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by Rieks van Klinken and Mic Julien

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Foreword

In Australia the area affected by lippia is mainly in the Murray–Darling Basin, in south-east Queensland, New South Wales and Victoria. The industries most affected by lippia invasions are pastoral production and mixed farming enterprises. The environment in general, including several Ramsar and other protected wetlands, is also affected. In most situations biological control is the only realistic way of managing this weed, and the long-term purpose of this research is to find effective and safe means of biological control.

Native range survey work relied on classic field survey techniques across the native range of lippia, supplemented by culturing and preliminary testing of potential agents in the laboratory in Buenos Aires. This work was done by an entomologist from the US Department of Agriculture's South American Biological Control Laboratory and a plant pathologist from the University of Bahia Blanca. Genetic work principally involved sequencing of *P. canescens* and *P. nodiflora* samples collected from around the world. Special effort was directed at sampling *P. nodiflora* from Australia, Africa, Asia and the Americas to help determine its native range distribution.

This project was funded in Phase 1 of the National Weeds and Productivity Research Program, which was managed by the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF) from 2008 to 2010. The Rural Industries Research and Development Corporation (RIRDC) is now publishing the final reports of these projects.

Phase 2 of the Program, which is funded to 30 June 2012 by the Australian Government, is being managed by RIRDC with the goal of reducing the impact of invasive weeds on farm and forestry productivity as well as on biodiversity. RIRDC is commissioning some 50 projects that both extends on the research undertaken in Phase 1 and moves into new areas. These reports will be published in the second half of 2012.

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Dr Rieks van Klinken is a research scientist with CSIRO Ecosystem Sciences. He has been working on invasive plants for the past 14 years, mostly in northern Australia, and currently leads the tropical invasive plants group in Brisbane.

Mr Mic Julien worked at CSIRO on the biological control of tropical invasive plants for more than 30 years. He is now a Retired Fellow with CSIRO Ecosystem Sciences in Brisbane.

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Executive Summary

Studies of the ecology, biology and management of lippia, *Phyla canescens*, have been supported by the New South Wales Department of Primary Industries, the Weeds Cooperative Research Centre and Australian Wool Innovation. This is a long-term project, and an agreement was developed with the Australian Weeds Research Centre through the Department of Agriculture, Fisheries and Forestry to support the research for the period from March 2009 to May 2010. The paper presented here is an edited version of the final report submitted to DAFF as part of the agreement.

The area affected by lippia in Australia

In Australia the area affected by lippia is mainly in the Murray–Darling Basin, in south-east Queensland, New South Wales and Victoria. The industries most affected by lippia invasions are pastoral production and mixed farming enterprises. The environment in general, including several Ramsar and other protected wetlands, is also affected. In most situations biological control is the only realistic way of managing this weed, and the long-term purpose of this research is to find effective and safe means of biological control.

In brief, the project has three objectives:

- to continue work on natural enemies of lippia—arthropods and pathogens—by conducting further survey work and detailed field and laboratory studies on high-priority species in the weed's native range in South America

- to build on the experience and knowledge gained by project staff in Argentina and Australia, to the point where the first of the natural enemies with greatest potential can be imported into Australian quarantine for detailed testing

- to complete the genetic work that underpins the biological control program. This work will also determine the genetic structure of *P. canescens* in Australia and its origin (or origins) in South America, which will help direct the search for and selection of agents.

Research methods

Native range survey work relied on classic field survey techniques across the native range of lippia, supplemented by culturing and preliminary testing of potential agents in the laboratory in Buenos Aires. This work was done by an entomologist from the US Department of Agriculture's South American Biological Control Laboratory and a plant pathologist from the University of Bahia Blanca. Genetic work principally involved sequencing of *P. canescens* and *P. nodiflora* samples collected from around the world. Special effort was directed at sampling *P. nodiflora* from Australia, Africa, Asia and the Americas to help determine its native range distribution.

Main findings

The main findings resulting from this research are as follows:

- Molecular work has shown that Australian populations previously identified as *P. nodiflora* and thought to be exotic contain both native and exotic genotypes. This has implications for the management of lippia—especially in the selection of biological control agents—and for conservation of native *P. nodiflora* genotypes in Australia.

- The most important potential biological control agents have been identified for further study following comprehensive native range surveying.

- We propose that the leaf-feeding beetle *Kuschelina bergi* be imported for host specificity testing in quarantine in Australia.

- Research has highlighted the importance of plant taxonomy when assessing potential biological control agents.

Potential biological control agents have been identified for lippia. The first of these should be available for release within two years, contingent on funding.

We recommend that funding for research into lippia biological control continue to be provided and that the research focus on conducting the detailed host specificity tests required to confirm that the priority agents are safe to release in Australia.

Introduction

Studies of the ecology, biology and management of lippia, *Phyla canescens*, have been supported by the New South Wales Department of Primary Industries, the Weeds Cooperative Research Centre and Australian Wool Innovation. This is a long-term project, and an agreement was developed with the Australian Weeds Research Centre through the Department of Agriculture, Fisheries and Forestry to support the research for the period from March 2009 to May 2010.

Objectives

The lippia biological control project has three objectives:

- to continue work on natural enemies of lippia—arthropods and pathogens—by conducting further survey work and detailed field and laboratory studies on high-priority species in the weed's native range in South America

- to build on the experience and knowledge gained by project staff in Argentina and Australia, to the point where the first of the natural enemies with greatest potential can be imported into Australian quarantine for detailed testing

- to complete the genetic work that underpins the biological control program. This work will also determine the genetic structure of *P. canescens* in Australia and its origin (or origins) in South America, which will help direct the search for and the selection of agents.

Methodology

Native range survey work relied on classic field survey techniques across the native range of lippia, supplemented by culturing and preliminary testing of potential agents in the laboratory in Buenos Aires. This work was done by an entomologist from the US Department of Agriculture's South American Biological Control Laboratory and a plant pathologist from the University of Bahia Blanca. Genetic work principally involved sequencing of *P. canescens* and *P. nodiflora* samples collected from around the world. Special effort was directed at sampling *P. nodiflora* from Australia, Africa, Asia and the Americas to help determine its native range distribution.

Progress was reported to the National Lippia Working Group at a meeting in September 2009 at Narrabri, New South Wales. Several conference and journal papers have also been prepared: these are listed in the reference section.

Results

Plant taxonomy

The taxonomy of *Phyla* species in their native range in South America remains problematic.

In summary, only one *Phyla* species, *P. canescens* (lippia), occurs in the southern range of *Phyla* in Argentina, whereas other species also occur in the northern range. The Darwinion Herbarium in Buenos Aires recognises three species—*P. canescens, P. nodiflora* and *P. fruticosa* (which was *P. reptans*)—but we expect changes as a result of a current revision of the genus. Meanwhile, genetic research that is part of this project has developed a phylogeny for *P. canescens* sensu lato. that comprises four clades, all of which are represented in Australia. The first two clades include forms that the Darwinion Herbarium calls *P. nodiflora* and *P. fruticosa*, northern forms in Argentina. Clades 3 and 4 include the southern and parts of the northern native range of lippia in Argentina and Chile. Further, our genetic studies of *P. nodiflora* suggest that this species does not occur in Argentina—perhaps not in South America—but occurs naturally on all other continents except Antarctica.

Our genetic studies also show that there have been multiple introductions of lippia into Australia. There is, however, little genetic variation in lippia, and gene mixing makes it difficult to pinpoint with accuracy the source of Australian populations. Nonetheless, most Australian populations are from central Argentina. *P. nodiflora* includes a genotype native to Australasia. There are also exotic genotypes from Africa and elsewhere, some of which have naturalised. Potential biological control agents that have been recorded from other *Phyla* species in Argentina might still be sufficiently host specific for release in Australia since *P. nodiflora* is a sister group to these taxa.

Native range studies

Pathology

CSIRO and the Universidad National del Sur at Bahia Blanca collaborated in the work on pathology. A detailed final report is available on request.

Seventeen fungus species were found on *Phyla* species in surveys conducted during three-and-a-half years. Only three species were considered to have potential as biological control agents. Two, the rust *Puccinia lantanae* and the leaf-spotting fungus *Cercospora lippiae*, might be useful for classic control and are considered in this report; the third species, a group of *Colletotrichum* fungi, might have potential as bioherbicides, but such studies are outside the scope of this project.

The rust *Puccinia lantanae* was collected on *Phyla* species in northern Argentina from a range of forms. Inoculation tests have shown it to be capable of infecting *Phyla* species and genotypes other than those from which it was collected, suggesting that it may be oliophagous within *Phyla*. The microcyclic and autoecious conditions of this form of the rust that infects *Phyla* were confirmed, these being important factors for its consideration as an agent of classic biological control. This rust was not easily found in the field, which could be because of the prolonged drought in Argentina (similar to that experienced in Australia) during the period of the field surveys. When more field material has been collected and isolated host range tests will be conducted to determine if *Puccinia lantanae* is likely to infect the lippia genotypes that are invasive in Australia but not the native *Phyla nodiflora*.

Cercospora lippiae fungi are widespread geographically and across all forms of *Phyla*. Only four of the 16 locations were included in the plant genetic studies, but those four occur in clades 3 and 4. *Cercospora* are difficult to work with experimentally. We have isolated them from various regions, but further work is needed to determine the environmental conditions for successful sporulation of mycelia, germination of conidia and infection, thus allowing host range studies to be conducted.

Entomology

The entomological studies were a collaboration between CSIRO and the US Department of Agriculture laboratory in Buenos Aires. A detailed final report is available on request.

Sixteen insect species have been found on lippia—a far lower diversity than expected. Five of the species have potential as biological control agents; another seven have little potential because they have been collected very few times and in low numbers, and nothing is known about them; the remaining four species are known to be polyphagous.

Potential biological control agents

Four insects and a mite have potential as biological control agents:

- The leaf-feeding beetle *Kuschelina bergi* has been collected only on lippia and has been studied in the laboratory in Argentina. It can feed and develop on all forms of *Phyla* in Argentina but has a preference for *P. canescens*. We propose that this insect be brought to a quarantine facility in Australia for host specificity testing, beginning with Australian native forms of *P. nodiflora.*

- The leaf beetle *Longitarsus* sp. 2 is the second *Longitarsus* species found on lippia in the southern range in Argentina. The first, the northern species *Longitarsus* sp. 1, was studied and found not to be host specific. Successful techniques for rearing this insect are yet to be developed.

- The eriophyid mite is new to science. It is microscopic and appears to infest all forms of *Phyla* in Argentina. The mites are usually highly host specific, so there could be several species or forms specific to each form of *Phyla*. Collections have been sent to Dr Sebahat Ozman, an eriophyid specialist in Turkey, who has agreed to determine the number of species and describe them. Techniques for managing and host testing this mite are yet to be developed.

- Stem galler 1 is probably an agromyzid fly. Galls are formed on nodes near the base of stems, and the stem galler is found on prostrate stems and sometimes stems covered in substrate. There may be different fly species making galls on different forms of *Phyla* since there appear to be size differences between plant forms. Parasitic wasps have emerged from galls collected throughout the range of *Phyla* in Argentina. Techniques for rearing and studying this insect are yet to be developed.

- Stem galler 2 is another as yet unidentified gall former, this one found in northern Argentina. Galls are formed on a node half-way down erect stems, and the stem above is killed. The galls appear to be caused by an agromyzid fly, and parasites have also emerged from galls held in the laboratory. The range of this insect is not yet known.

All surveys were conducted during a period of intense and prolonged drought in Argentina, and it is probable that the drought restricted the numbers of many herbivorous arthropods and pathogens. Should the drought break and several ‘normal’ growing seasons occur, natural enemies not recorded or from the second group of rarely found insects might become more abundant. Among these rare insects are a weevil, a leaf-mining fly, two species of thrip (sap feeders) and four species of moth.

Progress was reported to the National Lippia Working Group at a meeting in September 2009 at Narrabri, New South Wales. Several conference and journal papers have also been prepared: these are listed in the reference section.

Recommendations

This is a long-term project in the late exploration phase and moving towards the host specificity testing phase, when potential agents will be imported into Australia for study. We recommend that funding be provided for detailed host specificity testing—initially of the Australian *Phyla nodiflora*—and the subsequent release of any host-specific agents.

References

Journal publications related to this project

Fatemi, M, Gross, CL, Julien, M & van Klinken, R (in prep.), 'On the origin and genetic variation of Lippia (*Phyla canescens*: Verbenaceae) in Australia—inferred from ITS rDNA phylogeny and ISSR analysis', *Biological Invasions*.

Fatemi, M, Gross, CL, van Klinken, R & Julien, M (in prep.), 'The enigma of *Phyla nodiflora* (Verbenaceae): ITS rDNA reveals a phylogeny', *American Journal of Botany*.

Julien, MH, De Clerck-Floate, R, Bourne, A & Gellender, M (submitted), 'Growth responses of the floodplain weed lippia (*Phyla canescens*) to damage: seeking its Achille's heel', *Weed Research*.

Macdonald, MJ, Whalley, RDB, Julien, MH, Sindel, BM & Duggin, JA (submitted), 'Flood-induced recruitment of the invasive *Phyla canescens* (Verbenaceae)', *Plant Ecology*.

Xu, C, Julien, MH, Fatemi, M, Girod, C, van Klinken, R, Gross, C & Novak, S 2010, 'Phenotypic divergence during the invasion of *Phyla canescens* in Australia and France: evidence for selection-driven evolution', *Ecology Letters*, vol. 12, pp. 32–44.

Xu, C, Schooler, SS & van Klinken, RD 2010, 'Clonal integration leads to over-investment in shaded leaves and a species-specific pattern of ramet growth and maternal subsidy for two invasive plants', *Journal of Ecology*, in press.

Conference presentations related to this project

Julien, MH, van Klinken, RD, Xu, C, Sosa, A, Traversa, G, Delhey et al. 2009 'Ecology, genetics and the potential for biological control of lippia, *Phyla canescens*', *Proceedings of the 15th Biennial NSW Weeds Conference*, 14–17 September 2009, Narrabri, New South Wales.

Sosa, AJ, Traversa, MG, Delhey, R, Kiehr, M, Cardo, MV & Julien, MH 2008, 'Biological control of lippia (*Phyla canescens*): surveys for the plant and its natural enemies in Argentina', in MH Julien, R Sforza, MC Bon, HC Evans, PE Hatcher, HL Hinz et al. (eds) *Proceedings of the XII International Symposium on Biological Control of Weeds*, CAB International, Wallingford, UK, pp. 211–15.

Julien, MH, Sosa, AJ, Traversa, G, Xu, C, Fatemi, M & Greizerstein, E 2008, 'The potential for biological control of Lippia, *Phyla canescens*', 5th International Weed Science Congress. Vancouver, Canada, June, Abstract and poster.

Final reports

Traversa, G, Delhey, R, Kiehr, M & Julien, J 2010, 'Biological control of lippia (*Phyla canescens*): native range surveys for plant pathogens', Final report submitted by CSIRO to the Department of Agriculture, Fisheries and Forestry, unpub.

Sosa, S, Julien, M, Telesnicki, M & Cardo, V 2010, 'Biological control of lippia, *Phyla canescens* (Verbenaceae): surveys for its natural enemies in southern South America', Final report submitted by CSIRO to the Department of Agriculture, Fisheries and Forestry, unpub.

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