Pest Manag Sci **59**:671–680 (online: 2003)
DOI: 10.1002/ps.700

Biological control of weeds: research by the United States Department of Agriculture–Agricultural Research Service: selected case studies†‡

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Abstract: Research by the USDA-Agricultural Research Service (ARS) on biological control of weeds has been practiced for many years because of its inherent ecological and economic advantages. Today, it is further driven by ARS adherence to Presidential Executive Order 13112 (3 February 1999) on invasive species and to USDA-ARS policy toward developing technology in support of sustainable agriculture with reduced dependence on non-renewable petrochemical resources. This paper reports examples or case studies selected to demonstrate the traditional or classical approach for biological control programs using Old World arthropods against *Tamarix* spp, *Melaleuca quinquenervia* (Cav) ST Blake and *Galium spurium* L/*G aparine* L, and the augmentative approach with a native plant pathogen against *Pueraria lobata* Ohwi = P *montana*. The examples illustrated various conflicts of interest with endangered species and ecological complexities of arthropods with associated microbes such as nematodes.
Published in 2003 for SCI by John Wiley & Sons, Ltd.

Keywords: *Tamarix* spp; *Diorhabda elongata deserticola*; *Melaleuca quinquenervia*; *Boreioglycaspis melaleucae*; *Fergusonina turneri*; *Galium spurium*; *Galium aparine*; *Cecidophyes rouhollahi*; *Pueraria lobata*; *Myrothecium verrucaria*; mycotoxins

1 INTRODUCTION

The issuance of Presidential Executive Order 13112, Invasive Species,[1] dated 3 February 1999, requires in part that relevant programs and authorities, subject to appropriations and Administration budgetary limits, be used '...to conduct research on invasive species and develop technologies...to...provide for environmentally sound control of invasive species....' Moreover, the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) is committed to conducting research that supports sustainable agriculture.[2] In this context, one goal is to reduce inputs derived from non-renewable petrochemical resources. In view of Executive Order 13112, and since herbicides account for 44% of the volume of petrochemical pesticides,[3] development of biologically based management of invasive exotic weeds is a priority. Biological control can and should be a major component in integrated weed management systems.

First, the definition of biological control should be clear. However, it has been defined in a number of ways over a history of more than 100 years. For their comprehensive history of biological control within the USDA, Coulson *et al*[4] selected the following broad definition: 'use/management of naturally occurring, introduced or genetically-modified natural enemies (predators, parasites/parasitoids and pathogens of pests) and other selected beneficial organisms (antagonists, competitors and allelopaths) and their products, to regulate populations and effects of pests (invertebrate pests of useful plants,

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† One of a collection of papers on various aspects of agrochemicals research contributed by staff of the Agricultural Research Service of the United States Department of Agriculture, and collected and organized by Drs RD Wauchope, NN Ragsdale and SO Duke

(*Received 20 November 2002; accepted 8 January 2003*)

animals, man, terrestrial and aquatic weeds, and plant pathogens).'

Biological control of weeds or other pests is not risk-free. Researchers often encounter constraints and perceived or real conflicts of interest. The case studies selected and reported herein are included not only because they represent recent work, but because they also illustrate some of the constraints and/or conflicts of interest associated with certain biological control projects. These cases further demonstrate the competent professionalism and high ethical standards of the ARS scientists responsible for pursuing their projects. In all of these selected cases, the targets are examples of weeds that are adventive and invasive in the USA. In addition, the projects have been selected to illustrate the traditional, classical approach and the augmentative approach.

1.1 The classical or introductory approach to biological control

The traditional approach means trying to find the center of origin of the target, with the associated implication of also finding the greatest diversity in natural enemies because of the longest histories of co-evolution. At the end of the process, this approach usually involves only a few inoculative releases with subsequent perpetual reproduction and natural dispersal without further input.

In 1991, classical biological control procedures were outlined for ARS scientists.[5] Some key features remain the same today. A target weed is selected by the scientist or clients, and a proposal is submitted for approval by the ARS National Program Staff Matrix Team. Then, approval is solicited from the Animal and Plant Health Inspection Service (APHIS) sponsored Technical Advisory Group (TAG) for Biological Control Agents of Weeds. The area of origin is identified and foreign countries are explored for host-specific natural enemies. The biology/taxonomy/ecology/host-range are characterized for candidate biological control agents in the home range or in approved quarantine facilities, and regulatory approval is sought for release of biological control agents in the USA.

Additional procedures, including steps for submission of petitions for release, are now quite well outlined in a recent (2001) publication by APHIS.[6] One of the recent improvements is that a host-plant test list is submitted for approval of TAG prior to conducting the research. This gives ample opportunity for the United States Department of Interior's Fish and Wildlife Service (FWS), as a member of TAG, to check for potential conflicts of interest related to threatened and endangered species. This is a crucial step toward achieving future successes, as the FWS is federally responsible for administering and monitoring programs affecting rare and endangered species. The submission of a proposal to TAG also gives the member agencies opportunity to judge and comment on losses caused by targeted invasive, exotic weed species within their jurisdictions.

In recent years, biological control has come under increasing scrutiny and even criticism.[7] As a consequence, monitoring releases and assessing risks of approved biological control agents have been given increased emphasis[8] to help counter conflicts of interest and non-target effects. One tenet of classical biological control is to reduce the competitive intensity of the target weed(s) so that native plant communities are not overwhelmed. Monitoring of losses due to the invaders, and changes in their density must be done prior to releases of biological control agents. This long-term pre- and post-monitoring of releases over all areas usually is a cooperative effort between ARS and several federal/state action agencies and is necessary to provide usable databases.

However, in the early phases of releasing biological control agents, research/implementation functions may not be clear and the lines of responsibility may overlap between federal/state research and action agencies. Sometimes private companies can also be involved in the implementation phase. Thus, to obtain maximum benefit from often scarce numbers of biocontrol agents, cooperation and communication are essential, especially in the early stages of a release program. In this review some case studies are presented to illustrate recent progress.

1.2 The augmentative or manipulative approach to biological control

This approach entails the augmentation[9] (and manipulation) of native (USA) biological control agents that may not have co-evolved with the target, especially if the target is exotic. Such a relationship may imply what is commonly called 'a new association' between the biological control agent and the target. The augmentative approach normally requires repeated applications, and is therefore the more expensive to maintain over a long period of time (perhaps several years).

This approach is usually applied to native plant pathogens isolated from a target weed or found to be pathogenic against a target weed regardless of source. A plant pathogen, as a candidate biological control agent, must be fully characterized biologically. First, 'Koch's postulates' must be proved, ie, the investigator is able to induce the plant disease by inoculating a target plant species and then is able to re-isolate and identify the microbe from the diseased tissue. The pathogens are further characterized as to dew requirements for spore germination and infection, temperature optimums for disease expression, stages of weed growth as related to susceptibility, and host-range. Further studies may be pursued to develop formulation and application technology.

Regulatory approval for native weed pathogens is far different from the approval that is required for exotic biological control agents. Basically, the regulation of native weed pathogens comes under the aegis of the Environmental Protection Agency (EPA). The EPA

will allow researchers to apply up to ten acres of area with an 'Experimental Use Permit.' Interstate movement of all biological control agents, including native pathogens, requires a permit from APHIS, and the permits also require approval of the State Department of Agriculture for the receiving state(s). Applications of a plant pathogen as a biological control agent for more than 10 acres requires an EPA label issued under special requirements for safety (toxicity) and efficacy. Research for granting this approval may be facilitated by cooperative efforts through IR-4, which is covered elsewhere in the series of papers in this issue.

2 *TAMARIX* SPP, TAMARICACEAE (SALTCEDAR): A CLASSICAL APPROACH TO BIOLOGICAL CONTROL

2.1 The problem

Saltcedars are exotic shrubs to medium-sized trees indigenous to the Old World (Palaeartic) and are invasive in riparian ecosystems of the Western USA. Their invasion of riparian ecosystems in the Western USA is arguably one of the worst ecological disasters to occur in this region. In their new home, the invaders replace native plant communities, degrade wildlife habitat, reduce biodiversity, alter stream channels, waste large quantities of ground water, contribute to a higher frequency of wildfires, and reduce recreational access use. Saltcedars have also contributed to the decline of many wildlife and fish species, including approximately 30 that are endangered or threatened. In addition, saltcedar costs the Western USA an estimated US $133–185 million annually in lost ecosystem services.[10]

2.2 The research organizations

Research on biological control of *Tamarix* spp is being conducted by the USDA-ARS Grassland Soil and Water Research Laboratory, Temple, Texas, and the USDA-ARS Exotic and Invasive Weed Research Unit, Albany, California. Both of these research units are involved with foreign exploration, quarantine host-range studies and implementation studies of insects approved for release. The USDA-ARS European Biological Control Laboratory (EBCL), Montferrier-sur-Lez, France, also contributes to foreign exploration for natural enemies of *Tamarix* spp and conducts research on biological characterization of candidate biocontrol agents. Some monitoring after release in the northern tier of states is done by the USDA-ARS Northern Plains Agricultural Research Laboratory at Sidney, Montana.

2.3 Research on *Diorhabda elongata* ssp *deserticola* (Coleoptera: Chrysomelidae), a candidate agent for biological control of *Tamarix* spp

2.3.1 Introduction

All beetles used were *Diorhabda elongata* Brullé subspecies *deserticola* Chen from the original range of *Tamarix* spp. that were collected in northwestern Xinjiang Autonomous Region, China, or from eastern Kazakhstan. Insects from China were collected in cooperation with the Sino/American Biological Control Laboratory, Beijing, under the administration of the USDA-ARS Office of International Research Programs (OIRP), Beltsville, Maryland. *Diorhabda elongata* was identified by Dr AS Konstantinov of the ARS Systematics Entomology Laboratory and later confirmed as *Diorhabda elongata* subspecies *deserticola* by IK Lopatin, Byelorussian University, Minsk, Belarus.[11]

2.3.2 Materials and methods

Initial selection (1992) of test plants was based on a commonly accepted taxonomic scheme of Cronquist[12,13] who put Tamaricaceae (exclusively Old World) and Frankeniaceae (Australia, Chile, Eurasia, Africa and North America) as a two-family group within the large order Violales of subclass Dilleniidae. A subsequent 1998 selection of test plants was based on more recent (1997) molecular systematics[14] that had drastically rearranged Cronquist's scheme of Violales. In this classification scheme, Tamaricaceae and Frankeniaceae were placed in the two-family Order Tamaricales, subclass Caryophyllidae.

The choice and no-choice host-range tests were conducted according to the following objectives: (1) selection of test plants according to the phylogenetic (or centrifugal) system of Harris and Zwölfer[15] and Wapshere[16] which is generally accepted by researchers in biological control of weeds worldwide, and (2) inclusion of the concept of 'critical' test plants, ie, those species taxonomically within the normally acceptable host-range on which no, or only low level, damage is allowable (examples: *T aphylla* and *Frankenia* spp). Some species of *Frankenia* are native to North America, mandating that they be included in host-range tests. Another taxonomic scheme by Rusanov[17] put Tamaricaceae in the Order Parietales, so, at the request of TAG, four families were added: Clusiaceae, Theaceae, Primulaceae and Plumbaginaceae.

Typical strategies were demonstrated for accomplishing host-range tests on *D elongata deserticola*. Under the phylogenetic testing system and within the closest circle of related plants, the ARS researchers tested nine accessions of *Tamarix ramosissima* from different areas of the USA. Then in four increasingly more distantly related concentric rings, they tested 11 accessions of three other *Tamarix* species from the USA plus two species of *Tamarix* and one of *Myricaria* from China, three species of *Frankenia*, 15 species of 11 families of the Order Violales, plus four species of more distantly related plant families; ten species of five families of saltcedar habitat associates, and five species from the Order Parietales. In the later tests (1999 and after), they tested nine species from several families in subclass Caryophyllidae plus nine species of agricultural and horticultural plants to answer concerns of growers near some proposed release sites.

2.3.3 Results

These host-range tests indicated several important points: (1) that *D elongata deserticola* is restricted to *Tamarix* spp; (2) that the North American *Frankenia* spp are poor hosts; (3) that all other tested species are non-hosts; and (4) that the beetle should be considered safe for release in North America.

2.3.4 Conflict of interest in the release of Diorhabda elongata deserticola

Despite the fact that saltcedar has no related congeners in North America, it has become one of the most controversial weeds targeted for biological control in the USA.[18] The fact that it is used for nesting by the endangered southwestern willow flycatcher (*Empidonax trailii extinus*) and a few other species, coupled with the uncertain post-control revegetation by native willow and cottonwood species, has led some environmental and endangered species advocacy groups to question the advisability of releasing the beetle, *D elongata deserticola*, into the environment.

DeLoach and Tracy[19] prepared a 'Biological Assessment' document for the United States Department of the Interior (USDI) FWS on the project and concluded that biological control was not likely to adversely affect the endangered southwestern willow flycatcher. The USDA FWS concurred by letter on June 3, 1999. Cumulative evidence indicates that the biological control of saltcedar would far outweigh any probable negative effects and that many declining plant and animal species (including probably 30 threatened or endangered species) would benefit.[20]

The approval of the USDI FWS and the USDA APHIS for release of *D elongata deserticola* in selected locales was given for caged release in 1999.[21] Further open field releases were approved and have been made in 2001–2002 with monitoring underway; initial results are encouraging (pers comm, R Carruthers, 2002, USDA-ARS, Albany, CA).

The guidelines and safeguards for biological control have been strengthened as a result of controversies concerning biological control in general and as noted earlier, intensive monitoring of weed biological control projects is now required for several years after initial releases.

3 *MELALEUCA QUINQUENERVIA* (BROAD-LEAVED PAPERBARK TREE): A CLASSICAL APPROACH TO BIOLOGICAL CONTROL

3.1 The problem

The broad-leaved paperbark tree, *Melaleuca quinquenervia* (Cav) ST Blake, was introduced into Florida, USA, from Australia in the early part of the 20th Century. Subsequently, *M quinquenervia* has greatly expanded its range in southern Florida where it now infests over 200 000 ha, including the ecologically important Everglades National Park. This invader causes extensive environmental and economic damage. *Melaleuca quinquenervia* grows up to 30 m in height, is a prolific seed producer, and displaces native plants (and animals). Biological control of exotic invasive weeds, including *M quinquenervia*, is an important component of a major federal and state program to restore the Everglades.[22]

3.2 The research organizations

The melaleuca project is organized so that foreign exploration, biological characterization and preliminary host-range tests of candidate biological control agents are conducted by the USDA-ARS Australian Biological Control Laboratory in Brisbane, Queensland, Australia. For detailed quarantine host-range studies, promising candidate agents are passed to the USDA-ARS that uses the quarantine facility in cooperation with the Florida Biological Control Laboratory of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI), Gainesville, Florida. Implementation research is conducted by the USDA-ARS Invasive Plant Research Laboratory at Fort Lauderdale, Florida.

3.3 Research on the psyllid *Boreioglycaspis melaleucae*,[23] a candidate biological control agent for *Melaleuca quinquenervia*

3.3.1 Introduction

The psyllid *Boreioglycaspis melaleucae* Moore is a member of the plant lice family, Psyllidae. It feeds mostly on the foliage of the target trees, but it will feed on stems when populations are high. This insect completes its entire life cycle above ground, an aspect of its biology indicating that it will have an impact on Melaleuca stands in areas that are seasonally or permanently flooded. The biological characterization and initial host-range testing of the psyllid on 42 plant species was conducted at the USDA-ARS Laboratory in Brisbane, Queensland, Australia. Then, final host-range studies[24] were conducted by the USDA-ARS in cooperation with the University of Florida at the Florida Biological Control Laboratory in Gainesville.

3.3.2 Objectives

The objectives of these studies were twofold: (1) to conduct various no-choice host-range tests from 1997 to 1999 on native, crop and ornamental species grown in Florida, and (2) to address a potential conflict of interest concerning the possibility that the psyllid might vector a plant pathogen to *Citrus sinensis* (L) Osbeck (sweet orange).

3.3.3 Materials and methods

The positive control plants of *M quinquenervia* were grown from seed collected in Florida, and the majority of the other test plant species were purchased or grown from seed obtained from nurseries throughout Florida. Some seeds and cuttings from landscape plants were collected in the field. Most plants were 1 m or less in height at testing.

The test plant list of 61 species was made up of 36 known Myrtaceae species in Florida, including all eight native species, plus 25 economically important non-myrtaceous species, including three citrus species and sugarcane. These 25 species represented 16 plant families.

The candidate psyllids came from Australia in six shipments and were colonized routinely on Melaleuca plants. Voucher specimens from all shipments and colonies were sent to eight museums: four museums in the USA and each of four museums in Mexico, Puerto Rico, Cuba and Canada.

All of the host-range tests were conducted in a quarantine greenhouse where temperature and relative humidity (RH) were measured every 5 min. Temperatures ranged from 19 to 33 °C and RH ranged from 42 to 97% as tests were conducted in winter and summer with a 16:8 h light:dark photoperiodic cycle. The conduct of the various no-choice host-range tests are described in detail by the authors in Reference 23.

3.3.4 Results and discussion

The host-range tests showed that *B melaleucae* is limited to breeding on one species of melaleuca, *M quinquenervia*. When the *B melaleucae* psyllids had free access to entire melaleuca plants up to 2 m tall, the insects severely damaged the plants, which then recovered slowly or died. Extensive tests on the citrus plants showed no short or long-term damage. Monitored over a 1- to 2-year period, non-host plants showed no damage after exposure to the psyllids. Furthermore, no pathogenic bacteria or phytoplasmas were discovered in the psyllids when submitted to and tested by BA Frederick, formerly of the USDA-ARS Northern Plains Agricultural Research Laboratory, Sidney, Montana.

The authors recommended the field release of *B melaleucae* because no melaleuca species are native to the New World, and this psyllid species is essentially monophagous on the target plant. Evidence also indicates safety for all other plants, including citrus. In July 2000, the release of *B melaleucae* in Florida was recommended by the TAG for Biological Control Agents of Weeds. Field releases were made near Ft Lauderdale, Florida, during the spring 2002.

3.4 Research on *Fergusonina turneri* (Diptera: Fergusoninidae), a candidate biological control agent for *Melaleuca quinquenervia*[25]

3.4.1 Introduction

Under study at the USDA-ARS Australian Biological Laboratory, is a gall-making fly, *F turneri* which is a potential biological control agent for *M quinquenervia*. *Fergusonina* sp has an obligate nematode, *Fergusobia quinquenerviae* Davies & Giblin-Davis (Nematoda: Tylenchida: Sphaerulariidae). The fly and nematode, as symbionts, together form galls in the leaf and flower bud tissue of *M quinquenervia*. Apparently, the nematode initiates gall formation before the *F turneri* eggs have hatched (Giblin-Davis, pers comm). Multiple fly larvae then feed and develop within the gall tissue. The galls stop meristematic growth of the stem and thus prevent the formation of flowers. The resulting reduction in seed production is predicted to be important in the management of *M quinquenervia* in Florida.

3.4.2 Objectives of study

The objectives of the study were the following: (1) to gain a better understanding of the field population dynamics of the gall-making fly as it relates to the phenology of its host *M quinquenervia*, and (2) to evaluate the potential of the gall-making fly *F turneri* as a biological control agent.

3.4.3 Materials and methods

Three distinctly different ecological study sites were selected in sub-tropical, eastern Australia (two with standing water in summer months and one free of standing water year-round). Monthly surveys were conducted from July 1997 to September 1999 on 50 trees per transect to count active galls on each tree. Researchers also counted and categorized leaf buds into four sizes and determined relative density. Other ecological factors were also recorded, eg tree density, tree size, other woody species present, mean daily rainfall and temperature. The climatic data were compared with those of Miami, Florida. Analyses of covariance were used to compare effects of bud density, rainfall, temperature and site on gall density.

Eggs, larvae and pupae of Hymenoptera were collected for gene sequencing. Gene sequences of the immature Hymenoptera were compared with adults that had been reared from *Fergusonina* sp galls. Adult representatives of the less common Hymenoptera species were reared from *Fergusonina* sp galls collected during the previous 2 years. The researchers sequenced the D2 expansion domain of the 28S rRNA, which ranged from 564 to 593 base pairs long, depending on the species. The samples were subjected to polymerase chain reaction (PCR) for amplification and subsequent sequencing.[26]

3.4.4 Results and discussion

Fergusonina turneri populations cycled annually, with galls peaking in August/September. Gall density was correlated with bud density and temperature, but not with rainfall. Comparison of climates in Australia with those in Miami, Florida, predicts that climate should not be a limiting factor in the establishment of *F turneri*. Galls form a moderately powerful metabolic sink. High gall densities should suppress seed production and reduce vigor of the tree; thus, this insect species is predicted to be an effective biological control agent of *M quinquenervia*.

Seven of the Hymenoptera species were primary parasitoids, including two species with specialized biologies for chewing through gall tissues to feed on *F turneri* immatures. The authors predicted that fewer parasitoid species would attack *F turneri* in

Florida, and that they would be less co-adapted than those in Australia. Fergusoninidae are not represented in the New World, so the association with this family of gall-making flies would be novel for the indigenous parasitoids. In the absence of its co-evolved natural enemies, *F turneri* should reach much higher population levels, and should have a potentially significant impact on *M quinquenervia* in Florida.

In an extension of this research on *F turneri*, the team at the Australian Biological Control Laboratory has matched the identity of immature forms of nine species of Hymenoptera parasites with their adult forms using a molecular technique. This will allow for more precise investigations of the parasite/host ecological interactions and evaluation of the implications for the role of potential parasites in Florida.

4 *GALIUM SPURIUM* RUBIACEAE (FALSE CLEAVERS): A CLASSICAL APPROACH TO BIOLOGICAL CONTROL

4.1 The problem

Galium spurium L, an annual plant native to Europe, is an introduced weed of canola (*Brassica rapa* L) and other crops in California, Colorado, and the prairie provinces (Alberta, Saskatchewan and Manitoba) of Canada. The target, *G spurium*, is a major and increasingly serious weed of canola and other crops; for example, in Alberta, it occurred in less than 1% of cereal and oilseed crops surveyed in 1973–1977, but in 18% of fields surveyed in 1997. The *G spurium* seed cannot be separated easily from Canola seed after harvest and so leads to contamination and downgrading of crop quality.[27] The weed has also been reported to be resistant to acetolactate synthase inhibitors and to quinclorac, two groups of herbicides with different modes of action.[28]

4.2 The research organizations

Research on biological control of *G spurium* has been an international effort. Exploration and research for a biological control agent for *G spurium* was conducted by the USDA-ARS EBCL in cooperation with and supported by the Canola Council of Canada. The Alberta Agricultural Research Institute (Canada) furnished additional funding and some of the test plants were grown in Alberta Research Council greenhouses in Vegreville, Alberta, and shipped via air freight to EBCL for testing. The Laboratoire LPRC/IGEPAM, Programme Protection des Cultures, CIRAD-AMIS, Montpellier, France, conducted tests for viruses in mite-infested and non-infested plants.

4.3 Research on a gall mite, *Cecidophyes rouhollahi* (Acarina: Eriophyidae), a candidate biological control agent for *Galium spurium*[29]

4.3.1 Introduction

This recently discovered and named mite, *Cecidophyes rouhollahi* Craemer,[30] was found at Carnon, on the Mediterranean coast near Montpellier, France, where it was causing severe damage to *G aparine* L, a closely related congener of *G spurium*. The mites cause the leaves of the target weed to roll up around the mid-veins. Heavily attacked plants become chlorotic, stunted and seed production is greatly reduced. Preliminary tests showed that the mite would also cause similar damage to *G spurium*. The mite, a new species, was described by MC Craemer of the Plant Protection Research Institute, Pretoria, South Africa.

In addition, an analysis of the origin, distribution, and status of *G aparine*, which has sometimes been considered to be native to North America, was necessary. Since *G spurium*, *G tricornutum* Dandy and *G aparine* are all attacked by the mite, *C rouhollahi*, their status as invaders of North America is important. *Galium spurium* and *G tricornutum* are generally considered to be exotic in North America, but disagreement exists on the status of *G aparine*.[31]

An earlier report in the literature[32] noted two types of virus-like particles in thin sections of *G aparine* leaf tissue infested with the gall mite identified as *C galii* (Karpelles). Of these, one was suggested to be a virus in the genus *Tobamovirus*, and the other was characteristic of the family Potyviridae. This report raised the concern that if *C rouhollahi* were to transmit virus to *G spurium* plants in the field then, conceivably, generalist aphids and leafhoppers could transmit the virus to other plant species.

4.3.2 Objectives

This project had three primary objectives: (1) to biologically characterize the mite *C. rouhollahi* as a candidate biological agent, including host-range testing, (2) to review available information on the origin, distribution, and geographic status of *G aparine*, and (3) to conduct tests on the mite-infested and non-infested *G spurium* in an attempt to detect viruses of the genus *Tobamovirus* and the family Potyviridae.

4.3.3 Materials and methods

All tests were conducted as no-choice tests by infesting potted test plants with mites. Test plants were selected on the centrifugal phylogenetic system with representation of related species at the levels of section, genus, tribe, subfamily and family. Four crop species outside the Rubiaceae, but found sympatrically with the target weed, were included in the tests. Test plants also included 23 *Galium* spp, eight tribes in three subfamilies of Rubiaceae, and four crop plants in other families. The final test plant list was approved by the USDA TAG and the Canadian Biological Review Committee.

Ten replicates of each test plant species were inoculated with mites and in each case compared after one month with ten infested controls of *G spurium*. In addition, an experiment with 20 inoculated and 20 non-inoculated plants was conducted to determine the impact of the mites on the target.

To determine the status of *G aparine* in Europe and North America, comparisons were made of known cytotypes in the section *Kolgyda* and of habitats where the plant occurs. An analysis was also made of the occurrence of specialized natural enemies in both continents.

To test for the presence of virus in mite-infested plants, samples were taken from infested and non-infested plants in the field and from the greenhouse. Optical and electron microscopy and reverse transcription-polymerase chain reaction (RT-PCR) tests were employed to check for viral particles in the plant tissue. Positive controls were provided by tobacco (*Nicotiana benthamiana* Domin) and yam (*Dioscorea alata* L) plants infected with known viruses. Mechanical inoculation of extracts from infected and non-infected plants of *G spurium* was made on indicator plants, such as tobacco.

4.3.4 Results and discussion

The host-range tests indicated that the mite readily attacked and reproduced only on three *Galium* spp, viz *G spurium*; *G aparine* and *G tricornutum* in the section *Kolgyda*. Two other *Galium* taxa are listed as federally endangered in the USA: they are *G californicum* ssp *sierrae* Dempster and Stebbins, and *G buxifolium* Greene. Efforts to obtain propagules for testing of these two taxa were unsuccessful; however, *G buxifolium* is closely related to *G catalineuse*, which was tested and did not support mite reproduction. Thus, damage from the mite to endangered *Galium* spp is considered to be unlikely.

The authors provide strong evidence through the literature that *G aparine* is European; none of the other related annual species in the section *Kolgyda* are considered native in North America, whereas 16 of these occur in Europe.[33] Cytological evidence is also presented with a much greater diversity of cytotypes of *G aparine* in Europe than in North America.[34] Most plants in North America are of one cytotype with $2n = 66$; this is a frequent, but not the most common, cytotype ($2n = 64$) in Europe.

The number of specialized natural enemies should give a clue as to origin. In a 1984 survey,[35] 47 arthropod species were found feeding on *G aparine* in North America and only seven in Europe. Most of those found in North America were generalists (polyphagous). A later, more comprehensive, survey[36] reported 27 monophagous or oligophagous insect species found on *G aparine* in Europe. Moreover, fruits of *G aparine* were reported from numerous Pleistocene deposits in Europe and rarely, if at all, in North America. Thus, the status of *G aparine* as a native plant species in North America is unproven, and the evidence leans toward European origin. The feeding and development of *C rouhollahi* on *G aparine* should not be a deterrent to approval for release in North America.

Flexuous rod shaped virus infection was suspected in plants tested by leaf dip in 1999, but could not be confirmed for any of 16 plants tested in 2000. No virus aggregates or virus-induced structures could be detected by electron or optical microscopy in any of the leaves collected from plants infested or not infested by *C rouhollahi*.

PCR tests with infested and non-infested *G spurium* leaf tissue were negative for the viruses, while controls of viral infected tobacco and yam tested positive. Mechanical inoculation of tobacco plants with crude plant extracts from mite-infested *G spurium* failed to show that any virus was transmitted to inoculated plants. Thus, it was possible to obtain greenhouse colonies of *C rouhollahi* free of plant viruses.

On the basis of these results, the USDA, APHIS TAG for Biological Control Agents of Weeds has recommended approval for release of *C rouhollahi*, and an import permit has been issued by the Canadian Food Inspection Agency, Plant Health and Production Division for release in Alberta.

5 *PUERARIA LOBATA*, LEGUMINACEAE (KUDZU): AN AUGMENTATIVE OR MANIPULATIVE APPROACH TO BIOLOGICAL CONTROL

5.1 The problem

The perennial vine, *Pueraria lobata* (Willd) Ohwi, native to eastern Asia, is one of about 15 species of *Pueraria* that occur worldwide, but none are native to the New World. Kudzu was introduced in the late 1800s into the Southeastern USA where it was widely promoted and plantings were subsidized for forage in overgrazed pastures and for erosion control.[37,38] By the early 1950s, this program was stopped, and by 1970, the vine was listed by USDA as a common weed in the southern USA.[39] Several herbicides, eg picloram, dicamba plus 2,4-D, and tebuthiuron, are effective, but must be applied yearly for up to 10 years for complete control. In 1993, kudzu was included in a report by Congress (OTA)[40] as one of the most harmful non-indigenous plant species in the USA. Finally, in 1998 Congress added kudzu to the Federal Noxious Weed List.

This vine now infests over 2.84 million ha from Florida to New York and westward to Oklahoma and Texas, with the greatest infestations in Alabama, Georgia, and Mississippi.[41] Losses in potential productivity are estimated to be $336 million per year.[42] Thus, this invasive vine is a good target for biological control.

5.2 The research organizations

This research on the biological control of kudzu was conducted by the USDA-ARS, Southern Weed Science Research Unit, Stoneville, Mississippi, in cooperation with the School of Biological Sciences, Louisiana Tech University, Ruston, Louisiana. The research on the characterization of macrocyclic trichothecene mycotoxins was also conducted at the Southern Weed Science Research Unit, Stoneville, Mississippi, in

cooperation with the College of Pharmacy, University of Minnesota, Minneapolis, Minnesota and the Department of Chemistry, University of Maryland, College Park, Maryland.

5.3 Research on a native fungal plant pathogen, *Myrothecium verrucaria*, as a candidate biological control agent for *Pueraria lobata*[43]

5.3.1 Introduction

The fungal plant pathogen *Myrothecium verrucaria* (Alb & Schwein) Ditmar: Fr was isolated from diseased sicklepod, (*Senna obtusifolia* L), another leguminous weed. Earlier research had demonstrated that this fungus has excellent potential for biological control of several weed species, including sicklepod and hemp sesbania (*Sesbania exaltata* (Raf) Rydb ex AW Hill), when applied with a silicone–polyether copolymer spray adjuvant, Silwet L-77 (OSI Specialties Inc, Charlotte, NC, USA), under no-dew conditions.[44]

The ARS biological control researchers pointed out that, in their review of pertinent literature, they found reports of some isolates of *M verrucaria* that produced non-specific phytotoxins and metabolites toxic to humans and livestock.[45,46]

5.3.2 Objectives

Experiments were undertaken with the following objectives: (1) to compare control of kudzu with *M verrucaria* at various concentrations of inoculum, different kudzu growth stages, and at various temperatures, (2) to determine efficacy of the fungus under field conditions, and (3) to test *M verrucaria* for unwanted mycotoxins.[47,48]

5.3.3 Materials and methods

Inoculum of *M verrucaria* was produced on potato dextrose agar in Petri dishes. Seedlings of kudzu in various stages, ranging from cotyledonary to the seven- to eight-leaf growth stage were sprayed with concentrations of conidia from 0 to 2×10^8 ml^{-1} suspended in water containing Silwet L-77 surfactant (2 ml liter^{-1}).[49] Growth chambers were employed to provide seven different constant day/night temperatures from 10 to 40 °C.

To test the efficacy of the fungus in the field, kudzu seedlings in the first-leaf growth stage were transplanted into 0.5-m^2 field microplots in two separate experiments conducted in June and August, 1998. The plants were sprayed with a conidial suspension (2×10^7 ml^{-1}) either in water alone or in water containing Silwet L-77 surfactant, until they were fully wetted. Plants were monitored for disease development at three 5-day intervals and then harvested for dry weight determinations. A field test was also conducted in July 1998, at a site heavily infested naturally with kudzu plants. Treatments were the same as those used in the microplots.

Tests were initiated to determine if the Stoneville isolate of *M verrucaria* produced any of 10 known macrocyclic trichothecenes (phytotoxins and mycotoxins) when cultured in solid rice or liquid (cornsteep liquor or soyflour-cornmeal broth) media. Extracts from *M verrucaria* cultures were compared with extracts from media without fungus for phytotoxicity against leaf discs of kudzu and duckweed (*Lemna pausicostata* Helgelm) and for cytotoxicity against four cultured mammalian cell lines. Kudzu, soybean and sicklepod plants that had been sprayed with fungal suspensions were extracted with chloroform + methanol, and the extracts were compared by HPLC with three known macrocyclic trichothecene toxins.

5.3.4 Results and discussion

The highest inoculation level of 2×10^8 conidia ml^{-1} gave at least 90% control of the largest 7- to 8-leaf stage seedlings tested. The greatest disease development and kudzu control occurred at the higher temperatures (30–40 °C), which suggests that this pathogen could be effective during summer in the Southern USA.

As with previous tests on other weeds, the silicone-based adjuvant, Silwet L-77, was required to 'activate' the *Myrothecium verrucaria* against kudzu. The fungus at a concentration of 2×10^7 conidia ml^{-1} plus adjuvant at 2 ml liter^{-1} produced 100% control of kudzu in the field 14 days after inoculation. Because of the need for 'activation' by the adjuvant, the fungus did not spread beyond the treated area. Thus, it would appear that *M verrucaria* may have great potential as a biological control agent for kudzu and merits further research.

Ten macrocyclic trichothecene mycotoxins were produced by *M verrucaria* grown on solid rice medium. At least four mycotoxins were detected when the fungus was grown on liquid media, either cornsteep liquor or soyflour-cornmeal broth. Five of the trichothecenes produced were both phytotoxic and cytotoxic, but none of the toxic metabolites could be detected in tissue of kudzu, sicklepod or soybeans after treatment with *M verrucaria*. Thus, the fungus appears to show efficacy and to pose little danger after application.

However, because of the high mammalian toxicity of the macrocyclic trichothecenes, as noted by the ARS researchers 'extreme care should be exercised when handling ... *M verrucaria* mycelium, spores, or suspensions for field application.' More research is indicated for finding other isolates and/or methods of producing the fungus without associated mycotoxins.

6 CONCLUSIONS

Despite difficulties and complexities, scientists involved in these case studies have professionally and successfully executed their research objectives with favorable outcomes, or, as a minimum, with solid recommendations as to future objectives toward reaching one goal implied in the spirit of Executive Order 13112, viz that of developing successful biological control agents to help suppress targeted invasive exotic

weeds. Obviously, conflicts of interest such as those involving the use of a targeted weed, saltcedar, by an endangered bird species, the southwestern willow flycatcher, will continue to emerge. Through active cooperation between scientific research organizations, environmentalists and regulators, future problems such as this one can be solved to the satisfaction of all parties.

Other concerns were more easily addressed, eg proving that a psyllid candidate biological control agent for melaleuca trees very likely will not vector plant diseases to citrus groves in Florida. The same cooperative process and thorough testing of biological control agents can also allay fears such as removing the possibility of generalist vectors feeding on associated crops and weeds and passing weed-borne viruses potentially vectored by the mites as biological control agents for *G spurium*. These mites were carefully evaluated and cleared, based on the absence of viral particles in the collected plant specimens.

Thus, overwhelming evidence exists that the ARS biological control scientists are confronting and solving conflicts of interest with transparency and responsibility. For example, the research team at Stoneville, Mississippi, should be recognized for their responsible investigation of mycotoxins and associated dangers to potential applicators in the augmentation of *M verrucaria* for control of kudzu.

Clearly, as seen in the case studies presented herein, development of biological controls is a long-term program; usually solutions require several years. The ARS scientists involved in biological control research are dedicating their lives and effort to the moral imperative of suppressing invasive, exotic weeds with associated benefits to the native plant community.

ACKNOWLEDGEMENTS

Appreciation is expressed to all of the federal and state or provincial government agencies that have supported this ARS research.

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