Annual Report 2016

Biological control of perennial pepperweed, *Lepidium latifolium*

Jointly prepared by:

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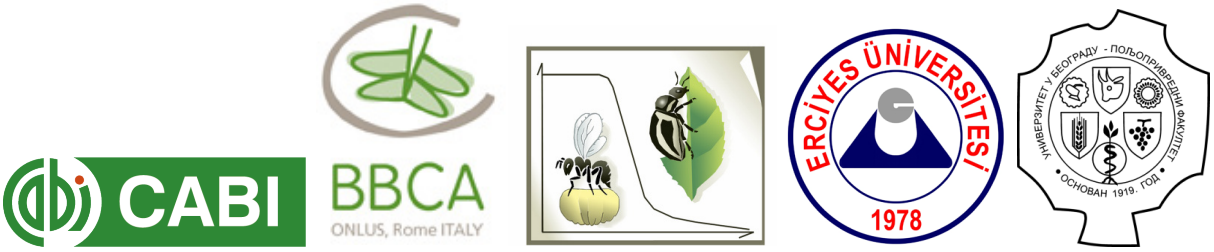
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Summary

1. In May 2016, field surveys were conducted in Kazakhstan. The main goal of this field trip was to survey for the eriophyid mite *Metaculus lepidifolii*. A total of 16 perennial pepperweed (*Lepidium latifolium*; PPW) sites were surveyed and eriophyid mites were found at eight of them. Morphological and molecular analyses revealed that the mites found at all sites are *M. lepidifolii*. Shoots infested with *M. lepidifolii* were collected at one of the sites and imported into the quarantine facility at CABI to establish a rearing colony. In addition, we dug up plants from nine PPW sites and dissected them for root herbivores. Two root-mining weevil larvae were found in one of the plants, but molecular analyses revealed that it is most likely a polyphagous species.

2. In 2016, an open-field test was set up with the gall-forming weevil *Ceutorhynchus marginellus* in Russia. Four native North American *Lepidium* species that had supported high adult development under no-choice conditions (*L. crenatum*, *L. eastwoodiae*, *L. huberi* and *L. virginicum*) and PPW were exposed at various distances from a central weevil release point. One month after the weevils had been released, all plants were inspected for galls. Plants without galls were dissected for larvae while plants with galls were covered with gauze bags and regularly checked for adult emergence. Larvae were found in PPW, *L. eastwoodiae* and *L. virginicum* and adults emerged from PPW and *L. eastwoodiae.* In summary, the data suggest that *L. crenatum*, *L. huberi* and *L. virginicum* will most likely not be attacked by *C. marginellus* when growing at greater distances (≥ 1.5 m) from PPW, but that *L. eastwoodiae* could act as an alternative host for this weevil. For the time being, we decided to postpone work with *C. marginellus* until we obtain feedback from the USDA-APHIS, Technical Advisory Group (TAG) on *Ceutorhynchus cardariae* petitioned for *Lepidium draba* control, which has a similar degree of host specificity as *C. marginellus.* A petition for field release of *C. cardariae* will be re-submitted in 2017/18. Since *C. marginellus* has been difficult to find in the field we will maintain a small rearing colony of this weevil at CABI.

3. In 2016, we continued working with the eriophyid mite *M. lepidifolii*. Unfortunately, *M. lepidifolii* from Kazakhstan imported into the quarantine facility at CABI died out 1.5 months after inoculation of plants and we could not begin host-range testing with this population. We assume that the fast decline of *M. lepidifolii* in our quarantine facility was due to predation by predatory mites occurring on the potted plants.

In collaboration with Erciyes University in Kayseri we continued our work with the Turkish population of *M. lepidifolii*. We conducted open-field and laboratory tests to quantify the attack by *M. lepidifolii* from Turkey on different PPW genotypes invasive in the USA and to compare it with the attack on PPW collected in Turkey. The results of these tests indicate that *M. lepidifolii* can establish on all four PPW haplotypes found in the USA. We also started to explore techniques for conducting host-range tests with *M. lepidifolii* under laboratory conditions. Since we observed that plants grown in standard potting soil were regularly colonized by predatory mites we assumed that this prevented *M. lepidifolii* from building up high populations on PPW and potentially also on test plants. We therefore washed plants and repotted them in sterilized soil shortly before inoculating them with *M. lepidifolii.* No-choice tests were conducted under laboratory conditions using 13 test species (2–4 replicates per species) and PPW as controls. These tests revealed that on some test plant species

(e.g. *Lepidium eastwoodiae*, *Boecheria hoffmannii* and *Raphanus sativus*) mites were only found on the inoculated leaves and the number of mites clearly declined two weeks after inoculation. On other species (e.g. *L. draba*, *L. virginicum* and *Euclidium syriacum*) mites could be found on the whole plant and the number of mites per plant increased over time. A logical next step would be to further investigate the acceptance of these test plant species under open-field conditions. However, owing to the current political situation in Turkey and because it appears to be difficult to obtain a permit to export living mites from Turkey, we decided to concentrate our work in 2017 on the *M. lepidifolii* population from Kazakhstan. We plan to re-collect *M. lepidifolii* and import it into our quarantine facility in order to conduct host-range tests with this population. Since *Lepidium draba* was heavily attacked by *M. lepidifolii* from Turkey under laboratory conditions, we also plan to survey populations of this species for eriophyid mites to investigate whether it is also attacked by *M. lepidifolii* from Kazakhstan under natural conditions. In addition, we will explore options for establishing open-field tests in Kazakhstan in 2018.

1. Introduction

Perennial pepperweed (PPW), *Lepidium latifolium* L., Brassicaceae, (syn.: *Cardaria latifolium*) is an herbaceous, semi-woody perennial that typically reaches 0.5–1.5 m in height and reproduces both vegetatively and by seed (Renz, 2000). Plants regrow early each year from a dense network of creeping, horizontal roots, flower in June/July, and set seeds in July/August. It is a prolific seed producer, capable of producing more than six billion seeds per acre of infestation (Miller *et al.*, 1986). Recent studies have shown that genetic diversity in the USA is very low, which is in strong contrast to the situation in the native range (Gaskin *et al*., 2012). In the USA, 99% of plants were found to be genetically identical. This lack of diversity in the US samples suggests that PPW, despite its success as an invader, is not well-positioned to adapt to new selection pressures, or to recruit pre-adapted genotypes that may vary in resistance or tolerance to disease or herbivory.

PPW is native to central Asia and is believed to have been introduced into the USA through California around 1900 as a contaminant of sugar beet seeds (Renz and DiTomaso, 1998). It is now widely distributed in the western USA, coastal New England, Mexico and Canada (Young *et al.*, 1995; Chen *et al.*, 2002). Although PPW has been recorded in the USA for more than 100 years, it only started invading a wide range of habitats throughout the western part of the country in the past three decades (Young *et al.*, 1998). It is especially prevalent in Nevada, Oregon, Utah and California, and declared noxious or prohibited in 15 US states (USDA, NRCS, 2017) and one Canadian province (Francis and Warwick, 2007).

PPW is often associated with mesic habitats, such as river banks, drainage ditches, and subirrigated pastures and hay meadows. However, it can invade a wide range of habitats including pastures, open fields, roadsides and residential areas (Young *et al.*, 1998). Downstream movement of seeds and root fragments along waterways and irrigation systems is the primary mode of spread for this weed. PPW is highly competitive and invasions result in dense monocultures and the subsequent loss of biodiversity through the exclusion of native vegetation. When dense stands establish along rivers and streams, the plant interferes with the regeneration of important native willow species and cottonwoods (Young *et al.*, 1995), reducing availability of cover and food for nesting waterfowl (Trumbo, 1994). It also displaces threatened, endangered and rare species such as the salt marsh harvest mouse (*Reithrodontomys raviventris*) (Trumbo, 1994) and Suisun Marsh aster (*Aster lentus*) (Skinner and Pavlik, 1994), and eliminates shading for fish and aquatic insects (Blank and Young, 1997). In areas that are not mown annually, semi-woody stems can accumulate over the years, negatively impacting on nesting habitats for birds and hindering the grazing and movement of livestock and wildlife (Renz, 2000). In addition, PPW can alter the habitat it invades by increasing its salinity (Blank and Young, 1997), thereby hindering regeneration of native flora. In agricultural settings, the species competes with crop plants and reduces agricultural yields (Eiswerth *et al.*, 2005). Furthermore, PPW contains few nutrients for livestock which decreases the quality of hay meadows infested with PPW and reduces livestock-carrying capacity in rangelands (Eiswerth *et al.*, 2005). Secondary plant compounds produced by PPW are reported to be toxic to livestock (Whitson, 1987). In Oregon, an economic impact of $110,000 was estimated for the 89,000 acres already invaded by PPW by 2012 (The Research Group, LLC, 2014). In the same study, it was estimated that if the

plant were to infest the total area suitable for it in the state (15,992,000 acres), a yearly economic impact of $5,329,000 could be anticipated.

PPW is difficult to control because of its large, stout root system. Mechanical or cultural control techniques usually provide no permanent reduction in populations (Young *et al.*, 1998; Renz and DiTomaso, 2006). Plants quickly resprout after mowing, and cultivation severs and transports root fragments that rapidly grow into new plants. When feasible, deep, repeated cultivation can suppress PPW. Grazing by sheep or goats may reduce weed density temporarily, but does not provide long-term control (Allen *et al.*, 2001). Application of herbicides containing chlorsulfuron is most effective for suppressing PPW; however, the substance is not registered in the USA for use in many of the invaded habitats, such as in areas adjacent to water (Renz and DiTomaso, 2006). Phenoxy herbicides, such as 2,4-D and clopyralid, will kill the shoots of PPW, but root crowns will quickly sprout new foliage. Applications may need to be repeated for up to five years to deplete the plant's abundant root reserves.

In 2004 and 2005, CABI in Switzerland had received modest start-up funding from the Idaho State Department of Agriculture, USA, through Dr Mark Schwarzländer at the University of Idaho to conduct preliminary literature and field surveys for insects, mites and fungi associated with PPW in Europe. In addition, BBCA (Biotechnology and Biological Control Agency) had carried out opportunistic surveys in conjunction with other weed biocontrol projects since 2002. Since 2006, CABI and BBCA have decided to share resources and collaborate closely in their effort towards developing biological control for PPW. Consequently, field surveys have been conducted cooperatively and results from both research teams are presented jointly in this report.

From field collections in various countries within the native range of PPW, more than 100 phytophagous organisms were sampled or reared, five of which were prioritized as potential biological control agents: the root-mining weevil *Melanobaris* sp. n. pr. *semistriata* (Col., Curculionidae), the gall-forming weevil *Ceutorhynchus marginellus* Schultze (Col., Curculionidae), the stem-mining flea beetle *Phyllotreta reitteri* (Heikertinger) (Col., Chrysomelidae), the eriophyid mite *Metaculus lepidifolii* Monfreda & de Lillo (Acari, Eriophyidae) and the stem-mining fly *Lasiosina deviata* Nartschuk (Dipt., Chloropidae) (Gerber *et al*., 2008). Investigations on these potential agents have focused on their biology, phenology, distribution and host range. Tests with *Ph. reitteri* revealed that this species is not specific enough to be further considered as a potential biological control agent and work with *L. deviata* was stopped owing to the absence of evident impact. Work with *Melanobaris* sp. n. pr. *semistriata* was suspended because of political instability in the region where this biological control candidate can be collected. In 2016, we concentrated our work on *C. marginellus* and *Metaculus lepidifolii*.

2. Work Programme for Period under Report

The following work plan was developed by CABI and BBCA for 2016 and proposed in the last annual report (Stutz *et al.*, 2016).

***Ceutorhynchus marginellus* (Col., Curculionidae)**

- Continue rearing in quarantine;
- Send weevils from the CABI rearing colony to Russia for a large-scale open-field test.

***Metaculus lepidifolii* (Acari, Eriophyidae)**

- Repeat the open-field test in central Turkey in collaboration with Erciyes University to quantify the attack of *M. lepidifolii* from Turkey on different US genotypes of PPW and on *Rorippa sinuata;*
- Conduct tests in parallel under laboratory conditions, exposing the same genotypes to verify results seen under open-field conditions.

Field surveys

- Conduct field surveys in Kazakhstan and/or Uzbekistan to collect mite strains better adapted to the most common US haplotype of PPW and to survey for new potential biological control agents.

3. Field Survey in Kazakhstan

A field survey was conducted in Kazakhstan from 13 to 27 May 2016. The main goal of this trip was to search for a new biotype of the eriophyid mite *M. lepidifolii* and to import it into the quarantine facility at CABI to establish a rearing colony. Surveys for *M. lepidifolii* were conducted in Kazakhstan because previous surveys had revealed that the most common PPW haplotype in the USA is also found in Kazakhstan (Gaskin *et al.,* 2013) and *M. lepidifolii* collected on these plants should be well adapted to the PPW populations invasive in the USA. Shoots of PPW were collected at a total of 16 sites and subsequently checked for eriophyid mites under a microscope in the field (Plate 1). Eriophyid mites were found at eight sites (Plate 1). Far more mites were found on most of the plants than during previous surveys in Turkey or Georgia. Shoots infested with mites were collected at one of the sites and imported into the quarantine facility at CABI to establish a rearing colony and develop methods for host-range testing (see section 5.1).

For each of the eight sites with eriophyid mites, plant material with mites was transferred into vials filled with either 70% or 95% ethanol for identification. Morphological and molecular identifications revealed that all samples contained *M. lepidifolii*. Molecular analyses revealed that *M. lepidifolii* collected in Kazakhstan belong to different haplotypes than those collected in Turkey or Georgia (p-distance: 1.7–2.2%). To identify the haplotype of the PPW populations on which mites were found, we collected silica-dried leaf samples from mite-infested PPW plants at six sites and forwarded them to Dr John Gaskin (US Department of Agriculture – Agricultural Research Service [USDA-ARS] Sidney, Montana, USA) for molecular analyses. These analyses revealed that the mites found in the western part of the area surveyed (Plate 1) occurred on PPW belonging to the same haplotype as the most common haplotype in the USA (haplotype 1 after Gaskin *et al.,* 2013) while the

mites collected in the more eastern part of the area surveyed (Plate 1) were found on PPW belonging to a haplotype not found in the USA (genetically close to haplotype 6 after Gaskin *et al.,* 2013).

In addition to surveying and collecting *M. lepidifolii* we also took the opportunity to search for new potential biological control agents: we dug up plants at nine PPW sites and dissected them for root herbivores. We found just two root-mining weevil larvae in one of the plants. One of the larvae was transferred to 95% ethanol and sent to our colleague Dr Ivo Toševski (Institute for Plant Protection and Environment, Department of Plant Pests, Zemun, Serbia) for molecular analysis. These analyses revealed that the larva is not the potential biological control agent *Melanobaris* sp. n. pr. *semistriata* but is genetically closer to *Baris lepidii,* a minor Brassicaceae pest. The other larva, together with the remaining part of the root, was imported into the quarantine facility at CABI and regularly checked for adult emergence, but no adult emerged.



Plate 1. PPW sites in south-eastern Kazakhstan surveyed for *Metaculus lepidifolii.* Sites with *M. lepidifolii* are represented with green dots, sites without *M. lepidifolii* with red dots (left). 'Field laboratory' to check the plant material for eriophyid mites (right).

4. *Ceutorhynchus marginellus* SCHULTZE (Col., Curculionidae)

Ceutorhynchus marginellus (Plate 2) is a gall-forming weevil native to Asia. The species was found at field sites in Kazakhstan, southern Russia, western China and eastern Turkey. The population used in host-specificity testing originates from southern Russia. *Ceutorhynchus marginellus* lays eggs in early spring. Galls are formed on leaf midribs, leaf stalks and stems, and adults emerge from mid-April onwards. The weevil has one generation per year.



Plate 2. Adult *Ceutorhynchus marginellus* on flowers of PPW (left) and *C. marginellus* gall on a leaf of PPW (right).

4.1. Overwintering and rearing

During winter, *C. marginellus* were kept in cylinders containing a small pot of sieved soil in an incubator set at 2°C and regularly provided with cut PPW leaves and shoots (see Stutz *et al*., 2016). In March 2016, weevils were taken out of the incubators and all surviving adults were counted. In contrast to last year when overwintering survival was 85.5%, winter survival was relatively low and of the 418 adults only 114 females and 89 males successfully overwintered (48.6%).

In spring and summer 2016, we continued to rear *C. marginellus* on potted PPW plants. In autumn, a total of 79 weevils, placed in cylinders as above, were transferred to an incubator set at 3°C for hibernation.

4.2. Large-scale open-field test in Russia

A large-scale multiple-choice open-field test was conducted in Russia with four critical test plant species (*Lepidium crenatum*, *L. eastwoodiae*, *L. huberi* and *L. virginicum*). These native North American species were chosen because previous tests revealed that they are particularly suitable for *C. marginellus* development under no-choice conditions. In addition, *L. crenatum* was attacked under multiple-choice cage conditions and *L. huberi* and *L. virginicum* were also attacked in a small-scale open-field test (plot size: 1.5 m × 1.5 m). The aim of the large-scale open-field test was to investigate whether *C. marginellus* would also attack these four non-target species when the plants are exposed at greater distances from a central release point.

METHODS Twelve potted plants of each of the four test plant species (*L. crenatum*, *L. huberi*, *L. virginicum* and *L. eastwoodiae*) and 48 plants of PPW were arranged at

distances of 1.5, 3.5 and 7.5 m in eight lines radiating out from a central point (Fig. 1, Plate 3). All potted plants were buried into the soil so that the upper edge of the pot was level with the surface of the soil. On 14 May, a total of 60 females and 45 males of *C. marginellus* were released at the central point. On 17 June, all plants were inspected for galls. If no galls were found during this first visual inspection, the plant was dissected and the number of larvae found during dissection was recorded. This was the case for 17 PPW, 12 *L. crenatum*, eight *L. eastwoodiae*, 11 *L. huberi* and nine *L. virginicum*. All remaining plants were individually covered with gauze bags and regularly checked for adult emergence.

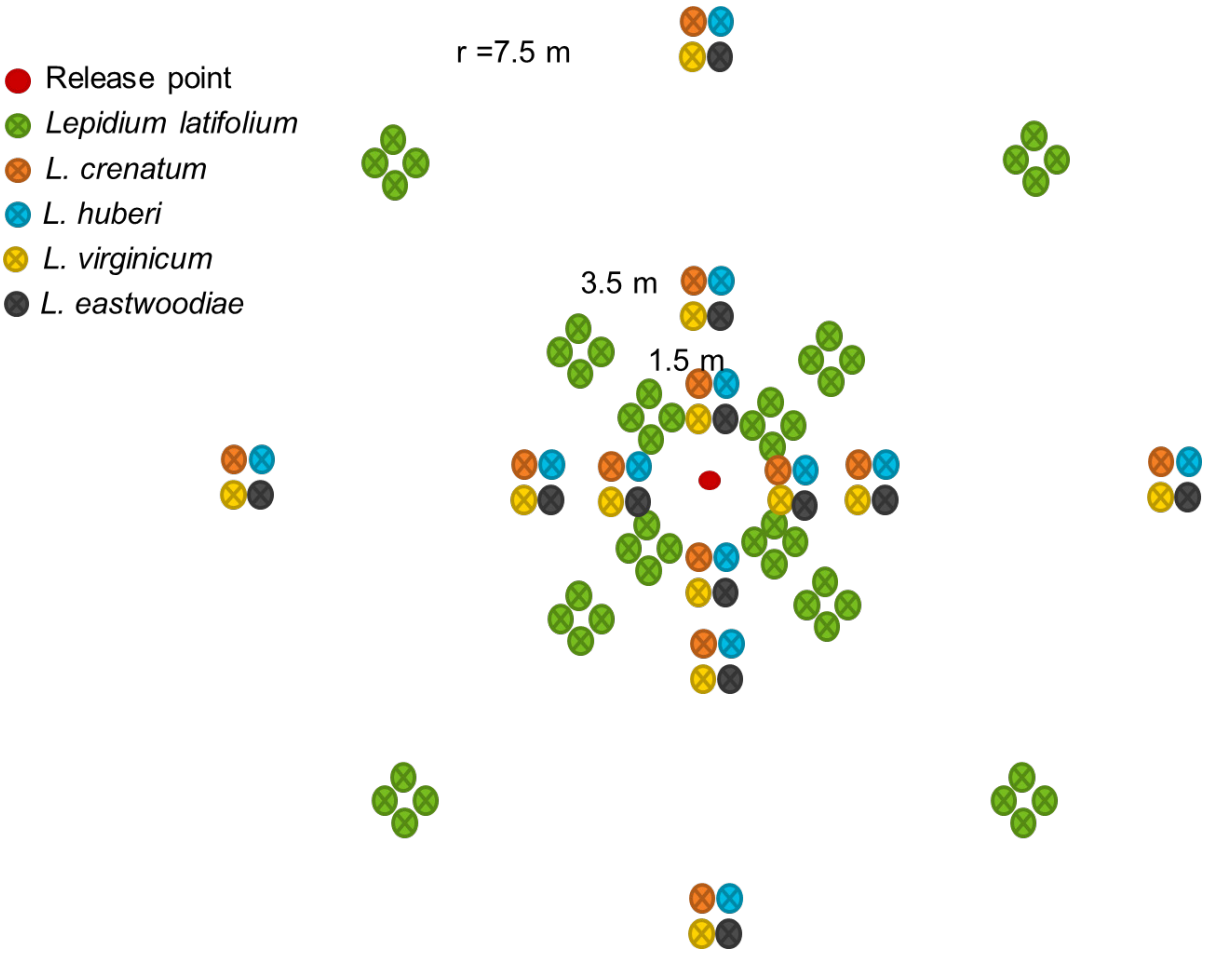


Fig. 1. Design of multiple-choice open-field test with *Ceutorhynchus marginellus.* Control and test plants were placed 1.5, 3.5 and 7.5 m from the central point where the weevils were released.



Plate 3. Set-up for multiple-choice open-field test with *Ceutorhynchus marginellus* in Russia.

RESULTS Larvae were found in PPW, *L. eastwoodiae* and *L. virginicum* and adults emerged from PPW and *L. eastwoodiae* (Fig. 2). No larvae were found in *L. crenatum* or *L. huberi*. In total, only four larvae were found in two of the *L. virginicum* exposed at 1.5 m and no adults emerged from the three plants covered with gauze bags. For PPW and *L. eastwoodiae*, the number of larvae and adults found per plant decreased with increasing distance from the release point (t = -3.6, P < 0.001) but for both species adults did emerge from plants exposed at 7.5 m from the release point. Fewer larvae and adults were found on *L. eastwoodiae* than on PPW, but this difference was only marginally significant (t = 2.0, P = 0.05).

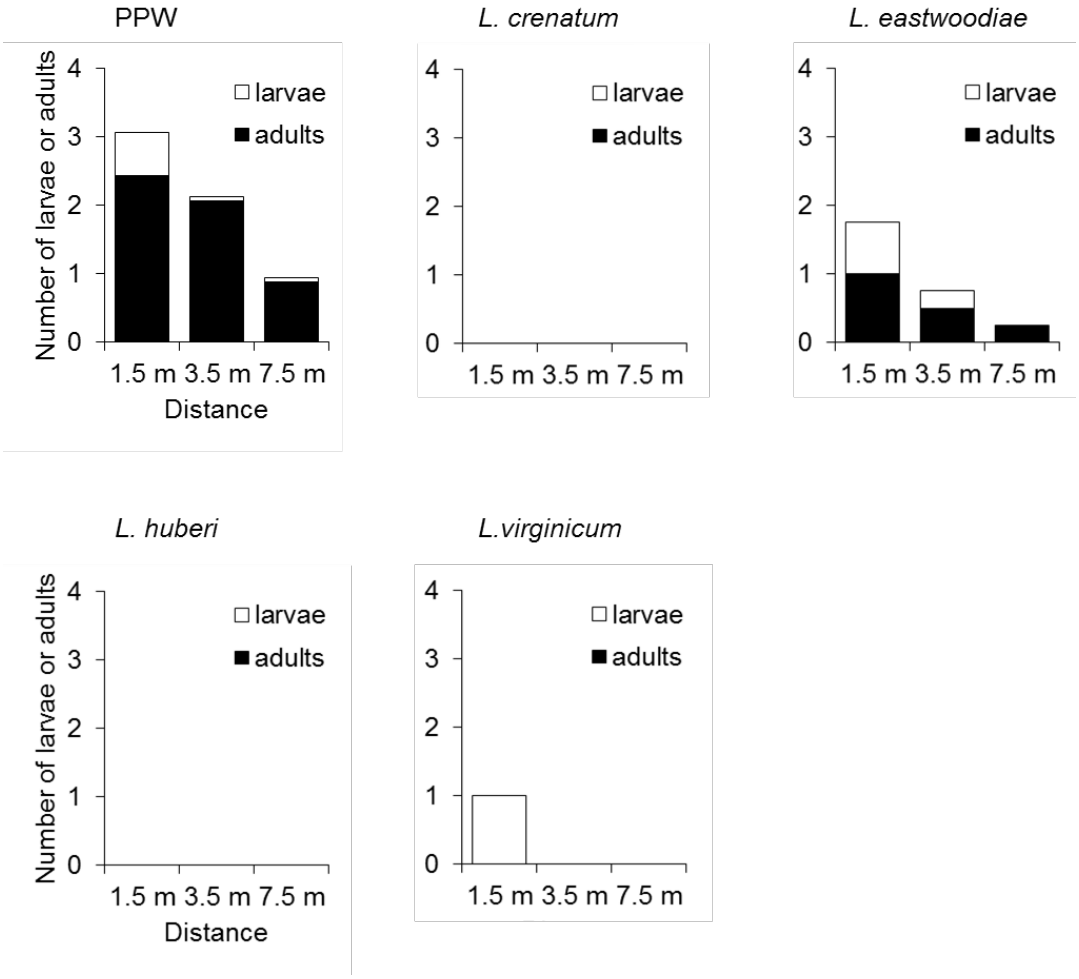


Fig. 2. Mean number of live larvae and adults of *Ceutorhynchus marginellus* found on PPW and *Lepidium* spp. test plants exposed to *C. marginellus* at increasing distances in a multiple-choice open-field test.

4.3. Conclusions and outlook

In host-specificity tests conducted at CABI between 2008 and 2014, adult development was recorded in 33 of 102 test species (32.4%) and in multiple-choice cage tests conducted in southern Russia attack was recorded in six of 26 test species exposed (23.1%). The results from the open-field tests conducted in 2016 indicate that *L. crenatum, L. huberi* and *L. virginicum* will most likely not be attacked by *C. marginellus* unless they are growing in close proximity to PPW. However, *L. eastwoodiae* exposed at 7.5 m from the release point was attacked by *C. marginellus* which indicates that this species might be attacked when growing further away from PPW. Since females reared from *L. eastwoodiae* had similar fecundity to females reared from PPW our results indicate that *L. eastwoodiae* could act as an alternative host for *C. marginellus*. For the time being, we decided to postpone work with *C. marginellus* until we obtain feedback from TAG on *Ceutorhynchus cardariae* petitioned for *Lepidium draba* control, which has a similar degree of host specificity as *C. marginellus.* However, since *C. marginellus* has been difficult to find in the field and the region where it was collected is under intensive reconstruction we will maintain a small rearing colony in the quarantine facility at CABI.

5. *Metaculus lepidifolii* MONFREDA & DE LILLO (Acari, Eriophyidae)

Metaculus lepidifolii is a newly described eriophyid mite species (Monfreda and de Lillo, 2012) that we have found on several occasions in central Turkey and Georgia and in 2016 also in Kazakhstan. It is of particular interest for biological control of PPW since eriophyid mites tend to be very specific. Our field and laboratory observations revealed that *M. lepidifolii* is a vagrant mite and does not induce gall formation. Under laboratory conditions, high *M. lepidifolii* densities have been observed to result in colour changes and wilting of leaves (Plate 4) and to greatly reduce plant growth.



Plate 4. *Metaculus lepidifolii* on PPW flowers collected in Kazakhstan (left) and leaves of potted PPW plants highly infested with *M. lepidifolii* (right).

5.1. Rearing of *Metaculus lepidifolii* from Kazakhstan under quarantine conditions

At the end of May, we successfully imported *M. lepidifolii* from Kazakhstan into the quarantine facility at CABI (see section 3). To establish a rearing colony, we inoculated potted PPW plants with *M. lepidifolii* by attaching field-collected plant parts infested with *M. lepidifolii*. Inoculation was successful on all plants and large mite populations built up until early July. Unfortunately, all eriophyid mites had disappeared from the plants by mid-July, which was before we could begin host-range testing. Since we found only a few dead eriophyid mites on the inoculated plants, but large numbers of predatory mites on some of them, we assume that the predatory mites had spread to all plants and that this has resulted in the rapid decline of *M. lepidifolii*.

5.2. Is PPW from the USA resistant to *Metaculus lepidifolii* from Turkey?

In an open-field test conducted in 2015, higher numbers of *M. lepidifolii* were found on PPW from Turkey than on PPW from the USA (Stutz *et al*., 2016). Since PPW plants from Turkey are genetically different from those invading the USA (Gaskin *et al*., 2013) we suspected that plants from the USA might be resistant to attack by *M. lepidifolii* from Turkey. In 2016, we therefore infested four PPW populations representing all four genotypes that are invasive in North America as well as PPW from Turkey with *M. lepidifolii* from Turkey under both open-field and laboratory conditions to investigate whether the different haplotypes indeed differ in their resistance to *M. lepidifolii.*

METHODS On 24 June, plants from five different PPW populations (Table 1) were randomly arranged in a field plot at Erciyes University, Kayseri, Turkey (distance between plants: 50 cm) and bouquets of cuttings of PPW infested with *M. lepidifolii* were attached to young leaves of each potted PPW with a paper clip (Plate 5). In addition, plants of the same five PPW populations were inoculated with *M. lepidifolii* and kept in laboratory conditions. On 17 July, all plants from the open-field test were harvested and mites extracted and identified. The plants kept in laboratory conditions were regularly inspected for mites and the mites were counted seven weeks after the inoculation with *M. lepidifolii*.

RESULTS Under both open-field and under laboratory conditions, *M. lepidifolii* was found on all PPW genotypes exposed. However, only slightly more than half of the PPW plants that were inoculated with *M. lepidifolii* were infested and only relatively low numbers of mites were found on them (Table 1). During regular observations of the plants kept under laboratory conditions we found relatively high numbers of predatory mites and relatively low numbers of eriophyid mites throughout the experiment.



Plate 5. Set-up for open-field test with *Metaculus lepidifolii* in central Turkey (left) and a bouquet of PPW cuttings infested with *M. lepidifolii* placed on a potted PPW plant (right).

Table 1. Results of laboratory and open-field tests exposing one PPW haplotype (PPW_TR) from Turkey and four PPW haplotypes from the USA to *Metaculus lepidifolii* from Turkey.

Population code	Laboratory test			Open-field test			
	No. plants inoculated	No. plants attacked	No. mites/plant (mean ± SE)[b]	No. plants inoculated	No. valid replicates[c]	No. plants attacked	No. mites/plant (mean ± SE)[d]
PPW_TR	3	2	26.7 ± 14.8	10	7	4	6.3 ± 3.8
PPW_US1	5	1	1.0 ± 1.0	10	9	4	2.3 ± 1.5
PPW_US4	6	4	15.0 ± 5.6	5	5	4	7.8 ± 4.0
PPW_US5	6	3	5.8 ± 3.3	5	5	3	1.0 ± 0.4
PPW_US9[a]	5	4	7.0 ± 2.5	5	5	2	2.2 ± 1.8

[a] Most common haplotype in the USA.
[b] The number of mites was estimated by visually inspecting all plants seven weeks after inoculation.
[c] Number of plants that were still alive at the end of the experiment (no plants died in the laboratory test).
[d] Mites were extracted and counted three weeks after inoculation.

5.3. Host-range tests with *Metaculus lepidifolii* from Turkey under laboratory conditions

We started to explore techniques for conducting host-range tests with *M. lepidifolii* under laboratory conditions. For our initial experiments (described in section 5.3.1), we used plants potted in standard potting soil for inoculation with *M. lepidifolii*. However, these plants were regularly colonized by predatory mites, which most likely drastically decimated the population densities of *M. lepidifolii* on PPW and potentially also on test plants. We were therefore looking for ways of reducing the number of predatory mites on the plants and decided to repot plants in sterilized soil shortly before infesting them with *M. lepidifolii* for subsequent test (described in section 5.3.2).

5.3.1. Host-range tests with plants potted in standard potting soil

METHODS PPW leaves infested with at least 30 mites were attached to leaves of potted test plants of 18 test species (1–4 replicates per test plant species; Table 2) and PPW. All plants were subsequently checked at one-week intervals for the presence of eriophyid and predatory mites and the date when eriophyid mites were found for the last time was recorded. The experiment was stopped after seven weeks.

RESULTS In general, *M. lepidifolii* survived longer on PPW than on test plants and on half of the test species no mites were found three weeks after inoculation (Table 2). On some species (*Barbarea orthoceras*, *Boechera hoffmannii*, *Euclidium syriacum* and *Streptanthus inflatus*) a few mites were found until 4–7 weeks after inoculation. However, the number of eriophyid mites per plant also decreased on control plants one to two weeks after inoculation and numbers of eriophyid mites remained low on them for the rest of the experiment. Although an effort was made to remove all predatory mites that were observed during the weekly inspections, predatory mites were found on most of the plants throughout the experiment.

Table 2. Host-range tests conducted with *Metaculus lepidifolii* from Turkey under laboratory conditions using plants potted in standard potting soil.

Plant species	No. of plants tested	No. of days that mites were found on plants (range)
Lepidium latifolium (PPW)	29	0–48
Barbarea orthoceras[a]	1	48
Boechera hoffmannii[a]	2	6–33
Descurainia incana[a]	1	20
Euclidium syriacum	2	0–41
Eutrema salsugineum[a]	2	0
Physaria fendleri[a]	1	0
Sisymbrium linifolium[a]	1	0
Streptanthus anceps[a]	3	6
Streptanthus glandulosus subsp. *niger*[a]	2	6–18
Streptanthus albidus subsp. *albidus*[a]	2	0–18
Streptanthus crassicaulis[a]	1	18
Streptanthus flavescens[a]	1	18
Streptanthus heterophyllus[a]	1	20
Streptanthus inflatus[a]	4	0–33
Streptanthus pilosus[a]	1	0
Streptanthus polygaloides[a]	1	5
Streptanthus tortuosus[a]	1	20
Thelypodium sagittatum[a]	1	6

[a] Plant species native to North America.

5.3.2. Host-range tests with plants potted in sterilized soil

METHODS Test and control plants were grown from seeds in standard potting soil for approximately seven weeks and subsequently repotted in soil that had been heat-sterilized at 80°C for approximately 14 hours. Before repotting the plants, all soil attached to the roots was removed and the roots and leaves were carefully washed using a black soap solution. After repotting, the plants were kept apart from any other plants that might be colonized by predatory mites. The plants were allowed to recover from repotting for at least one week before being inoculated with *M. lepidifolii*. For inoculation, PPW leaves infested with at least 30 eriophyid mites were attached to leaves of potted test plants of 13 test species (2–4 replicates per test plant species; Table 3) and control plants. The numbers of eriophyid mites were counted one, two, four and five to six weeks after inoculation or until the plants died. The distantly related *T. vulgare* (Asteraceae) was only inspected one week after inoculation. A subsample of the eriophyid mites found on test- and control plants was morphologically identified to verify that they are indeed *M. lepidifolii*.

RESULTS One week after inoculation, no mites were found on *T. vulgare* but mites were found on all of the other more closely related test plant species (Table 3). On some of the species (e.g. *Lepidium eastwoodiae*, *Boecheria hoffmannii* and *Raphanus sativus*) *M. lepidifolii* was almost exclusively found on the inoculated leaves and the number of mites clearly declined two weeks after inoculation. On other species (e.g. *L. draba*, *L. virginicum* and *Euclidium syriacum*) *M. lepidifolii* could be found on the whole plant and the number of mites per plant increased over time. On

most of the plants the number of mites decreased after 5–6 weeks, which was probably due to an increased infestation with thrips and a decrease in plant quality. All eriophyid mites that were morphologically identified were confirmed to be *M. lepidifolii*. No predatory mites were observed on any of the plants.

Table 3 Host-range tests conducted with *Metaculus lepidifolii* from Turkey under laboratory conditions using plants repotted in sterilized soil.

Species	No. of plants tested	No. of mites per plant (mean ± SE)			
		week 1	week 2	week 4	week 5–6
Lepidium latifolium (PPW)	5	112 ± 74	158 ± 92	229 ± 193	113 ± 93
Lepidium campestre	3	133 ± 33	50 ± 25	50 ± 0	27 ± 4
Lepidium draba	3	267 ± 88	80 ± 60	367 ± 318	133 ± 88
Lepidium eastwoodiae[a]	3	167 ± 44	7 ± 7	7 ± 7	0.3 ± 0.3
Lepidium virginicum[a]	3	667 ± 67	367 ± 219	3433 ± 3283	1740 ± 1631
Barbarea orthoceras[a]	2	100 ± 0	155 ± 145	175 ± 125	55 ± 45
Boecheria hoffmannii[a]	4	200 ± 41	16 ± 11	7 ± 6	0.3 ± 0.3
Euclidium syriacum	2	100	250 ± 50	225 ± 25	100
Nasturtium officinale	3	47 ± 27	72 ± 64	23 ± 15	-
Planodes virginicum[a]	2	150 ± 50	200 ± 0	200 ± 0	100
Raphanus sativus	3	43 ± 28	11 ± 10	0 ± 0	-
Sinapis alba	3	30 ± 12	77 ± 62	113 ± 94	-
Streptanthus inflatus[a]	2	210 ± 190	408 ± 393	101 ± 99	20
Tanacetum vulgare	2	0 ± 0	-	-	-

[a] Plant species native to North America

5.4. Conclusions and outlook

In contrast to the open-field test in 2015, where attack by *M. lepidifolii* was much lower on PPW plants from the USA than on those from Turkey, we did not find any indications that PPW from the USA is resistant to *M. lepidifolii* from Turkey during the laboratory and open-field tests conducted in 2016.

The results from the host-range tests conducted under laboratory conditions indicate that predatory mites can severely limit population growth of *M. lepidifolii*. Since *M. lepidifolii* does not induce galls, they have no refuge to hide from predatory mites and are therefore fully exposed to them. Using plants repotted in sterilized soil has proven to be a good method to eliminate predatory mites and to conduct host-range tests under laboratory conditions.

In the absence of predators under laboratory conditions, high mite populations built up on several test plant species. A logical next step would be to expose these species under open-field conditions in Turkey. However, owing to the current political situation in Turkey and because it appears to be difficult to obtain a permit to export living mites from Turkey we decided to stop working in Turkey and to concentrate our work in Kazakhstan. We plan to re-collect *M. lepidifolii* from a PPW population in Kazakhstan that belongs to the same haplotype as the most common PPW haplotype invasive in the USA and import it into our quarantine facility in order to conduct host-range tests. Since *M. lepidifolii* from Kazakhstan are genetically different from those collected in Turkey, they might also differ in their host range. Because *Lepidium draba* was heavily attacked by *M. lepidifolii* under laboratory conditions, we also plan

to survey populations of this species for eriophyid mites to investigate whether it is also attacked by *M. lepidifolii* under natural conditions. In addition, we will explore options of establishing open-field tests in Kazakhstan in 2018.

6. Work Programme Proposed for 2017

Based on results obtained during the field season 2016, we propose that work in 2017 should focus on the following:

***Ceutorhynchus marginellus* (Col., Curculionidae)**

- Maintain rearing in quarantine.

***Metaculus lepidifolii* (Acari, Eriophyidae)**

- Collect *M. lepidifolii* in Kazakhstan;
- Survey *Lepidium draba* populations in Kazakhstan for *M. lepidifolii;*
- Ship *M. lepidifolii* to US quarantine facility(ies) if requested;
- Establish a colony of *M. lepidifolii* at CABI in Switzerland;
- Investigate host range under quarantine conditions at CABI in Switzerland;
- Start exploring options for establishing open-field tests in Kazakhstan in 2018.

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