Host Specificity of *Bangasternus orientalis* Capiomont (Coleoptera: Curculionidae) Introduced into the United States for Biological Control of Yellow Starthistle (*Centaurea solstitialis* L., Asteraceae: Carduae)

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ABSTRACT The weevil *Bangasternus orientalis* Capiomont was selected as a candidate for the biological control of *Centaurea solstitialis* L. (yellow starthistle) in the United States. Its potential host range was studied during 1982, 1983, and 1984 in Rome, Italy, using populations collected from *C. solstitialis* in Thermi near Thessaloniki and Kozani in northern Greece. Adults laid eggs only on members of the genus *Centaurea* and the thistle *Onopordum acanthium;* eggs laid on *O. acanthium* hatched but 1st instars died before entering buds. There was no significant difference in the number of eggs laid on various U.S. and Italian strains of *C. solstitialis* included in the tests. Eggs were not deposited on plant species of economic importance such as *Cynara scolymus, Helianthus annuus, Carthamus tinctorius,* and *Lactuca sativa.* Larvae completed their larval development only on *C. solstitialis* from Greece and on U. S. and Italian populations of *C. solstitialis* among the 60 plant species, varieties, and strains (in 7 families) tested. This restricted host range suggested introduction of this weevil as a biological control agent against yellow starthistle in the United States. Releases of *B. orientalis* were made in California, Washington, Idaho, and Oregon in 1985 and it became established in all states in 1989.

KEY WORDS Coleoptera, *Bangasternus orientalis,* yellow starthistle, weed, weevil, biological control

YELLOW STARTHISTLE, *Centaurea solstitialis* L., is an herbaceous plant of Eurasian origin that is adventive in many parts of the world, including the United States of America, where it reaches high densities in several climatic and vegetational zones, especially in California (3,200,000 ha infested), Idaho (81,000 ha), Oregon (400,000 ha), and Washington (54,000 ha) (Maddox and Mayfield 1985, Maddox et al. 1985, Roché and Roché 1988, Callihan et al. 1989). This weed is a serious pest of rangelands, recreational areas, and abandoned croplands, and it is spreading at an alarming rate into new areas (Callihan et al. 1989). Yellow starthistle displaces native vegetation, its spiny capitulae deters grazing by livestock, it is a nuisance to people working or playing on infested lands, and it is poisonous to horses (Cordy 1978). Yellow starthistle is typically a winter annual that propagates only by seeds that usually germinate in the fall, depending on rainfall. Floral buds undergo a series of maturation stages before they terminate in yellow inflorescences (Maddox 1981) that typically open June through August in southern Europe.

Researchers have surveyed the entomofauna of yellow starthistle in southern Europe since 1950 for potential biological control candidates (Zwölfer 1965, 1969; Zwölfer et al. 1971; Sobhian and Zwölfer 1985; Clement and Mimmocchi 1988; Clement et al. 1988). Approximately 23 capitulum infesting insect species, including the weevil *Bangasternus orientalis* Capiomont (Sobhian and Zwölfer 1985), and 7 species associated with other parts of yellow starthistle were discovered in the Mediterranean region (Clement 1990). Five insect species, *Urophora sirunaseva* (Hering) (Diptera: Tephritidae), *Chaetorellia australis* Hering (Diptera: Tephritidae), *Eustenopus villosus* (Boheman) (Coleoptera: Curculionidae), *Larinus curtus* Hochhuth (Coleoptera: Curculionidae), and *B. orientalis,* have been released in the United States to control yellow starthistle (Maddox et al. 1986, White and Clement 1987, Maddox et al. 1990, Turner et al. 1996) and all are established. The biology and phenology of *B. orientalis* reported by Sobhian et al. (1992) was based on studies in northern Greece. Overwintered adults appear on bolting yellow starthistle during mid-May. Oviposition starts at the end of May and lasts ≈3 mo. Eggs are laid singly, on or near young buds, and are covered with a protective cap of frass produced by the females. Newly hatched larvae mine into flower heads from eggs laid on branch tips. Adults do minimal damage to yellow starthistle by feeding on leaves, but the larvae consume developing seeds. A single larva is capable of destroying the entire contents of a yellow starthistle flower bud, completing its development inside the bud. Females oviposit and feed on buds of sizes categorized by Maddox (1981) as stages Bu 1, Bu 2, and Bu 3; a few eggs are found on

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bud stage Bu 4 and flower bud stages F-1 and F-2. Two species of egg parasitoids, *Pterandrophysalis levantina* Nowicki (Trichogrammatidae) and an unidentified species of Mymaridae, emerged from eggs collected in the field near Thermi in northern Greece (Sobhian et al. 1992). High eggs and larval mortality (up to 98.7 and 84%), largely the result of predation, does not allow the weevil to build up large populations in its natural environment.

One of the most important criteria determining the introduction into a new country of phytophagous organism for the biological control of weeds is that they should be safe, sufficiently host specific to pose no threat to any cultivated or socially important plant. The objective of this article is to provide background information of specific laboratory tests that were conducted at the overseas laboratory with *B. orientalis* prior its introduction and release in the United States.

Materials and Methods

Host Specificity Tests. Tests were conducted during 1982, 1983, and 1984 with 60 plant species or varieties in 7 families (Table 1). The plants included genera and families closely related to *Centaurea* (order Asterales), plants in other orders of the superorder Rosidae, and plants attacked by other species of *Bangasternus*, such as *C. diffusa* DeLamarck, *C. calcitrapa* L., *C. paniculata* L., *C. scabiosa* L., and *C. maculosa* DeLamarck. Engler (1964) and Heywood (1978) were used as guides in constructing our list of test plants. Native American *Cirsium*, and *Centaurea* species such as *Cirsium undulatum* (Nuttal) Spreng, *C. douglasii* Decandolle, *C. andrewsii* (Gray) Jepson, and *Centaurea americana* Nuttall were included in the tests. Adults for host specificity tests were collected in Thermi (Thessaloniki) and Kozani in northern Greece during May. The weevils were sexed and kept in a refrigerator at 8–10°C until collections in Greece were completed. They were transported from Greece to Rome, where they were caged separately on young bolting plants of *C. solstitialis*, allowing them to feed on fresh plant material for 48 h to recover from the stress of the travel to Rome. Healthy adults were used to conduct the various host specificity tests.

Single Plant, No-Choice Oviposition Tests: 1982–1984. The experiments were conducted in a quarantine greenhouse (shaded on the top) under natural light during June and July 1982–1984. For the tests, mean temperature was 24.8 ± 5.9°C (range, 16–36°C), mean relative humidity was 65.6 ± 19.6% RH (range, 32–91%), and a photoperiod ≈16:8 (L:D) h. The insects were confined on potted plants covered with a transparent plastic cylinder (20 cm diameter, 70 cm high) that had 4 holes (10 cm diameter) covered with organdy on the side of each cylinder to allow air circulation. Each cage was capped with organdy cloth held in place by a rubber band. Experiments were conducted from late May to late July each year. Five replicates of each plant species were caged with 2 pairs of *B. orientalis* adults. Weevils were allowed to feed and oviposit on test plants until they died. All exposed buds of test plants, including the control, were checked to record the number of eggs laid. Longevity of caged adults also was recorded. Host specificity data were analyzed by analysis of variance (ANOVA) and means were separated by a Student–Newman–Keuls a posteriori test.

First Instar Survival Test. These trials were conducted in a shaded quarantine greenhouse under natural light between May and June 1982–1984. For the tests, mean temperature was 24.9 ± 6.2°C (range, 15–35°C); mean relative humidity was 65.7 ± 18.9% RH (range, 30–92%); and a photoperiod of 16:8 (L:D) h. The 1982 test was from 2 June to 30 July; 1983, 1 June to 31 July; and 1984, 3 June to 31 July. Infested buds were dissected and the numbers of larvae were recorded. Eggs for 1st-instar survival tests were obtained from 10 females each year. Males and females were caged with *C. solstitialis* (bearing young buds of categories Bu 1 and Bu 2) (Maddox 1981). Each day, newly laid eggs were recorded and removed from the plants and placed in hatching containers (Rizza 1977). Instead of using cotton as described by Rizza, the authors used plaster of paris as a hatching surface to avoid injury to neonate larvae. Egg development was monitored daily using a stereoscope. When the head capsule was visible through the mature eggs they were transferred to test plants with a moistened camel's-hair brush. Each test plant received 20 mature eggs distributed over 10 flower buds (2 eggs per flower bud), each bud serving as a replicate. We observed in the field that only 1 larva can develop in 1 flower bud. We decided that no more than 2 eggs per bud were necessary to avoid competition and stress between 1st instars using the same flower bud. Infested buds were marked to facilitate reexamination and to allow us to record the number of hatching eggs. Data of larval survival test are expressed with means and standard deviation.

Multiple Choice Test: 1984. Plants for this test were those that received eggs from tests of no-choice oviposition conducted in 1982, 1983, and 1984. The objectives were to see if *B. orientalis* adults would select nonhost plants for oviposition and to determine the degree of larval development. The degree of preference between American and European strains of yellow starthistle was also measured. Selected groups of test plants, each containing a control plant, were potted together in large plastic pots (80 cm diameter, 70 cm high). Each group of plants in a pot was covered by a cylinder cage as described previously. Four groups of plants were prepared, and each group was replicated 3 times. Ten pairs of *B. orientalis* were placed in each cage. Weevils (n = 180) collected in Thermi, Greece, in mid-May 1984 were used for this test. The experiment was carried out in a shaded quarantine greenhouse under natural light from 15 May to 30 July. All buds per test plant, with and without eggs, were recorded. During the test, the mean temperature was 24.4 ± 6.3°C (range, 16–36°C), the mean relative humidity was 65.3 ± 19.8% RH (range, 33–91%), and a photoperiod of 16:8 (L:D) h. The longevity of weevils used in these tests was recorded. Multiple choice

Table 1. Results of *B. orientalis* oviposition–no–choice host specificity tests, 1982, 1983, 1984 (mean ± SD followed by range)

Test plant species	No. replicates	Total exposed floral buds per plant		Total floral buds attacked per plant		Total eggs laid per plant		Adult Longevity Days	
				1982					
Centaurea solstitialis Greece (Control)	5	55.00 ± 4.74	49–61	51.60 ± 4.04	46–56	82.60 ± 4.93a	78–90	46.6 ± 4.83	39–51
Centaurea solstitialis Goldendale (WA)	5	46.00 ± 12.24	27–61	42.00 ± 12.51	24–59	61.60 ± 5.41b	56–70	41.8 ± 3.35	39–47
Centaurea solstitialis Walla Walla (WA)	5	40.00 ± 12.21	22–53	39.60 ± 12.22	21–52	63.40 ± 15.95b	43–80	46.4 ± 5.46	37–51
Centaurea solstitialis Yakima (WA)	5	39.80 ± 15.45	17–59	31.40 ± 8.96	17–39	51.40 ± 15.63c	35–75	44.8 ± 5.26	38–52
Centaurea solstitialis Concord (CA)	5	44.00 ± 5.91	37–51	38.00 ± 6.52	30–46	48.00 ± 11.36d	35–65	40.8 ± 2.39	38–44
Centaurea solstitialis Bracciano (Italy)	5	50.00 ± 4.18	45–56	39.60 ± 2.41	37–43	47.80 ± 7.73c	40–58	38.0 ± 4.64	32–44
Centaurea solstitialis Salerno (Italy)	5	4.00 ± 9.43	46–67	42.00 ± 2.74	38–45	62.00 ± 5.52e	54–69	42.4 ± 5.37	37–51
Centaurea solstitialis Bari (Italy)	5	47.00 ± 5.43	39–53	44.00 ± 5.79	37–50	97.80 ± 0.84f	97–99	46.4 ± 4.39	39–50
Centaurea solstitialis var. *schowii* (Italy)	5	57.00 ± 2.73	54–61	44.40 ± 2.70	40–47	48.00 ± 2.92g	44–51	44.2 ± 4.55	37–48
Centaurea diffusa Greece	5	54.40 ± 1.22	52–55	5.00 ± 2.45	2–8	6.00 ± 2.74h	2–9	28.2 ± 2.39	25–31
Centaurea jacea Italy	5	44.0 ± 2.91	39–46	20.60 ± 2.30	18–24	28.60 ± 5.59i	24–38	44.0 ± 4.36	39–49
Centaurea maculosa Italy	5	48.00 ± 6.85	39–57	4.60 ± 0.55	4–5	6.00 ± 2.92l	2–9	25.2 ± 3.03	21–29
Centaurea calcitrapa Italy	5	54.60 ± 13.37	39–75	14.00 ± 2.45	11–17	15.80 ± 2.68m	13–19	24.8 ± 4.97	19–30
Centaurea calcitrapa Greece	5	58.00 ± 6.12	49–66	20.00 ± 3.16	17–25	20.60 ± 3.51m	17–26	29.6 ± 3.65	24–34
Centaurea cyanus Italy	5	54.00 ± 10.01	42–65	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	17.4 ± 2.30	15–21
Centaurea paniculata Italy	5	46.00 ± 7.68	37–55	0.40 ± 0.89	0–2	0.40 ± 0.89n	0–2	20.2 ± 1.48	18–22
Centaurea cineraria Italy	5	66.00 ± 5.70	59–72	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	16.2 ± 3.11	13–21
Centaurea americana USA	5	5.80 ± 1.30	4–7	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	17.6 ± 1.67	15–19
Centaurea scabiosa Italy	5	42.00 ± 2.91	39–46	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	17.0 ± 2.24	14–20
Helianthus annuus USA	5	1.80 ± 0.83	1–3	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	17.2 ± 1.48	15–19
Helianthus tuberosus USA	5	3.80 ± 1.92	2–7	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	16.8 ± 2.17	15–20
Zinnia elegans Italy	5	9.40 ± 5.45	0–14	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	18.4 ± 1.95	16–21
Carthamus tinctorius USA	5	7.00 ± 3.53	4–13	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	16.0 ± 1.58	14–18
Cynara scolymus USA	5	4.00 ± 1.00	3–5	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	16.0 ± 1.58	14–18
Cynara scolymus Italy	5	3.00 ± 1.58	1–5	0.00 ± 0.00	0–0	0.00 ± 0.00n	0–0	15.8 ± 1.92	13–18
				1983					
Centaurea solstitialis Greece (control)	5	82.00 ± 8.24	69–89	82.00 ± 8.25	69–89	142.00 ± 34.02	99–190	49.0 ± 7.84	39–60
Centaurea alba Italy	5	45.80 ± 3.03	42–50	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.0 ± 1.22	15–18
Carduus nutans USA	5	15.00 ± 3.16	10–18	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	16.0 ± 2.35	12–18
Carduus pycnocephalus Italy	5	35.00 ± 6.67	28–45	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.6 ± 2.30	15–21
Carduus acanthoides USA	5	44.20 ± 13.64	29–64	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	14.8 ± 2.77	12–19
Carduus thoermeri USA	5	22.00 ± 4.84	15–26	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	16.0 ± 2.35	13–18
Cirsium lanceolatum Italy	5	2.00 ± 0.70	1–3	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.6 ± 2.30	14–20
Cirsium undulatum USA	5	2.40 ± 1.14	1–4	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.4 ± 2.88	13–21
Cirsium douglasii USA	5	2.20 ± 0.83	1–3	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.2 ± 1.30	14–17
Cirsium andrewsii USA	5	2.60 ± 1.34	1–4	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.6 ± 1.67	15–19
Onopordum acanthium USA	5	7.00 ± 3.08	4–11	0.00 ± 0.00	0–0	1.4 ± 0.55	1–2	21.0 ± 4.53	17–28
Onopordum illyricum Italy	5	4.20 ± 2.28	2–8	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.6 ± 1.95	15–20
Sylibum marianum Italy	5	4.20 ± 2.49	2–8	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.6 ± 1.95	13–18
Chrysanthemum leucanthemum Italy	5	25.80 ± 3.56	20–29	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	16.0 ± 1.87	14–19
Achillea millefolium Italy	5	27.80 ± 10.18	17–39	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.8 ± 2.95	13–21
Tanacetum vulgare Italy	5	30.00 ± 3.80	26–36	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.0 ± 1.22	14–17
Anthemis tinctoria Italy	5	26.40 ± 6.02	21–36	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.2 ± 2.28	13–18
Artemisia vulgaris Italy	5	34.20 ± 7.69	25–45	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.0 ± 2.35	14–20
Senecio jacobea Italy	5	10.00 ± 4.63	6–17	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.0 ± 2.65	15–22
Lactuca sativa Italy	5	34.00 ± 7.34	25–44	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.2 ± 1.30	14–17
Lactuca sativa var. Great Lakes USA	5	26.80 ± 8.10	14–35	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.4 ± 1.14	14–17
Leontodon crispus Italy	5	5.40 ± 3.78	2–10	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.4 ± 2.70	11–18
Taraxacum officinalis Italy	5	7.60 ± 3.50	2–11	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.4 ± 1.52	16–20
Tagetes erecta Italy	5	5.00 ± 2.34	3–9	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	16.6 ± 1.14	15–18
Calendula officinalis Italy	5	15.00 ± 4.47	10–21	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.8 ± 2.28	15–21
				1984					
Centaurea solstitialis Greece (control)	5	135.60 ± 21.96	99–156	146.00 ± 12.35	128–160	181.80 ± 7.19	173–190	54.8 ± 3.89	49–59
Antirrhinum majus Italy	5	24.40 ± 7.20	15–35	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.4 ± 1.14	17–20
Linaria vulgaris Italy	5	15.00 ± 4.18	10–21	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	20.0 ± 2.12	18–23
Linaria dalmatica USA	5	9.00 ± 2.35	6–11	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	20.8 ± 2.16	19–24
Silene vulgaris Italy	5	25.40 ± 4.62	19–32	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.8 ± 2.58	14–21
Viola bertolinii Italy	5	14.00 ± 3.94	11–20	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.2 ± 0.83	16–18
Ranunculus auricomus Italy	5	44.80 ± 12.15	27–56	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	17.0 ± 1.58	15–19
Papaver somniferum Italy	5	1.00 ± 0.00	1–1	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	16.4 ± 1.51	14–18
Euphorbia esula Italy	5	5.40 ± 1.52	4–8	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	15.4 ± 0.89	14–16
Euphorbia lathyris Italy	5	4.20 ± 2.28	2–8	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	14.8 ± 1.48	13–17
Euphorbia characias Italy	5	2.60 ± 0.89	2–4	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	18.8 ± 1.48	17–21
Euphorbia virgata USA	5	3.00 ± 1.22	2–5	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0	20.8 ± 2.28	18–24

Two males and 2 females were used per test plant. Five replicates were made per each tested plant. Means followed by the same letter are not significantly different ($P < 0.05$, Student–Newman–Keuls a posteriori test).

Table 2. Results of *B. orientalis* larval survival test, 1982, 1983, 1984 (mean ± SD followed by range)

Test plants	Total replicates test plants	Total eggs used	Total eggs hatched		Total larvae found/plant species	
			1982			
Centaurea solstitialis Greece (control)	10	20	2.0 ± 0.0	2–2	1.0 ± 0.0	1–1
Centaurea solstitialis Goldendale (WA)	10	20	1.7 ± 0.67	0–2	1.0 ± 0.0	1–1
Centaurea solstitialis Walla Walla (WA)	10	20	1.8 ± 0.63	0–2	0.9 ± 0.0	0–1
Centaurea solstitialis Yakima (WA)	10	20	1.9 ± 0.32	1–2	1.0 ± 0.0	1–1
Centaurea solstitialis Concord (CA)	10	20	1.8 ± 0.63	0–2	0.9 ± 0.0	0–1
Centaurea solstitialis Bracciano (Italy)	10	20	1.9 ± 0.32	1–2	0.9 ± 0.0	0–1
Centaurea solstitialis Salerno (Italy)	10	20	2.0 ± 0.0	2–2	0.9 ± 0.0	0–1
Centaurea solstitialis Bari (Italy)	10	20	1.9 ± 0.32	1–2	1.0 ± 0.0	1–1
			1983			
Centaurea solstitialis Greece (control)	10	20	1.8 ± 0.63	0–2	1.0 ± 0.0	1–1
			1984			
Centaurea solstitialis Greece (control)	10	20	1.8 ± 0.63	0–2	1.0 ± 0.0	1–1

Two eggs were used per test plant. Ten replicates were made per each tested plant. Only 1 larva can develop in 1 bud. Plants that did not have any larval survival are not listed in this table. For complete list of tested plants see Table 1.

test data were analyzed (ANOVA) and means were separated by a Student–Newman–Keuls a posteriori test.

Site Preference for Oviposition: 1983. Yellow starthistle plants, may have as many as 6 floral bud stages present at 1 time during the oviposition period of *B. orientalis.* Therefore, an experiment was conducted to determine the preferred bud stage for oviposition. We used the bud development classes of Maddox (1981) to identify the various bud stages during May and June. Potted plants of *C. solstitialis* were used for this test, and 1 pair of adults was caged on each test plant using the same plastic cylinders as in the no-choice oviposition test. Ten plants (replicates) with different bud stages were used in this trial, which was carried out from 25 May to 18 July in the same shaded quarantine greenhouse of previous tests. After 5 d floral buds were examined and eggs were recorded and removed. Data of adult oviposition site preference test are expressed with means and standard deviation.

Results

No - Choice Oviposition Tests: 1982–1984. The total number of exposed floral buds, floral buds attacked, total eggs laid, and adult longevity on each plant species, varieties, and strains are presented in Table 1. Adults laid eggs only on members of the genus *Centaurea* and the thistle *Onopordum acanthium.* Significantly more eggs were laid on *C. solstitialis* plants (all populations) than on other test plants in the 1982 (Table 1). The U.S. yellow starthistle plants were readily accepted, with plants from WallaWalla, WA, receiving the highest number of eggs per replicate (63.40 ± 15.95; range, 43–80). Populations from Italy were also accepted for oviposition, and the 1 from Bari had the highest mean number of eggs per replicate (97.80 ± 0.84; range, 97–99). There was no significant difference in number of eggs laid on *C. solstitialis* from WallaWalla versus *C. solstitialis* from Goldendale, WA, or *C. solstitialis* from Yakima, WA, versus *C. solstitialis* from Bracciano, Italy (Table 1). Other species in the genus *Centaurea* (*C. diffusa, C. jacea, C. maculosa,* and *C. calcitrapa* from Italy, and *C. calcitrapa* from Greece) received fewer eggs. There was no significant difference in number of eggs laid on *C. calcitrapa* from Greece versus *C. calcitrapa* from Italy (Table 1.). The only plant outside the genus *Centaurea* to receive eggs was *O. acanthium* with a mean of (1.4 ± 0.55). Eggs were not deposited on plant species of economic importance such as *Cynara scolymus, Helianthus annuus, Carthamus tinctorius, Lactuca sativa,* and the U.S. endangered species of *Cirsium, C. undulatum, C. douglasii,* and *C. andrewsii.* Adults fed little or not at all on nonhost plants and were shorter lived (15–20 d) compared with weevils caged on the natural host (39–51 d).

First Instar Survival Test: 1982, 1983, 1984. Results of this test are summarized in Table 2. In total, 657, 509, and 530 eggs were collected in 1982, 1983, and 1984, respectively, for a total of 1,696. Mean percentage of egg eclosion was 86.5%. This egg production was sufficient for larval survival tests. Larvae completed their larval development only on *C. solstitialis* from Greece and on U.S. and Italian populations of *C. solstitialis* among the 60 plant species, varieties, and strains tested. In the 1982 test, 1st instars completed their larval development only on the different population of *C. solstitialis.* Larval survival was 95% (76 out of 80) in this test. Survival was 100% in the 1983 and 1984 tests.

Multiple Choice Test: 1984. In all test-plant groups the plants receiving the most eggs were the *C. solstitialis* controls from Greece and the U.S. and Italian ecotypes of yellow starthistle. Significantly ($P < 0.05$) more eggs were laid on *C. solstitialis* from Greece than on any other test plant. No eggs were found on *O. acanthium* (Table 3). *B. orientalis* laid more eggs on *C. diffusa, C. calcitrapa, C. jacea,* and *C. maculosa* in this test than it did on these test plants under no-choice conditions. No large differences were noted in comparing adult longevity of *B. orientalis* caged with the various test-plant groups (Table 3).

Adult Oviposition Site Preference: 1983. The weevil oviposited heavily on floral buds Bu-1 and Bu-2. Stages

Table 3. Results of *B. orientalis* oviposition–multiple–choice host specificity tests, 1984 (mean ± SD followed by range)

Test plants	Replicates	Total exposed floral buds per test plant		Total floral buds attacked per test plant		Total eggs laid per test plant		Adult Longevity Days	
				Group I					
Centaurea solstitialis Greece (control)	3	100.00 ± 14.18	89–116	91.67 ± 15.53	79–109	143.33 ± 160.1a	127–159	61.7 ± 3.79	59–66
Centaurea solstitialis Goldendale (WA)	3	106.67 ± 6.81	99–112	65.67 ± 21.57	41–81	90.00 ± 18.52b	72–109		
Centaurea solstitialis Walla Walla (WA)	3	96.67 ± 6.11	90–102	58.33 ± 5.86	54–65	66.67 ± 27.79b	35–87		
Centaurea diffusa Greece	3	95.00 ± 18.73	80–116	7.33 ± 0.58	7–8	9.00 ± 1.00c	8–10		
Centaurea solstitialis Salerno (Italy)	3	90.00 ± 9.85	79–98	66.00 ± 6.56	59–72	70.00 ± 16.70d	55–88		
				Group II					
Centaurea solstitialis Greece (control)	3	100.67 ± 7.37	95–109	92.00 ± 7.21	86–100	129.67 ± 33.53a	97–164	64.7 ± 6.66	59–72
Centaurea solstitialis Yakima (WA)	3	66.67 ± 7.09	59–73	58.67 ± 8.33	52–68	62.33 ± 6.66b	58–70		
Centaurea solstitialis Concord (CA)	3	70.00 ± 10.44	58–77	55.67 ± 4.51	51–60	65.67 ± 3.51c	62–69		
Centaurea solstitialis Bracciano (Italy)	3	68.33 ± 10.02	57–76	49.00 ± 3.61	46–53	67.00 ± 7.55d	59–74		
Centaurea solstitialis Bari (Italy)	3	65.67 ± 9.45	55–73	33.33 ± 16.80	15–48	59.33 ± 12.50d	45–68		
				Group III					
Centaurea solstitialis Greece (control)	3	67.00 ± 31.48	41–102	56.33 ± 30.11	32–90	103.33 ± 57.85a	54–167	62.0 ± 4.58	58–67
Centaurea solstitialis va. *schowii* (Italy)	3	66.00 ± 9.17	58–76	23.33 ± 5.51	18–29	26.33 ± 5.41b	21–32		
Centaurea jacea Italy	3	45.67 ± 1.53	44–47	10.00 ± 2.00	8–12	12.33 ± 3.51c	9–16		
Centaurea maculosa Italy	3	71.67 ± 5.86	65–76	9.00 ± 3.61	5–12	13.00 ± 5.29d	7–17		
Centaurea paniculata Italy	3	49.00 ± 9.54	38–55	1.00 ± 1.00	0–2	2.00 ± 2.00b	0–4		
				Group IV					
Centaurea solstitialis Greece (control)	3	92.00 ± 14.73	76–105	78.33 ± 15.31	69–96	132.67 ± 14.84a	120–149	62.0 ± 3.00	59–65
Centaurea calcitrapa Italy	3	85.33 ± 21.55	63–106	23.33 ± 3.21	21–27	26.67 ± 4.16b	22–30		
Centaurea calcitrapa Greece	3	43.00 ± 7.00	38–51	33.33 ± 7.37	25–39	34.33 ± 7.37b	26–40		
Onopordum acanthium USA	3	5.00 ± 2.00	3–7	0.00 ± 0.00	0–0	0.00 ± 0.00	0–0		

Ten males and ten females per replication. Three replicates made per plant species. Means followed by the same letter are not significantly different ($P < 0.05$, Student–Newman–Keuls a posteriori test).

Bu-3 and Bu-4 received few eggs, whereas no eggs were laid on F-1 and F-2 floral buds. Stage Bu-1 had 31.50 ± 14.20 exposed buds per plant replicate of which 22.20 ± 11.62 were attacked, receiving 65.30 ± 47.66 eggs per replicate. Stage Bu-2 had 8.00 ± 5.70 buds per replicate and 5.80 ± 3.88 were infested, receiving 13.90 ± 7.11 eggs per replicate. Stages Bu-3 and Bu-4 were much less attacked, receiving 3.10 ± 2.69 and 0.60 ± 0.84 eggs per replicate (Table 4).

Table 4. Bud size preference for ovipositional site of *B. orientalis* on yellow starthistle, 1983 (mean ± SD)

Flower bud category	No. buds/plant	No. buds attacked/plant	No. eggs laid/plant
Floral bud stage Bu-1	31.50 ± 14.20	22.20 ± 11.62	65.30 ± 47.66
Floral bud stage Bu-2	8.00 ± 5.70	5.80 ± 3.88	13.90 ± 7.11
Floral bud stage Bu-3	3.80 ± 2.74	1.50 ± 0.97	3.10 ± 2.69
Floral bud stage Bu-4	3.10 ± 1.52	0.40 ± 0.52	0.60 ± 0.84
Anthesis stage F-1	2.20 ± 1.14	0.00 ± 0.00	0.00 ± 0.00
Anthesis stage F-2	1.70 ± 0.82	0.00 ± 0.00	0.00 ± 0.00

One male and one female used per replicate. Ten replicates made.

Discussion

Although some adults in this study oviposited on plants from which they have not been recorded, such as *Centaurea diffusa*, *C. jacea*, *C. maculosa*, *C. calcitrapa*, and *O. acanthium*, they deposited a majority of their eggs on *C. solstitialis*. Moreover, *C. solstitialis* was the only test plant to support larval development; there was no larval survival on *C. diffusa*, *C. jacea*, *C. maculosa*, *C. calcitrapa*, and *O. acanthium*. First instars that hatched from eggs placed on floral buds of other plant species died after hatching or after feeding on the nontarget floral bud. These studies confirm the findings of 1984 open-field host specificity tests in Greece (Maddox and Sobhian 1987). Oogenesis studies showed that *B. orientalis* females were incompatible with host plants other than *C. solstitialis*, their eggs atrophying after being switched from *C. solstitialis* to safflower or native *Cirsium* species (Maddox and Sobhian 1987). While checking for adults or eggs on various plant genera (*Centaurea*, *Carduus*, *Onopordum*, *Cirsium*, and *Galactites*) in the field in northern

Greece (Campobasso, unpublished data) as well as in the laboratory, we found that *C. solstitialis* is the only host of *B. orientalis* capable of supporting larval development. The results of these 1982–1984 host specificity studies in Rome, and field studies in Thessaloniki, Greece, at the same time (Maddox and Sobhian 1987), supported release of this weevil against yellow starthistle in the United States in 1985. The weevil was released and become established in California, Oregon, Washington, and Idaho (Grossman 1989, Turner 1996).

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