WATER PENNYWORT

by

Willie Cabrera Walsh and Mariano Maestro

Cooperators: Djamila Djeddour, Marion Seier and Dick Shaw, CABI-Europe, Egham, Surrey, UK.

Introduction

Water pennywort (*Hydrocotyle ranunculoides* L.f.) is a stoloniferous aquatic perennial that forms dense interwoven mats on stagnant or slow moving waters. It is native to the Americas, but has become invasive in the UK, other parts of Europe, and Australia. For further information on the weed see Annual Report 2010.

Citations for natural enemies of *Hydrocotyle* spp. in North America are limited, and reduced to generalist species (Stegmaier 1966, Center et al. 1999). In South America, however, a specialized natural enemy, the weevil *Listronotus elongatus* (Hustache) was studied, and several other more or less specialized natural enemies have been found and evaluated (table 1). *L. elongatus* is the most common and widespread herbivore on *H. ranunculoides*, and is currently the main organism being evaluated for quarantine studies in England. Previous works anticipated that it could be specific to the plant species (Cordo et al. 1982).

Cooperation with CABI-Europe began in September 2009 to study water pennywort's natural enemies and ecology in its native range. We report advances in our understanding of the plant's ecology, and laboratory studies of the host range of two of its natural enemies.

Materials and methods

We focused on the ecology of *H. ranunculoides* and *L. elongatus*, and specificity work on the latter. Monthly sampling of *H. ranunculoides* patches provided us with the No. of adult and larval *Listronotus*, other insects, and dry biomass of sample. We also separated and recorded the dry weight of other plant species present in the samples (competitors). From each sample we pick 10 leaves at random, and we record the number of *Listronotus* and pathogen lesions, and width of the leaves (as a measure of size). All this data were analyzed to understand the fluctuations in the plant's abundance and biomass, and the impact of its natural enemies, whether herbivores, pathogens and competitors.

Specificity studies of adult *L. elongatus* were approached in a three-stage process, beginning with a simple no-choice, cut-leaf feeding test on many plant species, devised to select a smaller group of the test plants that underwent significant feeding. This test was followed by a whole-plant against cut-leaf no-choice test on these selected species, in order to condense further the test-plant list. Finally, a multiple choice test in semi-field conditions was performed on a yet more reduced selection of test plants. For this, wild *L. elongatus* were allowed to invade a mixed *Hydrocotyle* patch, where after two months, damage was evaluated in 30 randomly selected 10 x 10-cm squares.

The moth, *Paracles quadrata* (Lepidoptera: Arctiidae), has also been found to be widespread and sometimes abundant. Mature larvae can produce heavy defoliation. First instars of *P. quadrata* were subjected to no-choice tests on its host plant and *H. modesta, H. bonariensis,* and *H. leucocephala*. Ten larvae were placed on leaf bouquets wrapped in tissue paper round the petioles, and soaked in Hoagland's solution. Each bouquet had 10 – 15 leaves, and they were replaced twice a week, or before if needed (i.e. they were too damaged to provide food). Survival and duration of each stage was registered and compared.

Results

Ecology of H. ranunculoides. *L. elongatus* is the most common herbivore on *H. ranunculoides*, but two unidentified species of mining flies (Chloropidae and Ephydridae, table 1) have been collected, that may be specific and need further studies.

Table 1. List of herbivores found feeding on *Hydrocotyle ranunculoides* in Argentina

Species	Taxa	Location	Abundance	Host range
COLEOPTERA				
Listronotus elongatus	Curculionidae	throughout plant's range	high	specific
Listronotus cinnamomeus	Curculionidae	east-central Argentina	medium	also on *Limnobium laevigatum*
Ochetina bruchi	Curculionidae	throughout plant's range	medium	also on *Limnobium laevigatum,, Ludwigia* spp*, Pistia stratiotes*
Helodytes striatus	Curculionidae	east-central Argentina	rare	Oryzeae and Paniceae
Neohydronomus sp	Curculionidae	east-central Argentina	rare	unknown
DIPTERA				
Hydrellia sp. 1	Ephydridae	east-central Argentina	high	unknown
	Ephydridae	east-central Argentina	high	unknown
Monochaetoscinella sp.	Chloropidae	east-central Argentina	high	unknown
Parasphaerocera sp.	Sphaeroceridae	east-central Argentina	medium	unknown
	Chironomidae	throughout plant's range	high	polyphagous, detritivores
Hedriodiscus chloraspis	Stratiomyidae	throughout plant's range	high	polyphagous, detritivores
Stratiomys spp.	Stratiomyidae	throughout plant's range	high	polyphagous, detritivores
HEMIPTERA				
Rhopalosiphum nymphaeae L.	Aphididae	throughout plant's range	medium	polyphagous
LEPIDOPTERA				
Paracles quadrata	Arctiidae	northeast to central Argentina	medium	unknown
Spodoptera eridania	Noctuidae	northeast Argentina	rare	polyphagous
Spodoptera marima	Noctuidae	northeast Argentina	rare	polyphagous
Condica sutor	Noctuidae	east-central Argentina	rare	polyphagous
GASTEROPODA				
Biomphalaria tenagophila(Orbigny)	Basommatophora: Planorbidae	throughout plant's range	high	polyphagous
Pomacea spp.	Mesogastropoda: Ampullariidae	throughout plant's range	medium	polyphagous

The dynamics of *H. ranunculoides* (duration, biomass and size variations in any given population or patch) may be considered from several points of view. This species was always found in the Otamendi canal, indicating the "population" is constantly present. Patches, however, frequently dwindle until they disappear, in spite of being a perennial species, and new ones are generated. Our data suggest any given *H. ranunculoides* patch will last between 3 and 8 months (Fig. 1). This patch dynamics does not seem to be season related, as it has been observed at different times of year. However, biomass variations in individual patches suggest *H. ranunculoides* starts to grow and expand toward the end of winter and spring, peaking in early summer. The lowest biomass points as well as the highest feeding and pathogen damage levels were detected between February and April (late summer and autumn), when no patches with low damage levels ("green") could be found. Conversely, the highest biomasses were measured in spring and early summer (Fig. 1). *H. ranunculoides* coexisted with 10 other plant species, and their biomass increased in parallel with the target plant. Any a priori motives to consider them competitors (i.e. that they might negatively affect each other's growth) are not supported by the data. However, it is an indicative fact that patches recovered only with a winter in between suggesting the cold-tolerant *H. ranunculoides* can only regain lost surface when the other species are eliminated by frost.

Sample biomass, was not significantly correlated to average damage levels of *L. elongatus* or *Cercospora*. This in itself is not surprising since biomass was found to vary considerably from month to month, but not following any seasonal patterns. However, sample biomass was positively correlated to weevil numbers. The number of larvae and adults of *L. elongatus* were positively correlated to *H. ranunculoides* biomass. This could indicate that the weevil displays a density-dependent behaviour, probably moving away from damaged sections, and concentrating in the denser sections of the plant patch. At the same time, significant biomass, feeding and pathogen damage differences across the patch suggest that the feeding and pathogen damage are the cause of these biomass reductions within a single patch. Also, the field collections consistently yielded *L. elongatus* and *Cercospora* lesions, while the other common natural enemies were erratic. All this suggests that the former are the key natural enemies regulating *H. ranunculoides* populations.

Specificity of *Listronotus elongatus*. In the no-choice test only six species of *Hydrocotyle* out of the 29 plant species tested showed significant feeding levels (10 to 100 times less than *H. ranunculoides*). Feeding less than 1/100 of the standard (mean *H. ranunculoides* levels) were considered negligible (Table 2). The six *Hydrocotyle* species and three of the low damage species were analyzed statistically.

Table 2. Test-plant list for *Listronotus elongatus*, and no-choice feeding results

Species	Common name	Environment & growth	Feeding
APIACEAE			
Hydrocotyle ranunculoides	water pennywort	perennial aquatic creeper, rooted/floating	standard
H. bonariensis	largeleaf pennywort	perennial hydrophilic to dryland creeper, dunes	significant
H.bowlesioides		perennial dryland creeper	significant
H. leucocephala		perennial dryland creeper	significant
H. modesta		perennial hydrophilic creeper	significant
H. verticillata		perennial hydrophilic creeper	significant
Ammi majus		annual dryland erect	negligible

A. viznaga		annual dryland erect	negligible
Apium graveolens	celery	annual dryland erect	low
A. leptophyllum		annual dryland creeper	negligible
Bowlesia incana		annual dryland creeper	0
Conium maculatum	hemlock	annual dryland erect	negligible
Coriandrum sativum	coriander	annual dryland erect	negligible
Daucus carota	carrot	annual dryland erect	negligible
Eryngium ebracteatum		perennial dryland erect	low
E. elegans		perennial dryland erect	0
E. horridum		perennial dryland erect	0
E. coronatum		perennial dryland erect	negligible
E. mesopotamicum		perennial dryland erect	0
Foeniculum vulgare	fennel	annual dryland erect	negligible
Lilaeopsis carolinensis		perennial aquatic creeper, rooted/floating	low
L. attenuata		perennial aquatic creeper, rooted/floating	negligible
Petroselinum crispum	parsley	annual dryland erect	low
ARALIACEAE			
Fatsia japonica	fatsi	perennial dryland erect	0
Hedera helix	ivy	perennial dryland creeper	0
Schefflera arboricola	umbrella tree	perennial dryland erect	0
S. elegantissima		perennial dryland erect	0
S. umbellifera		perennial dryland erect	0
Tetrapanax papyrifer	rice-paper tree	perennial dryland erect	0

Feeding levels were significantly higher on *H. ranunculoides* and *H. modesta*. The remaining four *Hydrocotyle* species formed a second group of damage levels, followed by a third and fourth group formed by the three species of Apiaceae outside the genus.

Survival analysis in this test reveals that survival was significantly highest on *H. ranunculoides*, with an average of 12.56 weeks, followed by *H. modesta* in a second group, with 9.2 weeks. All other plant species had lower survival averages. It is interesting to note that in sixth place, forming part of the fourth statistical group, with a mean survival of 4.72 weeks, was the no food test group. This means survival was higher with water alone than for all the test groups that fed on species outside the *Hydrocotyle* genus.

Only four plant species, all *Hydrocotyle*, were accepted for oviposition by *L. elongatus* (Fig. 12). Oviposition was significantly higher on *H. ranunculoides* (total = 1741) than on H. modesta (total = 415) ($H = 20.253$; $P < 0.000$; $N = 30$). *H. verticillata* and *H. bonariensis* were not tested because only four and three eggs, respectively, were laid in them in the course of the test (Fig. 3).

The multiple choice trial was reduced to *H. ranunculoides*, *H. modesta*, and *H. bonariensis*, the only species that got significant feeding with functional plants. Preference for *H. ranunculoides* was significantly higher in all aspects: the number of damaged leaves was 3.2 times higher for H. ranunculoides. When these data were made relative to the number of leaves per samples this tendency was multiplied tenfold. Finally, the number of lesions per quadrat was in average six times higher on *H. ranunculoides* than on *H. modesta* (Fig. 16c). *H. bonariensis* did not receive any feeding at all in this experiment.

Specificity of *Paracles quadrata*. This moth has also been found to be widespread and sometimes abundant, although its appearance has been quite sporadic. First instars typically squeletonize the *H. ranunculoides* leaves by eating the upper cuticle and mesophyl. Mature larvae eat the whole leaf, producing heavy defoliation. According to our no-choice test, some significant development can be achieved on *H. bonariensis*, although survival is much reduced and larval stage takes significantly longer (Table 3). *H. bonariensis* is a very common species in the field that often grows next to *H. ranunculoides.* However, *P. quadrata* has not been collected on other *Hydrocotyle* species in the field.

Table 3. *Paracles quadrata* no-choice test: 10 larvae per container, 4 replications

Host	%Survival	Days to adult (mean ± SD)
H. ranunculoides	40	35.57 ± 14.14
H. bonariensis	16.7	46.2 ± 12.99
H. modesta	2.5	43
H. leucocephala	0	

Conclusions

So far, the evidence indicates that *L. elongatus* is a specific and highly damaging candidate for the biological control of water pennywort. Some aspects would need further study, such as specificity tests on plant species specific to the Old World that could not be tested in South America, and perhaps in depth climate matching studies. Results are very promising indeed.

There are at least four other natural enemies that deserve some attention: notably the petiole and stolon mining *Hydrellia* and the leaf mining *Monochaetoscinella*, both still unidentified, and probably undescribed. The moth *P. quadrata* may also be interesting, although its specificity is questionable. Finally, the rust must also be studied further for its specificity and compared to the rusts found on other species of *Hydrocotyle*.

Significant accomplishment

L. elongatus is a specific and highly damaging agent for the biocontrol of *H. ranunculoides*.

Field Trips

- October 26 to 30, 2010: Santa Fe, Chaco and Formosa. Cabrera.
- Nov 27 to Dec 3, 2010: Entre Ríos, Corrientes and Santa Fe. Cabrera, Shaw and Djeddour.
- Feb 15 and 16, 2011**:** southern Buenos Aires province. Cabrera and Maestro.
- May 1 to 5, 2011: Chaco and Formosa. Cabrera and Maestro.
- Monthly trips to the Paraná Delta. Cabrera and Maestro.

Future plans

- Determine geographical and seasonal distribution of *L. elongatus*
- To obtain colonies of other *Hydrocotyle* herbivores such as the chloropid and ephydrid fly.

Literature Cited

Center, T.D., F.A. Dray Jr., G.P. Jubinsky, and M.J. Grodowitz. 1999. Insects and other arthropods that feed on aquatic and wetland plants. USDA-ARS, Technical Bulletin No. 1870.

Cordo, H. A., Loach, C. J. de and Ferrer, R. 1982. The weevils *Lixellus*, *Tanysphiroideus* and *Cyrtobagous* that feed on *Hydrocotyle* and *Salvinia* in Argentina. Col. Bull. 36, 279-286.
Stegmaier, Jr., C.E. 1966. Host Plants and Parasites of *Liriomyza munda* in Florida (Diptera: Agromyzidae). The Fla Entomol. 49: 81-86.

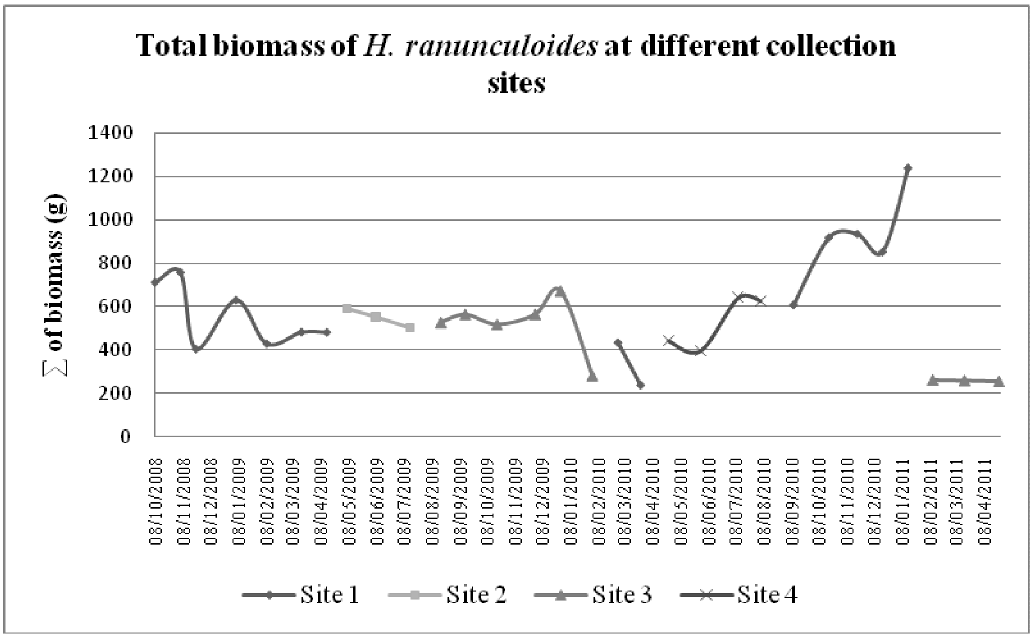


Figure 1. Population and biomass fluctuations *H. ranunculoides*: different segments indicate different patches, depicting the periodical extinction of patches

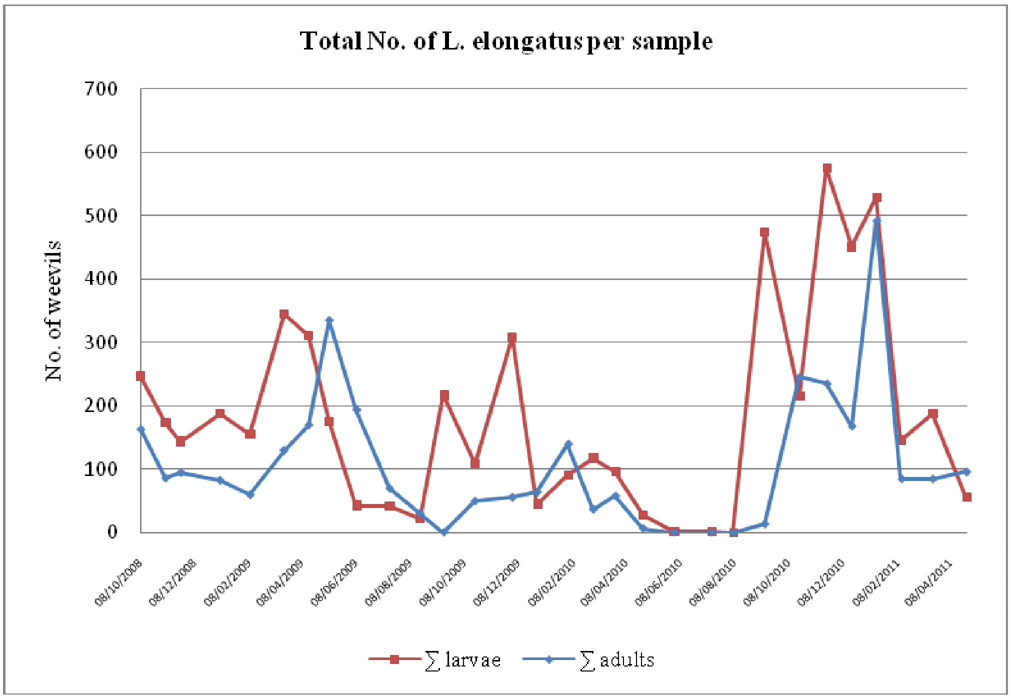


Figure 2. Seasonal fluctuations in *Listronotus elongatus* abundance.

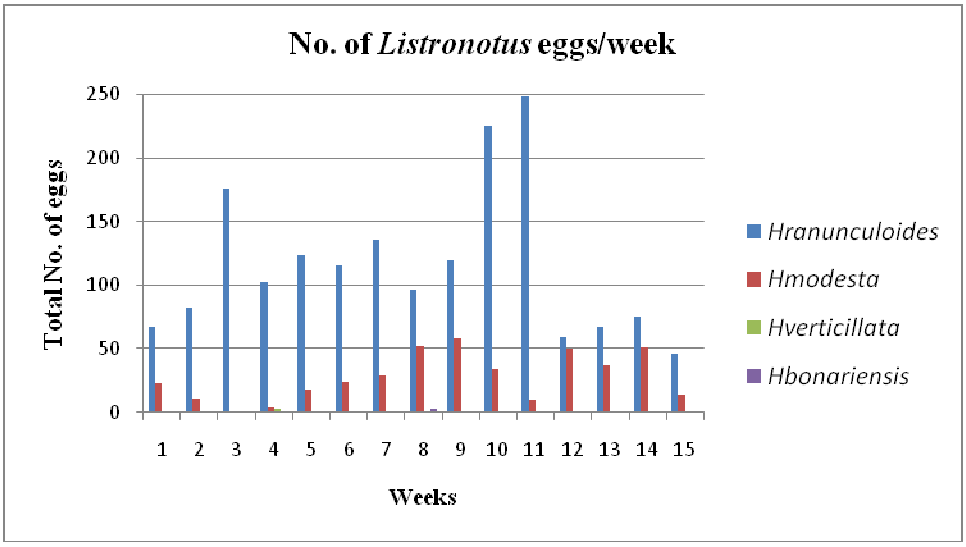
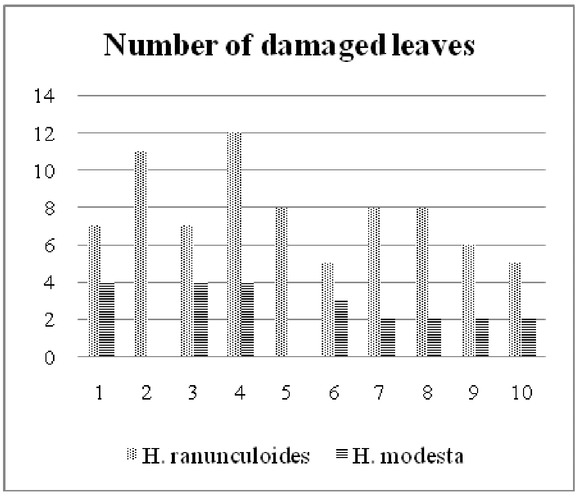
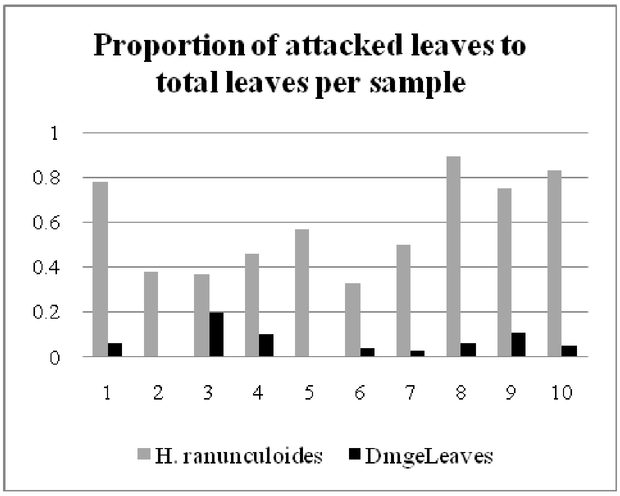


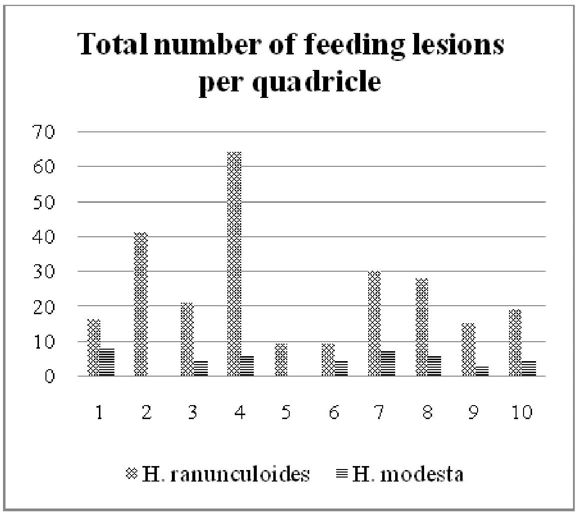
Figure 3. Number of *L. elongatus* eggs per week in the only four plant species accepted for oviposition.



a



b



c

Figure 4. Multiple-choice test in semi-field conditions: totals per quadrat.