

## Greenhouse and field evaluations of the rubber vine rust, *Maravalia cryptostegiae*, on Madagascan and Australian Asclepiadaceae

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**Abstract.** An isolate of the rust, *Maravalia cryptostegiae*, from northern Madagascar, released in Australia in 1993 for control of rubber vine (*Cryptostegia grandiflora*, Asclepiadaceae), has shown a lower level of virulence in the field from that determined by pathogenicity tests under greenhouse conditions in the United Kingdom. These tests indicated that rust isolates collected from species and variants of *Cryptostegia* from different regions of Madagascar were of equal virulence. The selected isolate was also shown to sporulate on species of related Asclepiadaceae genera, including *Gonocrypta grevei* (native to Madagascar) and *Cryptolepis grayi* (recently described from northern Queensland). Sporulation density, however, was highly variable on *G. grevei* and extremely low on *C. grayi*. It was postulated that these plants are not natural hosts and would prove to be immune in the field situation. Wind tunnel experiments in the UK, to simulate field inoculum levels, and observations in an experimental garden in Australia, have supported this hypothesis. These results are discussed in relation to recent studies with three rust strains.

### Introduction

*Cryptostegia grandiflora* Roxb. ex R.Br. (Asclepiadaceae: Periplocoideae), commonly known as rubber vine, is an aggressive woody climber which has become a major invasive weed in northern Queensland and now poses a threat to all tropical Australia (McFadyen and Harvey 1990; Parsons and Cuthbertson 1992). The taxonomy and distribution of the genus *Cryptostegia*, which is native to Madagascar, has been revised recently by Marohasy and Forster (1991). In addition to *C. grandiflora*, the genus comprises one other species, *C. madagascariensis* Bojer ex Decne., which has been subdivided into three varieties. Following surveys for natural enemies in Madagascar (Evans 1993) and host range studies in the United Kingdom (Evans and Tomley 1994), a strain of the rust fungus, *Maravalia cryptostegiae* (Cummins) Ono, from northern Madagascar was imported into Australia in April 1993 and released later that year. However, this strain proved to be selectively pathogenic to the *Cryptostegia* variants present in Australia, attacking the target rubber-vine weed only mildly. This contrasted strongly with the results of the greenhouse host-range studies which showed that *C. grandiflora*, as well as the three varieties of

*C. madagascariensis* and some collections of the closely-related Madagascan asclepiad *Gonocrypta grevei* H. Bn., were highly susceptible to this strain (Evans and Tomley 1994). A second strain was collected subsequently from south-west Madagascar and sent to Australia in December 1994. This paper compares the behaviour of these strains both in the greenhouse and the field, and attempts to simulate natural infection with the aid of a wind tunnel.

### Materials and methods

#### Greenhouse studies

The following rust isolates, maintained in liquid nitrogen at the International Mycological Institute (IMI), were used: IMI 331455 ex *C. madagascariensis* var. *septentrionalis* Marohasy and Forster, Diego Suarez, N. Madagascar, collected Oct. 1988; IMI 366461 ex *C. grandiflora*, Lake Andranoke, Tulear, S.W. Madagascar, collected 9 Oct. 1994; IMI 331462 ex *Gonocrypta grevei*, Ejeda, S.W. Madagascar, collected 11 Mar. 1994.

#### Pathogenicity studies

Each rust strain was inoculated onto four-month-old plants of *C. grandiflora* (ex Queensland and S.W.

Madagascar), *C. madagascariensis* (ex N. Madagascar and Kenya) and *G. grevei* (ex S.W. Madagascar) in an IIBC quarantine greenhouse chamber fitted with negative pressure, following the methodology described by Evans and Tomley (1994). In Australia, only the cryptostegia-rust combinations were tested and the plants were inoculated by spraying the underside of leaves ( $1.5 \times 10^{-6}$  spores/ml). Plants were examined three weeks later and the symptomatology was compared and assessed according to previously delimited categories and susceptibility ratings (Evans and Tomley 1994).

#### Wind tunnel studies

Twenty, five-month-old plants of the three host species tested above were inoculated with their respective rust isolate. Each host-rust combination was inoculated, incubated and maintained in separate quarantine chambers during the course of the experiment in order to prevent cross-contamination. Ten, three-month old plants of each of these species, grown and maintained in a separate propagation greenhouse, were used for each treatment, which also included two large, five-year-old plants of the Australian endemic asclepiad, *Cryptolepis grayi* Forster; a species previously shown to be weakly susceptible to the rust under greenhouse conditions (Evans and Tomley 1994).

Each of the rust strains was tested over successive days in a computer-controlled wind tunnel. Heavily rusted plants (three weeks after inoculation) were placed at one end of the wind tunnel, immediately in front of a turbulence screen and the 'bait' plants were grouped randomly 6 m away. In addition, three 5 cm diameter Petri plates containing distilled water agar were exposed in front of the bait plants to measure spore deposition rates. A constant wind speed of  $1 \text{ ms}^{-1}$  ( $3.6 \text{ km h}^{-1}$ ) was maintained for seven hours with both the rust strains from *Cryptostegia* species, after which the bait plants were removed, incubated for 24 h at 100% relative humidity and, 24-28°C, and then transferred to a quarantine greenhouse chamber.

Between treatments, the wind tunnel was sprayed thoroughly with industrial methylated spirits to remove rust inoculum. Observations of the rusted plants revealed that the spore chains remained largely undisturbed at the steady wind speed employed, whilst examination of the agar plates failed to detect spore inoculum. The third treatment, with the *G. grevei* rust strain, was modified, therefore, to test the effect of

wind gusting on spore release and dispersal, in which wind speeds of  $4.5 \text{ ms}^{-1}$  (about  $16 \text{ km h}^{-1}$ ) were generated for 13 seconds. All plants were examined after three weeks and the total number of pustules per plant was recorded with the aid of a stereoscopic microscope. Results were analysed using one-way ANOVA and least significance differences test.

#### Field studies in Madagascar

During surveys undertaken in March 1987 and October 1988, samples of rust were collected from throughout the natural range of the genus *Cryptostegia*, as well as from related Asclepiadaceae. Infected leaf material was dried in a plant press for 3-4 days, and transferred to waxed packets for transport to the UK for comparative studies on the morphology and pathogenicity of these isolates. Additional material was collected by the senior author in 1994 and also received from GTZ scientists based in S.W. Madagascar.

#### Field studies in Australia

The two *Cryptostegia* species, as well as *Cryptolepis grayi* and the Australian endemic *Gymnanthera nitida*, were inoculated as described above with both rust strains, in an experimental garden in Brisbane.

Large-scale field release of the rust strains was made either by deploying infected potted plants at selected sites throughout the geographic range of rubber-vine weed, or by brushing or spraying inoculum, collected with an aspirator, onto natural weed infestations. Potted plants were watered daily for at least 4-5 weeks. Strain IMI 331455 was released over a prolonged period from April 1993 until March 1994, whilst large scale distribution of strain IMI 336461 was undertaken during the 1994-1995 summer wet season.

## Results and discussion

#### Rust morphology

An analysis of the material collected on Asclepiadaceae in Madagascar, which included a detailed comparison of the main spore forms (Evans 1993), revealed that a morphologically indistinguishable rust, assigned to the species *Maravalia cryptostegiae*, occurs not only on all species and variants of the genus *Cryptostegia*, but also on *G. grevei* and on an unidentified species of *Cryptolepis*. Six additional *Maravalia* taxa were

delimited on various Asclepiadaceae, including *Maravalia pentopetiae* Evans (Evans and Punithalingam 1996), based on teliospore morphology.

#### Rust pathogenicity

Early indications of specificity were obtained following observations on a collection of Asclepiadaceae maintained in an experimental garden in S.W. Madagascar, in which heavily rusted rubber-vine was growing adjacent to, and occasionally intertwined with healthy *G. grevei* plants (H.C. Evans and J. Marohasy personal observation). However, comparative cross-infectivity studies were not possible until fresh isolates of the rust from *G. grevei* and *C. grandiflora* were obtained in 1994. The results of these cross inoculations (Table 1) show that the two cryptostegia strains can cross-infect with only minor differences in pustule number and size. Defoliation of *C. grandiflora* was thought to be a characteristic reaction of this species to rust infection, rather than a side effect of the rust strain *per se*. In contrast, the *G. grevei* rust strain (from S.W. Madagascar) failed to sporulate or sporulated poorly on *C. madagascariensis* (from N. Madagascar) but sporulated abundantly on both *G. grevei* and *C. grandiflora*. The behaviour of the *C. madagascariensis* strain on *G. grevei* was highly variable, from no sporulation, but with extensive chlorosis, to abundant sporulation.

The initial greenhouse tests in Australia, with strain IMI 331455, revealed that *C. grandiflora* plants were severely defoliated within seven days of sporulation, whilst *C. madagascariensis* plants retained their leaves despite heavy sporulation. In the experimental garden, natural spread from inoculum sources resulted in heavy infection of *C. madagascariensis* over summer, leading to defoliation by autumn. However, plants of *C. grandiflora* were only slightly defoliated and

infection was classified as light to moderate. The results with the second strain (IMI 366461) were in sharp contrast; inoculated *C. grandiflora* proved to be highly susceptible with dense sporulation but no premature leaf fall, whilst *C. madagascariensis* shed its leaves seven days after light to moderate pustule formation. As reported previously (Evans and Tomley 1994), no symptoms were recorded on *Gymnanthera nitida*, whilst *Cryptolepis grayi* showed a hypersensitive reaction, with limited chlorosis and restricted pustule development after 21 days.

In the field, the northern Madagascan strain caused only weak infection in 75% of the release sites and dispersal was confined to the immediate vicinity of the release point. The rust failed to survive the dry season. Conversely, the strain from S.W. Madagascar (IMI 366461) induced severe infection and subsequent defoliation of rubber-vine, and spread up to 20 km from the initial inoculum source within four months. From surveys undertaken in October 1994, towards the end of the dry season, it appears that the rust is surviving well.

#### Wind tunnel studies

The results (Table 2 and Fig. 1) support the hypothesis that each rust strain is more pathogenic on its original host, which was clearly demonstrated in the field situation but not in the greenhouse host-range tests. However, at steady, prolonged wind speeds, infection of the bait plants was extremely low, suggesting that spore dispersal is inefficient under these conditions. This was confirmed by the absence of rust spores on any of the exposed agar plates. Due to the relatively low infection rates and poor pustule formation, it was not possible to analyze the results statistically (Table 2), although there is an overall trend of decreasing pathogenicity away from the original

**Table 1.** Greenhouse cross-infectivity studies with strains of *Maravalia cryptostegiae* from Asclepiadaceae hosts. Figures in parentheses indicate variation within individual leaves or plants. 6 - resistant; small or abortive pustules, marked leaf chlorosis; 7 - moderately susceptible, larger and denser pustules than 6 (>1/cm<sup>2</sup>); 8 - highly susceptible, numerous (>15/cm<sup>2</sup>), fully expanded pustules, chlorosis but no premature leaf fall; 9 - highly susceptible, as for 8 above, but premature leaf fall with or without chlorosis.

Rust strain	Test plant and susceptibility rating		
	<i>C. madagascariensis</i>	<i>C. grandiflora</i>	<i>G. grevei</i>
IMI 331455 ex <i>Cryptostegia madagascariensis</i>	8	9	(6) 7 (8)
IMI 366461 ex <i>C. grandiflora</i>	(7) 8	8 (9)	8
IMI 331462 ex <i>Gonocrypta grevei</i>	(6) 7	8	8

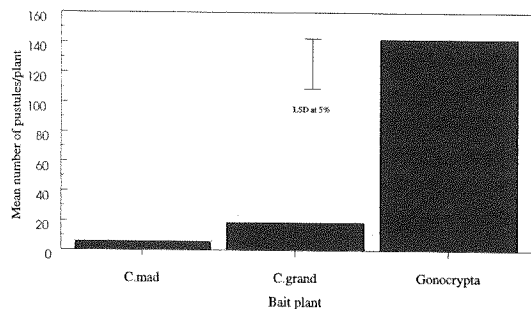
**Table 2.** Infection of Asclepiadaceae plants exposed to two strains of *Maravalia cryptostegiae* in a wind tunnel.\* - exposed to 7 h of wind at 3.6 km hr<sup>-1</sup>. \*\* - 10 plants/treatment. No infection was recorded on *Cryptolepis grayi*, 50 leaves examined/treatment.

Rust strain	<i>C. madagascariensis</i>		Test plant*		<i>G. grevei</i>	
	% infection**	No. pustules	% infection	No. pustules	% infection	No. pustules
IMI 331455 ex <i>Cryptostegia</i> <i>madagascariensis</i>	70	30	50	15	0	0
IMI 366461 ex <i>C. grandiflora</i>	40	10	60	28	20	8

host, for example, the strain from *C. madagascariensis* failed to infect *G. grevei*. However, the wind turbulence treatment (single gust of 16 km h<sup>-1</sup> for 13 seconds), proved to be highly efficient in dislodging and dispersing the spores, as evidenced by the substantial amounts of inoculum trapped on the agar plates and the high infection rates and pustule development on the bait plants (Fig. 1). A total of 383 spores were deposited on the plates, typically in clumps of between 3-30 (mean = 8). Infection rate and pustule production were significantly higher ( $p = 0.01$ ) with the *G. grevei* rust on its natural host compared with the two *Cryptostegia* species. Although sporulation was more pronounced on *C. grandiflora*, compared to *C. madagascariensis*, it was not significantly higher, probably because the results were skewed due to high pustule formation on a single plant of the latter species. No pustules were recorded on any of the *Cryptolepis grayi* leaves in any of the treatments, which lends support to the statement that "... the rust poses no threat to the endemic Australian flora" (Evans and Tomley 1994).

## General discussion and conclusions

The rust *Maravalia cryptostegiae* exists in Madagascar in a number of pathogenic strains or pathotypes. However, greenhouse host-range tests proved to be insufficiently sensitive to delimit clearly the strain differences, probably due to massive inoculum pressure and optimal conditions for infection. Under such conditions, a strain may either artificially extend its host range or increase its infection potential (pathogenicity) on a 'secondary' host which would normally exhibit resistance in the field situation. There is every indication that the *C. grandiflora* rust strain can infect *G. grevei* sharing the same habitat in Madagascar, and *vice versa*, since there was a certain degree of cross-infectivity in the wind-tunnel experiments, which are much more relevant to the field situation than the 'artificial' greenhouse inoculations. Thus, there must be an on-going exchange of genetic material between these sympatric strains which suggests that they are still evolving towards a more rigid host specificity, or narrower host range, as in the more highly evolved rust genera. *Maravalia* is now viewed as a primitive rust genus, following a recent cladistic analysis (Evans 1993), and is well represented in Madagascar as evidenced by the results of the survey. Natural host genera of *M. cryptostegiae* comprise *Cryptostegia*, *Gonocrypta* and *Cryptolepis*, and it is probable that species of all these genera will show some degree of susceptibility to any one strain or pathotype. In hindsight, therefore, it could have been predicted that the newly discovered Australian asclepiad *Cryptolepis grayii* would show some degree of susceptibility to the rubber-vine strains under optimal greenhouse conditions. The wind tunnel studies suggest that this species will prove to be immune in the field and also emphasize the need to interpret rationally the sometimes ambiguous results of



**Fig. 1.** Infection of Asclepiadaceae bait plants exposed to inoculum of *Maravalia cryptostegiae* ex *Gonocrypta* in a wind tunnel. *C. mad* - *Cryptostegia madagascariensis*; *C. grand* - *C. grandiflora*. Mean number of pustules/plant after exposure to a single wind gust (13 secs at 16 km/h). No infection was recorded on *Cryptolepis* (50 leaves examined/treatment).

host-specificity tests carried out in an artificial or *in vitro* situation (Olckers *et al.* 1995; Evans 1995). Without such critical analysis, potentially useful biocontrol agents may be rejected from weed management programmes.

The use of the wind tunnel could prove to be an extremely valuable tool in predicting both 'natural' host ranges, as well as dispersal patterns of fungi that are being considered for release as biocontrol agents of weeds. Initially, it was considered that a steady wind current (3-4 km h<sup>-1</sup> as being a reasonable approximation of air movement in the tropics) would be sufficient to gradually dislodge the rust spores from the pustules over a period of time. However, lack of deposition on the trap plates and low infection of the bait plants indicate that these conditions were not conducive to spore dissemination and that sudden gusts of wind (even as short as 13 seconds) are required to disrupt and disperse the spore columns. The agar-plate results demonstrate clearly that the spores move through the air and impact in clumps rather than singly. The distinctive hemilioid shape of *M. cryptostegiae* spores may be important in the aerodynamics of dispersal, as well as in the successful impaction on the underside of the host leaf; a critical factor since there are no infection sites (i.e. stomata) on the upper surface. McCartney *et al.* (1983) stressed the importance of gusts (measured at five times the mean wind speed) in the dispersal of mildew spores within barley crops and considered also that the impaction efficiency is likely to be dependent on the wind speed at liberation. This appears to be substantiated by the findings from these exploratory wind tunnel experiments.

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