Research Activities

Annual Report

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Biotechnology Biological Control Agency
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Contents

Cover photo: Massimo Cristofaro (BBCA) setting up an experimental plot for an open field test at BBCA facilities.

About us

Biotechnology and Biological Control Agency (BBCA) is a non-profit private foundation established in 2000 in Rome, Italy. Its mission is to conduct applied scientific research on invasive species. The main research areas of BBCA include classical biological control of invasive plants and insects, screening and formulation of natural bio-insecticides, evaluation of synthetic pheromones and other novel lure traps. The BBCA approach is focused on explorations of the native areas of the invasive species to search for the origin of the invasion and collect related natural enemies; select and prioritize potential biocontrol agents and hence study their biology, evaluate their specificity, and assess their impact on the target through laboratory and field tests. More recently, BBCA has been engaged in the development of integrated management strategies for weeds and insect pests that combine classical biological control with other approaches, such as mechanical control of weeds, or Sterile Insect Technique (SIT) and mass trapping for insect pests.

The team is led by Dr Francesca Marini and includes research scientists, temporary research assistants, technicians, and MSc and PhD students. In addition, BBCA can rely on an extensive multidisciplinary network of researchers and scientists, thanks to the relationships of cooperation developed with science institutions and universities around the world.

Weeds

1. Cheatgrass (*Bromus tectorum*)

Cheatgrass (*Bromus tectorum*) is a winter annual grass of Eurasian origin, introduced to North America as a contaminant in seed and straw. It is responsible for extensive ecological and economic damage in western North America, where it is considered invasive. It forms dense stands and outcompetes native grasses. It produces large, dense fire loads of dead grass that increase the likelihood of ignition, rate of spread and the length of the wildfire season, thereby shortening the interval between recurring fires. In addition, its sharp awns can injure livestock and animal eyes and mouths.

- In cooperation with: Dr B. Rector (USDA-ARS-WRRC, Albany – CA).

In 2023, as a part of the biological control program established by USDA ARS, BBCA conducted explorations and collections in the native range of the target (i.e. Europe and Western Asia) to identify potential biological control agents against the target. Three plant pathogens, a smut and two rusts, were found in late May 2023 in Armenia and Greece, respectively. The samples collected were sent to Dr M. Tancos and Dr M. Fulcher (USDA-ARS-NEA-FD-WSR, Ft. Detrick - MD) for further studies. Regarding the arthropods, particular attention was given to the following candidate agents:

- the gall midge *Stenodiplosis tectori*, a species recently described whose adults were observed to emerge from cheatgrass seed heads (Rector *et al.*, 2022). Based on the knowledge available for the other close relative species, the midge larvae are supposed to develop and feed inside the seeds, eventually affecting their germination. Additional field observations and surveys are planned to confirm this hypothesis and collect data regarding the biology of the midge. Determining the life cycle of the midge will be crucial for planning any future research activities, such as the establishment of a colony in controlled conditions, as well as the host range testing and impact assessment. In addition, collections are planned to send individuals at the preimaginal stage to the USDA-ARS-WRRC laboratory in Albany, CA.
- a crown root galling midge. Recorded for the first time in Greece in late April 2023, its larvae potentially feed on cheatgrass stems, preventing them from growing, and hence from producing seeds. Despite the efforts, no adults emerged from the pupae collected, and the species has not been identified yet. The molecular analyses performed by Dr M-C. Bon (USDA-ARS-EBCL, France) identified some of the pupae collected as belonging to the *Mayetolia* genus. However, no match was found with any of the *Mayetolia* species for which CO1 sequences are currently available (i.e., *M. hordei*, *M. destructor* and *M. avenae*). Additional collections will be performed next season with the main goal of obtaining adults and completing the identification of the species.
- a seed feeder weevil (*Pachythychius* sp.), recorded during explorations performed in Greece. Adults are morphologically and genetically similar to *P. hordei*, a pest for barley and wheat These similarities were identified through morphological and molecular analyses conducted by Dr M. L. Chamorro (USDA-ARS, c/o Smithsonian Institution - National Museum of Natural History, Washington, DC), and Dr M-C. Bon (USDA-ARS-EBCL, France). However, the field surveys conducted in the area where the weevil was originally found on cheatgrass did not reveal its presence in wheat and barley field crops, particularly of its larvae developing in the seeds. This lack of evidence does not allow the possibility that it is a different subspecies to be ruled out. Therefore, additional collections will be

performed to morphologically and genetically compare weevils collected from wheat and/or barley and from cheatgrass, respectively.

- the eriophyid mite *Aculodes marcelli*, a species recently described and known to be present at least in Bulgaria and Serbia (Vidović *et al.*, 2022). Although cheatgrass plants infested by the mite appear otherwise healthy, the feeding activity of *A. marcelli* on the seeds could affect their germination. This hypothesis is supported by the fact that *A. marcelli* mites was observed congregating on cheatgrass seed surfaces, while generalist eriophyid mite species remain on the leaves. A preliminary host range test was performed in 2023 at the University of Belgrade, Serbia, with the support of Dr B. Vidović. Four different cheatgrass American populations (from California, Colorado, Idaho, and Utah, respectively) were exposed to the mite in open field conditions for approximately two months (i.e. from the seedling stage until plant senescence). The mite exhibited different performance on the four American populations, reaching higher densities on the cheatgrass populations originating from Idaho and Utah. Some seeds of these latter populations were tested for their germinability, and no effect on seed germination was recorded for both populations. Therefore, the potential impact of *A. marcelli* on the target remains unclear, and additional tests may be required.

The four prioritized candidate biological control agents against cheatgrass. From left to right: the seed galling midge *Stenodiplosis tectori*; pupae of the crown root galling midge *Mayetiola* sp.; the seed weevil *Pachytichius* sp.; the eriophyid mite *Aculodes marcelli*.

Additional reading:

Rector BG, Gagné RJ, Perilla López JM, Tonkel KC, Bon M-C, Guermache F, Cristofaro M (2021). Taxonomic description of *Stenodiplosis tectori* n. sp. (Diptera: Cecidomyiidae), a seed parasite of cheatgrass, *Anisantha tectorum*, based on morphological and mitochondrial DNA data. *Insects* 2021, 12(8), 755.doi:10.3390/insects12080755

Vidović B, Anđelković N, Jojić V, Cvrković T, Petanović R, Marini F, Cristofaro M, Rector BG (2022). A New *Aculodes* species (Prostigmata: Eriophyidae) described from an invasive weed by morphological, morphometric and DNA barcode analyses. *Insects* 2022, *13*, 877. doi:10.3390/insects13100877

- In cooperation with: Dr J. Lucero (Texas A&M University), Dr A. Faust (University of Montana), and Dr P. Weyl (CABI, Switzerland).

A biogeographic study has been initiated to understand the ecological processes regulating the cheatgrass invasion. The primary aim is to evaluate the influence of biotic factors (i.e. plant-fungal interactions, insect herbivory, and cattle grazing) and abiotic factors (i.e. fire and mechanical soil disturbance) on cheatgrass in its native and non-native ranges, to figure out how they contribute to its invasion. BBCA, in collaboration with CABI, is responsible for the experiments conducted in the cheatgrass native area. Three experimental fields were set up, two in northern Greece (in collaboration with the University of Western Macedonia), and one in southern Hungary (with the support of the CABI satellite station), respectively. In late spring,

plots were marked out, and soil samplings and vegetation surveys were carried out to quantify the diversity at the site in general, and within each subplot where the different factors will be considered. At the same time, cheatgrass seeds were collected from the surrounding area of each site. The seeds were then sown at the experimental fields in October, once all treatments (i.e. fire, mechanical soil disturbance, the exclusion of grazing and the treatments against insects and pathogens) were applied. The experimental fields will be maintained for three years, by applying the treatments against insects and pathogens four times per year and performing soil samplings and vegetation surveys every spring. The findings of this biogeographical study may contribute to the understanding of how to control the spread of cheatgrass by either mimicking the population controls that naturally regulate the target in its native range or by disrupting the positive feedback present in the non-native range.

Setup of the field experimental plots in cheatgrass native range. From left to right: Stefan Toepfer (CABI) marking out of plots; Philip Weyl (CABI) collecting cheatgrass seeds; Fokion Papathanasiou (University of Western Macedonia) and his students conducting the vegetation survey; Francesca Marini (BBCA) applying one of the treatments (i.e. the fire).

2. Giant reed (*Arundo donax*)

Giant reed (*Arundo donax*) is a perennial grass native to Mediterranean Europe, purposefully introduced and cultivated in many of the subtropical and warm temperate areas of the world for a variety of purpose, including ornamental, erosion control along ditches and drainage canals. Giant reed spreads vegetatively through both rhizomes and canes, making it an extremely invasive species. It replaces native vegetation and grasses, thereby altering the ecology and successional processes of riparian areas. It blocks flood control channels, and costumes water, lowering groundwater tables. Furthermore, it changes fire regimes in invaded areas.

- In cooperation with: Dr P. Moran (USDA-ARS-WRRC, Albany – CA).

Lasioptera donacis is a leaf miner midge approved for release in the USA and Mexico as a biological control agent against *A. donax* (USDA-APHIS 2016). *Lasioptera donacis*, like other midges of the genus *Lasioptera*, is believed to be sapromy-cophagous, feeding on the fungus that grows in the leaf sheath galleries in a symbiotic association with reed and larvae. Indeed, the larval infestation of the midge in its native range is always associated with the presence of the saprophytic fungus *Arthrinium arundinis*, which is believed to provide a trophic resource for the larvae in the Arundo leaf, and hence indispensable for the establishment of the midge. In 2013, BBCA provided support to the USDA-ARS-WRRC in Albany, CA, where studies about the larval-fungal interaction are underway. This support included the collections and shipments of *L. donacis* larvae and pupae at the quarantine in Albany. Additionally, BBCA contributed to the development of a method to confine single midge females on Arundo canes to obtain single oviposition, useful to conduct the studies mentioned above.

Method for the single oviposition of the leaf miner *Lasioptera donacis*. From left to right: Francesca Di Cristina (BBCA) setting up the single female confining cages on giant reed at the BBCA facilities; detail of the confining cage; female of *L. donacis* ovipositing on giant reed; larvae of *L. donacis* in Arundo leaf sheath galleries obtained from a single female.

3. Ice plants (*Mesambryanthemum* spp.)

The two annual ice plant species, *Mesembryanthemum crystallinum* and *M. nodiflorum* are geographically native to the southern part of the African continent (mainly South Africa and Namibia). They can also be found in arid coastal habitats of the South Mediterranean, Morocco and the Canary Islands, where their status as indigenous plants is disputed. Both species are considered serious weeds in California and Mexico, where they form dense monocultures that negatively impact native biodiversity, leading to biodiversity loss and alteration ecosystem functioning. *Mesembryanthemum crystallinum* is a salt accumulator, meaning it accumulates salt in its bladder cells, which is then leached into the topsoil after plant death. This increased salinity promotes the establishment of halophyte species such as ice plants, while prevents the growth of those of the other less salt-tolerant species. Similar mechanisms and ecosystem results are also assumed for *M. nodiflorum*.

- In cooperation with: Dr P. Moran (USDA-ARS-WRRC, Albany – CA).

In 2023, BBCA conducted field surveys with the main goal of identifying potential biological control agents for these target weeds. The survey area was selected based on the results of the genetic analyses performed on several populations collected by BBCA in the Mediterranean and Canary Islands over the past couple of years. These analyses identified a close genetic match between the Canarias and Moroccan populations and the target weeds in California. Three insect species were found in association with the target weeds in the Canary Islands and along the Atlantic coast of Morocco (at the same latitude). In particular, two root feeding weevils, *Temnorhinus mixtus* and *T. brevirostris*, were found on *M. nodiflorum* in Morocco and Fuerteventura Island, while an undetermined flower feeding caterpillar was recorded on *M. crystallinum* in Tenerife Island. In addition, a large population of *Ammocleonus hieroglyphicus*, a broad nose weevil, was found on *M. crystallinum* near Alexandria (Egypt). Further field observations will be conducted in 2024, both in Morocco and the Canary Islands, and official cooperation with the Canary Islands Plant Health Institute will be established.

Potential biological control agents against ice plants. From left to right: adult and pupal case of the root boring weevil *Temnhorinus mixtus* on *Mesembryanthemum nodiflorum*; a flower feeding noctuid moth and the broad-nose weevil *Ammocleonus hieroglyphicus* on *M. crystallinum*.

4. Medusahead (*Taeniatherum caput-medusae*)

Medusahead (*Taeniatherum caput-medusae*) is an annual grass of Eurasian origin, considered invasive in the western United States. The invasion of medusahead is associated with a number of adverse effects on wildlife habitat and rangeland quality, including a reduction in biodiversity, and threats to ecosystem functioning. Moreover, this weed poses a major threat to the value of grazing lands, contributing to the acceleration of fire cycles over vast areas by providing tinder to ignite and carry fire to larger plant species.

- In cooperation with: Dr B. Rector (USDA-ARS-WRRC, Albany – CA).

Aculodes altamurgiensis is an eriophyid mite species occurring on medusahead (De Lillo *et al.*, 2018) on which BBCA has been worked in close cooperation with Dr B. Vidović (University of Belgrade, Serbia) since its discovery. In nature, the mite has never been recorded in association with other sympatric grass species. and its specificity for medusahead was confirmed by a preliminary host range test, which also pointed out that *A. altamurgensis* exhibited different performance (i.e. the ability to reach higher population density) on medusahead plants with different origins (Cristofaro *et al.*,2020). In natural conditions, the mite was found on medusahead plants at all stages of growth, and it was observed to congregate on and in the seeds when the plants started to senesce. Although infested medusahead plants do not show any symptom referable to the presence of the mite, these observations suggest a possible impact of *A. altamurgensis* on medusahead seeds. Therefore, an impact assessment of *A. altamurgensis* on medusahead will be conducted, with a particular focus on plants with different American origins. The test will allow to compare the germination rate of the seeds obtained from medusahead plants exposed to the mite with those kept mite-free. The experiment will be conducted under controlled conditions to exclude the effect of any other eriophyid mite species on test plants (i.e. plants exposed to *A. altamurgensis*) which could be present under open field conditions, and to avoid the use of acaricide on control plants (i.e. kept mite-free), which could affect their fitness.

Collections of the eriophyid mite *Aculodes altamurgensis* in Italy and Serbia. From left to right: Francesca Di Cristina (BBCA), Enrico de Lillo (University of Bari) and Francesca Marini (BBCA), Dragica Smiljanić and Biljana Vidović (University of Belgrade).

Additional reading:

de Lillo E, Vidović B, Petanović R, Cristofaro M, Marini F, Augé M, Cvrković T, Babić E, Mattia C, Lotfollahi R, Brian GR (2018). A new *Aculodes* species (Prostigmata: Eriophyoidea: Eriophyidae) associated with medusahead, *Taeniatherum caput-medusae* (L.) Nevski (Poaceae). *Systematic and Applied Acarology*, 23(7):1217-1226. doi:10.11158/saa.23.7.1

Cristofaro M, Roselli G, Marini E, de Lillo E, Petanović R, Vidović B, Augé M, Rector BG (2020). Open field evaluation of *Aculodes altamurgensis*, a recently described eriophyid species associated with medusahead (*Taeniatherum caput-medusae*). *Biocontrol Science and Technology*, *30*(4), 339–350. doi:10.1080/09583157.2019.1711021

- In cooperation with: Dr R. Sforza, Dr J. Kashefi and Dr M-C. Bon (USDA-ARS-EBCL, France).

During field explorations conducted in Greece in 2017, the new phytophagous eurytomid *Tetramesa amica* was recorded on medusahead (Lotfalizadeh *et al*., 2020). Adults are phytophagous and lay eggs into medusahead stems. Oviposition and larval development induce a plant response, which produces stem galls from which adults emerge. *Tetramesa* species are typically species-specific gall-inducers, and they may significantly impact their host plants by reducing the flowering head production and the seed weight. To date, the wasp has been found at low density and in a very restricted area in North-eastern Greece. Therefore, additional explorations will be conducted to determine the natural distribution of *T. amica* in the Western Palearctic region and identify the areas where it is more abundant. Moreover, given the current state of knowledge about the wasp, further studies are needed to characterize its biological traits, including the duration of immature stages, number of generations, fecundity, and longevity of adults, as well as host specificity.

From left to right: *Tetramesa amica* and its galls on a stem of meduasahead; Francesca Marini (BBCA) and Javid Kashefi (USDA-ARS-ECBL) conducting field collections and observations in North-eastern Greece.

Additional reading:

Lotfalizadeh H, Rasplus J-Y, Cristofaro M, Marini F (2020). *Tetramesa amica* and its parasitoid *Eurytoma amicophaga* (Hymenoptera, Eurytomidae): two new species associated with medusahead, *Taeniatherum caput-medusae* (Poaceae). *ZooKeys* 1005: 133–149. doi:10.3897/zookeys.1005.56353

5. Rusk skeletonweed (*Chondrilla juncea*)

Rush skeletonweed (*Chondrilla juncea*) is a perennial spindly weed of the family Asteraceae, native to Eurasia and the Mediterranean regions. It was introduced to the USA where it has become a significant invasive species due to its great potential to spread rapidly and form dense infestations given favorable conditions. To date, three distinct genotypes have been identified in the USA. Each genotype differs slightly in the appearance of the rosette leaves and branching, as well as in its resistance to biological control agents, the effectiveness of which depends also on local conditions. Therefore, a biological monitoring program is still ongoing to select additional candidates.

- In cooperation with: Dr P. Pratt (USDA-ARS-WRRC, Albany – CA).

In 2023, BBCA performed explorations and collections in the native range of the target (i.e. Europe and Western Asia) with the goal of identifying potential biological control agents against the target.
The rust fungus *Puccinia chondrillina*, a plant pathogen successfully released for the biological control of rush skeletonweed in North America, was collected in early June in Bulgaria and sent to Dr M. Tancos and Dr M. Fulcher (USDA-ARS-FD-WSR, Ft. Detrick - MD) who are investigating whether there is any European population of *P. chondrillina* that is more aggressive to the American rush skeletonweed genotypes and less susceptible to the rust strain already released. Additional collections will be eventually performed next year to expand the range of rust populations screened.
For the arthropods, explorations were conducted in Armenia, in close cooperation with Dr M. Kalashian (National Academy of Sciences of Armenia, Yerevan, Armenia), and Dr M. Volkovitsh and Dr M. Dolgovskaya (Russian Academy of Sciences, St. Petersburg, Russia). Special emphasis was given to the following candidate agents:

- an undetermined sawfly. Sawfly larvae were found and collected in Armenia in July 2023. Despite the efforts, none of the larvae collected survived up to the adult stage, and the species has not yet been identified. However, molecular analyses on some of the larvae collected are currently underway.
- the seed feeder moth *Schinia cognata*. Larvae and adults were collected in Armenia in July 2023, and kept alive at the facilities of the Russian Academy of Sciences (St. Petersburg, Russia) for the establishment of a colony. Nevertheless, despite the majority of the larvae reaching the adult stage, none of the adults, both those collected in the field and those obtained from the larvae, mated, or laid eggs, and they died by the end of August.

Additional collections for both candidates will be carried out next year, with a focus on Greece, where both species occur and the climatic conditions a more similar to those in California. The mail goals will be to complete the identification of the sawfly species and perform some preliminary host range testing to prioritize the potential agents available. Potentially, collections and shipments at the USDA-ARS-WRRC laboratory in Albany, CA, of both insects will be organized.
Finally, collections of the crown moth, *Oporopsamma wertheimsteini*, were performed in the Gorovan Sands desert in Central Armenia. The root cases with pupae were sent to Montana State University (Bozeman, MT), where Dr J. Littlefield is currently conducting host range testing to evaluate the potential of the moth as biological control agent for rush skeletonweed.

From left to right: Margarita Dolgovskaya (Russian Academy of Sciences) and Mark Kalashian (National Academy of Sciences of Armenia) performing collections of potential biological control agents of rush skeletonweed in central Armenia; *Schinia cognata* larva and adult on rush skeletonweed flowers; a sawfly larva on a rush skeletonweed leaf.

6. Russian thistle (*Salsola tragus*)

Russian thistle (*Salsola tragus*) is an invasive annual forb in the western USA native to Central Asia. It has been targeted for classical biological control in the USA, and two moths have been already introduced (*Coleophora klimeschiella* and *C. parthenica*). Although both species have been established, widespread control of Russian thistle remains incomplete.

- In cooperation with: Dr B. Rector (USDA ARS-WRRC, Albany – CA).

In the past years, BBCA has worked in close cooperation with Dr L. Smith (USDA-ARS-WRRC, Albany – CA) on the evaluation of the eriophyid mite *Aceria salsola* as biological control agent for Russian thistle (Smith *et al*., 2009; Marini *et al*., 2021). A petition for the release of the mite has been submitted and it is expected to be approved in 2024. In expectation of the approval, BBCA will monitor the field sites where the mite is known to occur naturally to select the best sites from which to collect the mite for shipment to the USDA ARS-WRRC in Albany, CA, which will be responsible for the eventual release of the agent.
During the explorations conducted in 2023 in the native range of the target, BBCA recorded the presence of weevil larvae on Russian thistle roots. The larvae appear to be gregarious and form a sand cocoon on the root surface on which they feed, resulting in weak and stunted plants. Molecular analyses performed by Dr M-C. Bon (USDA-ARS-EBCL, France) on some larvae and adults collected in Armenia, revealed two different weevil species, possibly belonging to the same genera. Unfortunately, neither of them matches any of the species for which sequences are available. Morphological identifications of the adults are currently ongoing. Due to the potential negative impact of the larvae on the target, it is crucial to clarify the identity of these two weevil species to prioritize them as potential candidates for Russian thistle biological control.

From left to right: the impact the eriophyid mite *Aceria salsoale* on Russian thistle; Enrico de Lillo (University of Bari), Lincoln Smith (USDA-ARS- WRRC), Alessandra Paolini and Francesca Di Cristina (BBCA) at the experimental field set up for the host specificity testing of *A. salsolae*; Silvia Barlattani (BBCA) inoculating eriophyid mites on a test plant.

Additional reading:

Smith L, Cristofaro M, de Lillo E, Monfreda R, Paolini A (2009). Field assessment of host plant specificity and potential effectiveness of a prospective biological control agent, *Aceria salsolae*, of Russian thistle, *Salsola tragus*. *Biological Control* 48, 237–243. doi:10.1016/j.biocontrol.2008.11.007

Marini F, Vidović B, Lonis S, Wibawa MI, de Lillo E, Kashefi J, Cristofaro M, Smith L (2021). Comparison of the performance of an eriophyid mite, *Aceria salsolae*, on nontarget plants in the laboratory and in the field. *Biological Control*, 152, 50 104455. doi:10.1016/j.biocontrol.2020.104455

7. Sahara mustard (*Brassica tournefortii*)

Sahara mustard (*Brassica tournefortii*) is a biannual plant species of the Brassicaeae family, well adapted to very dry climates, such as deserts and semi-desert ecotypes. It was introduced to the USA and Australia, where it has had negative impacts on agricultural and protected native systems over the past century. The species has continued to expand its range in the USA and Australia, and it has recently become invasive in Chile, New Zealand, South Africa, and South Korea.

- In cooperation with: Dr R. Sforza, Dr M-C. Bon and Dr J. Kashefi (USDA-ARS-EBCL, France).

In 2023, BBCA supported the USDA-ARS-EBCL in Montpellier, France, in the field survey conducted in the northern part of Egypt with the main goal of searching for potential biological control agents for the target. The survey area was selected based on the results of the genetic analyses performed of several Sahara mustard populations, collected in the past years by BBCA and cooperators in the native and invasive range of the target weed. In particular, these analyses indicated that the origin of the Sahara mustard invasion in the USA should be sought in Morocco and northern Egypt (Winkler *et al*., in press).
Two weevils, *Rhytideres plicatus* and *Ceutorhyncus pallidactylus*, were found during the survey conducted in early March 2023. Both weevils were observed attacking the root system of the plants, as external feeder and root crown borer, respectively. Due to the potential of both candidates, additional surveys were planned for 2024. However, the political situation in the region collapsed in the fall of 2023, forcing to postpone any travel until the region is deemed safe again.

From left to right: René Sforza (USDA-ARS-EBCL) and Francesca Di Cristina (BBCA) conducting field surveys to search for potential biological control agents in native range of the Sahara mustard; root crown boring larvae of the weevil *Ceutorhyncus pallidactylus* on Sahara mustard in North Egypt.

Additional reading:

Winkler DE, Amer WM, Augé M, Bon M-C, Chapin KJ, Cristofaro M, François O, Garmon JD, Gaut BS, Kashefi J, Marini F, Sforza RFH, Smith L, Huxman TE (2024). Human-mediated dispersal routes structure Sahara mustard genomics via date palm agriculture. *Molecular Ecology*. In press

8. Stinkwort (*Dittrichia graveolens*)

Dittrichia graveolens, also known as stinkwort, is an annual plant species of the Asteraceae family, native to the Mediterranean basin, and considered invasive in arid habitats of the West of the USA. It is a very late species, flowering in the late summer, or even in the fall. Its seeds are long-lived and can be dispersed by wind, or accidentally carried by animals and vehicles. The plant is not palatable to animals and can be poisonous to livestock. It can also cause allergic contact dermatitis in humans.

- In cooperation with: Dr P. Moran (USDA-ARS-WRRC, Albany – CA); Dr R. Sforza, Dr. M.C. Bon and Dr J. Kashefi (USDA-ARS-EBCL, France).

Preliminary opportunistic field surveys revealed a broad community of insects, mainly noctuid moths, associated with the target weed. The richest biodiversity was recorded by Dr R. Sforza (USDA-ARS-EBCL, France) in Cyprus. Although some of the insects collected have not yet been identified, most of them should be considered oligophagous. Additional surveys will be conducted in the fall of 2024, in the area of Cyprus and in the southeastern Mediterranean basin, to complete the screening of the entomofauna associated with the target and identify any potential biological control agents.

From left to right: René Sforza (USDA-ARC-EBCL) conducting field surveys on stinkwort in Cyprus; cardboard tube containing material collected in Cyprus; a noctuid moth larva feeding on the target weed; leaf and flower feeder noctuid larvae on stinkwort in Cyprus.

9. Tree of heaven (*Ailanthus altissima*)

Tree of heaven (*Ailanthus altissima*) is a tree native to China and considered a serious weed wherever it occurs outside of its native range. In addition to the direct impacts, it hosts invasive pest species, such as the brown marmorated stink bug (*Halyomorpha halys*) and the spotted lanternfly (*Lycorma delicatula*). Due to the limited effectiveness of chemical and mechanical methods, a biological control program was initiated in the early 2000s. However, to date, no biological control agents have been released for Tree of heaven in North America or elsewhere.

- In cooperation with: Dr R. Sforza, Dr M.C. Bon and Dr J. Kashefi (USDA-ARS-EBCL, France), Dr S. Stutz (CABI, Switzerland), Dr B. Vidović (University of Belgrade, Serbia).

During the surveys conducted as part of the biological control program for *Ailanthus altissima*, an eriophyid mite, *Aculus taihangensis*, was found to be infesting Tree of heaven in both Europe and the USA (de Lillo *et al*., 2017 and 2022; Marini *et al*., 2023). Since 2016, field observations, impact and host range tests have been conducted with promising results. Histological observations and impact tests showed that the feeding activity of the mite damaged Tree of heaven leaves, reducing the fitness of new sprouts, and severely damaging seedlings, reducing their biomass by about 80%. The host range of *A. taihangensis* has been explored, and so far 18 nontarget plant species, selected either for their phylogenetic and ecological similarity to the target weed or for their economic importance, were tested under open field conditions and none of them appear to be potential hosts of this mite (Marini *et al*., 2021). In 2023, additional screening of North American test plant species was conducted under controlled conditions. Seedlings of non-target species were grown from seed and exposed to the mite for six weeks, then harvested, and the mites extracted. Thousands of mites were found on the Tree of heaven seedlings, while far fewer mites, if any, were found on the nontarget species. Symptoms of the mites (i.e. deformed leaves) were observed on all Tree of heaven seedlings, while no obvious damage was observed on any of the nontarget species exposed to the mites throughout the whole experimental period. Preliminary data are encouraging and support *A. taihangensis* as a promising candidate for the biological control of Tree of heaven, with the potential to contribute to an effective integrated management strategy with long-term benefits in the invasive range. Host specificity testing of *A. taihangensis* will continue in 2024 by conducting laboratory and open field tests with native North American test plant species. In addition, North American Tree of heaven populations will be also included in these tests to verify if they are suitable hosts for the mite.

From left to right: Silvia Barlattani (BBCA) evaluating an impact experiment set up with the mite *Aculus taihangensis*; the impact of the mite on Tree of heaven; experimental field for testing the host specificity of *A. taihangensis*.

Additional reading:

de Lillo E, Panzarino O, Loverre P, Valenzano D, Mattia C, Marini F, Augé M, Cristofaro M (2017). New eriophyoid mites from Italy. IV. Mites associated with weed plants. *Systematic and Applied Acarology* 22, 2256–2272. doi:10.11158/saa.22.12.15

Marini F, Profeta E, Vidović B, Petanović R, de Lillo E, Weyl P, Hinz HL, Moffat CE, Bon M-C, Cvrković T, Kashefi J, Sforza RF, Cristofaro M (2021). Field assessment of the host range of *Aculus mosoniensis* (Acari: Eriophyidae), a biological control agent of the tree of heaven (*Ailanthus altissima*). *Insects* 12, 637. doi:10.3390/insects12070637

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10. Yellow starthistle (*Centaurea solstitialis*)

Yellow starthistle (*Centaurea solstitialis*) is an early maturing winter annual species. Native to Eurasia, it was introduced to North America as a seed contaminant. It has persistently spread across the country, and it is now listed as one of the most costly invasive weeds in western North America. A biological control program for yellow starthistle has been underway since the late 1960's, and six insects and one rust pathogen have been already released in the USA. However, although most of them have become established, the widespread control of *C. solstitialis* remains incomplete.

- In cooperation with: Dr B. Rector (USDA-ARS-WRRC, Albany – CA).

Since many years, BBCA has worked in close cooperation with Dr L. Smith (USDA-ARS-WRRC, Albany – CA) and has supported the biological control program for yellow starthistle by performing explorations and collections of potential biological control agents in the native area of the target, and conducting host range testing under open field conditions to evaluate them for their specificity for the target. In 2013, BBCA focused its efforts on the seedhead weevil *Larinus filiformis*, whose adults feed on young yellow starthistle buds and females lay eggs into the seedheads. Larvae feed inside the flower heads and destroy all seeds until they pupate.
In late June 2023, weevil adults were collected from two different locations in Bulgaria and sent to the quarantine facilities of the USDA-ARS-WRRC in Albany, CA, where a weevil colony is maintained, and host specificity testing is ongoing under controlled conditions. In addition, an open field host range test was planned to be conducted in Bulgaria at the facilities of the University of Plovdiv, with the support of Dr V. Harizanova and Dr A. Stoeva. The aim was to assess the risk of ten non-target species of being attacked by *L. filiformis*. Some of these test species need to be at least two years old and/or overwinter at very low temperatures to be able to bloom next spring. For these reasons, they were germinated in the fall of 2022 and allowed to grow under natural conditions until the following year. Annual nontarget species were then germinated at the end of the winter in 2023, and the experimental field was setup in early spring. However, most of the plants did not grow enough to flower and the experiment could not be performed, because no flowers would be available to be exposed to the weevil. Nevertheless, host range testing has been continued at the USDA-ARS-WRRC quarantine in Albany, CA, with very promising results, confirming the specificity of this weevil for the target weed.

Setup of the experimental field for the host specificity testing of the weevil *Larinus filiformis* in Plovdid, Bulgaria. From the left to the right: Francesca Di Cristina (BBCA) and Vili Harizanova (University of Plovdiv), Francesca Marini (BBCA), and Massimo Cristofaro (BBCA).

Insect pests

1. Bagrada bug (*Bagrada hilaris*)

Bagrada hilaris, commonly known as the bagrada bug, is an invasive insect pest, native to Africa, India, Pakistan, and parts of Asia. It was first reported in California (USA) in 2008, and subsequently spread in southwestern USA, Hawaii, Mexico, Chile, and Argentina. Additionally, this pest has also been reported in southern Europe (Malta and the Island of Pantelleria in Italy). Although *B. hilaris* has a wide range of hosts, it primarily feeds on crucifers. To date, there are no mitigation programs in place that do not involve pesticides. Therefore, much attention has been recently paid to identify more sustainable and effective control strategies. In this context, several egg parasitoids, belonging to the genera *Trissolcus*, *Gryon*, and *Ooencyrtus*, are currently under consideration as potential agents in classical biological control programs against bagrada bugs.

- In cooperation with: Dr R. Sforza and Dr M-C. Bon (USDA-ARS-EBCL, France); Dr. A. Cemmi (ENEA-Casaccia, Italy).

Classical biological control strategies for managing pentatomid pests are focused on the use of egg parasitoids. Egg parasitoids, both in the native and invasive range of the pest species, are detected, collected, and monitored by using sentinel eggs. This involves exposing egg masses of the target pest in the field for a few days, which can be parasitized by the egg parasitoids. The two main types of sentinel eggs used are newly oviposited or frozen eggs, which persist in the field for 72 hours and 3-4 days, respectively. The brief exposure period is not the only limiting factor in the use of sentinel eggs. Indeed, for example, manipulated eggs are less attractive. On the other hand, the use of live fertilized sentinel eggs of the alien species in a newly invaded area may result in the unintentional release of additional pests. In this context, BBCA has focused its research activities on evaluating the feasibility of applying irradiation techniques in support of classic biological control to manage *B. hilaris*. In particular, the effects of irradiation on the biology (i.e., fertility, longevity, and mating behavior) of bagrada bug males were evaluated with respect to unirradiated males. The results obtained enabled the identification of the appropriate dose to obtain sterilized eggs (Cristofaro *et al.*, 2022). The next step will be to evaluate whether sterile eggs of *B. hilaris* would be a suitable substrate for the oviposition and larval development of one of the egg parasitoids already identified, *Gryon aetherium*.

From left to right: Francesca Di Cristina (BBCA) collection of bagrada bugs in Pantelleria Island, Italy; aggregated bagrada bugs on their host; tube full of bagrada bug collected in Pantelleria Island; Chiara Elvira Mainardi and Chiara Peccerillo (BBCA) conducting laboratory bioassays at the quarantine of the USDA-ARS-EBCL, France.

Additional reading:

Cristofaro M, Sforza RFH, Roselli G, Paolini A, Cemmi A, Musmeci S, Anfora G, Mazzoni V, Grodowitz M (2022). Effects of gamma irradiation on the fecundity, fertility, and longevity of the invasive stink bug pest *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae). *Insects* 2022, 13, 787. doi:10.3390/insects13090787

2. Brown marmorated stink bug (*Halyomorpha halys*)

The brown marmorated stink bug (BMSB), *Halyomorpha halys*, is an invasive phytophagous species native to Eastern Asia. Due to its wide host plant range, it is considered among the most harmful agricultural pests in the USA and Europe. In addition, it is considered a nuisance insect in urban areas, where it invades buildings, especially in search of shelters for overwintering, and emits an unpleasant smell if disturbed. To date, the management of *H. halys* relies mainly on chemical insecticides, particularly pyrethroids and neonicotinoids. However, due to the increasing demand for limiting the use of broad-spectrum insecticides, more environmentally friendly solutions, and less hazardous techniques in integrated pest management (IPM) are currently being developed.

- In cooperation with: Dr K. Hoelmer (USDA-ARS-BIIRU, Newark, DE); Dr M-C. Bon (USDA-ARS-EBCL, France); Dr G. Anfora and Dr G. Roselli (University of Trento, Italy).

Egg parasitoids have been successfully used as inundative or augmentative biological control agents against a wide range of pests. *Trissolcus japonicus* is a small scelionid egg parasitoid wasp that oviposits and completes its larval development in a single egg of BMSB. The presence of adventive populations of *T. japonicus* has been documented in both the USA and Europe. The research activities of BBCA were focused on evaluating the performance of different BMSB sterile eggs as a suitable substrate for the oviposition and larval development of the egg parasitoid *T. japonicus*. The identification of the best method for sterilizing the BMSB eggs will improve their use as sentinel eggs for monitoring the dispersal of *T. japonicus*, and will promote their use as a suitable oviposition substrate for the parasitoid multiplication. The sterile eggs evaluated were obtained by three methods: refrigeration, mating sterile irradiated males and fertile females (SIT eggs), and direct irradiation. The results obtained showed that SIT eggs are a suitable oviposition substrate for *T. japonicus*, supporting the potential use of the irradiation techniques in combination with classical biological control for the management of BMSB (Roselli *et al.*, 2023). The objective for 2024 will be to assess the suitability of BMSB SIT eggs as a substrate for oviposition and larval development of *T. japonicus* in choice conditions.

From left to right: Gerardo Roselli (University of Trento) checking *Halyomorpha halys* rearing at the facilities of FEM in Trento, Italy; *H. halys* adults in the laboratory cage; sterile *H. halys* eggs; *T. japonicus* female emerging from an egg of *H. halys*.

Additional reading:

Roselli G, Anfora G, Sasso R, Zapponi L, Musmeci S, Cemmi A, Suckling DM, Hoelmer KA, Ioriatti C, Cristofaro M (2023). Combining irradiation and biological control against brown marmorated stink bug: are sterile eggs a suitable substrate for the egg parasitoid *Trissolcus japonicus*? *Insects* 2023, *14*, 654. doi: 10.3390/insects14070654

3. Cattle fever tick (*Rhipicephalus* spp.)

Cattle fever ticks (CFT), *Rhipicephalus* (*Boophilus*) *annulatus* and *R.* (*B.*) *microplus*, pose a significant threat to the economic security of the USA cattle industry as vectors of *Babesia bigemina* and *B. bovis*, as well as *Anaplasma marginale*, which cause bovine babesiosis and anaplasmosis, respectively. The Cattle Fever Tick Eradication Program (CFTEP), established in 1906 between the federal government and affected states, was successful in eradicating these tick species in the USA by 1943, and consequently, the threat posed by bovine babesiosis. However, CFT and bovine babesiosis remain endemic in neighboring Mexico and they represent a continuous threat to the USA livestock industry. To mitigate this risk, the CFTEP operates a permanent quarantine zone in south Texas along the border with Mexico.

- In cooperation with: Dr J. Goolsby and Dr L. Maestas (USDA-ARS-CFTRU, Edinburg, TX), Dr J Kashefi and Dr M-C. Bon (USDA-ARS-EBCL, France), Dr M. Piscitelli (ENEA-Casaccia, Italy).

Amongst the natural enemies of ticks, some parasitoids, such as wasps, have been well studied. For example, *Ixodiphagus hookeri* is a well-known parasitoid of ticks. These wasps reproduce sexually, and their females mate with the males immediately after emerging. Gravid females oviposit their eggs inside the ticks, and wasp larvae consume the entire internal of the engorged ticks. Adults emerge from the tick hosts 30–57 days after the oviposition. This wasp was first described as parasitizing *Rhipicephalus sanguineus* in Texas, as well as other species belonging to the genera *Dermacentor*, *Amblyomma*, *Hyalomma*, Haemaphysalis and *Ixodes*. Since 2022, BBCA has been involved in performing field collections of different physiological stages of different tick species, with the main goal of identifying any potential parasitoids. Once collected, the ticks were confined on mice at the ENEA veterinary facilities to allow them to fully engorge, and then kept under controlled conditions for monitoring the potential emergence of parasitoids. Unfortunately, so far, the project has been unsuccessful, but additional field collections will be conducted in 2024. At the same time, the use of different approaches is currently under consideration.

From left to right: Franca Di Cristina (BBCA) collecting ticks using dry-ice traps; ticks collected; Marta Piscitelli (ENEA) at the ENEA veterinary facilities.

4. Spotted wing drosophila (*Drosophila suzukii*)

The spotted wing Drosophila, *Drosophila suzukii*, is an invasive and polyphagous pest of Eastern Asian origin that attacks several commercial soft-skinned fruits and berries in Europe and the Americas. Females lay eggs into ripe soft-skinned fruits/berries, and larvae feed on the fruit flesh, causing damage and promoting the infection of microorganisms, which results in reduced fruit quality and economic losses. The fruits are infested close to the harvesting time, making the control with broad-spectrum insecticides difficult. Therefore, several approaches have been proposed to manage *D. suzukii* infestations, including biological and cultural control, the use of biorational pesticides, and mating disruption. However, most of these methods appear to be partially effective and/or unsustainable.

- In cooperation with: Dr D. Porretta (University La Sapienza, Italy), Dr G. Anfora and Dr G. Roselli (University of Trento, Italy).

In collaboration with the University of Rome, BBCA has explored the potential use of satyrization as a tool to control the abundance of *D. suzukii*. Satyrization is a form of sexual interaction between males of one species with females of another species, which induces fitness costs in one or both interacting species, resulting in a drastic alteration of the population dynamics. A series of experiments conducted with males of *D. melanogaster* (i.e. courtship tests, spermathecae analysis, and multiple-choice experiments) demonstrated that *D. melanogaster* males were capable of courting and mating with *D. suzukii* females, resulting in a reduction of their offspring. Moreover, a reduction in the total courtship time of *D. suzukii* males was observed in the presence of *D. melanogaster* males (Cerasti *et al*., 2023). These findings are encouraging for the potential application of satyrization as a control method for *D. suzukii*. This approach could be applied both alone and in combination with other area-wide control strategies. Next step will be to assess the suitability of a cross-specific Sterile Insect Technique (SIT) program against spotted-wing drosophila, which combines satyrization with the SIT, which is a species-specific and environmentally friendly pest control method. SIT is based on the mass rearing, sterilization, and inundative releases of sterile insects (generally males) of the same pest species. In this case, it would be *D. melanogaster* males that would be sterilized by irradiation, thus solving the issues of applying SIT directly on *D. suzukii*. Indeed, *D. suzukii* males require a high irradiation dose to induce sperm sterility, which has significant negative effect on their fitness and competitive ability.

Additional reading:

Cerasti F, Mastrantonio V, Dallai R, Cristofaro M, Porretta D (2023). Applying satyrization to insect pest control: the case of the spotted wing Drosophila, *Drosophila suzukii* Matsumura. *Insects* 2023, 14, 569. doi:10.3390/insects14060569

5. Red palm weevil (*Rhynchophorus ferrugineus*)

The red palm weevil (RPW), *Rhynchophorus ferrugineus*, is a weevil that feeds on various palm species within the Arecaceae family. Originally from Southeast Asia, it has rapidly spread to the Middle East and, subsequently, to the Mediterranean basin, Australia, China, Japan, California, and more recently to the Caribbean, where it has become a serious pest. The control of RPW is particularly challenging due to the biology of this pest species. Indeed, its life cycle does not allow for early detection of the damage caused by the endophagous larvae, which live and feed exclusively inside the palm. By the time the first symptoms of the attack are visible, such as the loss of the vegetative apex and the umbrella-like bearing of the leaves, the infestation may be well-established, making it more difficult to control.

- In cooperation with: Dr A. Cemmi (ENEA-Casaccia, Italy), Dr S. Anelli and Dr A. Biddittu (Parco Nazionale Isola di Pantelleria, Trapani, Italy)

Since 2013, the RPW has been a target of BBCA research activities, with the main goal of developing more effective management strategies based on a territorial approach. The Sterile Insect Technique (SIT) is a species-specific and environmental-friendly pest control method, based on the mass rearing, sterilization, and inundative releases of sterile insects (generally males) of the same pest species. This approach could enhance the probability of successful RPW control in those areas that are physically and ecologically isolated, especially if combined with other strategies, applied in an area-wide context. The results of the screening conducted by BBCA on the effects of irradiation doses on the biology of RPW, as well as on the after-mating sperm competition, pointed out that RPW females consistently select the sperm of the last male with whom they mated before ovipositing (Musmeci *et al.*, 2018). In 2023, a study was conducted to investigate whether wild RPW sterile males (irradiated at different doses) could sexually compete with wild-type non-irradiated males (previously captured using field traps). Laboratory bioassays under both no-choice and choice conditions assessed sexual performance in terms of number of copulations, duration of mating, and time elapsed until the first mating. The results showed that irradiation does not negatively affect the mating performance of sterile males, thereby confirming their ability to compete successfully with non-irradiated males in both no-choice and choice conditions (Cristofaro *et al.*, 2023). In 2024, the research activities will be focused on determining the optimum overflooding ratio to be applied in open field conditions. In particular, laboratory and semi-field confined bioassays will be conducted to evaluate the optimal number of sterile males to be released to suppress a red palm weevil population in a selected isolated territory.

From left to right: field trap used for the collection of red palm weevil wild males; wild males confined in a petri dish to be sterilized by irradiation; adult female of red palm weevil.

Additional reading:

Musmeci S, Belvedere S, Sasso R, Arnone S, Cristofaro M, Nobili P, La Marca A, De Biase A (2018). Last-male sperm precedence in *Rhynchophorus ferrugineus* (Olivier): observations in laboratory mating experiments with irradiated males. *Bulletin of Entomological* 2018, 108, 93–100. doi: 10.1017/S0007485317000840

Cristofaro M, Fornari C, Mariani F, Cemmi A, Guedj M, Ben Jamaa ML, Msaad Guerfali M, Tabone E, Castellana R, Sasso R, Musmeci S (2023). Effects of irradiation on mating behavior of red palm weevil, *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthoridae). *Insects* 2023, 14, 661. doi:10.3390/insects14070661

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