National blackberry biological control program in partnership with the community

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Summary Additional strains of the leaf-rust fungus *Phragmidium violaceum* are being released as part of a national program to enhance biological control of European blackberry. Suitable sites for release have been identified through an Expression of Interest process involving the community. Stakeholders participating in the program are asked to provide voucher blackberry (*Rubus fruticosus* L. agg.) specimens, which are identified and used to generate a national map of blackberry taxa. Releases are made either by project officers or participants, who are provided with 'kits' comprising guidelines, rust inoculum and the necessary material to make their own release. Molecular tools are being employed to assess establishment and persistence of the strains over time at specific sites, while their impact on blackberry growth parameters is measured using fungicide exclusion techniques.

Keywords European blackberry, *Rubus fruticosus*, biocontrol, rust fungus, *Phragmidium violaceum*.

INTRODUCTION

A national program has been established to coordinate the large-scale release of additional strains of the leaf-rust fungus *Phragmidium violaceum* (Schultz) Winter to enhance biological control of European blackberry (*Rubus fruticosus* L. agg.) across temperate Australia. Blackberry is one of the most important weeds of southern Australia, infesting almost 9 million hectares (James and Lockwood 1998) and is listed as one of the 20 Weeds of National Significance (WoNS). In natural ecosystems, dense infestations reduce biodiversity and wildlife habitat as well as the conservation value of public lands, parks and reserves. In agricultural areas, blackberry thickets replace pasture and exclude livestock. The weed also causes access problems in forests and reduces timber production by competition and preventing natural regeneration.

Phragmidium violaceum coevolved with European blackberry in Europe, but has been present in Australia for more than 20 years following prior unauthorised and authorised introductions. It is most damaging to young, emerging leaves of blackberry during active shoot growth, but causes limited or no disease symptoms on old leaves (Evans and Bruzzese 2003). Since its introduction to Australia, the rust has provided useful control of blackberry in areas with climatic conditions optimal for disease development (Mahr and Bruzzese 1998), but its effectiveness has been limited due to resistance of some blackberry biotypes (Evans *et al.* 2005).

The biological control program for European blackberry was re-started in the late 1990s to identify rust strains capable of infecting the range of weedy blackberry biotypes present in Australia (Morin *et al.* 2006). It was also recognised that the fitness and impact of *P. violaceum* in Australia could be enhanced by increasing the genetic diversity of the rust fungus through the release of additional strains that would recombine with the existing population. Eight additional strains capable of infecting the range of Australian blackberry biotypes and genetically different from the existing population of the rust fungus in Australia were sourced in southern France, an area with a Mediterranean climate, and tested for host-specificity (Gomez *et al.* 2006, Morin *et al.* 2006). These additional strains were approved for release in Australia in 2004.

The main objectives of the national program are to undertake coordinated releases of the eight additional rust strains in partnership with the community and to document their establishment, persistence and impact on blackberry at selected sites. The project commenced in June 2006 and is a research partnership between CSIRO, the Victorian Department of Primary Industries and the University of Tasmania. This paper summarises key activities undertaken as part of the project and progress so far.

MATERIALS AND METHODS

Expression of Interest (EOI) In late winter 2006, the project conducted a major EOI process within communities affected by blackberry across NSW, ACT, SA, Qld and WA to identify suitable sites for releases. A similar process had been undertaken in the state of

Victoria in the previous year. Another EOI process was carried out in spring 2007 to identify suitable release sites in Tasmania and bushfire affected areas in eastern Victoria. Not all sites nominated were guaranteed selection for release of the additional rust strains due to limited availability of inoculum or site characteristics that may have precluded establishment success.

Releases An information package and guidelines to assist community participants in making their own releases were developed and distributed. Release kits containing mass-produced inoculum of each of the additional strains, a spray bottle and other necessary material to make a release were sent via post or given to participants during field days.

Blackberry vouchers All stakeholders who sent completed EOI forms have been contacted and asked to collect voucher blackberry specimens following guidelines provided. Voucher specimens have been pressed and are identified using the blackberry lucid key (Evans *et al*. 2004) and lodged in National Herbarium collections.

Monitoring establishment and persistence The additional strains were released in spring 2006 at four sites in NSW and two sites in Victoria. Intensive sampling and molecular characterisation of recovered rust isolates will be carried out in subsequent years. Published methods for identifying and sequestering microsatellite sequences were improved upon to identify microsatellite DNA markers specific for *P. violaceum*.

Monitoring impact Field experiments were set up in 2005 at three sites in Victoria, and in spring 2006 at four sites in WA and two sites in NSW. The additional rust strains were released at all sites and fungicide was applied at 2–3-weekly intervals to exclude the rust disease in half the plots. Disease development and blackberry growth parameters are being assessed in mid- and late-season each year. Additional field experiments were set up at three sites in NSW in autumn 2007 in preparation for the next growing season.

Preliminary work was also undertaken during the 2006/07 growing season to devise an efficient methodology to measure the impact of the additional rust strains on potted blackberry plants exposed to outdoor optimal conditions for disease development (provided by overhead irrigation).

RESULTS AND DISCUSSION

Of the 263 EOI forms distributed in 2006 to land managers in blackberry-affected regions of NSW, ACT, SA, WA and Qld, 222 completed forms were received. Seven completed forms from Victoria were also received in 2006. These are in addition to the 205 forms received following the EOI process performed in that State prior to the initiation of the national project. The spring 2007 EOI process targeting Tasmania and eastern Victoria was still ongoing at the time this paper was written.

Sites with extensive and dense stands of actively growing blackberry, located in full sun or with limited tree canopy were given priority for release of the additional rust strains. Participants were advised not to physically or chemically control blackberry in areas where releases were conducted, for several years, to allow the additional strains to establish locally and then naturally spread to adjacent sites in the region. Sites under compliance orders for blackberry control were excluded from the release program.

A total of 83 release kits, containing the eight additional strains, were distributed to participants in spring 2006 (NSW and ACT (32), SA (17), Qld (2), WA (32)). Project Officers and community members in Victoria also released selected rust strains at 75 sites. Fifty-four of the participants who received a release kit provided feedback by returning a completed release details form. Ninety-six percent of these participants confirmed that rust symptoms developed on inoculated blackberry within one month of the release. Presence of rust symptoms soon after release was also reported by land managers from 34 of the 75 Victorian sites where releases were made in 2006. These results demonstrated the effectiveness of our devised release strategy.

Eighty participants from NSW, ACT, SA, Qld and WA have so far sent blackberry voucher specimens collected at their release sites. Voucher specimens have also been collected at the Victorian release sites. A national map of blackberry taxa will be produced, as this is considered a major knowledge gap in the fight against this WoNS.

Microsatellite DNA markers were successfully developed to track establishment and persistence of the additional rust strains over time. This approach was taken because previously developed molecular diagnostic tools were not sufficiently robust and reliable (Morin *et al.* 2006) and the SAMPL technique developed by Gomez *et al.* (2006) is technically and labour intensive. Nineteen unique microsatellite loci were identified and screened for allelic variation on various rust isolates. Initial results revealed unique allelic variation at some loci that can be used to differentiate between the additional European strains released and the existing population of the rust in Australia.

It is too early to report on the field experiments initiated to measure impact of the additional strains as the intention is to assess their cumulative effect over several seasons. It is noteworthy that the rust disease was not very severe at most sites in the 2005 and 2006 growing season as a result of the drought. Re-release of the additional strains is being contemplated for experimental sites in NSW and Victoria. A more controlled, manipulative experiment involving standardised blackberry plants in large pots placed in a shade house with overhead irrigation, is being performed in 2007 to provide impact data to complement field experiments.

The project is greatly benefiting from the participation of land managers, who facilitate the release of the additional rust strains at a large number of sites across the entire range of blackberry in Australia. The release, establishment and persistence or introgression of the additional rust strains with the existing rust population will increase the overall genetic diversity of this biological control agent. This in turn, may enhance the rust's capability to adapt to its variable host and different environmental conditions. This should lead to the rust having a greater impact on blackberry across the Australian landscape, which will assist in containing present infestations and reducing spread.

There is no single solution to deal with the blackberry problem in Australia. Consequently any improvement of biological control through the release of additional rust strains will be most welcome, particularly at sites where implementation of other control methods is inappropriate or impractical. However, biological control is not a silver bullet, but rather complements existing control techniques.

Biological control will not be effective across the full range where blackberry infestations occur in Australia and an integrated weed management approach will continue to be necessary. The additional rust strains will not have a major impact in areas where the growth rate of blackberry and infection potential of the rust are limited by sub-optimal environmental conditions such as high temperatures, summer drought and in areas where blackberries grow under dense tree cover.

For more details visit our website: http://www.ento.csiro.au/weeds/blackberry/index.html

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