Biotechnology and Biological Control Agency

Biological Control Research Activities
Biannual Report
2020-2021
Cover photo: Mark Volkovitsh (Russian Academy of Sciences, St. Petersburg - Russia) and Francesca Di Cristina (BBCA onlus, Rome - Italy) taking note during field exploration in Armenia.
BBCA onlus
Biological Control Research Activities

Biannual Report
2020-2021

Massimo Cristorafo & Francesca Marini
mcristofaro55@gmail.com  fra.rini.bbca@gmail.com
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Introduction

This report serves to document research conducted under the Specific Cooperative Agreement No. 58-2030-8-032-F, between USDA, ARS, WRRC, Invasive Species and Pollinators, Albany, CA and Biological Control Agency (BBCA), Rome, Italy. During the Fiscal Years 2020-2021 (October 1st, 2019 – September 30th, 2021), BBCA has been working on the following target weeds:

- Rush skeletonweed (*Chondrilla juncea*) - in cooperation with Montana State University (Bozeman, Montana - USA), University of Idaho (Moscow, Idaho - USA), Russian Academy of Sciences (Saint Petersburg - Russia), and Armenian National Academy of Sciences (Yerevan - Armenia);
- Russian thistle (*Salsola tragus*) - in cooperation with USDA-ARS, Exotic and Invasive Weeds Research Unit (Albany, California - USA);
- Yellow starthistle (*Centaurea solstitialis*) in cooperation with USDA-ARS, Exotic and Invasive Weeds Research Unit (Albany, California - USA) and Agriculture University (Plovdiv - Bulgaria).
Target Weeds

1.1 Rush skeletonweed (*Chondrilla juncea*)

*Chondrilla juncea* (rush skeletonweed) is an herbaceous biennial or perennial plant native in Eurasia. It was accidentally introduced into several regions around the world as a seed and fodder contaminant and it is currently reported as an invasive weed in Argentina, Australia, Canada, New Zealand, South Africa, and the USA. Without control measures, rush skeletonweed can produce a monoculture of interconnected plants, that replaces desirable forbs, grasses, and shrubs. Competing for water and nitrogen, it can severely reduce crop yield and forage for livestock and wildlife, and its wiry branches interfere with harvest.

1.1.1 *Oporopsamma wertheimsteini* (Lepidoptera, Tortricidae)

*Oporopsamma wertheimsteini* (Lepidoptera: Tortricidae) is a moth distributed throughout Iran, Central Asia, Asia Minor, and the Balkans (Fazekas 2009; Fazekas and Lescar 2009). It has one generation per year and two quiescent periods (i.e., aestivation as pupal and hibernation as egg or first instar larva). At the early stage, larvae feed on sprouting rosettes and then on the root crown of the plant, where they form a silken tube from which they continue to feed until they pupate (Fig. 1). Adults emerge in fall, and they lay eggs in the soil immediately after (Hasan, 1978). During the period of the larvae activity (i.e., from early spring to early summer), it has been observed a reduction of 80% of the populations of *C. juncea* heavily infested. Moreover, in the case of small diameter plants and weaken larger thicker rooted plants, the larvae were able to kill the plants (Hasan and Wapshere 1977; Littlefield L., pers. comm.). The moth has been reported as monophagous on *C. juncea* in its native range (Hasan and Wapshere 1977; Fazekas 2009; Fazekas and Lescar 2009) and the preliminary host specificity tests carried out in the 1970s supported this narrow host range (Hasan and Wapshere 1977).

![Fig. 1 – Collection of *Oporopsamma wertheimsteini* pupae infesting *Chondrilla juncea* root crowns (on the right) in Gorovan Sands desert in Central Armenia.](image)

Work performed in 2020 and 2021

Since 2014, BBCA perform every year collections in Gorovan Sands desert in Central Armenia with the support of Dr. M. Volkovitsh (Russian Academy of Sciences, Saint Petersburg - Russia) and Dr. M. Kalashian (Armenian National Academy of Sciences, Yerevan - Armenia) to provide root cases with pupae of *O. wertheimsteini* to Dr. J.
Littlefield (Montana State University, Bozeman, Montana – USA). Unfortunately, in 2020, due to the travel restrictions enforced by the COVID-19 outbreak, it was not possible to perform any field collection. However, thanks to the general improvement of the pandemic situation and the suppression of the limitations to travel in some countries, a field collection was accomplished in June 2021. About 200 O. wertheimsteini root cases were collected and sent to Dr. J. Littlefield, from which seventy-three adults emerged. Adults were placed in oviposition tubes and a thousand plus eggs were harvested. Larvae were then used for impact studies on those non-target plants on which some feeding and development occurred in previous tests (Littlefield L., pers. comm.).

Work program proposed for 2022
Additional collections of O. wertheimsteini root cases in collaboration with Dr. M. Volkovitsh and Dr. M. Kalashian have been planned for early summer 2022. As in the past years, the collections will be focused in Gorovan Sands Desert in Central Armenia and the pupae collected will be sent to Jeff Littlefield, MSU, Bozeman, MT.

1.1.2 Sphenoptera foveola (Coleoptera, Buprestidae)
Sphenoptera foveola is a root borer distributed from the Volga River and the Caspian Sea eastward through the deserts of Kazakhstan, and possibly northern of Turkmenistan and Uzbekistan, as well as on southern of Russia adjacent to Kazakhstan (Volkovitsh et al., 2008). Adults feed on green stems, whereas larvae feed externally on roots within of a latex case (Fig. 2). As the adults, larvae feed exclusively on plants of the Chondrilla genus and at high density can cause significant damage on the plants, often killing the above-ground portion of smaller plants and sometimes larger stems of bigger plants. The larval feeding induces a copious secretion of latex from the roots, representing a major loss of nutrients and energy, and hence stresses the plant, resulting in reduced growth, flowering, and general loss of competitiveness (Volkovitsh et al., 2008).

Fig. 2 – Adult and larvae, inside their latex cases, of Sphenoptera foveola feeding on stem (on the left) and roots (on the right) of Chondrilla juncea, respectively, in Kulanbasy desert in Southeastern Kazakhstan. (Photo credit: M. Volkovitsh).
Work performed in 2020 and 2021
Since 2016, BBCA performs every year collections in the Kulanbasy desert hills in the Almaty province of Southeastern Kazakhstan, to provide adults to Dr. M. Schwarzlaender (University of Idaho, Moscow, Idaho - USA) and Dr. M. Dolgovskaya (Russian Academy of Sciences, St. Petersburg - Russia), who are evaluating host-specificity and impact of this buprestid on rush skeletonweed, and studying some aspects of its biology, such as survival and fecundity. Unfortunately, since March 2020, the travel restrictions enforced by COVID-19 outbreak did not allow us to travel to Kazakhstan, and we could not perform any field collection during the past two years.

Work program proposed for 2022
A new field collection in collaboration with Dr. M. Volkovitsh, and with the support Dr. Roman Jashenko (Institute of Zoology, Almaty – Kazakhstan), has been already scheduled for May 2022. Adults collected will be sent to both Dr. M. Schwarzlaender and Dr. M. Dolgovskaya, in order to support them in the evaluation of this potential biological control agent against rush skeletonweed.
1.2 Russian thistle (*Salsola tragus*)

*Salsola tragus* (Russian thistle) is an annual herb native to southeastern Russia and western Siberia. It was introduced into the US as a contaminant in flaxseed and now it is widespread throughout western North America, including virtually all arid and semi-arid regions of California. Russian thistle is primarily present in disturbed semiarid agricultural environments, rangeland and nonagricultural areas, such as vacant residential lots and railroad or highway rights-of-way. The plant is also commonly known as tumbleweed. In fact, once it is mature and dry, the above-ground portion of the plants detaches from its root or stem and rolls away due to the force of the wind, reducing highway safety, promoting and spreading fire through grasslands. In agricultural areas, it can reduce the yield and quality of numerous crops. Moreover, it depletes soil moisture, interferes with tillage operations and it can threaten native plant ecosystems.

Among several natural enemies occurring on Russian thistle in the Mediterranean Region, special emphasis has been done to the eriophyid mite *Aceria salsolae*. The mite is highly specific for its host, and it can strongly damage *S. tragus* plants (Smith, 2005; Smith et al., 2009). Although in 2004, USDA-APHIS Technical Advisory Group (TAG) recommended approval of *A. salsoale* as biological control agent of *S. tragus*, it has not been released yet, because in 2009 APHIS denied a permit. Therefore, additional tests were carried out to assess the risk of *A. salsolae* to nontarget plant species, demonstrating that the mite is capable of very low reproduction on some nontarget plant species under some laboratory conditions, but that it is not likely to multiply on any of these plants under field conditions. Furthermore, the mite does not appear to cause any significant harm to these nontarget plants when it does occur on them. In conclusion, these studies answered to the concerns raised by APHIS and demonstrated that *A. salsolae* is not expected to pose a risk to any nontarget plants in the contiguous USA (Marini et. al, 2021).

1.2.1 *Gymnancyyla canella* (Lepidoptera: Pyralidae)

*Gymnancyyla canella* is a stem and seed borer moth associated with *Salsola kali* and *S. tragus*, distributed in Europe, North Africa and Asia Minor. The moth is univultine and it overwinters at the pupal stage. Adults oviposit their eggs on the stem and the newly hatched larvae immediately started to feed inside of the tender green stems. Larvae spin a silken web similar to the spider-webs, presumably to protect themselves from parasitoids and predators. When the plant starts to develop fruits, the larvae mine them and destroy the seed embryos, inducing an important impact on the plant sexual reproduction. Once mature, larvae leave the plant and pupate in the sand. Preliminary host range bioassays carried out at the end of 1990s at the USDA ARS EBCL (Montpellier - France) by Dr. R. Sobhian suggest that the moth is specific for its host having a narrow host range.

**Work performed in 2020 and 2021**

Since a few years, BBCA is involved in the collection of *Gymnancyyla* spp. pupae (Fig. 3) to be sent to Dr. L. Smith (USDA-ARS, Albany, California - USA). One of the best sites for the collection of *Gymnancyyla* spp. was in Eastern Sicily (Italy); however, three years ago a heavy rainstorm destroyed most of the Salsola plants present there. Therefore, we spent the last 2 years looking for new sites, but unfortunately no heavily infested site has been
found so far, and only few Gymnancyla pupae were collected and sent to the USDA ARS quarantine, in Albany, California.

Fig. 3 – Winter collection of Gymnancyla pupae from the sand under dry Salsola plants. Plants are identified and selected according to the presence of holes in the center of seed-capsules, which indicate mature larvae left the plant to pupate in the sand.

Work program proposed for 2022

In concert with Dr. L. Smith and Dr. P. Moran (USDA-ARS, Albany, California - USA), additional collections of Gymnancyla spp. pupae have been planned for spring 2022. Based on previous observations, we will focus our searching efforts on S. kali along the coasts of Central Italy and Eastern Sicily, and on S. tragus in Slovakia and Bulgaria. Both dry and alive pupae will be collected. The first ones will be sent to the quarantine of USDA ARS in Albany (California), where additional host range tests will be performed; whereas dry specimens will be given to Dr. A. Zilli (British Museum, London - England) for morphological identification and to Dr. M-C. Bon (USDA ARS EBCL, Montpellier - France) for molecular analyses to identify potential cryptic species.

1.2.2 Conorhynchus kindermanni (Coleoptera: Curculionidae)

Work performed in 2020 and 2021

During previous explorations carried out with the support of Dr. L. Gültekin (University of Erzurum, Erzurum - Turkey), a weevil, Conorhynchus kindermanni, was recorded on Russian thistle near Igdir, Eastern Turkey. Weevil larvae freely move in the soil, reaching lateral branches of nearby Salsola roots, and once mature they pupate into a hard soil-case cocoon (Gültekin N. et al., 2021). In 2021, during an expedition in Armenia, we observed an impressive damage on Salsola roots due to the simultaneous presence of several weevil larvae of different ages, which supposedly could be larvae of C. kindermanni (Fig. 4).

Work program proposed for 2022

In concert with Dr. L. Smith and Dr. P. Moran (USDA-ARS, Albany, California - USA), additional exploration and collections have been planned for late spring and early summer of 2022. A meeting with Dr. Levent Gültekin and Dr. Neslihan Gültekin (University of Erzurum, Erzurum - Turkey) is already planned for the beginning of April 2022 to discuss future cooperation in biological observations and field collections of C. kindermanni and Chromonotus vittatus, another weevil also associated with Russian thistle. Potentially experiments could be setup and performed in Eastern Turkey, by Dr. Levent Gültekin and
Dr. Neslihan Gültekin, and Armenia, in cooperation with Dr. M. Kalashyan (Armenian National Academy of Sciences, Yerevan - Armenia).

Fig. 4 – Weevil larvae from *Salsola* roots found in 2021 during a field exploration in Armenia.
1.3 Yellow starthistle (Centaurea solstitialis)

Yellow starthistle (Centaurea solstitialis) is an annual rangeland weed originating from the Mediterranean region. It became established in North America in the mid-1800s and today it primarily infests annual and perennial grasslands, pastures, shrub-steppe, open woodlands, and disturbed habitats such as hayfields, orchards, vineyards, roadsides and abandoned areas. The plant is toxic to horses and the thorny spines that surround the flower heads of C. solstitialis interfere with grazing by livestock and recreation. Moreover, yellow starthistle displaces native vegetation in grasslands and woodlands, with consequent reduction of biodiversity.

1.3.1 Larinus filiformis (Coleoptera, Curculionidae)

Larinus filiformis is a seed head weevil, occurring in Armenia, Azerbaijan, Turkey, and Bulgaria. It is univoltine and overwinters as adult. Adults feed on young buds and females lay eggs in the seed heads from mid-June to mid-July. Larvae feed inside the flower heads and destroy all seeds until they pupate. Field observations suggest that the weevil can destroy from 25 to 75% of capitula at natural field sites (depending on the sample date). Preliminary host specificity experiments on adult feeding indicate that the host range of weevil is restricted to a relatively small number of plants within the Cardueae (Gültekin et al., 2008).

Work performed in 2020 and 2021

Since 2011, BBCA is involved in studying the host range of L. filiformis. From the beginning the project was showing to be very challenging, because it is difficult to synchronize all the plant species at the correct phenological stage (flower bud), suitable for the weevil oviposition. The last open field test was performed in 2019 at the American Farm School in Thessaloniki, Greece. The experiment aimed to measure the risk to 10 non-target species of being attacked by the weevil. Unfortunately, even though at least 4 Larinus spp. were identified among the specimens collected from the flower heads of the plants exposed, none of them was morphologically and genetically identified as L. filiformis, and the experiment was not conclusive. Therefore, a new open field test was planned for the following year (2020). For logistic reasons, we moved the field garden at the Agricultural University Plovdiv, in Bulgaria. Test plants were grown from seeds at BBCA facilities, starting at the end of September 2019. At the beginning of November 2019, the plants obtained were moved in Plovdiv, transplanted in the soil and arranged in 10 columns x 12 rows (Fig. 5). At the same time, young rosettes of C. solstitialis were collected from a wild site (around 60 km Southeast of Plovdiv) and included in the field garden. However, the travel restrictions due to COVID-19 outbreak forced us to put on hold any activities and even though the field garden was maintained by our Bulgarian cooperators throughout the year, it was impossible to perform the experiment both in 2020 and 2021.

Work program proposed for 2022

Due to the long stop forced by the pandemic, no more plants remain at the experimental garden in Plovdiv. Therefore, we rescheduled the experiment for 2023, starting to grow test plants from seed in fall 2022, setting up the field garden by the beginning of the winter 2022 and performing the collection of L. filiformis adults to be released at the field garden in late spring 2023. From the end of June until beginning of August 2023, mature flower heads from test plants will be collected every week. Each capitulum will be
dissected and the insects found (larvae, pupae or adults) will be preserved in ethanol and provided first to Enzo Colonnelli for morphological identifications and then to Dr. Marie Claude Bon (ARS-USDA-EBCL, Montferrier-sur-Lez, France) for molecular identifications.

Fig. 5 – Setting up of the field garden at the Agricultural University Plovdiv, in Bulgaria, for the risk assessment of 10 nontarget species of being attacked by *Larinus filiformis*.

### 1.3.2 *Ceratapion basicorne* (Coleoptera, Curculionidae)

*Ceratapion basicorne* is a weevil native to Europe and western Asia (Alonso-Zarazaga, 1990; Wanat, 1994). It is univoltine and overwinters as adult. Adults feed on leaves of yellow starthistle rosettes and they mate soon after new adults emerge and after hibernation ends in the following spring. Eggs are laid inside leaves, and larvae tunnel down the leaf petiole and develop inside the upper root and basal stem (root crown), where they pupate. Adults emerge from the plant in early summer when it bolts. Adults feed briefly on yellow starthistle foliage then aestivate and hibernate until the following spring (Clement et al., 1989, Smith and Drew, 2006). Under controlled conditions, feeding of the weevil larvae caused up to a 23 percent reduction in size, and in a field study of naturally occurring yellow starthistle plants in Turkey, plants infested by *Ceratapion* had 15 percent lower seed fertility than uninfested plants (Uygur et al., 2005). The weevil is highly specific for the target weed, and even though it has been reported to develop occasionally on the nontarget plants in laboratory no-choice experiments (Smith, 2007), additional field experiments support the conclusion that this weevil species poses no significant risk for any nontarget species (Smith et al., 2006; Cristofaro et al., 2013).

**Work performed in 2020 and 2021**

*Ceratapion basicorne* has been approved for the release during 2019. For this reason during 2020 and 2021 we planned to return back at the site in Greece near Kilkis (40°56′10″N; 022°50′37″E; 180m) where we made an excellent collection in the middle of May 2019. Unfortunately, Europe was in full lockdown during the full spring 2020 (because the Covid-19 pandemic) and the borders re-started to be open only in July. For this reason, BBCA was not able to perform any field work during the spring 2020. We returned back at the site near Kilkis during May 2021. Unfortunately, for the second year in row there was something wrong: despite to the perfect synchronization of the dates, the phenology of YST was different from 2019: most of the plants were not at the rosette stage and the diameter of the root crown was much smaller than the size recorded in
2019. For this reason, during 2021 we recorded in the YST only one larva of *C. basicorne* inside, while in 2019 the number was ranging from 5 to 12 in each root, and they were mature larvae or pupae. In conclusion, very few adults emerged from the root material collected in 2021 and only in the first 10 days after the collection; the small infested roots with young larval instars became too hard to allow the larva to finish the life cycle and most of the weevils did not emerged. In any case, the few emerged adults were sent to the quarantine facility in Albany, where they arrived alive and in good conditions.

**Work program proposed for 2022**

To avoid the problems recorded in 2021, we will perform 2 travels in Greece during the summer of 2022, respectively at the beginning and in the middle of May. Moreover, at the end of March 2022 we will visit our Turkish cooperator Levent Gultekin, to verify with him if he has the possibility to get the permit from the Turkish authorities, in order to collect (officially) *C. basicorne* adults and send them to us. Finally, we will try to collect *C. basicorne* from 2 sites in Rome province, respectively 50 km North and 60 km South of Rome.
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