partheniicola* to infect parthenium weed was 12 h, a quite long period that rarely happen in the field. The requirement for a long dew period (high humidity) in the success of pathogen biocontrol agent was also reported by Politis and Bruckart (1986) for the infectivity of *P. carduorum* on musk thistle and in the recent review by Auld and Morin (1995). Fortunately, *P. abrupta* var. *partheniicola* was also able to infect parthenium weed after a shorter leaf wetness of 6 h, with considerable rust infection with a 9 h leaf wetness period. The plants grown on at day/night temperatures of 18/13°C were infected more severely than those grown on at 30/26°C. This may be due to the adaption of the rust to its origin from upland areas in central Mexico (Evans 1987). Our finding suggests that *P. abrupta* var. *partheniicola* as a biocontrol agent is best applied late in the afternoon when the temperature is expected to be around 15°C, and when the dew period may be expected to last for 6 h or more. These conditions are most likely to occur during the Australian autumn (May or June).

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