Biological control of blackberry: progress towards finding additional strains of the rust fungus, *Phragmidium violaceum*

John K. Scott[1,2,3], Mireille Jourdan[2] and Katherine J. Evans[1,4,5]
[1] Cooperative Research Centre for Australian Weed Management
[2] CSIRO European Laboratory, Campus International de Baillarguet, 34980 Montferrier sur Lez, France
[3] Present Address: CSIRO Entomology, Private Bag, PO Wembley, Western Australia 6014, Australia
[4] Department of Applied and Molecular Ecology, Adelaide University, Waite Campus, PMB 1, Glen Osmond, South Australia 5064, Australia
[5] Present address: Tasmanian Institute of Agricultural Research, New Town Research Laboratories, 13 St. Johns Avenue, New Town, Tasmania 7008, Australia

Summary Improved identification of blackberry, *Rubus fruticosus* L. agg., in Australia has enabled a new search for effective strains of the rust *Phragmidium violaceum*, suitable for use as a biological control agent against this weed. A strain of rust has been selected for further study from surveys in Portugal in areas that match the climate of selected blackberry infested regions of Australia. A 'trap garden' of Australian clones of blackberry was established in France. Rusts rapidly colonised the garden. Cultures of these rusts are being studied to determine their degree of difference to rusts already in Australia, and their relative effectiveness against the weed.
Keywords Biological control, blackberry, *Rubus* species, *Phragmidium violaceum*.

INTRODUCTION

One of Australia's most important weeds subjected to classical biological control is the European blackberry, *Rubus fruticosus* L. agg. Work in the 1980s identified a strain of the rust fungus, *Phragmidium violaceum* (Schultz) Winter, a plant pathogen that is native to Europe and the Middle East, that was safe for release in Australia. While this work was being done in France, a strain of the fungus, presumably introduced illegally, appeared on blackberry in Australia in 1984. This fungus soon spread and provided useful control, but not in all situations, possibly due to climatic reasons or for genetic differences within and among blackberry populations. The selected strain of the rust fungus was subsequently approved for release and then distributed widely in 1991 and 1992 (Amor *et al.* 1998, Bruzzese and Lane 1996). A decade later the much-improved understanding of genetic variability in blackberry (Evans *et al.* 1998, 1999) and the continued need for improved control of blackberry have led to renewed investigations. Progress on the European based component of this work is reported here.

MATERIALS AND METHODS

Two lines of investigation have been adopted of the work in Europe. Initially the aim was to collect rust from areas climatically matched to regions infested with blackberry in Australia. Subsequently, emphasis moved to the setting-up of a trap garden in France comprised of the genotypes found in Australia and to assessing the rusts that colonised these plants.

Surveys Climate matching was used as the primary site-selection criterion because it was not possible logistically to survey the entire Mediterranean region and because the species identification of blackberries in this region is often difficult (Weber 1999). The CLIMEX program (Sutherst and Maywald 1985) was used initially to identify Portugal as a suitable area that matched selected blackberry infested regions in Australia. Climate stations in the Mediterranean region were matched to Manjimup in Western Australia (chosen as an example of a blackberry infested area from Mediterranean climates in Australia). Coimbra, Evora, Lisbon and Porto in Portugal were the only matching sites in the entire Mediterranean region when the Match Index (MI) was set above 0.74 (max. and min. temperature, total rainfall and pattern, and relative humidity were given equal weighting). G.I.S. techniques were then used to more precisely identify study areas within Portugal, using the climate factors of mean daily temperature, mean relative humidity at 9 a.m. and mean annual rainfall as defined by the climate at Manjimup (see snig.cnig.pt for these Portuguese databases). Surveys were then made to find rust outbreaks on blackberry in the identified regions.

Trap garden Australian blackberries of known genotypes were imported into France as virus-free and rust-free tissue cultures from which plants were produced. The plants were grown on in the glasshouse before transplanting into the trap garden at the CSIRO European Laboratory. By way of comparison to the

Portuguese survey, Montpellier, the nearest climate station, has a match index to Manjimup of MI = 0.66. This is a relatively low level of match despite ranking 51st of the 281 Mediterranean sites used in this MI comparison (neighbouring climate stations usually match above 0.7).

The trap garden consisted of four blocks set 7 m apart. Each block contained a random distribution of the same 21 *Rubus* clones (19 genotypes, including *R. anglocandicans* Newton (G32) the most widespread form in Australia). The plants (i.e. four rooted stems of the same clone) were contained in pots, buried to soil surface level in plastic-lined trenches partly filled with soil. The plants were spaced 1.5 m apart. The trap garden was placed to maximise drainage and exposure to dominant winds from the NW and NE.

Because of European quarantine requirements, all plants, in the glasshouse or the trap garden, were maintained free of flowers and prevented from vegetatively propagating. All prunings were burnt and used soil was autoclaved.

The plants were monitored for presence of rust at weekly intervals. Native *Rubus* growing nearby were also monitored to determine the phenology of rust presence.

Collection, isolation, multiplication and disease susceptibility We studied rusts from the trap garden, Portugal and reference isolates obtained from Australia. Leaves with uredinia were collected from infected plants and urediniospores were harvested in the lab (or directly in the trap garden) using a cyclone collector. The rust isolates (except C23, a strain collected from Portugal) were purified by single uredinium isolation using a sterile artist's paintbrush for transfer of urediniospores, and multiplied on detached leaves from their original clone according to the method of Bruzzese and Hasan (1986), slightly modified. Isolate V2 (collected on *R.* ?*leucostachys*, from Foster, Victoria, April 1997) was sent from Australia by K. Evans. Genotype and rust clone codes follow those of Evans *et al.* 1999, 2000.

Detached leaves from Australian and Portuguese *Rubus* plants were inoculated by a suspension of urediniospores (0.25 mg mL^{-1}) in a settling tower designed by Burgerjon (1956) and incubated at 20°C in a phytotron. Twenty-eight days after inoculation the number of pustules were counted and the diameter of uredinia and weight of urediniospores were measured. At the same time, the disease susceptibility was rated at the macroscopic level, according the following scale: I = immune, no macroscopic symptoms, R = resistant, small chlorotic or necrotic spots and no uredinia, MR = moderately resistant, larger chlorotic or necrotic spots present and small uredinia occasional, MS = moderately susceptible, numerous uredinia but restricted sporulation, S = susceptible, numerous uredinia and abundant sporulation.

RESULTS

Rusts collected in Portugal Blackberry plants (most likely to be *R. ulmifolius* Schott) were examined at 23 sites in five regions in southern Portugal that were identified by the G.I.S. technique. One population of plants (site P23, 28°23'43"N; 008°45'08"E, Comporta, Portugal) showed severe rust infection in November 1999 and 2000, and spores were collected for further study.

Australian genotypes (G32, G28, G21, G7) were tested for susceptibility to this rust, but overall few sori were produced when compared with the degree of infection caused by the so-called illegal strain (V2) on G32 (Table 1, K-W statistic $X^2 = 16.0$, $P<0.01$). Sori of V2 strain on G32 were twice the size of sori produced by C23 and correspondingly, the weight of V2 spores was 14 times that of C23. Overall, the four Australian blackberry genotypes were judged moderately resistant or immune to the Portuguese rust (Table

Table 1. Susceptibility of the Australian genotypes to V2 and C23 (mean ± S.D.). A control using G32 and water and a test using G7 and C23 produced no infection.

Rubus genotype	G32	G32	G28	G21
Rust strain	V2	C23	C23	C23
Susceptibility rating	S	MR	MR	MR
Average number of sori cm^{-2} (n = 7 leaves)	3.4 ± 2.25	0.9 ± 0.78	0.3 ± 0.27	0.2 ± 0.23
Diameter of sporulating and non-sporulating uredinia (mm) (number measured)	0.68 ± 0.22 (348)	0.31 ± 0.13 (187)	0.29 ± 0.10 (52)	0.37 ± 0.18 (27)
Total weight of spores (mg)	19.8	1.4	0.3	0.1

S = susceptible, MR = moderately resistant, and I = immune.

1). Therefore, further research concentrated on the trap garden, although it is still possible that other Australian blackberry genotypes, that were not available at the time, will prove susceptible to the Portuguese rust.

Trap garden in France Since 1999, 27 *Rubus* clones representing 23 genotypes were imported and grown into some 1000 plants at the CSIRO European Laboratory. Planting of the trap garden started May 2000 with six genotypes. The trap garden was completed March 2002 with 19 genotypes randomly placed in each of four blocks (Table 2). Genotype G32 was the most vigorously growing genotype.

Phragmidium violaceum colonised plants in the first spring following establishment of plants. The first rust uredosori were observed 19 June 2000 and 8 June 2001, with teliosori forming in August both years. All genotypes showed evidence of rust presence, but some, including the most widespread genotype found in Australia (G32), produced severe rust infestations. Rust collections were made in 2000 and 2001 on 27 plants covering 13 genotypes (Table 2).

Genetically uniform cultures of the rust were established from 15 samples (noted as * in Table 2). DNA was extracted from urediniospores of 12 isolates and dried DNA pellets shipped to Australia for RFLP analysis according to Evans *et al.* (2000).

DISCUSSION

The use of CLIMEX and G.I.S. provided a rationale for limiting the region surveyed for additional rust strains, in a situation where wider surveys would have been too expensive and time consuming. However, blackberry diversity is highest in temperate Europe (Weber 1999) and relatively low in Mediterranean regions. In southern Portugal, for example, *R. ulmifolius* appears to be the only species present (Monasterio-Huelin 1998). Further tests are required of *P. violaceum* strain C23 to establish its range of pathenogenicity and whether it might still prove effective in the field, despite its low disease rating when compared with isolate V2 under controlled environment conditions.

The trap garden worked as planned with rust infection appearing on the plants within months of the plants being established in the garden. None of the plants held in the glasshouse showed rust development although only 50 m separated the two groups of plants.

Two 'lineages' of *P. violaceum* are possibly present in Australia; the descendants of the 'illegal' strain(s) and descendants of strain F15. An analysis by Evans *et al*. (2000) characterised the DNA phenotypes of 19 collections of urediniospores from throughout Australia including F15, which originally came from Chalon-sur-Saône in France, by using RFLP techniques. Thirteen DNA phenotypes were identified from the

Table 2. Results of collections of blackberry rust from the trap garden in 2000 (0) and 2001 (1). Strains that have been cultured from single spore origins (i.e. purified) are noted *.

Taxon or putative taxon of *Rubus fruticosus* agg.	Genotype	Clone	Year planted	Collections			
				Block 1	Block 2	Block 3	Block 4
R. anglocandicans	32	9607	2000	0*	0*, 1	0*	0*, 1
R. leucostachys	2	EB18	2000		0*, 1		
R. leucostachys	6	EB19	2000			1	0*, 1
R. leucostachys	7	EB16	2000		0*		
R. leucostachys	9	1669	2000		0*, 1	1	
R. leucostachys Foster biotype	21	9721	2000	0*, 1	1		0, 1
R. sp. (*R.* ?*leucostachys*)	39	971606	2000		0*, 1		
R. phaeocarpus	19	15734	2000			1	0*
R. rubritinctus	18	SR18	2000		1		0*
R. sp.?	14	SR43	2000		0	1	0*, 1
R. sp. Tasmania	16	981901	2000	0*			
R. vestitus	28	EB21	2000	1	0*	1	0, 1
R. cissburiensis	29	EB14	2001				
R. erythrops	25	EB20	2001				
R. laciniatus	37	SR14	2001				
R. laciniatus	37	EB22	2001				
R. leightonii	38	JH1660	2001				
R. polyanthemus	36	961107	2001				
R. rubritinctus	18	SR10	2001				
R. ulmifolius	10	WP11-3	2001			1	
R. vestitus	27	1655	2001				

Australian material, none of which corresponded closely to rust strain F15. It now appears that the illegal strain dominates throughout the distribution of blackberry, and further research is required to determine the fate of strain F15 and its descendants, following its introduction in the early 1990s. This study also demonstrated that genetic variation occurs in the rust fungus.

Some *Rubus* clones in the trap garden showed evidence of the rust disease two months later than other clones in 2001, and the degree of infection varied, indicating possible genetic variation in the rust pathogen – host-plant interaction. Preliminary DNA profiles obtained for the rusts from the trap garden suggest that they are genetically different to rust isolates characterised recently in Australia (Evans *et al.* 2000). The DNA analysis will be repeated, using both RFLP and AFLP markers, to confirm these results. Assuming the trap-garden isolates are genetically different to the Australian isolates, they might add useful variation to the existing population of *P. violaceum* in Australia, when imported as additional strains for improved biological control. Most importantly, genetically-different rust strains will be bioassayed for their virulence phenotype across a range of *Rubus* genotypes (the so-called differential set (Evans *et al.* 2001)) to ensure that desirable biological properties are introduced into the existing population of *P. violaceum.*

The trap garden proved useful for selecting directly for virulent rust strains, whereas the utility of climate-matching for rust-strain selection is yet to be demonstrated. Even so, ideally, further trap gardens should be placed in regions of high diversity of *Rubus* species, for example southern England (Edees and Newton 1988), which is a highly likely region of origin of the blackberry found in Australia. Likewise, a trap garden in southern Portugal would test the presence of rust strains adapted to the Mediterranean climate and attacking Australian blackberry. However, quarantine requirements for these trap gardens would make this a major undertaking. The elimination of the trap garden in France started in 2002 (two blocks are being removed) because two years at least will be needed to verify complete removal.

Future work includes determination of the virulence phenotype of the European strains on a differential set of Australian *Rubus* genotypes identified by Evans *et al.* (2000), and to find different physiological races to those of the rust observed in Australia. Host range tests will also be required in quarantine in Australia and approval obtained from the relevant authorities before any additional strains can be released in Australia.

ACKNOWLEDGMENTS

Funding for the work on blackberry in France comes from CSIRO, Western Australian Department of Agriculture, Conservation and Land Management, Western Australia and the CRC for Australian Weed Management. We thank Delphine Allorent and Sylvie Richarte for their participation in the project.

REFERENCES

Amor, R.L., Richardson, R.G., Pritchard, G.H. and Bruzzese, E. (1998). *Rubus fruticosus* L. agg. *In* 'Biology of Australian weeds.' Vol. 2., eds F.D. Panetta, R.H. Groves and R.C.H. Shepherd, pp. 225-246. (R.G. and F.J. Richardson, Melbourne).

Bruzzese, E. and Hasan, S. (1986). The collection and selection in Europe of isolates of *Phragmidium violaceum* (Uredinales) pathogenic to species of European blackberry naturalised in Australia. *Annals of Applied Biology* 108, 527-533.

Bruzzese, E. and Lane, M. (1996). 'The blackberry management handbook.' (Keith Turnbull Research Institute, Victoria).

Burgerjon, A. (1956). Pulvérisation et poudrage en laboratoire par des préparations pathogènes insecticides. *Ann. Epiphyties* 7, 675-684.

Edees, E.S. and Newton, A. (1988). 'Brambles of the British Isles.' (The Ray Society, London).

Evans, K.J., Jones, M.K., Mahr, F.A. and Roush, R.T. (2000). DNA phenotypes of the blackberry biological control agent, *Phragmidium violaceum*, in Australia. *Australasian Plant Pathology* 29, 249-54.

Evans, K.J., Jones, M.K. and Roush, R.T. (2001). Biological control of blackberry: characterization of host and pathogen. Biennial Conference of the Australasian Plant Pathology Society, p. 344.

Evans, K.J., Symon, D.E., Hosking, J.R., Mahr, F.A., Jones, M.K. and Roush, R.T. (1999). Towards improved biocontrol of blackberries. Proceedings of the 12th Australian Weeds Conference, pp. 325-329.

Evans, K.J., Symon, D.E. and Roush, R.T. (1998). Taxonomy and genotypes of the *Rubus fruticosus* L. aggregate in Australia. *Plant Protection Quarterly* 13, 152-6.

Monasterio-Huelin, E. (1998). *Rubus* L. *Flora Iberica* 6, 16-71.

Sutherst, R.W. and Maywald, G.F. (1985). A computerised system for matching climates in ecology. *Agriculture Ecosystems and Environment* 13, 281-299.

Weber, H.E. (1999). The present state of taxonomy and mapping of blackberries (*Rubus*) in Europe. *Acta Botanica Fennica* 162, 161-168.