

# Potential Alternatives to Classical Biocontrol: Using Native Agents in Invaded Habitats and Genetically Engineered Sterile Cultivars for Invasive Plant Management

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## ABSTRACT

The development of an effective approach to control and eradication of invasive species has become a major challenge to scientists, managers, and society. Biocontrol has been widely utilized to control exotic plants in the past few decades with some degree of success. However, there have been an increasing number of controversies pertaining to this approach, largely due to the potential environmental risks when introduced natural enemies attack non-targeted species. Here we present two alternatives in addition to classical biocontrol of invasive plants using case studies, and discuss the possibility that there may be more than one formula for a success. One strategy is to search for native agents (other than introduced from elsewhere) in the invaded ranges to manage invasive plants that have been difficult or risky to control or eliminate with classical biocontrol methods. Another new approach is to use traditional breeding or modern transgenic technologies to produce sterile cultivars for economically important exotic plants used in horticulture and forestry.

**Keywords:** exotic plants, native biological agent, sterile cultivars

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## INTRODUCTION

Biological invaders are a growing environmental issue which has received worldwide attention, largely due to the threat posed to biodiversity and economics (Liu *et al.* 2005; Guo and Ricklefs 2010; Pyšek and Richardson 2010; Hulme 2011). While most early studies focused on the impacts of invasive species on local ecosystems, recent studies focus on methods for the control and eradication of these species (Van Driesche *et al.* 2002; Pyšek and Richardson 2010; Yu *et al.* 2011). Among the commonly used control measures, biocontrol methods are increasingly popular because they are more environmentally friendly; they do not rely on physical (mechanical) and/or chemical (herbicides) means; and have been successful worldwide (Morin *et al.* 2009; Van Driesche *et al.* 2010). Classical biocontrol, which typically utilizes introduced natural enemies of the invasive plant to maintain and/or reduce the coverage to densities lower than would occur in their absence. Classical biocontrol methods originated as early as about 100 years ago when natural enemies of agricultural pests were introduced from their native ranges (Debach 1974). A major

challenge in biocontrol is the species- and habitat-specific nature of each situation which makes developing effective broad spectrum controls extremely difficult.

Classical biocontrol has been widely used to control invasive exotic plants (McFadyen 1998; Muller-Scharer and Schaffner 2008; Van Driesche *et al.* 2010). It was founded on two ecological principles: (1) one organism can be used to control another, and, (2) some biocontrol agents have a limited host range (Müller-Schärer and Schaffner 2008). Around the world, classical biocontrol has often been highly successful in some countries. For example, in South Africa, 63 species of biological agents have become successfully established on 44 invasive exotic plant species since 1913, and 25% of the target exotics have been completely controlled (Moran *et al.* 2005). The introduction of the defoliating Klamath weed beetle (*Chrysolina quadrigemina*) from Europe won the battle in controlling St. John's wort (*Hypericum perforatum*) in the U.S. in the 1940s (Hall *et al.* 1980).

However, there have also been increasing doubts about classical biocontrol due to its potential risks and unknown long-term consequences. First, the imported agents may

attack non-target native species (e.g., Messing and Wright 2006). Host-specific agents may exhibit substantial non-target effects not only through direct host shifting but also through indirect interactions. Second, the introduced agents may have long-term negative impacts on food chains in invaded ecosystems, facilitate emerging resistance, and cause environmental pollution (Messing and Wright 2006). Third, introduced agents may spread to other areas and become invasive there (e.g., South American cactus moth, *Cactoblastis cactorum*; Simberloff *et al.* 2005). Finally, classical biocontrol sometimes requires a long period of agent screening in its home range prior to introduction and release. Therefore, more ecologists are now recommending classical biological control only as a last resort (Louda and Stiling 2004; Simberloff *et al.* 2005).

In contrast to classical biocontrol, some native organisms (other than introduced from elsewhere) in invaded habitats may be useful biocontrol agents for some invasive plants under certain conditions (Guo 2006; Parker *et al.* 2006). One important advantage of using such agents may be the reduced risk of unpredicted and undesired effects on non-target species. Because native species have coevolved with many existing species and local consumers, there may be sufficient ecosystem resistance to invasion from native natural enemies. Although caution is always needed to make sure selected native control agents do not show any invasiveness in nearby and similar habitats, screening native natural enemies may still save some time and expense in comparison to assessing foreign agents in the foreign country and quarantine procedures. A recent meta-analysis of 63 manipulated field studies including 100 exotic plants, revealed some promising cases where native herbivores suppressed exotic plants (Parker *et al.* 2006). Efforts using native agents to control invasive plants in the field of weed science have been reported (Sheldon and Creed 1995; Newman 2004). However, there has been limited discussion within the ecological communities regarding the potential for native agents to control invasive species, particularly for those that have been difficult to control or eliminate using existing introduced non-native predators and herbivores.

Another new approach to control of invasive plants is to use traditional breeding or modern transgenic technologies to produce sterile cultivars of economically important exotic plants used in horticulture and forestry. Although traditional breeding techniques were used to create sterile forms of some invasive ornamental plants, the effort is minimal (Li *et al.* 2004). The use of transgenic plant technology to produce sterile forms of exotic ornamental plants has just begun (Li *et al.* 2004). For example, Yi Li and his colleagues are using transgenic technology to produce “super-sterile” cultivars of economically important ornamental plants that are invasive. One type of these “super-sterile” plants is pollen-sterile but produces normal-sized fruit for wildlife consumption and ornamental appeal (Chen *et al.* 2008). Because spread by these plants is mediated by seeds, their sterile forms should represent less invasive potential.

It is clear that novel control agents and diverse technologies are needed to control invasive species (Simberloff *et al.* 2005), which requires bridging gaps between disciplines by bringing ecologists, molecular biologists, and biocontrol researchers together to share information and address potential alternative approaches to biocontrol. In the field of controlling invasive species, there may be more than one formula for a success. The coauthors of this study have multidisciplinary backgrounds, and would like to direct ecologists and managers’ attention to two promising approaches to invasive plant management: (1) case studies using native biocontrol agents (herbivores and/or plants) to control invasive plants that are difficult to manage using existing approaches, and (2) use of advanced molecular techniques to develop modified cultivars when possible for new planting of commercial and horticultural plants.

## CASE STUDIES USING NATURAL NATIVE ENEMIES IN INVADDED AREAS TO CONTROL INVASIVE PLANTS

It has been long suggested that only a small number of introduced species became invasive (Alpert 2006; Callaway and Maron 2006; Williamson 2006). This is largely due to the resistance of native enemies in the invaded habitats which limit the establishment or spread of most invaders (Darwin 1859). These suggestions, originating more than a century ago, constitute a theoretical basis for a management plan that uses natural agents in areas impacted by invasive plants. In the past, there were a few well-known case studies using this approach. For example, approximately 20 native pathogens and 40 native insects have been used as biocontrol agents for weeds, with an average success rate of approximately 55 and 17%, respectively (Julien and Griffiths 1998). Increasing the population of an indigenous shield beetle (*Cassida rubiginosa*) was a feasible way to control creeping thistle plants (*Cirsium arvense*) in Europe (Bacher and Schwab 2000). A native insect (herbivorous weevil, *Euhrychiopsis lecontei*) was successfully used as a biocontrol agent of an exotic aquatic plant, Eurasian water-milfoil (*Myriophyllum spicatum*), in North America (Creed 1998; Newman 2004).

Here, we would like to present two recent case studies that may be unfamiliar to some ecologists (Yu *et al.* 2008, 2011). One is using an indigenous insect, parasitoid wasp (*Chouioia cunea*), against the invasive exotic, fall webworm (*Hyphantria cunea*) in China (Yang *et al.* 2006). The fall webworm has invaded large areas of northern and northeastern China since the 1970s, feeding on the foliage of as many as 175 species including ornamental plants, agricultural crops, and forest trees. After the failure of decade-long efforts using chemical and other biocontrol approaches, scientists started to focus on finding a native natural enemy of this invasive insect. Surprisingly, 27 natural enemy species of the invasive webworm were discovered in the impacted areas, among these, the endoparasitic wasp was most promising (Yang *et al.* 2006). The wasp can parasitize pupa of the fall webworm and its larvae can consume all tissues of the invasive insect. The native parasitoid wasp has been successfully mass-produced and released in invaded areas (Yang *et al.* 2006). Five years after release of the parasitic wasps, the population of the invasive webworm declined rapidly and has remained at low levels, which no longer damage local trees and horticulture plants (Yang *et al.* 2006). Because the wasp was known to attack 16 other pest moth species in the area, its potential impacts on non-target species and other post-release issues are being assessed.

Recently, promising progress has been made using a native parasitic weed to control an invasive weed found in south China. Three parasitic dodders (*Cuscuta* spp.) have potential use in controlling an aggressive invasive plant, *Mikania micrantha* H. B. K. (Wang *et al.* 2004). *Mikania*, known as the “mile-a-minute” weed, is a perennial species native to Central and South America, which has had enormous negative economic and environmental impacts in southern China since the 1980s (Li *et al.* 2000) (Fig. 1). Numerous prevalent control techniques have been applied, however, these techniques have met with limited success in controlling *Mikania*. In 2000, it was first observed that the dodders, common agricultural weeds in the region which parasitize various crops and some wild plants, eliminated *Mikania* in some habitats. Since then, preliminary studies have been conducted on the potential effects of these native dodders on invasive *Mikania*, including field surveys, species introduction, taxonomy, physiology, growth, and risk assessment (Wang *et al.* 2004). Recently, a field survey was conducted at four sites where one dodder (*C. campestris* Yuncker) was deliberately introduced to control *Mikania* between 2000 and 2005 (Fig. 2). The results showed that *Mikania* covered 80% of the plots at the untreated site, but only 10% at the *Cuscuta*-treated site four years after treat-



**Fig. 1** Invasive *Mikania micrantha* rapidly grew and produced abundant beige fruits in Neilingding National Natural Reserve, Guangdong, China. It climbed and smothered native species including trees, such as *Litchi chinensis*, *Euphoria longan*, *Syzygium jambos*, *Boehmeria nivea* and *Macaranga tanarius*, as well as shrubs and herbs, altering community structure, and composition and reducing local diversity. On the right, *Macaranga tanarius*, *Litchi chinensis* and *Boehmeria nivea* were covered and smothered to death.

ment (Fig. 3). After the death of *Mikania*, some early-succession native species came back and increased their coverage. The dodder's cover continued to decline, which indicates that the dodder was not able to parasitize these native species (Yu *et al.* 2011). Moreover, not only the dodder could restrain exotic *Mikania* (Yu *et al.* 2009), it also enhanced soil nutrient resources beneficial to native species in the invaded communities (Yu *et al.* 2008). These findings suggested that *Cuscuta* may be an effective measure against *Mikania* and be valuable for the restoration of invaded communities. However, a rigorous risk assessment of dodder's 'non-target' effects needs to be further conducted.

Over two decades ago, Murdoch *et al.* (1985) suggested that different theories might easily produce very different or even conflicting advice for practitioners seeking a biocontrol agent. Use of native agents to control invasive plants, which are otherwise difficult to control by traditional approaches, may provide a promising alternative to the use of introduced biocontrol agents. However, key information is needed to decide whether a native organism can be used as a biocontrol agent, which includes feeding preference of the agent, host range expansion, trophic cascades, agent effectiveness, environmental regulation of the agent, and risk assessment of native agents which are inoculated or released at higher densities than usual. Natives may colonize non-target species that are in their ancestral diets (Ding and Blossey 2005), when the density of their populations is augmented. Thus, it is critical to evaluate the full host range and non-target effects of a potential native agent prior to significant field applications.

### USING PLANT BIOTECHNOLOGY TO CONTROL THE SPREAD OF INVASIVE PLANTS BEFORE THEY ARE PLANTED

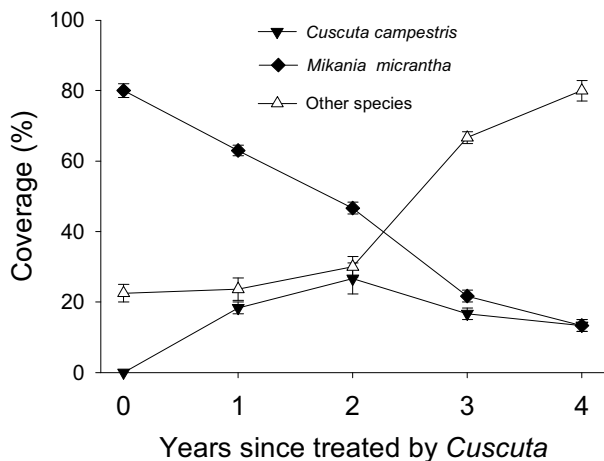
Ornamental horticulture is a significant source of invasive plants. In the United States, more than 50% of all invasive plants and 85% of invasive woody species were introduced intentionally for landscape use. In many cases, ornamentals with invasive characteristics are popular and economically important. For example, the invasive winged euonymus and Japanese barberry accounted for approximate \$20 million in annual sales in Connecticut (Fig. 4) (Li *et al.* 2004). These plants generally produce abundant fruits and seeds that are distributed by birds and other animals. They adapt to various environments, grow quickly, form dense thickets that



**Fig. 2** The obligate parasite, dodder (*Cuscuta campestris*), parasitized *Mikania micrantha* in Neilingding Natural Reserve, Guangdong, China. (A) an early phase, (B) about 1.5 years later, and (C) a large-scale view.

block out native vegetation, and lack serious pest problems. Therefore, once they become established in natural habitats, it is often difficult to control their expansion by current means, including chemical and mechanical methods, as well as classical biocontrol technologies (Li *et al.* 2004).

One effective strategy to address invasion of these economically important plants is to neutralize their invasive characteristics before they are planted in the landscape (Li *et al.* 2004). Plant gene transfer technology has been used effectively to produce seedless fruits (Li 1998; Fig. 4). One of the widely used techniques for the production of seedless fruit is to express an auxin (a plant hormone) biosynthetic



**Fig. 3** Coverage changes of the invasive *Mikania micrantha*, with use of the obligate local parasite dodder, *Cuscuta campestris*, and native plant species in the communities without (0 year) and with *Cuscuta*-treatment (from 1 to 4 years) in Neilingding Natural Reserve, Guangdong, China. Other species including *Pueraria lobata*, *Lantana camara*, *Thunbergia grandiflora* existed prior to the *Mikania* invasion and reappeared after the introduction of *Cuscuta*. Data are means  $\pm$  SE (n=3).



**Fig. 4** The spectacular fall color of winged euonymus (A) and Japanese barberry (B). Although they are exotic and invasive, they are extremely popular landscape ornamentals in many regions of USA. Dr. Yi Li's laboratory has developed a seedless fruit technology and used it to produce tomato and other fruits without seed: Conventional tomato plants with seeded fruits (upper panel), Biotech-improved ones with normal- or bigger-sized fruits but no seeds (lower panel). Photographs provided by Dr. Yongqin Chen and Mr. Litang Lu.

gene specifically in the ovary or developing fruit (Duan *et al.* 2006). Because the overproduction of auxin in the plant is restricted in reproductive organs, no obvious side effects have been observed. Plant gene transfer technology also offers tools to produce sterile plants. Targeted expression of a cytotoxin gene in anther, pollen, stigma or ovary has been shown to be highly effective in producing sterile plants (Goldman *et al.* 1994). Li's lab is currently using both a seedless fruit gene and a cytotoxin gene targeting reproductive organs to produce "super-sterile" (i.e., male and female sterile and with normal sized fruit production) cultivars of winged euonymus and Japanese barberry (Chen *et al.* 2008).

Transgenic plant technology can also be used to manipulate metabolic processes that are important for plant growth and reproduction and subsequently their invasiveness. For example, we may use plant gene transfer techniques to reduce the invasiveness of certain exotic plants by suppressing or enhancing metabolic processes that lead to alterations in nutrient use or growth rate of a targeted organ or the whole plant at specific developmental stages. This, in

return, may provide additional tools to reduce the uncontrolled spread of invasive plants.

Using plant biotechnology to limit/control the invasiveness of economically important landscape plants offers several distinct advantages. Production of sterile or seedless cultivars via gene transfer techniques can be faster and therefore more cost effective compared to traditional control approaches. Transgene escape has been the subject of public concern; the "super-sterile" cultivars, with the combination of male-sterility, female-sterility, and seedless traits in a single plant, make transgene escape extremely unlikely. In addition, the sterile cultivars allow introduction of highly valuable exotic plants without concern of invasion.

There are disadvantages associated with plant biotechnology approaches. First, the delivery of sterile and seedless genes into many of the ornamental plants may be technically difficult because no genetic transformation methods have yet been developed for these species. As a result, this technology cannot be used in many invasive plant species. Second, although ornamental plants are not used for food, and introduced genes would be well contained because of their sterile nature, concerns still exist about "genetically modified" plants. Third, sterile technologies can be effective in eliminating or reducing the invasiveness of modified plants, but have no utility in preventing the spread of unmodified plants that currently exist in natural areas. It is our goal to develop plant biotechnology that will aid in eradicating existing invasive plants. Finally, the sterile technologies discussed here are not applicable to some invasive plants, such as kudzu and English ivy, whose invasiveness is dependent on rapid vegetative propagation.

## CONCLUSIONS

The research focus on biocontrol needs to be broadened and advanced. The use of native herbivores and/or plants as biocontrol agents may be a promising approach to invasive plant management, particularly for those that are extremely difficult to manage and/or show limited success using traditional approaches. Expanding research in these areas may also advance our insights on broader questions, such as the evolution of host choice, trophic cascades, and host range expansion in invaded ecosystems. It is important to note that biocontrol agents may work in some environments and not in others. Risk assessment must be conducted to identify the full host range and potential non-target effects prior to massive field application. For economically important commercial plants that can become invasive, genetically engineered sterile cultivars provide means to allow for their utilization, without the risk of invasion. Cross-disciplinary communication and collaboration among scientists, managers, and stakeholders is central to the identification of new approaches to biocontrol and in developing a new paradigm for invasive plant management.

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