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Biocontrol Ability of *Puccinia abrupta* var. *partheniicola* on Different Growth Stages of Parthenium Weed (*Parthenium hysterophorus* L.)

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A research was conducted to investigate the biological control ability of *Puccinia abrupta* var. *partheniicola* infected to parthenium weed (*Parthenium hysterophorus* L.) at different stages of growth in a glasshouse. The study also investigated the combined effect of the infection and the competitor plant, i.e. buffel grass (*Cenchrus ciliaris* L.), a pasture species usually found in the weed habitat in Central Queensland. The 2 x 3 factorial experiment was arranged in a completely randomized design with six replicates in each treatment. The parthenium weeds were planted with or without buffel grass. The plants were inoculated with *P. abrupta* var. *partheniicola* urediniospores either at the rosette, flowering or mature growth stage of development. As controls, an additional six non inoculated plants with and without buffel grass were planted. The results showed that *P. abrupta* var. *partheniicola* affected more on the younger plants than on the older ones. Its infection decreased the plant height. A higher reduction in plant above ground biomass was recorded because of the rust when the plants were inoculated at the rosette growth stage of development in the presence of competition. The impact of the rust was greatest on the ability of parthenium to produce seeds.

Key words: *Puccinia abrupta* var. *partheniicola*, biological control, parthenium weed

INTRODUCTION

The successful of a pathogenic biological control agent released into the field to suppress the growth of a target weed depends on several factors, but of primary importance is the timing of application of the agent. If it is applied at the appropriate time and under the appropriate environmental condition there is a good chance that the pathogen will establish then build up to epidemic proportions (Sand & Miller 1993; Frantzen & Hatcher 1997).

The appropriate environmental conditions to encourage infection and disease development of the agent include an appropriate air temperature and sufficient moisture to of the weed vegetative tissues for spore germination, tissue infection, survival, latent period, and host resistance (Chen 2005). The most conducive condition for the infection and disease progress of rust fungus *Puccinia abrupta* var. *partheniicola* on parthenium weed is a low temperature (15 ^{o}C) and a moderate to long dew period (9 to 12 h) at the time of application (Fauzi *et al*. 1999), though a severe infection could still occur when a long dew period (9 h) was delayed for up to 2 days (Fauzi 2002).

The infection susceptibility of a host to a particular pathogen and the rate of its disease development may vary considerably depending on the age of the host (Fraser 1985). Some pathogens, such as the damping-off fungi, readily infect younger plants (seedlings), while other pathogens only prefer mature plants. Two weeks rosettes of musk thistle (*Cardus nutans* subsp. *leilophyllus*), for example, were more susceptible to *Puccinia carduorum* infection than older ones (Politis *et al*. 1984). Similarly, for globe artichoke (*Cynara scolymus* L.), in which plants in their second-to sixth-leaf growth stage were more susceptible than the older ones grown from seed (Politis & Bruckart 1986). Many older plants have an ability to resist pathogen attack. The presence of adult plant resistance in several crop species has been discussed by several authors (Russell 1977), but very little has been reported for weeds.

In opposition to those studies undertaken on the mechanism of crop resistance, the purpose of studying the infection mechanism in weeds is to find ways of allowing for infection to occur. Possibilities for manipulation of environment, to allow a better infection of the pathogen to target weed have been reported by Bruckart and Hasan (1991). Other studies have shown that certain pathogens, intended for use as classical biological control agents, often do not kill their target weeds, rather they stress them (Politis & Bruckart 1986) and reduce the plant size. By reducing the plant size, biological control agents are also likely to reduce the competitive ability of the host species (Hanley & Grooves 2002). Reduced competitive ability will reduce the population of the weeds (Mortensen 1986). Therefore, understanding the relationship between the biological control agent activities due to plant-plant competition is very important if control of a weed is to be fully understood.

The objective of this study was to investigate the biological control ability of *P. abrupta* var. *partheniicola* when being applied to parthenium weed at different growth stages. The study also investigated the combined effect of rust infection and that from competition from buffel grass (*Cenchrus ciliaris* L.), a pasture species usually found in the parthenium weed habitat in Central Queensland.

MATERIALS AND METHODS

Plant Preparation and Inoculation. Parthenium weed and buffel grass were sown simultaneously into trays containing a peat and sand (1:3, v/v) posting compost enriched with complete fertilizer. The 14 days old seedlings were then transplanted into 20 cm diameter plastic pots containing the black clay soil obtained from Gatton, Queensland. Each pot either contained one parthenium and one buffel grass or one parthenium alone. All plants were then grown at a 30/26 ^{o}C (day/night) in a naturally-lit glasshouse with a photoperiod of 13/11 h (day/night). To produce parthenium at the rosette, flowering, or mature stages, the weed alone or together with one buffel grass plant were sown in a staggered planting regime, with intervals of 3 or 6 weeks. When the appropriate growth stages had been attained, the plants were inoculated with freshly collected urediniospores (60,000 spores/ml) of *P. abrupta* var. *partheniicola*. Inoculation of urediniospores to plants were conducted at all 3 growth stages and took place at the same day. Immediately after inoculation, the plants were covered with previously misted black plastic bags and incubated in a controlled naturally-lit glasshouse with a day/night temperature regime of 18/13 $\pm$ 1 ^{o}C for 12 h. After this treatment, the plastic bags were removed and the plants returned to the naturally-lit glasshouse and allowed to grow. The whole experiment took 160 days.

Data Collection Disease Outcome. The appearance of disease symptoms was monitored daily starting from first day after inoculation. The number of pustules per plant was quantified every 5 days beginning from the appearance of the first disease symptoms until no further pustules were formed or when the pustules were so dense that pustules were beginning to coalesce. The disease progress was defined by plotting the area under the disease progress curve (AUDPC) and was calculated using the following equation:

$$AUDPC = \sum_{i}^{n-1}\left[\frac{Y_i + Y_{i+1}}{2}\right](t_{i+1} + t_i)$$

in which n is the number of assessment times, t is the time at which the assessment are made and Y is the number of pustule at each time t (Shaner & Finney 1977).

Plant Characters. At the age of 16 weeks, the height of each parthenium weed plant was measured, the number of mature flowers was determined and the dry weight of above ground biomass was calculated. Later, the number of germinable seed extracted from the flowers was determined. The germination tests were undertaken in 9 cm plastic Petri dishes lined with two moist filter papers. The dishes were incubated for 14 days at a 25/20 $\pm$ 2 ^{o}C (day/night) temperature regime in a germination incubator set on a 12/12 h photoperiod. A seed was counted as a germinant when 2 mm of radicle had protruded. The biological control ability of *P. abrupta* var. *partheniicola* was estimated by comparing the percentage of suppression of each measured characters in the presence and absence of the rust or the competitive plant.

Experimental Design and Statistical Analysis. The 2 x 3 factorial experiment was arranged in a completely randomized design with six replicates plants in each treatment. The experiment consisted of 18 parthenium growing in pots with competition and 18 plants growing in pots without competition. Within each treatment, six plants were inoculated with *P. abrupta* var. *partheniicola* urediniospores at the rosette stage, six plants at the flowering stage and six plants at the mature stage. As controls, an additional six non inoculated plants with and without buffel grass were planted.

Data obtained from all measured characters and disease outcomes (AUDPC) were analyzed by using a two way analysis of variance test. Data obtained from control treatment were not included in the ANOVA test, but were utilized to determine the biological control ability of the rust compared to the weed suppression by the competition alone. The term substitutive was used when only one factor has an impact, and the term multiplicative was used if both factors have an impact but without an interaction and synergistic when an interaction has occurred between the biological control agent and the competition on the plant characters.

RESULTS

Disease Outcomes. The first disease symptoms appeared 9 days after inoculation when parthenium was inoculated at the rosette and flowering growth stages, and 13 days when mature plants were inoculated (Table 1). Competition with buffel grass did not affect the time at which these first disease symptoms to appeared.

The disease progress of *P. abrupta* var. *partheniicola*, as shown by the AUDPC, was higher on the younger plants than the older ones. Plants inoculated at the rosette growth stage had a significantly higher AUDPC (thus disease progress) than those plants inoculated at the flowering or mature growth stages. The flowering plants had a significantly higher AUDPC compared to mature plants (Table 1).

Plant Characters. Application of *P. abrupta* var. *partheniicola* urediniospores to the rosette and flowering growth stages resulted in significant reduction in height of the plants, and were significantly lower than those inoculated at the mature stage, but did not differ from each others. Competition alone has a significant impact on reducing plant height. However, there was no interaction detected between the inoculation and the competition (Figure 1). Thus, the outcome of these two treatments was multiplicative to the plant height.

Table 1. The appearance of the first disease symptom and the AUDPC of *P. abrupta* var. *partheniicola* as affected by parthenium weed growth stage*

Growth stage	Appearance of first symptoms (days after inoculation)	AUDPC**
Rosette	9	2301.0 (47.54)c
Flowering	9	1085.8 (31.13)b
Mature	13	119.8 (9.00)a

*Data presented are averaged over the competition treatment with buffel grass. Data are the mean observations taken on twelve replicate plants. Values inside the bracket are the square root of AUDPC. **Values followed by the same letter are not significantly different at P = 0.05 according to a Student Newman Keuls' (S-N-K) test.

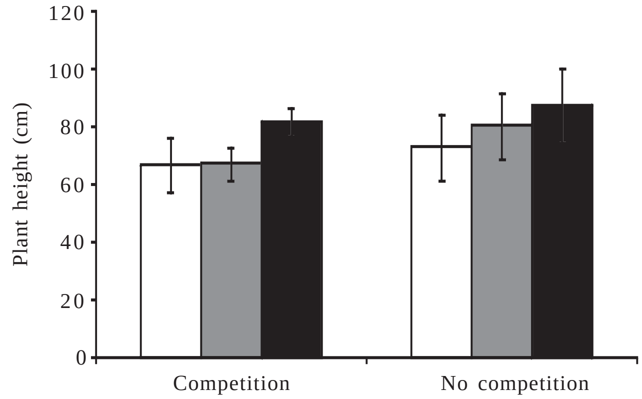

Figure 1. Effect of *P. abrupta* var. *partheniicola* inoculated at three different growth stages (□: Rosette, ▨: Flowering, or ■: Mature growth stage) on the height of parthenium weed in the absence and in the presence of competition with buffel grass. Tails on each bar are the standard deviation (n = 6).

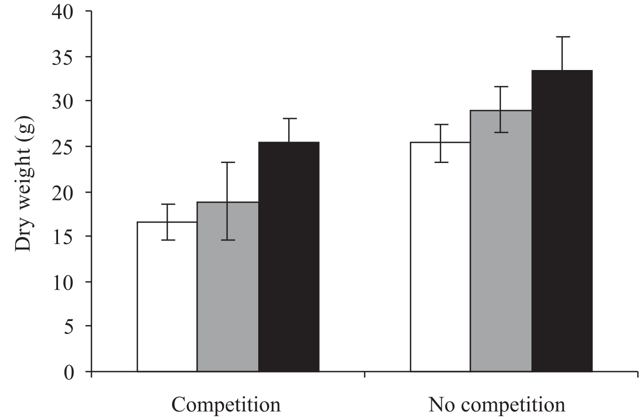

Figure 2. Effect of *P. abrupta* var. *partheniicola* inoculated at three different growth stages (□: Rosette, ▨: Flowering, or ■: Mature growth stage) on the above ground biomass of parthenium weed in the absence and in the presence of competition with buffel grass. Tails on each bar are the standard deviation (n = 6).

The rust also affected the dry weight of above ground biomass. The highest reduction in the biomass was recorded when the plants were inoculated at the rosette stage. This reduction was significantly less than those inoculated at mature stages. The competition from buffel grass also significantly reduced the plant biomass, but there is no interaction detected between growth stage and competition treatments (Figure 2). The effect of competition and the biological control agents was then multiplicative on the above ground biomass of the weed.

The number of mature capitula (hence seed) produced by the plants was significantly affected by the time of inoculation. Plants inoculated at the rosette stage produced significantly fewer mature flowers compared to those plants inoculated at the flowering stage, and both produced significantly fewer mature flowers than those inoculated at the mature stage. The competition with buffel grass also reduced the production of mature flowers. However, there was no interaction detected between competition and growth stage treatments (Figure 3). In this case, a multiplicative impact described the relationship between the competition and the pathogen treatments.

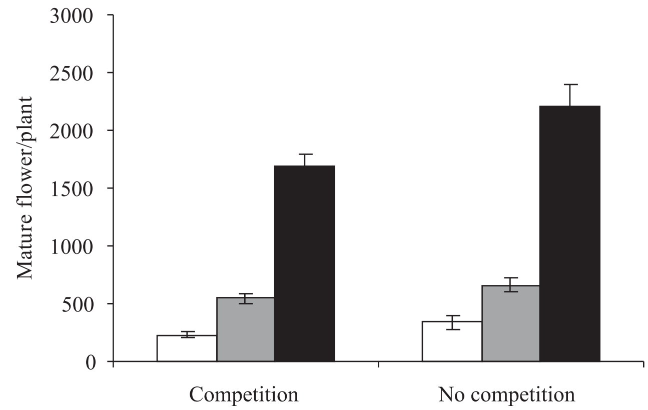

Figure 3. Effect of *P. abrupta* var. *partheniicola* inoculated at three different growth stages (□: Rosette, ▨: Flowering, or ■: Mature growth stage) on the number of mature flowers produced by parthenium weed in the absence and in the presence of competition with buffel grass. Tails on each bar are the standard deviation (n = 6).

The production of germinable seed was also significantly lower in the plants inoculated at the rosette growth stage than those inoculated at the flowering and at the mature growth stages. The highest number of germinable seed was recorded on inoculated mature plants and was significantly higher than that on inoculated flowering plants. Competition from buffel grass did not affect the germinable seed produced (Figure 4). The outcome of both treatments was then substitutive to the production of germinable seeds.

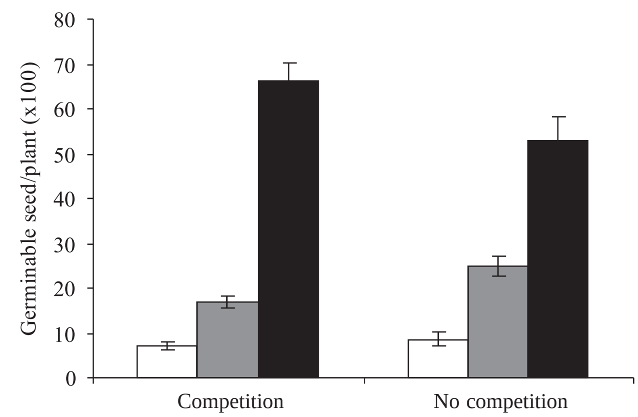

Figure 4. Effect of *P. abrupta* var. *partheniicola* inoculated at three different growth stages (□: Rosette, ▨: Flowering, or ■: Mature growth stage) on the production of germinable parthenium weed seed in the absence and in the presence of competition with buffel grass. Tails on each bar are the standard deviation (n = 6).

Biological Control Ability of *Puccinia abrupta* var. *partheniicola*. *Puccinia abrupta* var. *partheniicola* infection decreased the final plant height by up to 22% in the absence of competition and by up to 28% in the presence of competition from buffel grass when the rust was applied at the rosette stage. Lower reductions (12 and 6%) were recorded when the rust was applied to the plants at the mature growth stage in the presence and in the absence of competition, respectively. In comparison, the competition alone could only reduce plant height by 12%. A higher reduction in the above ground biomass (48%) was recorded due to the rust when the plants were inoculated at the rosette stage in the presence of competition, compared to only 20% reduction in the absence of competition or in the absence of the rust. The impact of the rust was greatest on the ability of parthenium to produce seeds. The production of seeds was reduced by up to 90% in the presence of competition and by up to 85% in the absence of competition. The impact was observed when the plants were inoculated at the rosette growth stage. The reductions in seed production were much lower (1%) when mature plants were inoculated in the absence of competition. The effect of the biocontrol agent on various plant characters are summarized in Table 2.

DISCUSSION

Parthenium weed plants at the rosette growth stage are the most susceptible to the infection by *P. abrupta* var. *partheniicola*. Adult plants showing some forms of resistance to infection from the rust. This adult resistance is implicated from the longer time needed for the appearance of the symptoms and the near zero disease progress following infection. As a result, when the rust was inoculated to the adult parthenium, occurred only small reduction in plant height (6%), no reduction in plant biomass (0%), and only a very small reduction in seed production (1%).

The age-related resistance had been observed in other plant species. As plants mature, some of them become more resistant to normally virulent pathogens (Rusterucci *et al*. 2005). Chakraborty *et al*. (1994) reported that 5- to 9-week-old seedlings of *Cassia* spp. were more resistant to *Alternaria cassiae* infection than younger ones (2-to 5-weeks old). This resistance might be correlated to leaf physiology with older plants having more physiologically diverse leaves than younger plants. This resistance of older parthenium leaves to *P. abrupta* var. *partheniicola* has been reported by Parker *et al*. 1994 that younger leaves were the most susceptible to infection. Considerable resistance to rust infection might be associated with the surface features of the leaves. The physical barriers such as hairs and cuticle could prevent biological control agents to infect the weeds (Auld & Morin 1995). This idea is supported by Guest and Brown (1997) who stated that 'adult plant resistance' could occur as a result of the reduced ability of the pathogen to penetrate to thicker, tougher cell walls; for example, the rust *P. graminis* could only infect younger barberry leaves with thin cuticles, while not being able to penetrate mature leaves with thicker cuticles.

Even though the rosette stage of parthenium is the most susceptible to *P. abrupta* var. *partheniicola* infection, the plants are far from being killed by the action of the rust. It is most likely that the rust acts to suppress the vegetative and generative growth of the weed, as shown by its ability to significantly reduce plant biomass and seed production. This suppression in turn will probably reduce the ability of the weed to compete with other plants (Politis & Bruckart 1986), increasing the growth of other wanted species (Yandoc *et al*. 2004), and reduce the amount of seed put back into the seed bank. This reduced ability to compete with other plants is clearly observed in the present study, especially in the reduction in plant biomass. The present study shows that the competition from buffel grass reduces plant biomass up to 20%, similar to the reduction when the plants were inoculated with the rust at the rosette growth stage. However, when both the competition and the rust are involved, the reduction in plant biomass could be as much as 48%.

The negative impacts of plant disease on plant growth development have been shown to inhibit the target weed's ability to compete with non-target plants (Fauzi & Murdan 2008). The interaction of *P. abrupta* var. *partheniicola* with the competition from buffel grass in the present study are mainly multiplicative, in which each factor has a significant impact on reducing plant growth. No interactions were detected between the two factors. Previous studies have shown that similar multiplicative effects in natural enemy-competition relationships are the most common found.

Puccinia abrupta var. *partheniicola* inoculated at the rosette stage of parthenium, in the presence of competition from buffel grass resulted in a high reduction (48%) in plant biomass. Both factors are contributing a similar amount (20%) of reduction. Sheppard (1996) suggested from reviewing several studies on competition-natural enemy interaction, that natural enemy rarely affect on the plant growth rate, but competition could have a dominant effect on plant growth. However, in the present study, competition was not dominant in reducing the above ground biomass of parthenium weed plants. This probably showed that buffel grass was too weak as competitor when in a 1 to 1 density. The competitive ability of buffel grass might be increased if the number of the grasses is increased, as shown in central Queensland that the grass competed well with parthenium (O'Donnel & Adkins 2005).

The most convincing effect of the rust was on the reduction of seed production. *P. abrupta* var. *partheniicola* inoculated at the rosette stage significantly reduced the ability of parthenium to produce seed in the presence (90%) or in the absence (85%) of competition from buffel grass. This may be

Table 2. Biological control ability of *P. abrupta* var. *partheniicola* inoculated onto parthenium weed at three different growth stages in the presence or in the absence of competition from buffel grass*

Plant growth stage	Uninfected plants with competition	Rust infected plant	
		No competition	Competition
Plant height (%)	12		
Rosette		22	28
Flowering		14	27
Mature		6	12
Above ground biomass (%)	20		
Rosette		20	48
Flowering		9	41
Mature		0	20
Seed production (%)	22		
Rosette		85	90
Flowering		70	75
Mature		1	24

*The results are for various plant characters and values are expressed as a percentage of reduction on plant characters from uninfected plants grown in the absence of competition from buffel grass.

due to the fact, that a heavy infection on the weed leaves will only leave small proportion of the green leaf area to undertake photosynthesis and to produce energy for seed production. This reduction in seed production, in the long term, will reduce the soil seed bank which in turn reduces the size of the weed population in central Queensland. The parthenium weed seed bank is very dominant in most sites in central Queensland, with parthenium weed seed accounting for 87% of the entire soil seed bank (Navie *et al*. 2004).

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