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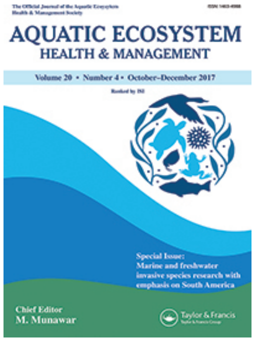

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Lessons from three cases of biological control of native freshwater macrophytes isolated from their natural enemies

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Classical biological control–or biocontrol–is a form of pest management comprising the release of specialized natural enemies (biocontrol agents) of an exotic pest. Classical biocontrol agents are scientifically selected from among the natural enemies the pest has in its native region. However, biological control is firmly resisted in many countries because of the belief that it is more risky than not doing anything, or using the more familiar chemical and mechanical control methods. In this review, three classical biocontrol projects from Argentina are described. These projects had the peculiarity that native insects were used to control two native aquatic plants–Water Hyacinth and Water Lettuce–in isolated water bodies where their natural enemies were absent. In two of these projects complete control of the weeds were achieved, and preliminary results are quite promising for the most recent project, as well. This article stresses that classical biocontrol can be applied in a weed's native range under special circumstances, and describes why such cases can be used to promote biological control of native invaders while circumventing the more resisted aspect of classical biocontrol: introducing exotic species.

Keywords: Water Hyacinth, Water Lettuce, native weeds, Neochetina, biocontrol

Introduction

Alien plants constitute threats to ecosystems, economic activities, and human welfare (Mack et al., 2000). Added to these effects, aquatic weeds also contribute to the spread of many human diseases around the world, including malaria, schistosomiasis, dengue, yellow fever, and many more (Mack and Smith, 2011). It is widely accepted that the most efficient way of dealing with invasives is controlling their entrance with competent phytosanitary policies, early detection and eradication (Simberloff, 2013; Aracena et al., 2014). Once an exotic weed is widespread, however, control efforts depend on economic considerations: When agricultural lands or areas of considerable conservation value are affected, chemical or mechanical control are normally used. When these conditions

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are not met, the weeds may often go ignored because of the prohibitive cost of these control measures.

Alternatively, a handful of countries resort to classical biological control (CBC) (Cruttwell McFadyen, 1998). Scientifically based CBC is a form of ecosystem manipulation that endeavours to accomplish a definitive solution to problems caused by alien invasive plants and arthropods. It consists in the release of biocontrol agents, which are specialised natural enemies from the invader's native area, with the objective of achieving their permanent establishment and reduction of the pest populations to levels acceptable for agriculture, public health, and/or the recovery of natural environments.

CBC of weeds is often resisted because of the widespread, although erroneous, notion that it is unsafe. However, even biocontrol detractors tend to agree that prevention of non-target effects in weed biocontrol has been successful (Barratt et al., 2006; Simberloff, 2012). Furthermore, the notion that chemical control methods are less risky and reversible is not supported by evidence (Thomas and Willis, 1998). Doing nothing also "feels" safer to decision-makers, but no logical analyses support this notion (Delfosse, 2005). Be that as it may, most countries resist or even prohibit weed biocontrol, with the notable exceptions of Australia, Canada, New Zealand, South Africa and the USA, countries where it has been applied routinely for many decades, often with stunning success (Winston et al., 2014). Biocontrol agents have been blamed for important community level impacts through non-target attacks, competition and indirect ecological interactions (Messing et al., 2006; Moran and Hoffmann, 2015). Yet a cursory analysis of the examples listed to expose the alleged mistakes of CBC reveals a surprising fact: the vast majority of the examples were accidental introductions, amateur introductions (not scientific, but carried out by farmers or other private entrepreneurs), or dated long ago (Simberloff and Stiling, 1996a,; Hoddle, 2003; Hays and Conant, 2007). Hence, these cases should not be attributed to modern scientific biocontrol, any more than intoxications due to self-medication can be attributed to medicine. Arguably, the impact of all scientific CBC on the environment has been negligible compared to the effects of other human activities that must also comply with preliminary risk assessments, such as engineering activities like mining, or the building of dams and highways; pharmaceutical research and production; industries; agrochemical production and use; exotic species trade, etc.

One way of surmounting this resistance is by subtracting the most resisted trait of CBC, which is the introduction of exotic biocontrol agents. This resistance derives from the misguided notion that introducing an exotic agent to control an exotic invader is adding fuel to the flames, ignoring–or disbelieving–the fact that the security protocols followed to minimize the risk of host shifts have been almost infallible to date (Marohasy, 1996; Futuyma, 2000; van Klinken and Edwards, 2002). Yet the only situation in which a form of CBC could be applied with native natural enemies on a native plant would be in the rare cases when the plant becomes a nuisance due to isolation from its natural enemies. In this review, three such cases are described, which were implemented by the Foundation for the Study of Invasive Species (FuEDEI, formerly USDA-ARS South American Biological Control Lab.) in Argentina. Two of the projects dealt with Water Hyacinth (*Eichhornia crassipes* (Mart.) Solms, Pontederiaceae) infestations: Los Sauces reservoir, La Rioja Province and El Ojo Lake, Buenos Aires Province; the other one was a Water Lettuce (*Pistia stratiotes* L., Araceae) infestation at the Vicente López Ecological Reserve (REVL) lake, Buenos Aires Province (Figure 1). In the three cases, preliminary samplings determined that the natural enemies normally found on these plant species in Argentina were absent or undetectable (Deloach and Cordo, 1983; Cabrera Walsh and Maestro, 2014; FuEDEI, unpublished).

However, some terms must be discussed at this stage; alien plants, exotic plants, non-native plants, naturalized plants, invasive plants, nonindigenous plants, are all terms used for "plant taxa in a given area whose presence there is due to intentional or accidental introduction as a result of human activity" (Richardson et al., 2000). There is no unanimous definition of native plants, however, so in this work the definition of the Natural Resources Conservation Service of the United States Department of Agriculture will be accepted. This reads "A plant that is a part of the balance of nature that has developed over hundreds or thousands of years in a particular region or ecosystem. Note: The word native should always be used with a geographic qualifier. Only plants found in this country

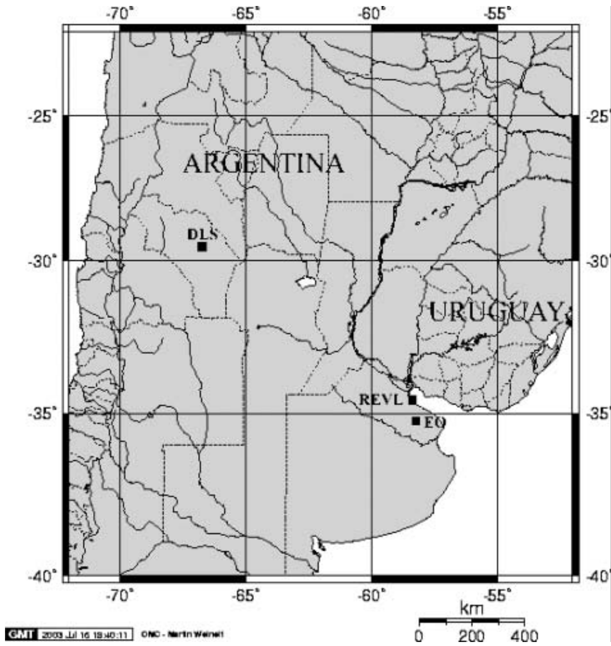

Figure 1. Partial map of Argentina showing location of experimental water bodies: DLS, Los Sauces Reservoir; REVL, Vicente Lopez Natural Reserve; EO, El Ojo Lake.

before European settlement are considered to be native to the United States" (https://www.nrcs.usda.gov. Last accessed 01/31/2017). Within these definitions, Water Hyacinth cannot be considered native to either of the water bodies, an artificial Andean reservoir and a Pampean lake, because it was introduced deliberately by man in recent times, and because neither site is part of the Paraná-Paraguay basin, where the plant occurs naturally. As for the Water Lettuce infestation, it was in an artificial lake very close to the lower Paraná Delta, where the plant is native. The lack of specific herbivores was probably due to its isolation within the urban matrix. In all cases, classical biological control was applied within the political frontiers of the native distribution of all the organisms involved, making their movement and collection less conflictive from the administrative and environmental stances.

Experiments

The natural enemies released in the three water bodies mentioned were *Neochetina bruchi* Hustache and *N. eichhorniae* Warner (Coleoptera: Curculionidae) for Water Hyacinth, and *Neohydronomus affinis* Hustache (Coleoptera: Curculionidae) and *Lepidelphax pistiae* Remes Lenicov (Hemiptera: Delphacidae) for Water Lettuce. All three Weevil species have been tested for specificity and released in many countries since the 70s, with no non-target effects detected to date (DeLoach, 1976; Van Driesche et al., 2008; Day, 2012). *L. pistiae* is a newly discovered species from Argentina (Remes Lenicov and Cabrera Walsh, 2013) that had also passed intensive specificity tests in the laboratory (Cabrera Walsh et al., 2014). The Weevil *N. affinis* has been released in many countries around the globe, often with great success (Neuenschwander et al., 2009), but *L. pistiae* was being considered to complement the effect of the Weevil in some areas invaded by Water Lettuce. Both herbivores were tested for compatibility to test whether competition between them could be expected to diminish the controlling effect of the Weevil where it was already present (Cabrera Walsh et al., 2014).

Vicente López ecological reserve

The lake is close to natural populations of Water Lettuce, but considering it drains into the

River Plate, it is assumed that the plants sprung from seeds, hence the lack of natural enemies. The plant was first observed in 2008, and had completely covered the lake by 2009. The herbivore species were released toward the end of 2011 in dome-shaped floating cages (enclosing 0.33 m^2) designed to test the effects on the host plant of both herbivores together and individually, and develop large populations of herbivores before they were released into the environment. This is important to prevent Allee effects (Taylor and Hastings, 2005). These experiments suggested that *L. pistiae* may complement the controlling effect of *N. affinis* in areas invaded by the weed (Cabrera Walsh and Maestro, 2014). Damage of both insects was observed throughout the lake by October 2011, and by November 2011 (56 days after release), 100% infestation was recorded. All the plant cover and biomass variables were significantly lower less than a year after the release of the herbivores (Figure 2). Plant cover dropped from 100% at the beginning of the experiment (late summer) to 7.5% in September, and it did not reach original levels again (Table 1). Covariance analysis controlling for the seasonal variations provided significant evidence that the plant population was diminishing consistently. Also, during the first stages of these experiments (autumn 2011 to spring 2012) *P. stratiotes* was the only floating macrophyte in the lake. After release of the herbivores, plant species richness increased dramatically as six other species were collected regularly in the samples. No feeding damage from the herbivores released was ever recorded on any of them (Cabrera Walsh and Maestro, 2015). Also, plant cover was reduced within two years to a fraction of that observed prior to the release of the natural enemies (Cabrera Walsh and Maestro, 2015), and remains so to this day (unpublished).

Los Sauces reservoir

It is not exactly known how Water Hyacinth arrived in this reservoir, but considering it is so far away from natural populations and no rivers flow into the lake from areas where Water Hyacinth occurs, it was almost certainly introduced deliberately. The plant had allegedly been introduced in 1956–1926 years after the dam was built-, and covered the whole lake by 1965 (Hunziker, 1966). This reservoir is close to the capital city of the province of La Rioja, a mountainous, dry region in western Argentina where fresh water is scarce (mean annual rainfall = 327mm). The reservoir is the main drinking water and irrigation source in the province, and it was threatened from the evapotranspiration and eutrophication caused by the Water Hyacinth invasion that completely covered it. The Weevil *N. bruchi* (153 males and 131 females) was released by former FuEDEI researchers in 1974. The Weevils were simply dropped on the plants by the shore. Four years after its introduction the population had increased to 3.5 adults per plant and Water Hyacinth was reduced to 25% cover, and 2 years after that to only 4 to 8% (Deloach and

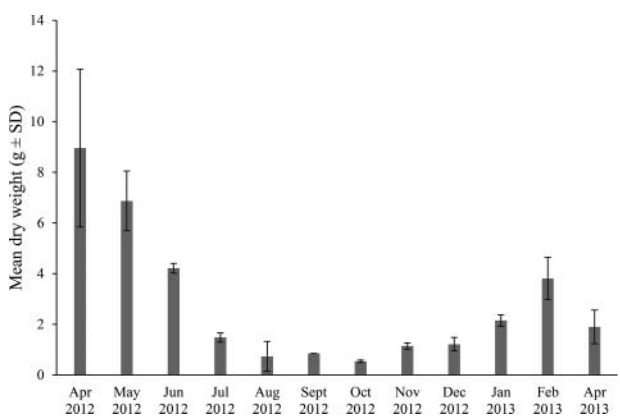

Figure 2. Evolution of *Pistia stratiotes* dry weight after release of *Neohydronomus affinis* and *Lepidelphax pistiae* in the REVL Lake.

Table 1. Summary of the biocontrol projects at the Los Sauces, Vicente López Natural Reserve (REVL) and El Ojo water bodies.

Site	Location	Coordinates	Agent release dates	No. insects released	Max. plant cover	Control level[1]
Los Sauces	La Rioja, Argentina	S 29°23′ W 66°58′	Mar 1974	284	100%	4–8%
REVL	Buenos Aires, Argentina	S 34°29′ W 58°28′	Sept and Dec 2011	700N.a.[2] 600L.p.[3]	100%	7.5–20%
El Ojo	Buenos Aires, Argentina	S 35° 00′ W 58°	Dec 2014 and Jan 2015	1378N.e.[4] 522N.b.[5]	60–70% (133 plants/m^2)	60–70% (87.5 plants/m^2)

[1]Present weed cover as last reported in literature or personal observations of authors. [2]*Neohydronomus affinis*; [3]*Lepidelphax pistiae*; [4]*Neochetina bruchi*; [5]*Neochetina eichhorniae.*

Cordo, 1983) (Figure 3, Table 1). Monitoring trips taken 30 years after the event show that the weed is still under complete control, with small populations of the plant and Weevil that coexist in a few inlets and sloughs around the reservoir (unpublished).

El Ojo Lake

Although El Ojo Lake is relatively close to natural Water Hyacinth populations, as in all Pampean lakes, this plant did not naturally occur. Local witnesses and aerial images show that the plant was not present in the lake until 2009. The plant was allegedly introduced deliberately by the owner of a plant nursery planning to make a profit by selling them for garden ponds. Three insect species were released in 2014, *N. bruchi, N. eichhorniae*, and *Taosa longula* Remes Lenicov (Hemiptera: Dictyopharidae) (which did not establish, possibly because it cannot adapt to the temperate climate). As in the case of the Vicente López Reserve, the insects were released in dome-shaped floating cages (each enclosing 0.75 m^2) to multiply the insect populations before release into the environment. Results are preliminary, and plant cover has not decreased significantly a year after release, but biomass (i.e. plant size) and mat consistency (the strength given by the chains of interwoven clones) have dropped significantly (Table 1). Weevil populations have grown steadily throughout the lake as well (Figure 4) (unpublished).

Discussion

Two of the projects described herein (Los Sauces and REVL) were very successful, as the target weeds were reduced to a fraction of their original densities and cover, and remain thus to this day. The latest project, El Ojo, is still too recent to tell if results will be as good, but preliminary results

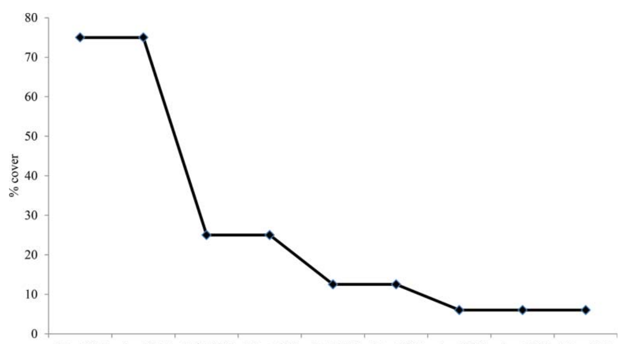

Figure 3. Water Hyacinth cover development at the Dique Los Sauces after release of *Neochetina bruchi* (graphed from data of DeLoach and Cordo, 1983, with permission of the authors).

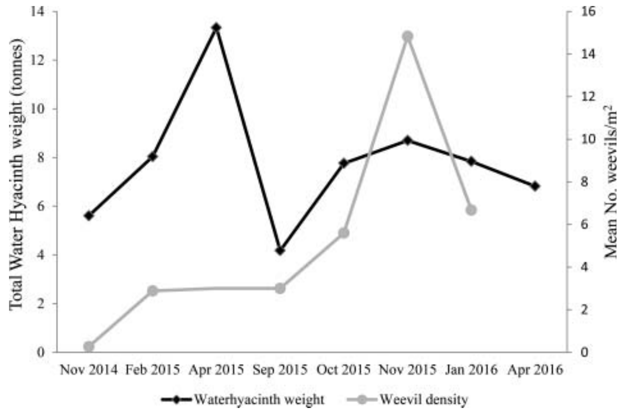

Figure 4. Development of the *Neochetina* population one year after release. Left axis is total estimated tonnes (fresh weight) of Water Hyacinth in the lake; right axis is mean no. of Weevils per m^2.

are auspicious. These projects do not only show that classical biocontrol can succeed whenever specialized natural enemies are absent, they also serve to illustrate a principle often invoked by biocontrol scientists: the enemy release hypothesis (ERH). The ERH states that organisms experience a decrease in regulation by natural enemies upon introduction to an exotic region resulting in a rapid increase in distribution and abundance. The mechanisms behind this effect can be varied and non-exclusive (Keane and Crawley, 2002; Colautti et al., 2004), but several of them have been validated as critical for invasion success (Heger and Jeschke, 2014). The water bodies described in these studies, at least both lakes located in the province of Buenos Aires, are climatically and biogeographically identical to areas where the plants possess their full suite of specialized natural enemies. They can also be expected to have a matching set of generalist herbivores, especially compared to the exotic range of Water Hyacinth and Water Lettuce around the world. So the lack of specialized natural enemies seems *a priori* to be the only different factor, and the only variable manipulated to reverse the invasion. This provides a substantial support both for the ERH and the efficacy of biocontrol.

The projects presented here are not the only instances where CBC was attempted using native insects on aquatic weeds. A similar project entailed the release of *Prokelisia marginata* (Hemiptera: Delphacidae) from Georgia, Virginia, and Rhode Island, to control *Spartina alterniflora* (Poaceae) in the northwestern coast of the U.S. (Grevstad et al., 2003, 2012). The impact of the insect on the weed populations has not been reported, however.

There is an additional situation that can teach the public and administrators of the advantages of biological control, namely, when an invasive weed is controlled by specific natural enemies that enter the region on their own or in other unexplained ways. Such situations have been observed with great positive impacts in Spain, with the alien plants *Opuntia maxima* Mill. (Cactaceae), *Agave americana* L. (Asparagaceae), and *Azolla filiculoides* Lam. (Azollaceae) (Deltoro et al., 2014). A similar situation has been reported for *Cytisus scoparius* (L.) Link (Fabaceae), with the accidental introduction of *Aceria genistae* (Nal.) Castagnoli s.l. (Prostigmata: Eryophyidae) in western North America (Andreas et al., 2013).

Conclusions

Biological control with native species, as well as species that arrived serendipitously, should be publicised to help administrators and the public to understand and witness the benefits of CBC. In addition, no discussion on the pros and cons of biocontrol can overlook the savings in agrochemical inputs and permanent control achieved through biocontrol (Jennings, 1997). Furthermore, successful biocontrol reduces the density of the target over several years, thus providing the potential for native species to re-establish, and limiting the impact of drastic chemical or mechanical measures that can leave niches bare and susceptible to other invasions–the “exotic species treadmill” described by Thomas and Reid (2007)–and polluted from the sudden nutrient input. Situations like the ones described in this work should be taken advantage of to help get CBC into the toolbox of invasive species managers and scientists.

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