THE ESTABLISHMENT OF *UROMYCES HELIOTROPII* SRED., A BIOLOGICAL CONTROL AGENT OF *HELIOTROPIUM EUROPAEUM L.*

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Summary. The results of releases of the rust fungus, *U. heliotropii,* against the summer annual weed common heliotrope, *H. europaeum,* in Australia are described. Of 19 separate releases of urediniospores made at 13 sites in NSW, SA, Vic. and WA over three years, 11 showed local spread of the disease to distances up to 500 m. Of these releases, 13 led to the production of teliospores. To date, six release sites have been monitored in the season following teliospore production and three have shown natural re-infection via the production of spermogonia and aecia. Releases have been most successful in South Australia.

INTRODUCTION

Common heliotrope, *Heliotropium europaeum* L. (Boraginaceae), is a summer-growing, herbaceous annual plant from Mediterranean Europe to the Middle East. The plant has become a weed in southern Australia where it often dominates fallow, disturbed or ploughed land from December to April when it can also be an important component of annual pastures. The foliage contains high levels of pyrrolizidine alkaloids (11), which can poison grazing animals and, as such, the weed causes substantial economic stock losses in years of high abundance (2). Infestations of the plant also take moisture from the soil in the months prior to sowing, therefore affecting subsequent crops (4). The seeds germinate on bare soil or in light vegetation when soil temperatures exceed 24°C following spring and summer rainfall (10). The plant produces flowers and seeds concurrently with vegetative growth, soon after germination, that create a persistent seed bank (3) of up to 300,000 seeds/m2, and dies in autumn or following water stress.

Uromyces heliotropii Sred., is a macrocyclic, autoecious rust fungus native to Eurasia (5). This rust is specific to *H. europaeum* and some closely related European species (8). During testing some limited development occurred on the Australian natives *H. crispatum* and *H. pressiana.* However, *H. crispatum* grows in winter in regions unsuitable for the rust, while the spores produced on *H. pressiana* proved non-viable (8). Studies in Montpellier (southern France) have shown that the pathogen can both kill young seedlings and reduce viable seed production of mature plants (7). The rust has two relatively long-lived spore types. The brown urediniospores are wind dispersed, being produced only during the summer, and lead to either the further production of uredinia on healthy plants or the production of black telia on stressed or senescing plants. The telia produce teliospores that can over winter on plant fragments that remain in the litter layer and re-infect plants the following season producing honey coloured spermogonia and then orange aecia. The environmental requirements for the germination and survival of these spores have been studied under laboratory conditions (6,7,9). AQIS and ANPWS approved release of *U. heliotropii* in early 1991. This paper documents the results of releases made to date, and discusses conditions for successful establishment.

METHODS

Table 1. Rust incidence following inoculations of common heliotrope with *U. heliotropii* urediniospores; (i) methods as described or modified (*) in text; (ii) maximum spread from release point; (iii) classes of disease intensity in the area of spread (see text); *i* = irrigated site; *nd* = data not yet available.

Site		Inoculation		Disease	Spread (m) (ii)	Intensity (iii)	Telia	Accia (yr+1)
		Date	Method (i)					
Gnowangerup WA		1/91	1,2	yes	30	a	yes	no
		1/92	1,2	yes	22	c	yes	no
		1/93	1	yes	4	c	yes	*nd*
Jugiong NSW,	area 1	1.91	1,2	yes	100	d	yes	yes
	area 2	3/92	2	no	-	-	-	-
	area 3	1/93	3	yes	80	b	yes	*nd*
Trangie NSW,	area 1	1/92	1	yes	500	b	yes	no
	area 2	2/93	3	yes	0	a	yes	*nd*
Parkes NSW		1/93	1,3	yes	0	a	no	*nd*
Temora NSW*i*		1/93	3	no	-	-	-	-
Young NSW		2/93	3*	yes	5	b	yes	*nd*
Barham NSW*i*		1/93	1,3	no	-	-	-	-
Dookie VIC		1/93	1,3	no	-	-	-	-
Tailem Bend SA,	area 1	1/92	1,2	yes	2	b	yes	yes
	*i*area 2	1/93	3	yes	*nd*	*nd*	yes	*nd*
Kadina SA		1/93	3	no	0	a	no	*nd*
Maitland SA		1/93	1,3	yes	10	b	yes	*nd*
Streaky Bay SA		1/93	1,3	yes	5	b	yes	*nd*
Minnipa SA*i*		1/92	1,2	yes	100	c	yes	yes

Releases of urediniospores were made from December to March in 1991, 1992 and 1993 at six sites in NSW, five sites in South Australia, and one site in each of Victoria and Western Australia (Table 1). With the aim of maximising the production of inoculum at the site, the plants selected for inoculation bore many green leaves. Inoculation took place in late-afternoon to early-evening with 2-9 week old spores collected from a laboratory culture. Three methods of release were used: (i) 30 mg of spores were brushed, using a paint brush, on to the upper surface of all leaves of 6-16 plants which were at least 15 cm high and with few mature seeds. The plants were watered underneath and then sprayed with a fine mist of water following inoculation. Small polythene covers (also sprayed with water on the inside) were placed over each plant for 12 hours; (ii) 30 mg of spores were mixed with 50 mL of water just prior to inoculation and applied using a hand-held aerosol sprayer to 6-8 plants. Small polythene covers were also placed over these plants for 12 hours; (iii) 200 mg of spores were mixed with 125 mL of talc and applied to pre-watered plants over a 1x5 m area using a hand held pump-action duster. Following inoculation plants were covered with a fine mist of water and a moistened clear plastic sheet which was again left until morning. Table 1 outlines where these

methods were used. No attempt was made to assess impact in the first year. Environmental data loggers were installed at the five longest studied sites (those in Table 2) for comparison of conditions for infection.

In the first season of inoculation, 10 uninoculated plants of similar size and development were chosen in the immediate surrounding area. Inoculated and uninoculated plants were then monitored fortnightly. For these plants, height was measured and the total number of leaves and the number of cymes bearing seeds counted. The distance of spread of rust symptoms was also monitored at the same time by examining surrounding plants and searching at increasing distances from the points of release until either no further spread was observed or the limit of the weed infestation was reached. During these examinations, rust intensity was assessed per site in four categories: (a) no plants infected; (b) few plants infected; (c) most plants infected; and (d) all plants infected. Where possible, other infestations of the weed within a 2-3 km radius were also examined. The presence of teliospores was also noted.

In late spring of the year following inoculation, each site was visited to clear or plough a core area to encourage germination of the weed. From December monthly visits were made to each site. Plant density was measured in the core area, and a random sample of 25 plants was collected to count the number of leaves, cymes and developing seeds. The percentage of plants infected by aeciospores and urediniospores was recorded on each visit.

RESULTS AND DISCUSSION

Of the three inoculation methods used, 1 was reliable, but proved time consuming per treated plant, 2 provided poor results in the field and proved impractical as the nozzle regularly froze up during application; while 3 was as effective per plant, but allowed many more plants to be treated for the same effort. A modified method 3 without an application of water or plastic sheeting also worked very effectively in a gully site at Young.

Of the 19 releases of urediniospores, 14 led to significant infection, 13 showed significant teliospore production and 11 had some spread of the pathogen within the season (Table 1). At three of the five sites where inoculation was unsuccessful, the spores used had very low viability. Subsequent tests showed that the spores over 5 weeks old, which were stored in a desiccator at 0 to -7°C, had less than 20% viability. At the other two sites, ineffective inoculation method was the cause at Jugiong in 1992 and early plant death from the leaf blotch, *Cercospora taurica* Tranzschel, prevented infection at Temora.

The distance of spread or the intensity of the disease were not associated with any of the plant characteristics measured at each site. At Parkes spread and telia production were prevented by an outbreak of *C. taurica* killing the infestation. At many sites spread was limited by a lack of available plants following initial infection. This resulted from either small weed infestation size (Jugiong in 1991, Gnowangerup in 1992 and Minnipa), or heavy plant death as a result of early (Gnowangerup in 1991) or late season (Jugiong in 1993 and Trangie in 1993) water stress. To combat this, controlled irrigation was used at four sites (Table 1). This encouraged the rust-weed interaction at Minnipa, however at two other sites infection failed due to other causes. The fourth site, Tailem Bend in 1993, still requires assessment. The rust showed >50 m spread at four sites. At Trangie in 1992 and Jugiong in 1993 patches of infection were associated with the prevailing wind and heavy rain soon after the first pustules were produced. At Jugiong in 1991 and Minnipa less directional spread and high infection intensity (Table 1) were associated

with late season rains in March. At Gnowangerup in 1991 aecia were observed in the same year as inoculation. Early water stress had caused the production of telia and a later cohort of the weed became reinfected.

Successful establishment, as indicated by the presence of spermogonia and aecia in the season following inoculation, has now been observed at three of the six sites that have been followed so far (Table 2). At Gnowangerup in WA re-infection in the following year has failed two years running despite the weed increasing in abundance size from one year to the next. At Jugiong (NSW) only a few aecia were found in the next year. Severe flooding had occurred during the preceding winter and common heliotrope covered only a quarter of the previous year's inoculation zone due to the growth of other vegetation The rust did not survive even though common heliotrope covered the surrounding hillsides. At Trangie (NSW) there was no re-infection despite an abundance of healthy plants early in the season (Table 2). The only success has been in South Australia. At Tailem Bend in 1992, 6.3% of plants produced telia in the release area in the first year. In 1993, 1.3% of plants of the next generation produced aecia leading to 24% of plants with uredinia by March (Table 2). The disease had spread 7 m. At Minnipa, 100 % of plants produced telia 1992, 6% had aecia in January 1993, and 19% had uredinia by March. The disease here had spread 400 m in one direction and 170 m in another. While a slow build up of the disease occurred at Tailem Bend, the disease situation at Minnipa was potentially epidemic, until flooding destroyed 44% of plants in February 1993. The disease is currently recovering at this site.

Table 2. Spread of *U. heliotropii* away from previous year's diseased area and characteristics of *H. europaeum* during the period of aecia observation at sites followed over two years (nd = no data available)

Site	Date	Aecia		Other spore types			Plant characteristics/m^2		
		% Plants infected	Spread (m)	Uredinia /telia	% Plants infected	Spread (m)	Plant density	Leaf density	Seed density
Gnowangerup WA	92	0	-	-	-	-	c.200	12200	3680
	93	0	-	-	-	-	212	4452	77168
Juiong NSW	92	<0.01	0	none	-	-	nd	nd	nd
Trangie NSW	93	0	-	-	-	-	272	9836	18170
Tailem Bend SA	93	1.3	0	U,T	24	5	892	21140	60656
Minnipa SA'	93	6	400	U,T	19	170	148	2738	8702

The spread of *U. heliotropii*, thus far, has contrasted markedly to the success of *P. chondrillina* released against skeleton weed. In that case only one year was required for the disease to cover south-eastern Australia (1). The local rate of spread of *P. chondrillina* within a site was comparable to the best cases with *U. heliotropii*, but this rust has so far failed to make the rapid jump to more distant weed infestations and most of the ground gained in one season is lost when common heliotrope re-appears. Common heliotrope is short lived compared to skeleton weed and furthermore, heliotrope infestations are more ephemeral in time. Therefore its pathogen is limited in the time it has available to multiply on one host before it must infect others, and often these do not appear for the next 7 months and are not necessarily close by. *U. heliotropii is*

dependent on its teliospores to infect future generations of the weed, while *P. chondrillina,* with a perennial host, is not.

The continuation of infection through the summer depends on the survival rate of spores in Australia. Previous seasons teliospores would not survive the summer and, urediniospore survival is less than a month at temperatures above 30°C (S. Hasan, unpublished data). Summer daytime temperatures can stay at these levels for extended periods, while humid conditions necessary for infection may often be separated by more than a month. However, the differences between sites in Table 2, must be caused by the conditions necessary for teliospore germination. While there are clear environmental differences between the sites in NSW, WA and South Australia, these have not been used, so far, to understand why releases in South Australia have been more successful. There was little evidence to suggest that teliospores can be dispersed away from the site of production. This biological control agent probably requires consecutive generations of hosts in the same area in order to show good local survival and explosive outbreaks. Unfortunately, observations suggest conditions which appear favourable for the rust can also produce destructive outbreaks of other natural enemies of the weed such as *C. taurica* and the moth *Utethesia pulchelloides* Hamps.

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