1. **Project Title:** Development of a New Biological Control Agent for Yellow Starthistle

2. **Principal Investigator:** Lincoln Smith, USDA, Agricultural Research Service, 800 Buchanan Street, Albany, CA 94710, Tel. 510-559-6185, fax 510-559-5737, link.smith@ars.usda.gov

3. **Cooperators and Other Participating Institutions:**
Levent Gültekin, Plant Protection Department, Faculty of Agriculture, Atatürk University, 25240 TR Erzurum, Tel.: +90.442.231.2095, lgultekin@gmail.com
Massimo Cristofaro, Biotechnology and Biological Control Agency (BBCA), Via del Bosco 10, 00060 Sacrofano (Rome), Italy; and ENEA C.R. Casaccia BIOTEC-SIC [Institute of New Technology for Energy and the Environment], Via Anguillarese 301, 00123 S. Maria di Galeria (Rome), Italy, Tel. +39-(0)338-909-3537, massimo.cristofaro.cas@enea.it

4. **Amount Requested, Project Leveraging:**

| | Requested | ARS provides | Atatürk U. provides | BBCA/ENEA provides |
|---|---|---|---|---|
| year 1 | **39,496** | 88,511 | 14,994 | 22,500 |
| year 2 | **35,051** | 139,661 | 14,994 | 22,500 |
| Total | **74,547** | 228,172 | 29,988 | 45,000 |
| % of total requested: | | 306% | 40% | 60% |

5. **Project Goals and Supporting Objectives**:
**Goal**: Test a new biological control agent to provide long-term control of yellow starthistle.
**Objectives**:
1. Test host plant specificity of *Larinus filiformis* (quarantine & field experiments).
2. Submit a petition to USDA-APHIS requesting permission to release *L. filiformis*.

6. **Project Justification/Urgency**

Yellow starthistle (YST, *Centaurea solstitialis*, Asteraceae) is a winter annual forb originating from the Mediterranean Basin that has invaded about 8 million ha of North American rangeland, primarily in California, Oregon, Washington and Idaho, and is spreading eastward (Fig. 1, Sheley *et al.* 1999, Pitcairn *et al.* 2006). YST first appeared sometime after 1824, apparently arriving in contaminated shipments of alfalfa seed during the California gold rush in the 1850s (Gerlach 1997). It started spreading explosively in the 1980s and is continuing to spread eastward. A 2002 survey in California indicated that the area infested by the weed had increased by 81% since 1985 (Pitcairn *et al.* 2006). YST is spreading eastward into the Sierra Nevada mountains and over into Nevada and New Mexico. Colorado and Montana have eradication programs to try to prevent establishment of the weed in their states. Computer modeling studies indicate that potential geographic range of yellow starthistle is likely to expand with climate change (Bradley et al. 2009).

Yellow starthistle is adapted to a relatively wide range of environmental conditions and tolerates a variety of soil types (Maddox *et al*. 1985). In California, it is widely distributed in the central valleys and adjacent foothills and is currently spreading in mountainous regions up to 2,100 m (7,000 ft) (Pitcairn *et al*. 1998, 2006). It is most abundant at sites with full sunlight and deep, well-drained soils where annual rainfall is between 25 to 150 mm (10-60 in.). Habitats include rangelands, pastures, vineyards, abandoned croplands, wilderness areas, nature preserves, alfalfa and small grain fields, and roadsides. YST is found from flood plains and riverbanks to grasslands, ridges, and mountain slopes. It is most competitive in habitats that have a cool wet season that allows the rosettes time to send their roots down deep, followed by a warm-to-hot summer with little or no moisture that allows the plant to mature and produce seed with little interspecific competition. YST is particularly successful in the Mediterranean/Pacific-influenced climates of California, Oregon and Washington and the intermontane grasslands of Idaho (Pitcairn *et al.* 1998, Piper 2001, Prather *et al.* 2003).

Yellow starthistle can invade natural areas and displace native plants, including natural wildlife forage, and often becomes the dominant vegetation (DiTomaso *et al.* 2006a). The plant has long sharp spines on the flower heads that repel cattle and people, and it contains repin, which causes an incurable "chewing disease" in horses (Kingsbury 1964, Cordy 1978, Akba *et al.* 1995). Infestations reduce the use of recreational areas by the public (Eisworth 2005). YST significantly depletes soil moisture reserves in annual grasslands in California (Dudley 2000, Enloe *et al.* 2004) and in perennial grasslands in Oregon (Borman et al 1992). Large YST populations transpire the equivalent of about 4 to 8 inches of rainfall for each three feet of soil depth (Gerlach *et al.* 1998). Native perennial species such as blue oak (*Quercus douglasii*) and purple needlegrass (*Nassella pulchra*) depend on summer soil moisture reserves for growth and survival. However, because YST uses deep soil moisture reserves, dense YST infestations cause these plants to experience drought conditions even in years with normal rainfall (Gerlach *et al.* 1998). Water consumption by YST is estimated to be worth $16 to $75 million per year in the Sacramento River watershed alone (Gerlach 2004). Direct economic impact of yellow starthistle on rangeland in California was estimated to range between $40 million and $1.4 billion, depending on the economic assumptions (Jetter *et al*., 2003).

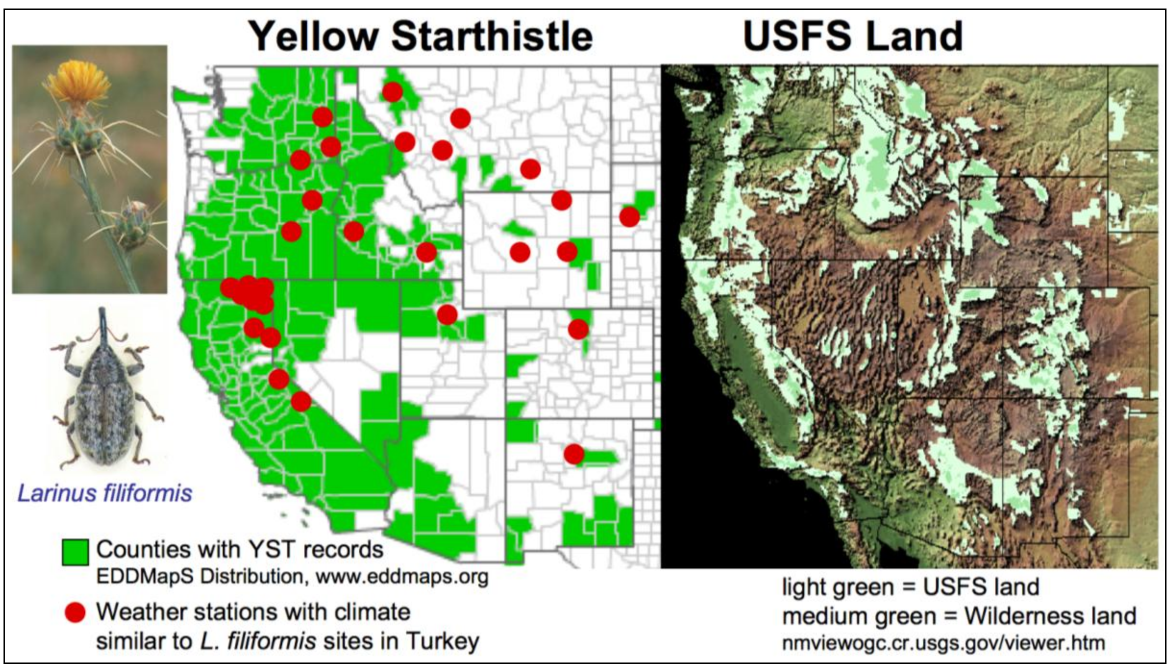


Figure 1. Comparison of known distribution of yellow starthistle to USFS land, and rough indication of the predicted range of the seed head weevil, *L. filiformis*, if it is released, based on similarity of overall climate to locations where the weevil occurs in Turkey (CLIMEX, Match Climates function).

Yellow starthistle plants can be killed by a number of herbicides, including aminopyralid, clopyralid and picloram (DiTomaso et al. 2006a). However, it is difficult to achieve lasting control because seeds in the soil can persit for several years (Joley et al. 2003). Herbicides are often not economical, and they may be prohibited in some habitats or jurisdictions. Mowing and controlled grazing by livestock can be effective when timed appropriately and repeated several times during a season (Thomsen *et al.* 1994, 1997, Benefield *et al.* 1999). Controlled burning can be effectively combined with follow-up herbicide treatments, but burning is difficult to plan and execute safely (DiTomaso et al. 2006b). Successful control by any of these strategies depends on persistent follow-up management for at least three years. Revegetation programs for yellow starthistle control are still experimental, expensive and require long time periods. Usually they employ planting native or introduced perennial grasses by seed drill, often integrated with other control methods such as herbicides (e.g., Callihan *et al.* 1986, Northam and Callihan 1988, Larson and McInnis 1989, Prather and Callihan 1991). Classical biological control, introducing species of insects or pathogens that attack only yellow starthistle, has the best prospect of economically reducing YST populations over large areas (Smith 2007a).

Research on biological control of YST began in the 1960s, ultimately resulting in the introduction of six insects that attack flower heads and one pathogen (Turner *et al.* 1995, Pitcairn et al. 2004, Woods et al. 2010). Of these, two insect species have become widespread and abundant: the hairy weevil, *Eustenopus villosus* (Coleoptera: Curculionidae), and the false peacock fly, *Chaetorellia succinea* (Diptera: Tephritidae) (Pitcairn *et al.* 2008). The latter species was an accidental introduction; however, subsequent testing indicated that it poses no significant risk to nontarget plants in this region (Balciunas and Villegas 2007). Insects infest about 63 to 88% of flower heads, which reduces seed production, and YST densities have decreased in much of Oregon and at some sites in California (E. Coombs, pers. comm., Pitcairn *et al.* 2002, 2005). However, some controlled field experiments failed to detect any significant reduction of the weed population caused by the seed head insects (Garren and Strauss 2009, R. Winston and M. Schwarzlaender unpubl. data). In any case, there is a generally perceived need to find additional agents to complement the established insects. Another introduced biological control agent, the rust pathogen, *Puccinia jaceae* var. *solstitialis*, has failed to persist at most release sites and usually has little impact on the weed (Fisher *et al.* 2007, Woods *et al.* 2010). Foreign exploration for new agents was expanded to Turkey, Bulgaria and southern Russia, resulting in discovery of additional prospective agents (Cristofaro *et al.* 2004a, Smith et al. 2005). The weevil, *Ceratapion basicorne*, from Turkey has been extensively tested, and a petition requesting permission for release was submitted to APHIS in 2006 (Smith *et al.* 2006, Smith 2007b). Although the Technical Advisory Group (TAG) unanimously recommended approval of *C. basicorne*, APHIS denied the permit, and this decision is currently being challenged. Several other agents have undergone preliminary testing: a flea beetle, *Psylliodes chalcomera*, (Cristofaro *et al.* 2004b, Smith unpubl. data); an eriophyid mite, *Aceria solstitialis*, (Monfreda *et al.* 2008, A. Stoeva et al. unpubl. data); a lace bug, *Tingis grisea* (Paolini *et al.* 2008); and a seed head weevil, *Larinus filiformis* (Smith *et al.* 2005, Gültekin *et al.* 2008a, b). Of these, only *L. filiformis* is likely to be sufficiently host-specific to meet current requirements for APHIS approval.

*Larinus filiformis* is is similar to the hairy weevil, *E. villosus*, which is considered to be one of the most effective agents in California (Pitcairn et al. 2002, 2008); however it is larger and occurs in a different region, in Turkey rather than in Greece, and at higher elevations than *E. villosus*. *Larinus filiformis* is very common in eastern Turkey, where it attacks about 75% of

YST flower heads (Gültekin *et al.* 2008b). Climate matching based on its known range in Eurasia suggests that *L. filiformis* will be well adapted to many areas where YST habitat occurs on USFS land (Fig. 1, Gültekin *et al.* 2008b). The adult is active June through July, and larvae complete development by October in Turkey. This enables adults to avoid winter predation by birds or mammals that may feed on flower heads. YST is the only known host plant in the field, and preliminary host specificity experiments are very promising (Gültekin *et al.* 2008b).

The project team is experienced working with *L. filiformis* in eastern Turkey and in conducting effective field host specificity experiments (Smith et al. 2006, 2009). A host plant test list has already been developed and previously has been used for *C. basicorne* (Smith 2007b). We are currently growing many of the test plants. An APHIS permit to work with this insect in the ARS quarantine facility in Albany, CA is already in hand.

**7. Approach**

**A. Test specificity of in quarantine greenhouse choice and no-choice experiments.**

We will determine the taxonomic range of plants that are susceptible to damage by *L. filiformis* following guidelines recommended by USDA-APHIS (1998). A list of 52 plant species to test has been developed based on phylogenetic relatedness to the target weed and other risk factors such as similar morphology or secondary chemistry and co-occurrence with the target weed (Smith 2007b). Seed to grow test plants will be obtained from our seed repository and from cooperators and commercial vendors. Plants will be grown for quarantine experiments using methods developed in our laboratory or by others experienced in growing these or similar plants. Some of these plants must grow for more than one year before flowering, but many of these are already being grown. Only plants with flower heads at a suitable developmental stage will be used in trials. Adult insects will be collected in eastern Turkey in May by L. Gültekin, hand carried by M. Cristofaro to Italy, and shipped under APHIS permit to the ARS quarantine facility in Albany, CA. Choice experiments will be conducted using a single gravid female inside screen cages (0.6 x 0.6 x 1.0 m), with 4 plants per cage (1 YST and 3 other nontarget species), inside the quarantine laboratory (*e.g.*, Balciunas and Villegas 2007). Each trial will last 2 to 3 days, and only trials in which oviposition occurs will be considered valid. In no-choice experiments, a gravid female will be enclosed on a plant inside a sleeve cage for 2 to 3 days. She will be held on YST for similar periods between each trial to confirm that she is still capable of feeding and ovipositing. In all experiments, data consist of number of adult feeding holes, number of eggs and area of plant tissue damaged. In cases where eggs hatch, plants will be maintained in the quarantine greenhouse and monitored to record larval survival, ultimate developmental stage attained, and amount of tissue damaged. We will perform at least four replicates of each nontarget plant species, and eight when there are signs of feeding damage or oviposition. Voucher specimens of adult insects will be deposited at the ARS laboratory in Albany CA, California Dept. of Food and Agriculture in Sacramento, and ARS Systematic Entomology Laboratory in Washington, D.C. Progeny from the experiments will be maintained in incubators to simulate winter conditions to enable breaking diapause to permit their use on plants that flower outside of the normal June - July oviposition period. We expect to complete testing all plants within two years.

**B. Field test of specificity in eastern Turkey**

We will test susceptibility of eight nontarget plant species in field experiments during two years in eastern Turkey (artichoke, safflower-oleic, safflower-linoleic, *Plectocephalus* *americana*, *Centaurea cyanus, Cirsium brevistylum*, *Cirsium occidentale*, *Onopordum acanthium*, *Saussurea salsa*, and YST as positive control). Because of confinement and artificial conditions during greenhouse experiments, it is likely that some nontarget plants may be damaged that would not normally be damaged in the field (Marohasy 1998). The best way to get more realistic results is to conduct field experiments (Clement and Cristofaro 1995, Briese 1999). The nontarget plant species of greatest concern are those most closely related to YST, especially threatened and endangered species, and those of economic importance. There are no native North American species within the genus *Centaurea* (Keil and Ochsmann 2006). The closest native relatives are *Plectocephalus americana* and *P. rothrockii*, and *Saussurea* spp. Safflower and artichoke are the most closely related agronomic crops. Bachelor's button (*C. cyanus*) is both an ornamental and an invasive alien weed, and has been an issue in recent APHIS decisions. We will test representatives of all these groups in a fenced field garden experiment conducted at Atatürk University in Erzurum, Turkey. Plants will be grown in large flower pots that will be set in the ground during the course of the experiment. This will allow us to start biennial species early enough the preceding summer or fall to enable them to flower for the experiment. Annual plants will be started in the greenhouse in late winter (YST) and spring (safflower, bachelor's button). Plants will be placed out in a cultivated garden after the risk of frost, plastic mulch will be used to control weeds, and drip irrigation will provide water as needed. Twenty replicates of each plant type will be planted in a pseudo Latin Square design, with 1-m spacing (total: 10 x 20 = 200 plants). At least 100 adult *L. filiformis* will be collected from the field and released in the garden in late May. Each week the presence of *L. filiformis* adults and developmental stage of plants will be monitored in the garden. If the adult *L. filiformis* population appears to decline, additional adults will be collected and released. Senescing flower heads will be harvested to prevent escape of seed and will be held in individual paper cups to rear out adults. All flower heads will be dissected in the fall. Data will consist of number of adult feeding or oviposition holes, number of larvae, area damaged, and number of viable seeds per flower head, and proportion of flower heads damaged. Phenology of flowering of each plant species will also be reported. Although statistical analyses, such as ANOVA, can be done on such data, the main objective is to accurately measure the frequency of attack (proportion infested with binomial estimate of standard error) and level of damage (mean and SE) (*e.g.*, Smith *et al.* 2006). This experiment will be conducted two years, but with possible addition of plant species that are found to be susceptible to damage in the quarantine choice and no-choice experiments. Eurasian species will be substituted for native N. American plant species if permit restrictions prevent us from sending them to Turkey. Frequent monitoring of plants will prevent the escape of seed, and all plants will be destroyed at the end of the experiment.

**8. Expected Products and Outcomes**

This research will result in sufficient data to submit a permit application ("petition") to USDA-APHIS Technical Advisory Group (TAG) for *Larinus filiformis*. This starts the regulatory process to obtain a release permit. After a permit is issued, ARS will provide insects to cooperators, such as USFS, CDFA, Oregon Dept. of Agriculture, U. Idaho, Washington State University, and Nez Pierce Tribe, to release, multiply and redistribute. Establishing this agent should increase overall attack rates on YST, especially in habitats with cold winters. Because this insect is larger than the other species, it consumes more seed, so the per-individual impact will be greater. Effective biological control will reduce the rate of spread of YST and should gradually reduce the density of plants in established infestations.

## Curriculum Vitae

**Lincoln Smith**, Research Entomologist, USDA Agricultural Research Service, Exotic and Invasive Weeds Research Unit, Albany, CA

Dr. Smith received a B.A. in Ecology from Amherst College (1975) and M.Sc. (1994) and Ph.D. (1998) degrees in Entomology, with specialization in biological control, from Cornell University. His research involved studying the ecological niche associations and dispersal behavior of parasitoids of house flies and stable flies at dairy farms. He conducted research for four years with the USDA-ARS in Savannah, Georgia on biological control of insects in stored corn. This work involved collecting temperature dependent life history data and developing computer simulation models of parasitoid-host population dynamics. He worked four years at the International Center for Tropical Agriculture (CIAT), in Cali, Colombia on an international project to find and evaluate natural enemies (mites and pathogens) of the cassava green mite. Predatory mites sent to Africa succeeded in bringing the pest under control over a large region.

In 1998, Dr. Smith returned to the U.S. to work on biological control of weeds at the USDA-ARS in Sidney, Montana. He evaluated the impact of previously introduced biological control agents on spotted and diffuse knapweeds.

In 2000, Dr. Smith took a new position at USDA-ARS, in Albany, California to conduct research on the discovery, evaluation and introduction of new arthropod agents for biological control of yellow starthistle and Russian thistle (tumbleweed). He has submitted petitions for the release of a gall mite on Russian thistle and a root weevil on yellow starthistle. He has had collaborative projects with scientists in France, Italy, Greece, Russia, Turkey and Uzbekistan.

He has written over 100 scientific publications, and has given over 130 scientific presentations, including 30 invited talks.

**Some Recent Publications:**


Smith, L., M. Cristofaro, E. de Lillo, R. Monfreda and A. Paolini. 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.

Fisher, A.J., D.M. Woods, L. Smith, W.L. Bruckart. 2007. Developing an optimal release strategy for the rust fungus *Puccinia jaceae* var. *solstitialis* for biological control of *Centaurea solstitialis* (yellow starthistle). Biological Control 42(2): 161-171.

Smith, L. 2007. Physiological host range of *Ceratapion basicorne*, a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). Biological Control 41: 120-133.

Smith, L. and A. E. Drew. 2006. Fecundity, development and behavior of *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of yellow starthistle. Environ. Entomol. 35(5): 1366-1371.

Smith, L., R. Hayat, M. Cristofaro, C. Tronci, G. Tozlu and F. Lecce. 2006. Assessment of risk of attack to safflower by *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). Biological Control 36(3): 337-344.

Smith, L. and M. Mayer. 2005. Field cage assessment of interference among insects attacking seed heads of spotted and diffuse knapweed. Biocontrol Science and Technology 15(5): 427-442.

**Levent Gültekin**, Associate Professor, Plant Protection Department, Faculty of Agriculture, Atatürk University, Erzurum, Turkey

Dr. Gültekin has been working on weevil (Curculionoidea) biodiversity in Anatolia since 1997, with special emphasis on the taxonomy, systematics and ecology of certain subgroups (Lixinae, Ceutorhynchinae and Baridinae); for some groups (tribe Lixini) he has expanded his work to the entire Palaearctic Region. He is a leading taxonomic expert on inflorescence inhabiting Lixini (*e.g.*, genera *Bangasternus*, *Rhinocyllus*, *Larinus*, *Lachnaeus*, *Eustenopus*). His work includes a combination of taxonomy, systematics and ecological characterization of species, and rearing of adults from larvae in host plants for confirmation of host plant linkage. He has successfully completed two nationally- and nine internationally-funded research projects mostly related to weevils and their ecology, taxonomy and systematics, and potential as biological control agents for weeds. In this context, he has published 46 scientific papers on weevils. Thirty of these were peer reviewed, and 16 of them were published in SCI journals. He has conducted studies at several natural history museums and research institutes across Europe, and has carried out collaborative research with several biological control scientists from BBCA-Italy, CABI-Switzerland, and USDA-ARS.

**EDUCATION & PROFESSIONAL EMPLOYMENT**

Since 2009: Associate Professor, Plant Protection Department, Atatürk University.
2002–2009: Assistant Professor, Plant Protection Department, Atatürk University.
2001: Ph.D. Institute of Natural and Applied Sciences (Entomology), Atatürk University.
1994–2001: Research Assistant: Plant Protection Department, Atatürk University.
1993–1996: Master´s: Institute of Natural and Applied Sciences (Entomology), Atatürk University.

**Some Recent Publications:**

Gültekin, L. M. Cristofaro, C. Tronci, and L. Smith. 2008. Natural history studies for the preliminary evaluation of *Larinus filiformis* (Coleoptera: Curculionidae) as a prospective biological control agent of yellow starthistle. Environ. Entomol. 37(5): 1185-1199.

Gültekin, L., R. Borovec, M. Cristofaro, L. Smith. 2008. Broad-nosed weevils feeding on *Centaurea solstitialis* L. in Turkey, with a description of the new species *Araxia cristofaroi* sp. n. (Coleoptera: Curculionidae: Entiminae). Annals of the Entomological Society of America 101(1): 7-12.

Gültekin, L., Cristofaro, M., Tronci, C. and Smith, L. 2008. Bionomics and seasonal occurrence of *Larinus filiformis* Petri, 1907 (Coleoptera: Curculionidae), potential biological control agent for *Centaurea solstitialis* L. in eastern Turkey. In Julien, M.H., Sforza, R., Bon, M.C., Evans, H.C., Hatcher, P.E., Hinz, H.L. & Rector, B.G. (eds.), Proceedings of the XII International Symposium on Biological Control of Weeds, April 22-27, 2007, Montpellier, France. CAB International, Wallingford, UK. pp. 150-153.

Gültekin, L. 2008. Taxonomic review of the stem-inhabiting trehala-constructing *Larinus* Dejean, 1821 (Coleoptera: Curculionidae): New species, systematics and ecology. *Zootaxa* 1714: 1-18.

Gültekin, L. 2007. Oviposition niches and behavior of the genus *Lixus* Fabricius (Coleoptera: Curculionidae, Lixinae). *Entomologica Fennica*, 18: 74-81.

**Massimo Cristofaro**, Senior Scientist, ENEA C.R. Casaccia BIOTEC-SIC, [Institute of New Technology for Energy and the Environment], S. Maria di Galeria (Rome), Italy

**EDUCATION**

B.S. University of Rome "La Sapienza", Dec. 1980, mark 110/110, with a thesis in applied botany concerning the number genes for ribosomal RNA of *Scilla peruviana* L. (Liliaceae).

**PROFESSIONAL EMPLOYMENT**

1992 - present Senior Research Scientist. ENEA C.R. Casaccia (National Agency for Energy and Environment), Rome, Italy. I have supervised 6 Master and 2 Ph.D. graduate students.

2000 - present Founder and President. Biotechnology and Biological Control Association onlus, Rome, Italy. BBCA conducts foreign exploration and evaluation of prospective biological control agents of weeds. It currently collaborates with ENEA, USDA-ARS, Russian Academy of Sciences in St. Petersburg; Atatürk University in Erzurum, Turkey; and the Agriculture University of Nitra, Slovakia.

1987 – 1991 Research Entomologist USDA-ARS Biological Control of Weeds Laboratory, Rome, Italy. Working on biological control of leafy spurge.

1982 – 1987 Biological Assistant. USDA-ARS Biological Control of Weeds Laboratory, Rome, Italy. Working on biological control of field bindweed, yellow starthistle, leafy spurge, curly dock and bedstraw.

1981 – 1982 Teacher. Natural Sciences at the Agricultural High School, Europa Unita of Grottaferrata in Rome, Italy.

**Some Recent Publications:**

Antonini G., Coletti G., Serrani L., Tronci C., Cristofaro M. & Smith L. 2009. Using molecular genetics to identify immature specimens of the weevil *Ceratapion basicorne* (Coleoptera, Apionidae). Biological Control 51: 152–157.

Smith, L., M. Cristofaro, E. de Lillo, R. Monfreda and A. Paolini. 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.

Paolini, A., Tronci, C., Lecce, F., Hayat, R., Di Cristina, F., Cristofaro, M. and Smith, L. 2008. A lace bug as biological control agent of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae): an unusual choice. In Proc. XII Int. Symp. on Biological Control of Weeds. CAB International Wallingford, UK. April 22-27, 2007, Montpellier, France. pp. 189-194.

Smith, L., M. Cristofaro, C. Tronci, R. Hayat. 2008. Refining methods to improve pre-release risk assessment of prospective agents: the case of *Ceratapion basicorne*. In Proc. XII Int. Symp. on Biological Control of Weeds. CAB International Wallingford, UK. pp. 321-327.

Tronci, C., Paolini, A., Lecce, F., Di Cristina, F., Cristofaro, M., Reznik S. and Smith, L. 2008. Impact of larval and adult feeding of *Psylliodes chalcomera* (Coleoptera: Chrysomelidae), on *Centaurea solstitialis* (yellow starthistle). In Proc. XII Int. Symp. on Biological Control of Weeds. CAB International Wallingford, UK. pp. 333-339.

Cristofaro, M., Hayat R., Gültekin L., Tozlu G., Tronci C., Lecce F., Paolini A., Smith L. (2006). Arthropod communities associated with *Centaurea solstitialis* L. in Central and Eastern Anatolia. VIII European Congress of Entomology, 17-22 Sept. 2006, Izmir, Turkey p. 148.

Smith, L., R. Hayat, M. Cristofaro, C. Tronci, G. Tozlu and F. Lecce. 2006. Assessment of risk of attack to safflower by *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). Biol. Control 36: 337-344.

**Budget**

| | Requested | | | | Contributed | | | | Requested | | Contributed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mo. | Year 1 | mo. | Year 2 | mo. | Year 1 | mo. | Year 2 | mo. | Total | Total |
| **Salary** | | | | | | | | | | | |
| Scientist ARS - L. Smith (PI) [1] | | 0 | | 0 | 2 | 22,893 | 3 | 34,340 | 0 | 0 | 57,233 |
| Scientist ENEA - M. Cristofaro [2] | | 0 | | 0 | 1 | 12,250 | 1 | 12,250 | 0 | 0 | 24,500 |
| Scientist Atatürk - L. Gultekin [3] | | 0 | | 0 | 3 | 8,586 | 3 | 8,586 | 0 | 0 | 17,172 |
| Technician - ARS [4] | 2 | 7,654 | 2 | 7,654 | 10 | 38,269 | 10 | 76,538 | 4 | 15,308 | 114,808 |
| Technician - BBCA [5] | 1 | 3,500 | 1 | 3,500 | 1 | 3,500 | 1 | 3,500 | 2 | 7,000 | 7,000 |
| Technician - Ataturk Univ. [6] | 3 | 4,548 | 3 | 4,548 | 0 | 0 | 0 | 0 | 6 | 9,096 | 0 |
| **Total Salary** | **6** | **15,702** | **6** | **15,702** | **17** | **85,499** | **18** | **135,214** | **12** | **31,404** | **163,480** |
| | | | | | | | | | | | |
| **Benefits** | | | | | | | | | | | |
| Scientist ARS - L. Smith (PI) [1] | | 0 | | 0 | 2 | 6,868 | 3 | 10,302 | 0 | 0 | 17,170 |
| Scientist ENEA - M. Cristofaro [2] | | 0 | | 0 | 1 | 5,250 | 1 | 5,250 | 0 | 0 | 10,500 |
| Scientist Atatürk - L. Gultekin [3] | | 0 | | 0 | 3 | 6,408 | 3 | 6,408 | 0 | 0 | 12,816 |
| Technician - ARS [4] | 2 | 2,296 | 2 | 2,296 | 10 | 11,481 | 10 | 11,481 | 4 | 4,592 | 22,962 |
| Technician - BBCA [5] | 1 | 1,500 | 1 | 1,500 | 1 | 1,500 | 1 | 1,500 | 2 | 3,000 | 3,000 |
| Technician - Ataturk Univ. [6] | 3 | 3,198 | 3 | 3,198 | 0 | 0 | 0 | 0 | 6 | 6,396 | 0 |
| **Total Benefits** | **6** | **6,994** | **6** | **6,994** | **17** | **31,507** | **18** | **34,941** | **12** | **13,988** | **49,278** |
| | | | | | | | | | | | |
| **Total Salary + Benefits** | **12** | **22,696** | **12** | **22,696** | **34** | **117,005** | **36** | **170,155** | **24** | **45,392** | **287,160** |
| | | | | | | | | | | | |
| **Travel** | | | | | | | | | | | |
| Domestic Travel - L. Gultekin [7] | | 3,200 | | 3,200 | | 0 | | 0 | | 6,400 | 0 |
| Foreign travel - BBCA [8] | | 4,000 | | 4,000 | | 0 | | 0 | | 8,000 | 0 |
| Foreign travel - L. Smith [9] | | 2,000 | | 0 | | 2,000 | | 0 | | 2,000 | 2,000 |
| **Total Travel** | | **9,200** | | **7,200** | | **2,000** | | **0** | | 16,400 | 2,000 |

| | Requested | | | | Contributed | | | | Requested | | Contributed |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | mo. | Year 1 | mo. | Year 2 | mo. | Year 1 | mo. | Year 2 | mo. | Total | Total |
| **Other Direct Costs** | | | | | | | | | | | |
| Major Equipment - L.Smith [10] | | 0 | | 0 | | 5,000 | | 5,000 | | 0 | 10,000 |
| Supplies - L. Smith [11] | | 0 | | 0 | | 2,000 | | 2,000 | | 0 | 4,000 |
| Supplies - BBCA [12] | | 150 | | 150 | | 0 | | 0 | | 300 | 0 |
| Supplies - L. Gultekin [13] | | 3,500 | | 1,500 | | 0 | | 0 | | 5,000 | 0 |
| **Total Other Direct Costs** | | **3,650** | | **1,650** | | **7,000** | | **7,000** | | **19,300** | **14,000** |
| | | | | | | | | | | | |
| Indirect Charge [14] | | 3,950 | | 3,505 | | 0 | | 0 | | 7,455 | 0 |
| Grand Total | | **39,496** | | **35,051** | | **126,005** | | **177,155** | | 74,547 | **303,160** |
| **Total requested** | | | | **74,547** | | | | | | | |
| | | | | | | | | | | | |
| **Breakdown by institution:** | | | | | | | | | | | |
| ARS | | 15,900 | | 13,455 | | 88,511 | | 139,661 | | 29,355 | 228,172 |
| BBCA/ENEA | | 9,150 | | 9,150 | | 22,500 | | 22,500 | | 18,300 | 45,000 |
| Atatürk University | | 14,446 | | 12,446 | | 14,994 | | 14,994 | | 26,892 | 29,988 |
| | | 39,496 | | 35,051 | | 126,005 | | 177,155 | | 74,547 | 303,160 |

Total contributed as percentage of total requested: ARS = 306%, BBCA/ENEA = 60%, Atatürk University = 40%.

**Budget Footnotes:**

1] Research Entomologist, USDA-ARS (GS-14); coordinate project, design and analyze experiments, supervise quarantine experiments, assist design and execution of field experiment, write publications.

2] Senior scientist, ENEA, Rome, Italy; assist design and execution of field experiments, collect and ship insects to ARS.

3] Associate Professor, Atatürk University, Erzurum, Turkey; collect and identify insects, supervise field experiment.

4] Biological technician, USDA-ARS (GS-7); grow test plants, conduct host specificity experiments in quarantine laboratory.

5] Biological technician, BBCA, Rome, Italy; assist execution of field experiments, and collection of insects.

6] Biological technician, Atatürk University, Erzurum, Turkey; collect insects, grow plants, conduct field experiment.

7] Travel inside Turkey to collect insects, 3 trips x 5 days, each year to collect *Larinus filiformis* adults for experiments (1 person, car rental, gasoline, hotel, subsistence).

8] 2 trips from Italy to Turkey to assist setup and takedown of host specificity field expt. (1 person, 5 d each) (including airfare, hotel); hand carry insects in May to Italy to ship to ARS.

9] 1 trip from USA to Turkey to establish host specificity testing field expt. (1 person, 7 d) (including airfare, hotel, per diem).
10] Amortization of climatic chambers, greenhouse, autoclave.
11] Laboratory & greenhouse supplies, utilities, communications.
12] Packaging and shipping of insects to ARS.
13] Drip irrigation system, fence, consumables for experiments (jiffy pots, plant pots, paper bags, etc.), greenhouse supplies, operation of climatic chambers.
14] 10% of total requested required by ARS for indirect expenses.

**Timetable**

| | Sum. | Fall | Winter | Spring | Sum. | Fall | Winter | Spring |
|---|---|---|---|---|---|---|---|---|
| quarantine host specificity -LS | X | X | | X | X | X | | |
| collect & ship insects to ARS - LG, MC | X | | | | X | | | |
| field host specificity, Turkey -LG, MC | X | X | | | X | X | | |
| data analysis -LS | | | X | | | | X | |
| submit TAG petition, publish results -LS | | | | | | | | X |

Responsible: LS- L. Smith, LG- L. Gültekin, MC- M. Cristofaro

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