Impact of foliar herbicides on pod and seed behaviour of rust-infected rubber vine (*Cryptostegia grandiflora*) plants

F.F. Bebawi, P.L. Jeffrey, J.R. McKenzie, J.S. Vitelli and **A.M. Lindsay**, Tropical Weeds Research Centre, Department of Natural Resources, PO Box 187, Charters Towers, Queensland 4820, Australia.

Summary

Field trials evaluated the impact of foliar herbicides on pod characteristics and germination behaviour of seed within pods of rust-infected rubber vine plants (*Cryptostegia grandiflora*). There were three herbicide treatments: triclopyr/picloram (Grazon DS®) at 1.05/0.35 g L^{-1}, metsulfuron (Brush Off®) at 0.09 g L^{-1} and control; three rust (*Maravalia cryptostegiae*) infection levels: low, medium and heavy; and three pod maturity stages: juvenile, immature and mature. The effects of herbicide and rust infection varied with pod maturity stages. On average, both herbicides reduced pod dry weight (DWT) and relative water content (RWC) by 22 and 91% respectively. Heavy rust infection on its own increased pod DWT and RWC by 7 and 15% respectively. The effects of herbicides, rust infection and pod maturity stage on seed germination varied with sites. A synergistic reaction between triclopyr/picloram and heavy rust infection resulted in complete inhibition of germination of both immature and mature seeds of rubber vine. On average, triclopyr/picloram and metsulfuron reduced seed germination by 77 and 42% respectively. In contrast to its effects on seed germination, the rust had no significant effect on seed viability. Maximum seed mortality (84%) was caused by triclopyr/picloram. Results suggest that early treatment with Grazon DS and Brush Off during the reproductive growth stages of rust-infected rubber vine plants may disrupt pod development, but may not kill all seeds within pods.

Introduction

Rubber vine (*Cryptostegia grandiflora* Roxb. R.Br.) costs primary industry in Queensland around 18 million dollars annually (Mackey 1996). This woody weed, of the family Asclepiadaceae, poses a major constraint to pasture and livestock production. It chokes out native pasture, prevents stock from gaining access to water and is listed as a poisonous plant (Everist 1974).

The Australian range of rubber vine currently covers more than 350 000 km^2 of northern and eastern Queensland. It includes the southern half of Cape York Peninsula and the region south of the Gulf of Carpentaria, and extending as far south as Brisbane (Humphries *et al*. 1991). Of greater significance is its potential distribution, which Chippendale (1991) estimated to be at least 20% of the Australian mainland. A land invasion of this scale can only be achieved by species that have prolific seed production and effective dispersal mechanisms plus suitable establishment conditions. Rubber vine certainly fits this category. In Queensland, large shrubs are capable of producing as many as 30 pods per plant, or an average of 670 and 410 pods ha^{-1} between April and August (Grice 1996). The number of seeds per pod ranges from 170 to 420 (Curtis 1946, Dale 1980, Vitelli 1989, Grice 1996). Consequently, as many as 8000 wind-dispersed seeds can be released from individual plants in a single reproductive episode. Pods are characteristically 10–12 cm long, 3–4 cm wide, produced in pairs, horizontally opposed and diverging from the tip of a short but common stalk, sharply three-angled, tapering into a long beak (Parsons and Cuthbertson 1992). Seeds have a white coma (fluffy hairs), 18.9 to 38 mm long at the micropylar end. These make wind the primary dispersal vector (Marohasy and Forster 1991). However, water too can play a substantial role in the distribution of rubber vine seed (Brown *et al*. 1996). These two dispersal mechanisms enable rubber vine to invade new sites solely from seed (Grice 1996).

If any control technique used for treating rubber vine infestations can reduce the amount of viable seed available for recruitment it will help to reduce rate of spread and reinfestation of treated areas. Such a reduction may occur when foliar herbicides are used if there is seed located on the plants at the time of application. Studies on some weed species have reported reduced seed viability following herbicide applications (Isaacs *et al*. 1989, Shaw and Hydrick 1993). Whether this is the case with rubber vine was not known prior to this study as it was the ability of the applied chemicals to kill mature plants or seedlings, which was assessed previously (Vitelli *et al*. 1994). The effect of herbicides on seed on the plant at the time of treatment was not recorded. The study carries on from a preliminary in-vitro study on the effect of herbicides on germinability and mortality of rubber vine seeds of different maturity stages (Bebawi and McKenzie in press).

In recent years, the efficacy of foliar herbicides in killing rubber vine has been complicated by the release of biological control agents, in particular a rust (*Maravalia cryptostegiae* (Cumm.) Ono) released into Australia in 1993 from Madagascar (Evans and Tomley 1996). The rust infects leaves and young stems of rubber vine (Evans and Tomley 1996). A high infestation can result in total defoliation (McFadyen 1985) and occasional dieback in seedlings (Vitelli 1995). McNellie (1996) reported that rust-infected rubber vine plants produce fewer flowers and exhibit significant reductions in total chlorophyll (a and b) content compared with rust free plants. She also found that while relative leaf water content decreases with light and moderate rust infection levels, it increases with severe infection levels.

Mackey (1996) expressed concerns that the defoliating effect of the rust pathogen might interfere with control operations, particularly as foliar applications of herbicides coincide with the season of rust activity, i.e. at the time rubber vine plants are actively growing.

Most recommendations on the efficacy and control aspects of foliar herbicides are based on the premise that herbicide translocation to the target point in the plant body occurs unimpeded under conditions when the plant is actively growing. Little is known about the efficacy of foliar herbicides under abnormal conditions, such as when the plant sustains high levels of rust infection. The introduction to Queensland of the rubber vine rust as a biocontrol agent in 1993 (Evans and Tomley 1996) has recently caused such abnormal conditions. This phenomenon is overwhelming, particularly during the wet season; the leaf canopy along creeks and water courses infested with rubber vine is gradually transformed by rust-infection from dark green to a golden-orange colour.

The important question now arises: How will the target area, already affected by one control tool (rubber vine rust) respond to the action of another control tool (foliar herbicides)? That is, will interaction between rust and foliar herbicides alter the efficacy of either control tool?

This study measured the impact of foliar herbicides on pod characteristics, and the germination and mortality of seed contained within pods of plants infected with varying levels of rust.

Materials and methods

A 3 × 3 × 3 factorial field experiment replicated three times in a randomized complete block design was established during May 1997 at Sandy Creek, 11 km west of Charters Towers and repeated at Larkspur, 20 km north of Charters Towers, Queensland. The two sites are situated in the semi-arid region of north-eastern Queensland. This area has a mean annual rainfall of 658 mm with 76% of this occurring during the three months of summer (Bureau of Meteorology 1988). Site description and environmental conditions during the trial are presented in Table 1.

In this experiment, factor A was foliar herbicide treatment (triclopyr / picloram, metsulfuron and control (no herbicide)), factor B was level of rust-infection (low, medium and heavy), and factor C was pod maturity stage (juvenile, immature, mature). The rust infection factor did not include a control because it was not possible to achieve rust free plots; no fungicide had yet been found that would control *M. cryptostegiae.*

Plots were randomly selected within each site. Plots were approximately 5 × 5 m and contained a minimum of 20 mature, 1.5–2.5 m tall, rust-infected rubber vine plants. The free standing population ranged between 12 000 and 26 000 plants ha^{-1} at all sites. Free stands of mature rubber vine plants were selected for two reasons. First, they were at the podding stage and second, because each plant was totally accessible for sampling purposes, unlike rubber vine climbing over trees.

Rust rating index

Prior to herbicide application, 100 leaves were randomly sampled per plot to determine a rust rating index. This index is derived by determining the percentage of rust pustules per leaf by the number of leaves infected with rust. The score was then used to rank the plots into light, medium, or heavy rust infection categories. Plots of rubber vine plants having the majority of leaves with a rust-infection cover of between 1 and 25%, 26 and 70%, and 71 and 100% were classified as light, medium and heavy, respectively.

Pod maturity stages

In each of the 27 plots, rubber vine pods were sorted and tagged according to their stage of maturity using a previously described classification system (Bebawi and McKenzie 1999). Pods were classified as juvenile, immature, or mature. No ripe pods were available at either site at the time the experiment was conducted. According to Bebawi and McKenzie's classification system, ripe pods are brown in colour. Juvenile, immature and mature pods are green in colour. Juvenile pods are rubbery in texture and not woody. Immature and mature pods are green and woody, but mature pods are more woody and display distinct brown fibrous woody patches on their surface.

Because the number of juvenile, immature and mature pods varied within plots and between plots, pod variables were determined on pod sub-samples from each plot. The number of pods of each class ranged between 8 and 45 pods per plot.

Spray equipment and herbicide application

Metsulfuron and triclopyr / picloram were the two foliar herbicides selected because of their high capacity to kill mature rubber vine plants (Vitelli *et al*. 1994). Metsulfuron and triclopyr / picloram were applied at rates of 0.09 g L^{-1} and 1.05 / 0.35 g L^{-1} respectively. All solutions contained 0.01% (v / v) non-ionic surfactant (BS 1000 (alcohol alkoxylate)). The herbicides were applied with a handgun fitted with a D6 nozzle and operating at 700 kPa. A positive displacement flowmeter (Manu MEK20LCD4) attached in-line to the spray unit recorded spray volume delivery. At each site, the plants were thoroughly sprayed to the point where the spray mixture dripped from the foliage. Spray volume ranged between 2500 and 15 000 L ha^{-1}, depending on plant density.

Measurements

Pod characteristics. Rubber vine pods were removed from plants 8 weeks after foliar herbicides had been applied because, by then, most of the herbicide treated pods had started to manifest signs of maturity. These signs involved a change in pod colour from green to greyish-brown, followed by initial splitting along the ventral axis of the pod. If the pods were not harvested then, they would split further and the seeds would be shed. Once harvested, the pod fresh weight (FWT) was recorded, with measurements taken on two basally united pods. Pods were then placed in size 2 (12 × 25 cm) Trojan Satchel paper bags and air-dried for 60 days, before being oven-dried to constant weight at 40°C. This temperature was chosen in order to prevent seed damage within the pod. Pod dry weight (DWT) was then recorded and the pod relative water content (RWC) calculated gravimetrically using the formula:

$$\% RWC = (FWT - DWT) / FWT \times 100$$

Statistical analysis of variance was performed on six sub-samples per treatment. Pairwise comparisons of means was performed using Duncan's Multiple Range Test. Because we had a non-parametric index for rust-infection levels we could only perform correlation analysis within rust treatments.

Seed germination. Seed germination tests were conducted on sub-samples of seed obtained from bulked seedlots of each of the 27 treatments. Six replicates of 50 seeds each were used per treatment. Seeds were placed in 9 cm petri-dishes filled with 10 mL de-ionized water. Petri-dishes were randomly stacked in containers with the lid sealed to reduce evaporation, then stored in black plastic bags because rubber vine seeds have a definite preference for germination in the dark (Sen 1968). The containers were then placed in a controlled environment glasshouse set at a 12 h / 12 h day and night temperatures of 30 ± 1°C and 20 ± 1°C, respectively.

Grice (1996) indicated that 90% of rubber vine seed germinate within 10 days of moisture becoming available. In our study, germinated seeds (identified by radicle emergence) were counted under green light and removed daily for 15 days and the cumulative germination percentages were calculated. Statistical analysis of variance was performed on arcsin transformed data which was later back-transformed. Pairwise comparisons of means were performed using Duncan's Multiple Range Test.

Seed mortality. Seed mortality was tested using incubation in tetrazolium (Moore 1985). The procedure was similar to that used for the germination test, but with three replicates only. Seeds were pre-soaked for 24 h in de-ionized water and incubated for 7 days in petri dishes, each of which contained 10 mL of 0.1% solution of tetrazolium (2,3,5-triphenyltetrazolium chloride). They were then cut longitudinally. Contents of viable seed were light to dark pink, while those of non-viable seed were dull brown. Statistical analysis of variance was performed on arcsin transformed data which was later back-transformed. Pairwise comparisons of means were performed using Duncan's Multiple Range Test.

Results

In this study, the term 'synergistic' or 'antagonistic' refers to interactions between the herbicide and rust treatment. If the interaction results in increased damage to the weed, the interaction is referred to as synergistic; if the interaction leads to less damage than would be expected from a simple addition of the separate impacts of the herbicide and the rust, the interaction is antagonistic.

The effect of herbicide treatment was evident first on foliage, then the pods and later on the stems. One week after herbicide application, the foliage was necrotic and olive-grey in colour. Three weeks after application, similar symptoms were manifest on the pods. After five weeks the stems changed from reddish-brown to dark brown, while most of the pods became completely grey in colour when compared with the green colour of the control pods.

Impact on pod DWT and RWC

Effects of herbicide and rust infection on DWT and RWC varied with pod maturity stages (herbicide × rust × maturity stage interaction significant at $P<0.05$) (Table 1). For example, with heavy rust-infection, the DWT of juvenile pods was significantly reduced by metsulfuron while that of immature and mature pods was not when compared with that of the heavy rust controls (Figure 1). The herbicide and rust treatment interacted synergistically on juvenile pods; metsulfuron caused a greater reduction in relative pod DWT with heavily infested pods than with lightly infested ones. On the other hand, the herbicide and rust treatment interacted antagonistically on immature and mature pods, so that the herbicide produced no significant reductions in relative pod DWT (Figure 1). On average, herbicides reduced pod DWT by 22% compared to the control and high rust infection increased pod DWT by 7% compared with low rust-infection. The DWT of mature pods was greater by 109% than that of juvenile pods (Figure 1).

The interaction between pod maturity, rust-infection level and herbicide impact on RWC was not the same for the two herbicides (Figure 2). For example, reduction in RWC of juvenile pods did not differ significantly from the reduction in mature pod RWC when metsulfuron was used. With triclopyr/picloram there was an antagonistic effect between the herbicide and rust intensity. This resulted in a significant increase in RWC of heavily infected mature pods compared with low-infected pods (Figure 2). Over all sites, herbicides reduced pod RWC by 91% whereas rust increased RWC by 15% and the RWC of mature pods was greater by 20% than that of juvenile pods (Figure 2).

Impact on seed germination

There was a highly significant interaction ($P<0.01$) between the effects of herbicide type, rust infection level, seed maturity and site on seed germination (Table 2). This interaction indicates that there was a different response in germination behaviour of rubber vine seeds at the Larkspur site from those of Sandy Creek. For example, the germination of immature rubber vine seeds lightly infected by rust was not significantly affected by metsulfuron at Sandy Creek whereas at Larkspur it was. However, over all sites, heavy rust-infection prevented germination of both immature and mature seeds when triclopyr/picloram was used, while metsulfuron allowed some germination of heavily-infected seed (Figure 3). This particular example indicates the occurrence of a synergistic reaction between triclopyr/picloram and heavy rust, resulting in complete inhibition of germination of both immature and mature seeds of rubber vine. Across all pod maturities, triclopyr/picloram and metsulfuron reduced seed germination by 77 and 42% respectively, compared with the control whereas germination of mature seeds was 2040 % (21-fold) greater than that of juvenile pods (Figure 3). The significant difference in germination response of mature seeds when compared with that of juvenile seeds may be attributed to the greater seed immaturity of juvenile seeds.

Impact on seed mortality

In contrast to seed germination, there were only significant interactions between the effects of herbicide type and pod maturity on seed mortality (Table 2). Rust had no significant effect on seed mortality. Under control conditions (no herbicides), 99.9% of juvenile seed was non viable compared with 56.2 and 21.1% for immature and mature seed respectively (Figure 4). Consequently only immature and mature seeds could be affected by

Table 1. Site description and conditions during foliar herbicide application on rust-infected rubber vine plants at two sites near Charters Towers during May 1997.

Site description					
Site	Location	Soil type	Treatment date	Time applied	Harvest date
Sandy Creek	S20°4', E146°10'	Sandy loam	1.5.97	11.00–17.30	27.6.97
Larkspur	S19°15', E146°12'	Silty clay loam	2.5.97	09.45–10.45	27.6.97
Conditions					
Site	Temperature (°C)	Relative humidity (%)	Wind speed (knots)	Wind direction	Conditions
Sandy Creek	26–33	43–52	0–7	SE/NW	Overcast/Sunny
Larkspur	24–26	50–55	1–2	SE	Sunny

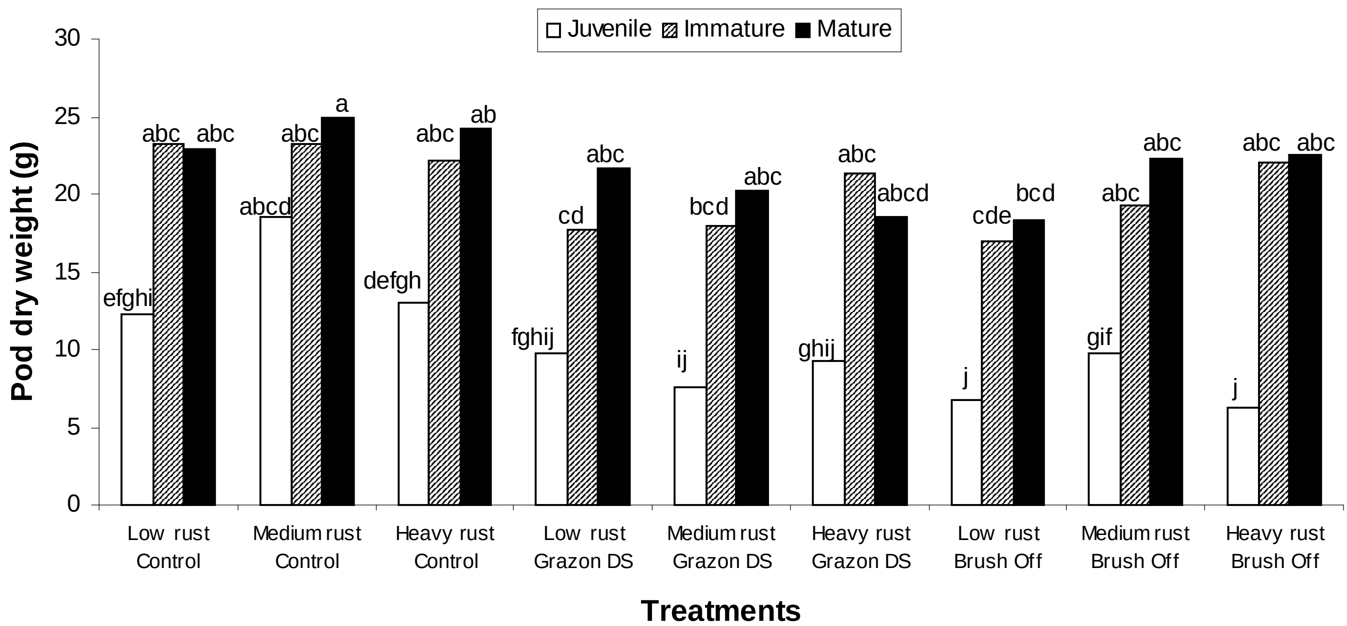

Figure 1. Mean pod dry weight as affected by herbicide, rust and pod maturity stage over both sites. Values followed by the same letter are not significantly different at P<0.05.

exposure to herbicides. Although maximum seed mortality (84%) was caused by triclopyr/picloram, there were, in fact, no significant differences between seed mortality caused by triclopyr/picloram and metsulfuron (Figure 4). Eight weeks after application, seed mortality of immature and mature seeds was 53 and 157% higher respectively than control seeds (Figure 4).

Discussion

The overall effects of heavy rust which resulted in significant increases in RWC of pods parallel those reported by McNellie (1996) for rubber vine leaves. Apparently heavily infected rubber vine plants tend to take up increased amounts of water; in our case this was also reflected in heavier pod DWT. Variations in germination behaviour with site may have resulted from the error inherent in the rust rating index system.

Our results indicate that the impact of foliar herbicides and rust can vary with the degree of pod maturity. For example, with pod DWT the interaction was synergistic in juvenile pods and antagonistic in immature and mature pods. The change in response as the pod matures may relate to differences in surface texture of pods; immature and mature pods are more woody than juvenile pods. However, unexpected results have also been reported by Evans (1992) when working with rubber vine rust inoculation. He found that height and dry weight of rubber vine seedlings decreased after two inoculations but increased after three inoculations and finally decreased after four inoculations. He attributed this anomaly in height and dry weight behaviour after the third inoculation to an increased rate

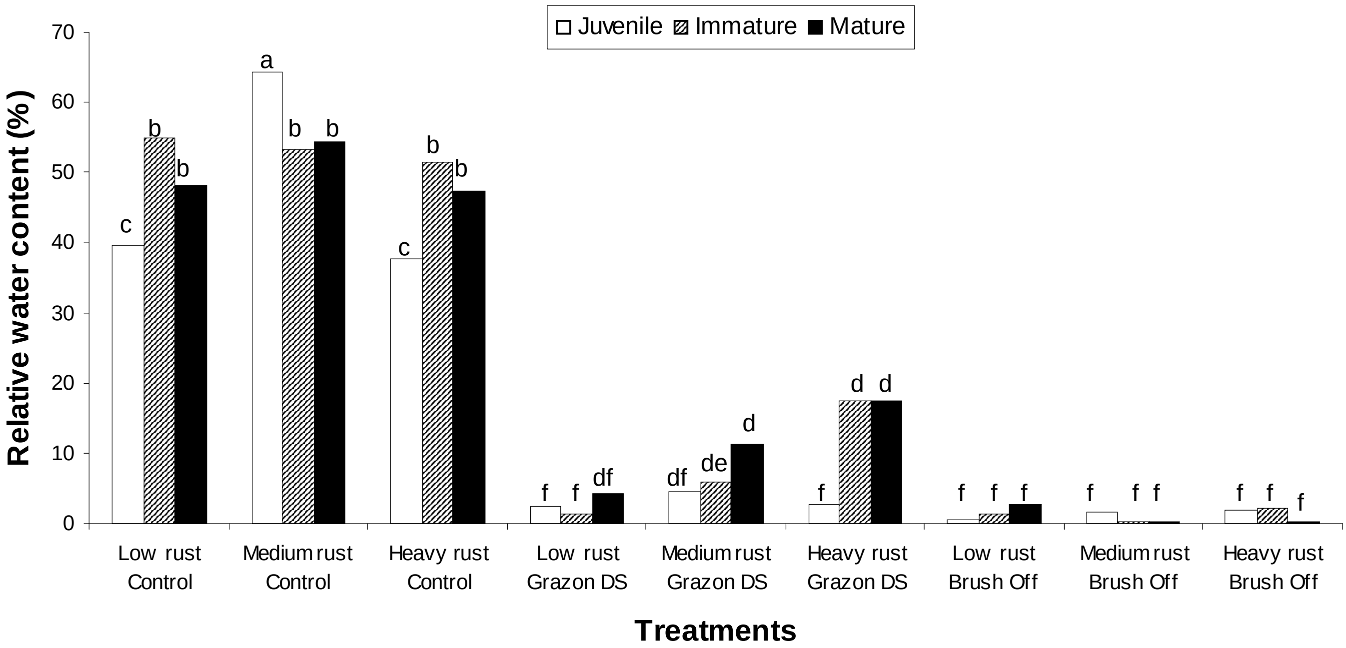

Figure 2. Mean pod relative water content as affected by herbicide, rust and pod maturity stage over both sites. Values followed by the same letter are not significantly different at P<0.05.

Table 2. Degrees of freedom and mean squares for the variance in rubber vine pod dry weight (DWT), relative water content (RWC), seed germination and seed mortality associated with main effects and interactions over both sites.

Source of variance	df	DWT (mean squares)	RWC (mean squares)	Seed germination (mean squares)	df	Seed mortality (mean squares)
Site (S)	1	131.2**	183.1	0.0001	1	0.2431
Replicates (pooled)	10	12.0	46.3	0.0682	4	0.1019
Treatments (T)	26	418.0**	6279.0**	1.0677**	26	0.6615**
T × S	26	31.5**	291.6**	0.2694**	26	0.1426
Herbicides (H)	2	728.9**	76418.5**	1.6988**	2	1.3320**
H × S	2	17.2	483.4**	0.3436	2	0.0686
Rust (R)	2	66.7*	567.6**	0.2047	2	0.1498
R × S	2	8.1	353.9**	0.2522	2	0.0060
H × R	4	4186.0**	445.0**	0.3429*	4	0.2300
H × R × S	4	10.5	8.3	0.2729	4	0.2174
Pods (P)	2	69.0**	899.0**	7.0964**	2	5.3870**
P × S	2	8.5	78.3	0.0096	2	0.1606
H × P	4	44.2*	219.0**	0.9377**	4	0.3418*
H × P × S	4	22.5	191.0*	0.2098	4	0.2609
R × P	4	39.0	435.0**	0.2227	4	0.0784
R × P × S	4	50.1*	375.0**	0.3256	4	0.0159
H × R × P	8	37.0*	274.0**	0.4682**	8	0.1075
H × R × P × S	8	16.4	413.0**	0.3200*	8	0.1577
Error (pooled)	260	17.0	57.5	0.1385	104	0.1381
Total	323				161	

* Significant at P<0.05 level of significance. ** Significant at P<0.01 level of significance.

of flushing, probably a compensatory response to defoliation by the rust. Work with other species of rust (*Puccinia canaliculata* Schw. Lagerh.) on yellow nutsedge (*Cyperus esculentus* L.) did not reveal similar positive effects on the shoot system of the plant (Bruckart *et al.* 1988). It is clear that the physiological responses of rubber vine to rust infection are complex. It is, therefore, not surprising that they may modify reactions of the plant to herbicides and that these modifications can also be complex.

The synergistic effects between triclopyr/picloram and rust on germination of mature seed suggests that the response to triclopyr/picloram, unlike metsulfuron, was not sensitive to high levels of rust infection, in fact its efficacy was markedly enhanced by severe rust-infection. Our results infer that herbicides can differ in their interactions with rust infection, their efficacy is not invariably diminished by heavy rust infection levels. Each herbicide should be judged on its merit. Results of maximum seed germination (95%) realized by mature seed in this study concurs with those of Dale (1980).

The seed mortality results indicated that rust-infection levels had no significant effect on mortality of juvenile, immature, or mature seed, which suggests that only pod maturity and herbicide treatments affected seed mortality. Triclopyr/picloram caused on average 84% seed mortality. If this percentage is related to the actual number of seeds per pod, which may approximate 362 ± 24 (Dale 1980), then triclopyr/picloram may kill 323 seeds out of a maximum of 386 seed. This is a very high kill, but not enough to stop significant invasion of new sites: 63 seeds would remain alive. Seed viability of other weed species has been reduced by herbicide applications (Hill *et al.* 1963, Taylorson 1966, Fawcett and Slife 1978) especially when herbicides were applied at, or near, flowering (Maun and Cavers 1969, Biniak and Aldrich 1986). Bruckart *et al.* (1988) also studied the impact of rust and foliar herbicides on tuber mortality of yellow nutsedge. They found that the application of low rates of the foliar herbicide bentazon (3-(1-methylethyl)-(1H)-2,1, 3-benzothiadiazin-4(3(H)-one 2,2-dioxide) did not enhance, but reduced, the efficacy of the rust agent by 50% as a biocontrol tool. In our study, rust alone did not appear to act as a control agent in relation to mortality of rubber vine seeds or germination behaviour, but rather, interacted differently with triclopyr/picloram and metsulfuron. Our results indicate that different herbicides can interact in different ways with varying levels of rust infection. A previous in-vitro study found that seed

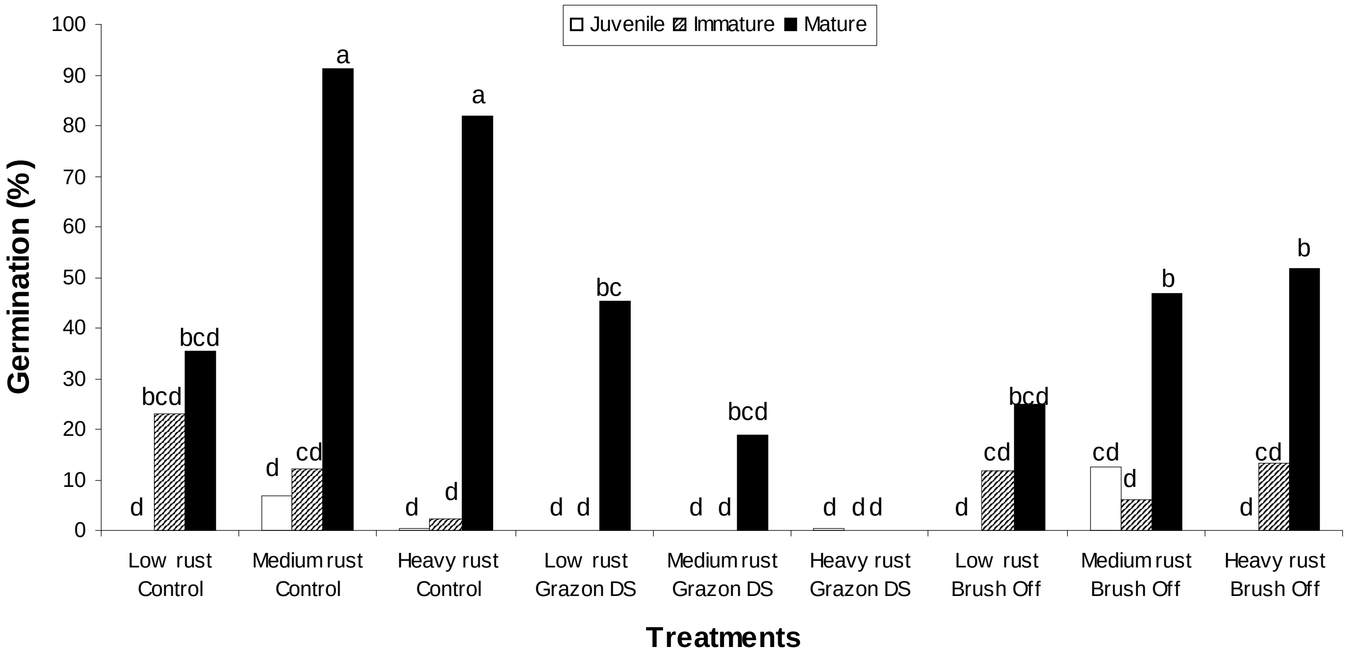

Figure 3. Mean germination of rubber vine seeds as affected by herbicide, rust and seed maturity stage over both sites. Values followed by the same letter are not significantly different at P<0.05.

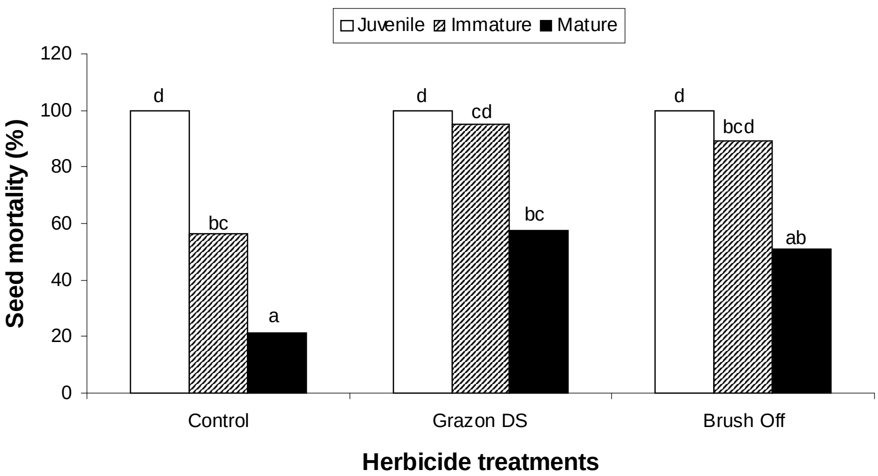

Figure 4. Mean mortality of rubber vine seeds as affected by herbicide and seed maturity stage at time of treatment over all rust and site treatments. Values followed by the same letter are not significantly different at P<0.05.

mortality was highest at the mature stage, with 44 and 65% of viable seeds killed following exposure to triclopyr/picloram and metsulfuron respectively (Bebawi and McKenzie in press). In contrast, ripe seeds suffered minimal mortality, averaging 6.7% irrespective of the chemical applied.

Conclusions

Because of the absence of rust free plots, this study was unable to determine definitely whether maximum efficiency of triclopyr/picloram and metsulfuron was realized in the presence of rust. We can tentatively conclude that both synergistic and antagonistic interactions may occur between the effects of herbicide treatment and the degree of rust infection of rubber vine plants. These interactions vary with the herbicide used and the maturity of pods. In the absence of chemical control, rubber vine pods of all maturity stages may continue to ripen and shed their seed. This would not only aggravate the current infestation problem but also expand the invasive area of rubber vine in Australia. Further studies are required to evaluate the effects of higher doses of triclopyr/picloram and metsulfuron and to screen other herbicides that may induce a 100% kill of seeds within pods on rust-infected rubber vine plants.

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