Control tools and technologies for established pest animals and weeds competitive grants program

Project

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Grantee

Full legal name of grantee	The Crown in right of the State of New South Wales acting through the Department of Primary Industries as an office of the Department of Industry
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Project Summary –

Species in the Noogoora burr complex[1] are problematic weeds in primary production, the environment and the Australian community. Nationally significant impacts on productivity and profitability are experienced in summer cropping (e.g. cotton, sorghum, maize and pulse industries), in pastures and in rangelands (particularly wool production). Noogoora burrs are also hosts of insect pests and pathogens such as Verticillium wilt (*Verticillium dahliae*), an important crop disease. They are a serious threat to riverine ecosystems, habitats and native species, impacting 36 vegetation communities, including 11 'Endangered Ecological Communities' in NSW alone. While a range of herbicides control Noogoora burrs in cropping systems, all cause off-target damage and are inappropriate for use in sensitive situations such as the riverine areas.

This project aimed to develop a bioherbicide to manage species in the Noogoora burr complex. Bioherbicides contain naturally occurring fungal pathogens that are specific to the target weed. Among their many benefits, they are safe to use in sensitive environmental and production areas and are highly cost effective when compared to traditional herbicide development. Having said this, bioherbicides require free water from dew or rain for the pathogens to develop and for plant death to occur. We overcome this global limitation through the use of a complex emulsion.

Our research builds on existing knowledge of the pathogen *Alternaria zinniae* and its ability to kill plants of the Noogoora burr complex. Initially, our research sought to better understand the taxonomy within the species complex since such uncertainty can constrain biological control efficacy. DNA barcoding and next-generation sequencing showed us that the Noogoora burr complex consists of two distinct genetic groups and a wide range of hybrids (not the 4 morphologically distinct species (morpho-types) originally described). The first group encompassed the species *Xanthium cavanillesii* and *X. italicum*, and the second encompassed *X. occidentale* and *X. orientale*. Fortunately, our bioherbicide is equally effective across these groups, supporting the validity and use of this bioherbicide against the Noogoora burr complex.

DNA barcoding also confirmed the presence of the Verticillium wilt pathogen in all groups of the Noogoora burr complex. Isolates identified belonged to a range of strains including the defoliating VCG1A and non-defoliating VCG2A Verticillium wilt.

The project was highly successful in achieving all its aims. It has progressed the potential to deliver an environmentally friendly bioherbicide alternative for the integrated management of Noogoora burr species. We anticipate partnership with land managers in testing this product as it moves towards a commercially viable product. Future research should extend the use of the complex emulsions to other pathogen/weed combinations to achieve control of other established weeds in primary production and environmental ecosystems.

[1] Four species comprising the Noogoora burr complex have been taxonomically described in Australia (*X. cavanillesii, X. italicum, X. occidentale* and *X. orientale*), while only a single species (*X. strumarium*) is defined in the Americas, the source of the burr. In practice, intra- and inter-specific variation exists, with some plants not readily conforming to any of the published taxonomic descriptions.

1 Evaluation of how Project achievements contribute to overall Program objective and outcome

The project has been highly successful in delivering on all activities. In summary, it developed a new control tool/technology, a bioherbicide for controlling species and/or hybrids within the Noogoora burr species complex (a well-established and troublesome complex of introduced weeds in Australia that cause a range of agricultural production, environmental (ecosystem and native species) and societal impacts). The bioherbicide was sourced from an existing, naturally occurring fungal pathogen agent (*Alternaria zinniae*). In a global first, we overcame the universal bioherbicide limitation of a dew point requirement (moisture is needed for pathogen disease development and plant death) using a complex emulsion sourced through collaboration with scientists from the University of Melbourne. When combined with the emulsion, *Alternaria zinniae* will kill all Noogoora burr species and hybrid plants under standard application conditions. The next steps include seeking a commercial partner to develop and release this emulsion/pathogen combination as a bioherbicide for the Noogoora burr complex. We expect to engage potential end-users across Australia (primary producers, environmental managers and weed professionals, for example) in the use of this product throughout the commercial development phase, and after the release of the product.

In specific detail, the project was able to obtain five samples (strains or isolates) of *A. zinniae* and screened these on Noogoora burr species and hybrids. The most virulent strain was highly efficacious on 3-4 leaf Noogoora seedlings and used for the development of the bioherbicide in this research. Future collection of other naturally occurring *A. zinniae* (under more favourable seasonal conditions), may be a useful avenue of research as there were differences in the virility of the five strains used.

The selected *A. zinniae* isolate was successfully mass-reared, and in combination with a complex emulsion, was shown to be effective in controlling seedlings of all species and hybrids in the glasshouse, and in the field. The use of the food-grade complex emulsion to enable infection of the target plant was pivotal to the success of this project. This development should result in a global change in the use of a range of other commercial bioherbicides as well as those under investigation, removing the limitations caused by the dew point requirement for pathogenic infection. Research on the development of specific emulsions for bioherbicides is required and is extremely promising. The use of these emulsions should enable the development of a range of bioherbicides which can be safely used in a range of sensitive areas.

Over the course of the project, samples from 115 populations of plants within the Noogoora burr complex were collected from five mainland states and territories of Australia. These populations included plants that fitted the four taxonomic species or groups in the Noogoora burr complex, but also included a range of plants which did not closely follow the taxonomic classifications and appeared to be hybrids. Plants were subsequently grown from the seed collected from these populations of species and hybrids, greatly simplifying the field collection process for obtaining young, green leaf material for DNA and pathogenic host analysis. This extensive collection has allowed us to identify the current distribution of species in the sampled areas and provided the foundation for the remainder of the project. Many more samples would be required to obtain an Australian distribution of this weed and its species.

DNA analysis of the collection has shown that the four species (*X. italicum*, *X. occidentale*, *X. orientale* and *X. cavanillesii*) fall into two genetic species (groups), in agreement with recent work from Europe. There were also a number of hybrid populations collected. Overall, this information will be central to the future classification of these species, although questions do remain, including why there appears to be only two genetically separable groups and yet four taxonomically separable

species. Also, there is currently little understanding of hybrids within this group and how genetically stable they may be. Use of both next generation sequencing and genetic barcoding has been valuable, with each approach supporting the results obtained from the other.

Determining whether all *Xanthium* species were hosts for Verticillium wilt in cotton has resulted in innovation in the techniques developed in this project and should greatly assist with detecting the host status of a range of other weeds. In addition, the finding that all species in the Noogoora burr complex were hosts of a number of *Verticillium dahliae* strains raises further questions around the potential for some of these additional strains to be present in other weeds, and in cotton. The techniques developed in this project will enable these possibilities to be further explored in future projects.