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1987 ANNUAL REPORT

USDA-ARS
BIOLOGICAL CONTROL OF WEEDS
LABORATORY - EUROPE
ROME - ITALY



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Cover photograph

By Tim McCabe, Information Service, ARS, USDA, Beltsville, Md.

Oxycesta geografica
(Lepidoptera: Noctuidae)

This moth was found in Romania. The female lays her eggs on the underside of a leaf of Euphorbia virgata W. et K. and the neonate larvae move to the top of the plant and make a silken tent which increases in size as the larvae grow. The later instar larvae feed outside of the tent, but return to the tent to rest. As the larvae mature they completely defoliate the plant on which the eggs were laid as well as adjacent plants. The mature larvae migrate away from the plants on which they have fed and pupate in dried leaves and plant trash.

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INTRODUCTION

The year 1987 was an active year for the Rome laboratory and good progress was made toward clearing a number of natural enemies for release in 1989 through 1991.

In addition to the screening program a lot of effort was put into collecting insects already cleared for release and colonization. A brief overview of the activities associated with each target plant shows that the following collections were made for sending to the U.S. against the named target plants.

Leafy spurge:	<u>Aphthona cyparissiae</u> , <u>Aphthona flava</u> , <u>Aphthona czwalinae</u> , <u>Bayeria capitigena</u> , <u>Oberea erythrocephala</u>
Yellow starthistle:	<u>Bangasternus orientalis</u>
Diffuse knapweed:	<u>Pterolonche inspersa</u>
<u>Convolvulus arvensis</u> :	<u>Tyta luctosa</u>

More importantly, there are a substantial number of insects in the "pipeline" being cleared for introduction. This is an impressive list and clearly indicates where the bulk of our research is concentrated. We are currently working on clearing 12 arthropods and all of them are promising candidates. A list of the target weeds, the natural enemies being screened for their control, and the probable release date of these natural enemies follows:

WEED	CANDIDATE NATURAL ENEMY	PROPOSED RELEASE DATE
Leafy spurge	<u>Aphthona abdominalis</u>	1989
	<u>Chamaesphecia crassicornis</u>	1989
	<u>Dasineura</u> sp.	1989
	<u>Oxycesta geographica</u>	1990
	<u>Simyra dentinosa</u>	1989
Diffuse knapweed	<u>Bangasternus fausti</u>	1989
	<u>Aceria centaurea</u>	1990
	<u>Larinus minutus</u> ^{1/}	1989
Yellow starthistle	<u>Eustenopus villosus</u>	1989
	<u>Larinus curtus</u>	1991
Musk thistle	<u>Psylloides chalcomera</u>	1989
	<u>Cheilosia corydon</u>	1991

If our research program runs smoothly in 1988 and there are no surprises it is conceivable, even possible, that 8 natural enemies will be ready for release in 1989, another 2 in 1990 and another 2 in 1991, all against the target weeds in our current list.

A new set of target weeds must be selected soon so the preliminary library, herbarium, museum work and exploration can be started so the weeds and candidate insects can be found and research started on these new insects to replace those which are now in the process of being cleared for introduction.

1/ This is a Canadian project, financed by Agriculture Canada with CIBC Delémont. We have a cooperative field trial in Thessaloniki, Greece being conducted by Delémont and ARS (Sobhian) personnel.

This is my 7th and last annual report because I am retiring shortly. Preparing the annual report has never been my favorite occupation but in a good year it provides a lot of satisfaction after it has all come together and we can see the progress we have made during the year. This is the staff's and Research Leader's opportunity to let our administrators see what they got and what they are going to get for the support they have given us. When I look back at this preface and the contents of the report, I feel like by leaving I am jumping out of a moving train and have a vague feeling that I would like to stay on board until we arrive at our destination and clear 4 or 6 or 8 insects for release. This will be done, I just won't be on board.

It has been an interesting trip with great traveling companions and lots of adventures and hard work. I want to thank all of you, (you know who you are), who made these last 7 years an unforgettable experience.

Paul H. Dunn,
Director.

LEAFY SPURGE PROJECT 1987

(P. Pecora, L. Fornasari, M. Cristofaro, M. Stazi)

Simyra dentinosa Freyer (Lepidoptera: Noctuidae)

Pasquale Pecora and Massimo Cristofaro

BIOLOGY NOTES

Adult emergence of Simyra dentinosa was recorded from 41 pupae, 21 of which were individually distributed into 250 cc. cardboard containers and kept in an outdoor insectary, and 20 were removed from their cocoons and put (10 each) into two plastic containers (15 x 15 x 20 cm.), buried within (6-8 cm.) cornmeal. These containers were kept in a controlled temperature cabinet ($15 \pm 1^\circ\text{C}.$) from mid-June until early November and then moved to the outdoor insectary until adult emergence was completed.

From the 21 pupae kept out-of-doors, 13 adults (8 females, 5 males) (62%) emerged during the second half of April; 4 (30%) of these had malformed wings. From pupae kept in cornmeal, 12 adults (5 females, 7 males) emerged in the last week in April and 7 (58.3%) were malformed. In the laboratory adults survived 4-10 days, sitting for hours on cage walls or on a plant with no activity. No mating was observed.

Thirty eggs, collected on Euphorbia seguierana at Volvi Lake on April 5, 1987, were measured for size (diameter only). Also, the pre-eclosion period and the percentage of egg fertility were recorded on a sample of 150 field-collected eggs, kept in an outdoor insectary.

The eggs, which were relatively flat, disclike, nearly circular, measured 0.87 ± 0.05 mm. ($n=30$) in diameter (range = 0.76-0.96 mm.). They were light yellow when first laid and turned dark brown in 3-5 days. The eggs were laid

in masses in more-or-less regular rows on the underside of single leaflets of E. seguierana. The number of eggs/mass ranged between 61-241 (n=28). The pre-eclosion period of 150 fresh laid field collected eggs, kept in a laboratory room (temp. $20^{\circ} \pm 3^{\circ}\text{C.}$) ranged from 16-19 days, and 95% were fertile.

HOST SPECIFICITY TESTS

The test conducted in 1986 showed that S. dentinosa larvae could develop only on plants in the genus Euphorbia. In 1987 host specificity studies were completed on plants recorded in the literature as being attacked by other Simyra spp., i.e. Triticum aestivum L., Secale cereale L., Phalaris canariensis L., Poa pratensis L., Dactylis glomerata, Zea mays L. (sweet corn) (Gramineae), Rheum rhabarbarum (Polygonaceae), Typha latifolia L., (Typhaceae), and several ornamental plants representing related superorders. These were: Sedum album L. (Crassulaceae), Helianthemum apenninum L. (Cistaceae), Fagopyrum tataricum (L.) Gaertn.; Asclepias syriaca L., A. speciosa Torr. (Asclepiadaceae); Alyssum argenteum All. (Cruciferae), Iris sibirica L. (Iridaceae).

Two larval survival tests were set up by using neonate larvae from eggs collected near Volvi Lake (Greece) on E. seguierana at the beginning of April 1987. In one experiment (Test A) single first instars were tested, using 10 larvae for each plant species. Each larva was placed in a 500 cc. cardboard cup which had a folded paper towel on the bottom to absorb any water and was covered with a plastic lid in which a 5 cm. diameter central hole had been made, and covered with nylon organdy to allow air exchange. A bouquet of fresh leaves of the appropriate test plant was placed in the cup, and was inspected and replaced twice weekly. At each inspection, the number of living

and dead larvae was recorded, and the amount of feeding was measured in mm^2 by using a transparent plastic grid.

Since the larvae of S. dentinosa are gregarious until the 4th instar, a more natural experiment (Test B) was made by using groups of neonate larvae on potted plants, which were caged within transparent plastic tubes (20 cm. diameter; 50 cm. height). Each potted plant represented one replicate and 10 first instar larvae were placed on each replicate. The species or varieties of each plant tested, as well as the control plant, were replicated twice. The larvae were left undisturbed except for the replacement of test plants when necessary. The silk webs formed by colonies of larvae between successive molts, were removed from the used test plants which had been replaced, and were saved in 500 cc. paper cups. Later the width of the head capsules contained in each silk web were measured, so the number of instars of S. dentinosa larvae which developed each of the various test plants was determined. Both experiments were conducted in quarantine, under natural day length and ambient temperature from April to May.

Complete larval development occurred only on the control plant (E. seguierana). Minimal feeding was recorded on Helianthemum apeninum on 3 larvae in test A, and 4 larvae in test B molted to the 2nd instar. On the other test plants no feeding was observed and the larvae died in 3-4 days without molting (Table 1).

Table 1. Larval development of Simyra dentinosa on different plant species.

Testing made at the USDA Rome Laboratory in 1987.

	<u>TEST A</u>							<u>TEST B</u>							
	No. Individuals Surviving To:							No. Individuals Surviving To:							
	STAGE							STAGE							
	I	II	III	IV	VI	Pupa	Adult	I	II	III	IV	VI	Pupa	Adult	
<u>Euphorbia seguierana</u> (Control)	10					7	7	6	20	20	20	20	15	15	13
<u>Triticum aestivum</u>	10								20						
<u>Secale cereale</u>	10								20						
<u>Phalaris canariensis</u>	10								20						
<u>Poa pratensis</u>	10								20						
<u>Dactylis glomerata</u>	10								20						
<u>Zea mays</u>	10								20						
<u>Rheum rhabarbarum</u>	10								20						
<u>Typha latifolia</u>	10								20						
<u>Sedum album</u>	10								20						
<u>Helianthemum apeninum</u>	10	3							20	4					
<u>Fagopyrum tataricum</u>	10								20						
<u>Asclepias syriaca</u>	10								20						
<u>A. speciosa</u> Torr.	10								20						
<u>Alyssum argenteum</u>	10								20						
<u>Iris sibirica</u>	10								20						

Chamaesphecia sp. (Lepidoptera: Sesiidae)

(P. PECORA, and M. STAZI)

From infested roots of Euphorbia virgata "group", collected at the end of October 1986 near the Danube delta in Romania, only a few adults of C. crassicornis emerged in July 1987. These adults laid only unfertile eggs, so no host specificity tests were made. Another collection of infested roots, containing various stages of larvae of C. crassicornis, was made in October 1987 in Romania. If adults emerge and we get fertile eggs, a larval survival test will be conducted in 1988 on several closely related Euphorbia spp. and plants of economic importance.

Taxonomic investigations on the gall midge

Dasineura sp. near capsulae

(P. Pecora)

A European gall midge, whose larvae produce capsule-like galls on Euphorbia spp., was selected in 1982 as a candidate for the biological control of leafy spurge in North America. Host specificity tests, made at the USDA's laboratories at Rome (Italy) and Albany (California) from 1983 to 1987, using test insects from a population which occurs at S. Rossore (Central Italy) on E. esula, demonstrated that this midge is able to develop only on plants in the genus Euphorbia (subgenus Esula).

In the midge complex associated with Euphorbia spp. in Europe, four species (Dasineura capsulae Kieffer, D. loewi (Mik), Perrissia corniflex Kieffer, P. euphorbiarum Kieffer) which produce capsule-like galls have been described (Kieffer 1901, Kieffer 1909, Houard 1908). Recently, Solinas and Pecora (1984) suggested, that in this complex, "only two good species (D. capsulae and D. loewi) may remain". One of these "good species", D. capsulae was recorded from E. cyparissias L., E. esula L., E. nicaeensis Allioni, E. pithyusa L. (Kieffer 1901, Houard 1908), E. falcata L., E. lucida Waldstein & Kitaibel (Buhr 1964) and the other, D. loewi, was found on E. seguierana Necker (Buhr 1964).

Adults originating from larvae which produced capsule-like galls on E. cyparissias and E. esula, and which emerged in 1983, were identified as Dasineura capsulae Kieffer by M. Solinas. Specimens of adult midges tested in quarantine at Albany, California, in 1986, whose larvae came from E. esula capsule-like galls collected at S. Rossore in mid-June 1985, were sent

by R.W. Pemberton, USDA/ARS Albany laboratory, to R.J. Gagné, USDA-ARS-SEL (Systematic Entomology Laboratory) for identification. The specimens examined by Gagné showed a shorter ovipositor than those illustrated by Solinas and Pecora (1984). R.J. Gagné stated that "these specimens belong to neither D. capsulae nor Bayeria capitigena, although they do belong to Dasineura in the broad sense". Since the taxonomy of the midge complex associated with Euphorbia spp. in Europe is not yet clear, R.J. Gagne suggested calling the midge from E. esula "Dasineura sp. near capsulae" until it is properly described and named. From the controversial results of the taxonomic determinations it seems that two species have been taken from the E. esula and E. cyparissias capsule-like galls, with one of the following scenarios:

- (1) That one of these species is an inquiline. Inquilines lay eggs in galls already initiated by other species. Their larvae outcompete and indirectly cause the death of the gallmaker;
- (2) That many differently-shaped cyathium galls are formed on Euphorbia spp. Possibly, cyathium and leaf capsule galls occurring at S. Rossore were produced by different species and were mixed during the collection to provide material for host specificity testing.
- (3) More than one species of gallmaker make the same kind of gall. Possibly the species, collected in 1982/83, has now been replaced by another species which now occupies the area.

In order to make an indepth study of the taxonomy of the complex of gall midges on Euphorbia spp., R.J. Gagné travelled to Europe June 6 to 27, 1987. The objectives of the trip were to study the types of gall midge species that feed on Euphorbia spp.; to collect galls from which to rear new specimens, for

making neotypes for types that are lost; and to meet with European colleagues to discuss various facets of this research. This trip was fruitful for collecting different kinds of galls for museum work, and for consulting with his European colleagues.

(a) Collection of biological material

R. J. Gagné collected cyathium and leafy capsule-like galls and rosette-like galls both at S. Rossore (Italy) on E. esula and at Scharrachbergheim, 15 kms. West of Strasbourg (France) on E. cyparissias.

(b) Museum Work

Dr. Gagné visited the Entomologische Institut, Eidgenössischen Technische Hochschule in Zurich, where the Bremi collection is deposited. Dr. H. Sauter, curator of the insect collection at this Institute, allowed Gagné to borrow certain types of the spurge midges. He also visited the College of St. Augustin at Bitche, where Abbé J.J. Kieffer described hundreds of species of gall midges, including several species from spurges. However, nothing remained of the Kieffer collection.

On the way back to Rome, Dr. Gagné stopped in Florence at the Museo Zoologico "La Specola", to see the collection of Rondani for the types of some species in the genus Dasineura, to which the spurge midges presumably belong. The types were not there; however, he found some additional types of spurge midges collected by Bremi in Zurich.

Dr. Gagné, Professor Solinas and I met in Perugia, and explored the possibility of the existence of two species of the capsule gall maker, one on E. esula and one on E. cypariassias, and we arrived at the aforementioned scenarios. Dr. Gagné stated that his major interest is to resolve the problem of the types, in order to have available names for those spurge midges that would be imported to North America as biological control agents of leafy spurge.

Aphthona abdominalis Duftschm.

L. Fornasari and M. Stazi

INTRODUCTION

This year additional testing was carried out to investigate, in different ways, the host specificity of this flea beetle in the larval and adult stages, and its ability to complete development on the six plants tested. Three tests were made: an oogenesis test, a host suitability test, and a larval survival test.

1. OOGENESIS TEST (LABORATORY)

MATERIALS AND METHODS

A no-choice test was conducted to assess the ability of A. abdominalis overwintering adults to produce and lay eggs on six test plant species. In addition to Euphorbia esula as control, the species screened were: E. supina, E. maculata, E. tirucalli, E. lathyris, E. marginata and Linum usitatissimum. The adults used in this test were collected in the Rome laboratory garden on September 9, 1986, and kept in outdoor cages with soil and wood chips as shelter. The experiment started on April 2, 1987. The test plants were presented as bouquets in cardboard cups (11 cm. diameter and 8 cm. high) covered with a fine mesh nylon screen to allow aeration. Eight A. abdominalis adults were put in each cup and 4 replications were made for each test plant. Twice a week the bouquets were checked for oviposition and replaced with fresh ones. As the adults died they were dissected to check for the presence and condition of the eggs in the ovarioles. The experiment was conducted in a laboratory room with natural light and the temperature ranging between 20 and 25°C. It ended when the last of the insects on the test plants died on June 19, 1987.

RESULTS

Oviposition occurred on the control (248 eggs) and on Euphorbia maculata (12 eggs). Eggs were found on stems of the plants in the bouquet and on the caps of the vials containing the bouquets. While the eggs laid on E. esula hatched normally, the eggs laid on E. maculata did not hatch (temperature was approximately 25°C R.H.=80-90%). No egg development was found during dissection of the adults which had fed on the other test plant species.

2. HOST SUITABILITY TEST (LABORATORY)

MATERIALS AND METHODS

The objective of this test was to see if A. abdominalis could complete development on the following test plants: E. maculata, E. supina, E. tirucalli, E. marginata. E. esula was used as control. On August 6, 1987 ten ovipositing adults were placed on each potted plant, placed under transparent plastic cylinders (60 cm. high x 19 cm. dia.) with the top covered with netting, and four screen covered holes (12 cm. dia) on the sides to allow air circulation. Four replications were made for each plant species tested and the control. Fifteen days later (on August 21) these adults were recollected and the tubes removed. On September 7 the plastic cages were replaced over the plants, and checked daily for emergence of new adults.

RESULTS

From September 29 to October 9, 16 adults were found on the control. On October 7, one adult was found on E. marginata.

3. LARVAL SURVIVAL TEST (LABORATORY)

MATERIALS AND METHODS

The objective of this trial, carried out in quarantine in June and July 1987, was to determine the ability of neonate larvae of A. abdominalis to develop on these test plants, on which adult feeding activity had been observed in the no-choice feeding tests made in the summer of 1984, 1986 and 1987. The following plant species were used: E. esula (from Italy) as control, E. marginata, E. maculata, E. supina, E. lathyris, E. tirucalli, E. serpyllifolia, Ricinus communis and Linum usitatissimum. In previous testing there was no feeding on L. usitatissimum, however, it was tested again because of its economic importance.

Larvae used in this test came from eggs laid by adults collected in the laboratory garden and caged in paper cups with E. esula bouquets as oviposition plants. The eggs were kept in a temperature cabinet at a constant temperature ($25^{\circ} \pm 0.5^{\circ}\text{C}$) on wet blotting paper in Petri dishes until eclosion (about 8 days). Five neonate larvae were put on the root collar of the plants at soil level and two weeks later transparent plastic tube cages (the same kind used in the host suitability test) were placed over the plants. Five replications were made of each test plant, thus 25 neonate larvae per test plant species were used. Later, the number of adults emerging from each of the plants was recorded.

RESULTS

A. abdominalis larvae completed their development only on the control, and on E. lathyris. The larval development took about 30 days. From the control 7 adults (=28% survival) emerged in \bar{x} 28.4 \pm 3.45 days. Seven adults (=28% survival) also emerged from E. lathyris, taking about the same time \bar{x} 28.4 \pm 3.64 days.

During the trial the temperature ranged from a minimum of 12°C to a maximum of 35°C. The mean temperature range measured at 2-hour intervals was 17-30°C. The mean relative humidity was 54-75% with a minimum of 26% and a maximum of 93%.

CONCLUDING REMARKS

This year testing was not extensive enough to define the host range of A. abdominalis. Next year additional plants will be tested and an open field test that we were unable to make in 1987 will be made.

Collection and Survey Trips

(P. PECORA, M. STAZI, M. CRISTOFARO, and A. LAREGINA)

Bayeria capitigena (Bremi) (Dipt.: Cecidomyiidae); (M. Stazi, M. Cristofaro, collectors):

Five shipments of tip galls containing various larval instars and pupae of B. capitigena were shipped to Albany, California. These galls were collected on E. esula at S. Rossore, Italy on May 11 (140 galls), May 22 (320 galls), May 29, (350 galls), June 3 (250 galls) and July 15 (220 galls). Twelve working days were required to make these collections.

Dasineura capsulae Kieffer (Dipt.: Cecidomyiidae); (M. Stazi, M. Cristofaro collectors):

About 8,000 mature larvae of D. capsulae were produced from 1100 galls collected on E. esula at S. Rossore in mid-June 1987. These larvae were transferred to 4500-ml. acrylic containers with a 3-4 cm. deep layer of moist peat moss and fine sand mixture to hibernate until the following spring. Two working days were spent to collect this stock of galls.

Oberea erythrocephala Schrank (Col: Cerambycidae); (M. Stazi, M. Cristofaro collectors):

Adults of this longhorned beetle, collected on E. esula at S. Rossore on June 3 (210 adults), June 15 (210 adults) and July 7 (97 adults), were shipped to Albany, California for field releases. Six working days were needed to collect these beetles.

Aphthona flava Guill. (Col.: Chrysomelidae); (M. Stazi, M. Cristofaro collectors):

Four shipments of this flea beetle were sent to Albany, California for field releases. These insects were collected on E. esula and E. cyparissias on June 15 (1900 adults), June 22-30 (3100 adults), July 7 (700 adults) and

July 13 (1500 adults). Ten days were spent for these collections.

Aphthona cyparissiae (Koch) (Col.: Chrysomelidae); (P. Pecora, A. Laregina collectors):

Two thousand eight hundred adults of A. cyparissiae were collected on E. cyparissias near St. Polten (Austria) between June 19 and July 2, 1987. In addition, 1000 adults of this flea beetle were collected on E. cyparissias and 200 on E. virgata in two localities near Gyor (Hungary) in the first week of July. These insects were shipped to Albany, California for field release. Eleven days were necessary for these collections.

Aphthona czwalinae (Weise) (Col.: Chrysomelidae); (P. Pecora, A. Laregina collectors):

Nine hundred adults were collected on E. esula in Eastern Austria on July 1-2, 1987, and 100 adults were collected in Hungary (Gyor area) on E. esula in the first week of July. Four days were spent collecting Aphthona cyparissiae and A. czwalinae.

A trip was made to Romania and Czechoslovakia in mid-October by P. Pecora and M. Cristofaro to collect Chamaesphecia crassicornis (Lep.: Sesiidae) and Oxycesta geographica (Lep.: Noctuidae), and to locate new sites infested with larvae of these species. One hundred fifty plants of E. virgata containing larvae of C. crassicornis in various instars were collected in the Braila area (Romania). Three hundred mature larvae of O. geographica were collected on E. virgata and E. stepposa in the same area. C. crassicornis larvae were also found on E. virgata at Sturovo in Czechoslovakia. In this locality 22% of the plants examined (n=120) were infested.

YELLOW STARHISTLE PROJECT - 1987

L. Fornasari

Eustenopus villosus (Boheman, 1836).

INTRODUCTION

The insect called Eustenopus abbreviatus Faust. and E. hirtus (Waltl) in previous reports is in reality Eustenopus villosus (Boheman, 1836) (determined by Dott. E. Colonnelli, Dipartimento di Biologia Animale e dell'Uomo, Viale dell'Universita', 32 - Roma, Italy). This is the most recent and hopefully final name, after a taxonomic revision of the genus that involved several specialists in different countries.

In 1987 emphasis was placed on assessing the potential of E. villosus as biological control agent for yellow starthistle (YST) in the U.S. Testing was confined to the host specificity of this weevil under no-choice (Test No. 1) and choice (Test No. 2) conditions, on plant species present in the list approved by the Technical Advisory Group on Biological Weed Control. In another study (Test No. 3) the ability of E. villosus larvae to develop in buds of various plant species was investigated. Adults collected in the field on Centaurea solstitialis in Greece were used for these studies. When they arrived at the Rome laboratory they were allowed to feed for at least 48 hours on YST buds (obtained from Greek plants) before copulating pairs, i.e. a male and a female, were selected for tests.

TEST 1 - ADULT FEEDING AND OVIPOSITION NO-CHOICE TEST.

METHODS

This test was conducted in a quarantine greenhouse (temp. = 22.5 ± 4.07 C, range = 14 - 33°C; R.H. = $60.7 \pm 17.76\%$, range = 28 - 88%, and natural lighting) on 21 plant species, listed in Table 1. Branches of each test plant were caged in black, nylon tulle sleeve cages (a system previously used in 1985 and 1986). Two couples of E. villosus were caged on each potted plant and there were 5 replicates of each test plant species. Weevils were permitted to feed and oviposit for 7-10 days, then moved onto another fresh caged plant and left for 7-10 days. This procedure was repeated until all the beetles died. All the exposed buds were dissected to estimate and record the feeding damage and count the number of eggs laid. In addition, dead adults were dissected to determine sex, and to observe the condition of ovaries and the number of eggs retained and not laid. This test started July 6 and finished August 27.

RESULTS

The results of this test are reported on Table 2 and the pattern of host preference is illustrated in Fig. 1.

Fig. 2 shows the total number of exposed and damaged or fed-on buds for each species. The highest numbers of damaged buds, after the control, were observed on Centaurea maculosa, C. paniculata, C. alba, C. scabiosa, C. cyanus and C. calcitrapa. If we consider (Fig. 2) the percentage of damaged buds, Safflower had the highest value (58.3%) after the control (87.8%), followed by Centaurea paniculata (57.3%), C. scabiosa (51.0%), C. alba (49.1%), C. cyanus (41.7%) and C. jacea (39.4%). On Gazania splendens 38.1% of the exposed buds

were fed on. A small percentage of buds Zinnia elegans (14.8%) and Achillea millefolium (0.9%) were fed on. In spite of the feeding on Carthamus tinctorius, no eggs were laid on this species under no-choice conditions, confirming data from previous tests on safflower by Clement and Mimmocchi (1986 Annual Report). They observed no development of the ovaries in E. villosus females fed on safflower under no-choice conditions and no oviposition. The 1986 tests also showed that under choice conditions "only minor feeding was recorded on safflower (only on the leaves) when YST buds were present".

Eighteen eggs were found (Fig. 4) on C. scabiosa, 8 on C. maculosa, 5 on C. napifolia, 3 on C. jacea, and 107 on the control. The damage of E. villosus was negligible to plants of economic (agricultural or ornamental) importance tested, i.e. safflower, Gazania splendens and Zinnia elegans (Fig. 3). The longevity of E. villosus was recorded and is shown in Fig. 5

In comparison to the control (\bar{x} 21.8 \pm 8.54 days), its longevity was considerable on Centaurea maculosa (\bar{x} 17.2 \pm 12.70 days), Carthamus tinctorius (\bar{x} 14.6 \pm 4.52 days) and Centaurea scabiosa (\bar{x} 13.3 \pm 6.54 days). Examination of the ovaries of the weevils in the test showed that well developed ovaries were present only in the females which fed on the YST control. There was no feeding or oviposition on Centaurea americana, but buds and flowers of this species were infested with flies, whose larvae were found feeding in the capitula. Because of this artifact, this test will be repeated next year using insect-free plants.

TEST 2 - ADULT FEEDING AND OVIPOSITION CHOICE TEST.

METHODS

This test, started July 6 and finished August 12, was carried out in a quarantine greenhouse (temp. = $22.2 \pm 3.95^{\circ}\text{C}$, range = 14 - 33°C ; R.H. = $61.7 \pm 17.62\%$, range = 31-88%, and natural lighting) using Cynara scolymus, Zinnia elegans, Calendula officinalis, Centaurea americana as test plants, and Centaurea solstitialis (Greece) as the control. A choice was given between the YST control and each of the four test plant species. Each treatment was replicated five times. Branches of YST and test plants were tied together and caged inside black, nylon tulle sleeve cages. Two couples of E. villosus were introduced into each cage. The test was terminated when a choice was no longer available i.e., when YST branches were completely destroyed by the beetles. All exposed buds were dissected to record feeding damage and number of eggs laid.

RESULTS

The results of this test are reported on Table 3. Nibbling occurred on three buds of Zinnia elegans and no feeding was recorded on the other test plants. The yellow starthistle control plants were heavily damaged with complete bud destruction, and eggs were laid only on the control. On Centaurea americana neither feeding nor oviposition were observed, but this species will be tested in 1988 again, because the buds were infested with larvae of indigenous flies.

TEST 3 - FIRST INSTAR LARVAL SURVIVAL TEST.

METHODS

This test was made between July 23 and August 24 in a quarantine greenhouse (temp. = $22.7 \pm 3.73^{\circ}\text{C}$, range = $15 - 32^{\circ}\text{C}$; R.H. = $61.0 \pm 18.0\%$, range = 28 - 87%, and natural lighting). These eggs were placed in 35 ml. plastic cups provided with a layer of moistened (not wet) plaster of Paris on the bottom.

The following plant species were used: Centaurea solstitialis (from Greece) as control, and the test plants Carthamus tinctorius, Cynara scolymus, Zinnia elegans, Calendula officinalis, Centaurea americana, C. cyanus, C. scabiosa, C. napifolia, C. jacea and C. maculosa.

Neonate larvae from eggs kept in a temperature cabinet at the constant temperature of 25°C were transferred with a fine brush into holes made in the buds of the test plants with a scalpel (30 buds/plant species). The holes were then covered with plant bud tissue. After about 25 days, or when the plant condition required it, these buds were dissected.

RESULTS AND DISCUSSION

This test was conducted to try to overcome the difficulties encountered in a similar study in 1986 (Clement and Mimocchi, 1986 Annual Report). Last year a test carried out placing fertile eggs into the test plants buds was not successful, because when the eggs hatched the larvae were confronted with altered plant tissue in the hole made to insert the egg into the bud. This kind of tissue is not encountered in a normal oviposition hole. Problems arose this year too, probably due to the fragility of first instar larvae, even though maximum care was taken in handling them. Very few larvae developed and reached the pupal stage on the control, thus the results obtained on test plants were not appraisable. More work is needed on technique.

CONCLUDING REMARKS

E. villosus studies confirmed that this weevil is a potential control agent of yellow starthistle. In spite of the rather broad feeding spectrum under no-choice conditions, normal oogenesis and oviposition occurred only on the control, and scarcely on Centaurea scabiosa, C. maculosa, C. napifolia and C. jacea under no-choice conditions. Appreciable feeding occurred only on the plants of the genus Centaurea. Neither oogenesis, nor oviposition were observed on safflower. Host specificity testing and studies on E. villosus are expected to be completed in 1988.

Collection and Survey Trips.

In mid-June a trip was taken to Sicily to look for Larinus curtus in the Palermo area, and to collect specimens of this weevil to be identified and reared at the Rome laboratory for future testing. Several localities were surveyed and about 300 adults were collected on Centaurea solstitialis subsp. showii.

At the end of June a trip was taken to Greece to collect Eustenopus villosus and to review the work going on with Larinus curtus and L. minutus (the last one is presently under study by C.I.B.C.). Six hundred and sixty E. villosus adults were collected in Doirani, to provide material for the 1987 Rome laboratory experiments. L. curtus was found in several sites and was particularly abundant in Oreokastro, near Thessaloniki, where three people collected 150 adults in about one hour. In that particular site YST is being crowded out by grassy plants, thus endangering the L. curtus population. For this reason, Dr. Sobhian is trying to develop a system for rearing large numbers of this weevil at our laboratory in Greece.

MEETINGS ATTENDED

· International Symposium on Fruit Flies of Economic Importance (Workshop on Research - Coordination CEC - IOBC). Rome, Italy, April 7-10, 1987.

Table 1.

List of plant species used in the no-choice adult feeding, oviposition and longevity test with E. villosus.

1. Centaurea solstitialis L. (Greece)
2. Silene vulgaris (Moench) Garcke
3. Antirrhinum majus L.
4. Zinnia elegans Jacq.
5. Aster principessa
6. Calendula officinalis L.
7. Achillea millefolium L.
8. Targetes erecta L.
9. Gazania splendens E.G. & A. Henderson
10. Carduus pycnocephalus L.
11. Cynara scolymus L.
12. Carthamus tinctorius L.
13. Centuarea scabiosa L.
14. Centuarea paniculata L.
15. Centaurea maculosa Lam.
16. Centaurea calcitrapa L.
17. Centaurea napifolia L.
18. Centaurea alba L.
19. Centaurea jacea L.
20. Centaurea cyanus L.
21. Centaurea americana Nutt.

Table 2. *E. villosus* 1987 adult feeding, oviposition and longevity no-choice test

Plant Species	Total No. eggs laid	Total No. feeding punctures	Feeding* Damage rating X \pm SD	Total No. of exposed buds	Total No. of damaged buds	Longevity (days) X \pm SD	Bud diameter Range (mm)
<i>Centaurea solstitialis</i>	107	440	2.25 \pm 0.452	304	267	21.8 \pm 8.54	7 : 9
<i>Silene vulgaris</i>	0	0	0	63	0	7 \pm 0	3 : 6
<i>Antirrhinum majus</i>	0	0	0	46	0	7 \pm 0	1 : 2
<i>Zinnia elegans</i>	0	4	0.57 \pm 0.534	27	4	7.9 \pm 2.39	10:12
<i>Aster principessa</i>	0	0	0	43	0	7 \pm 0	15:30
<i>Calendula officinalis</i>	0	0	0	33	0	7 \pm 0	12:17
<i>Achillea millefolium</i>	0	30	0.86 \pm 0.378	ca. 2,000 (27 corymbs)	19	8.3 \pm 2.83	2:2.5 (corymb 20:50)
<i>Targetes erecta</i>	0	0	0	28	0	7 \pm 0	9:12
<i>Gazania splendens</i>	0	12	0.60 \pm 0.548	21	8	7 \pm 0	14:17
<i>Carduus pycnocephalus</i>	0	16	0.80 \pm 0.447	43	10	7 \pm 0	4: 6
<i>Cynara scolymus</i>	0	0	0	5	0	7 \pm 0	60:80
<i>Carthamus tinctorius</i>	0	32	1	36	21	14.6 \pm 4.52	10:14
<i>Centaurea scabiosa</i>	18	69	1.67 \pm 0.500	98	50	13.3 \pm 6.54	10:13
<i>Centaurea paniculata</i>	0	110	2	164	94	12.1 \pm 4.93	5: 7
<i>Centaurea maculosa</i>	8	177	2	437	166	17.2 \pm 12.70	7:09
<i>Centaurea calcitrapa</i>	0	54	2	138	49	8.7 \pm 3.11	6:08
<i>Centaurea napifolia</i>	5	37	2	119	30	10.8 \pm 4.35	5:08
<i>Centaurea alba</i>	0	69	2	116	57	11.8 \pm 5.98	8:12
<i>Centaurea jacea</i>	3	46	1.62 \pm 0.517	99	39	9.2 \pm 4.08	9:11
<i>Centaurea cyanus</i>	0	64	2	120	50	8.1 \pm 2.63	4:06
<i>Centaurea americana</i>	0	0	0	18	0	7 \pm 0	16:20

*Based on a scale of 0 to 3: 0 = no feeding; 1 = very little feeding, no effect on bud development; 2 = very little feeding on a well developed capitula, but considerable damage to young buds; 3 = heavy damage, complete bud destruction.

Table 3. *E. villosus* 1987 adult feeding and oviposition choice test

Plant Species	Total No. eggs laid		Total No. feeding punctures		Feeding Damage rating ^{3/}		Total No. of exposed buds		Total No. of damaged buds	
	YST ^{1/}	T.P. ^{2/}	YST	T.P.	YST	T.P.	YST	T.P.	YST	T.P.
<i>Cynara scolymus</i>	6	0	33	0	3	0	47	5	27	0
<i>Zinnia elegans</i>	16	0	95	4	3	0.20+0.447	149	27	75	3
<i>Calendula officinalis</i>	10	0	193	0	3	0	120	44	92	0
<i>Centaurea americana</i>	14	0	49	0	3	0	70	26	44	0

^{1/} YST = Yellow starthistle

^{2/} TP = Test plant

^{3/} Based on a scale of 0 to 3: 0 = no feeding; 1 = very little feeding, no effect on bud development; 2 = very little feeding on a well developed capitula, but considerable damage to young buds; 3 = heavy damage, complete bud destruction.

Fig. 1. Feeding damage in the no-choice test.

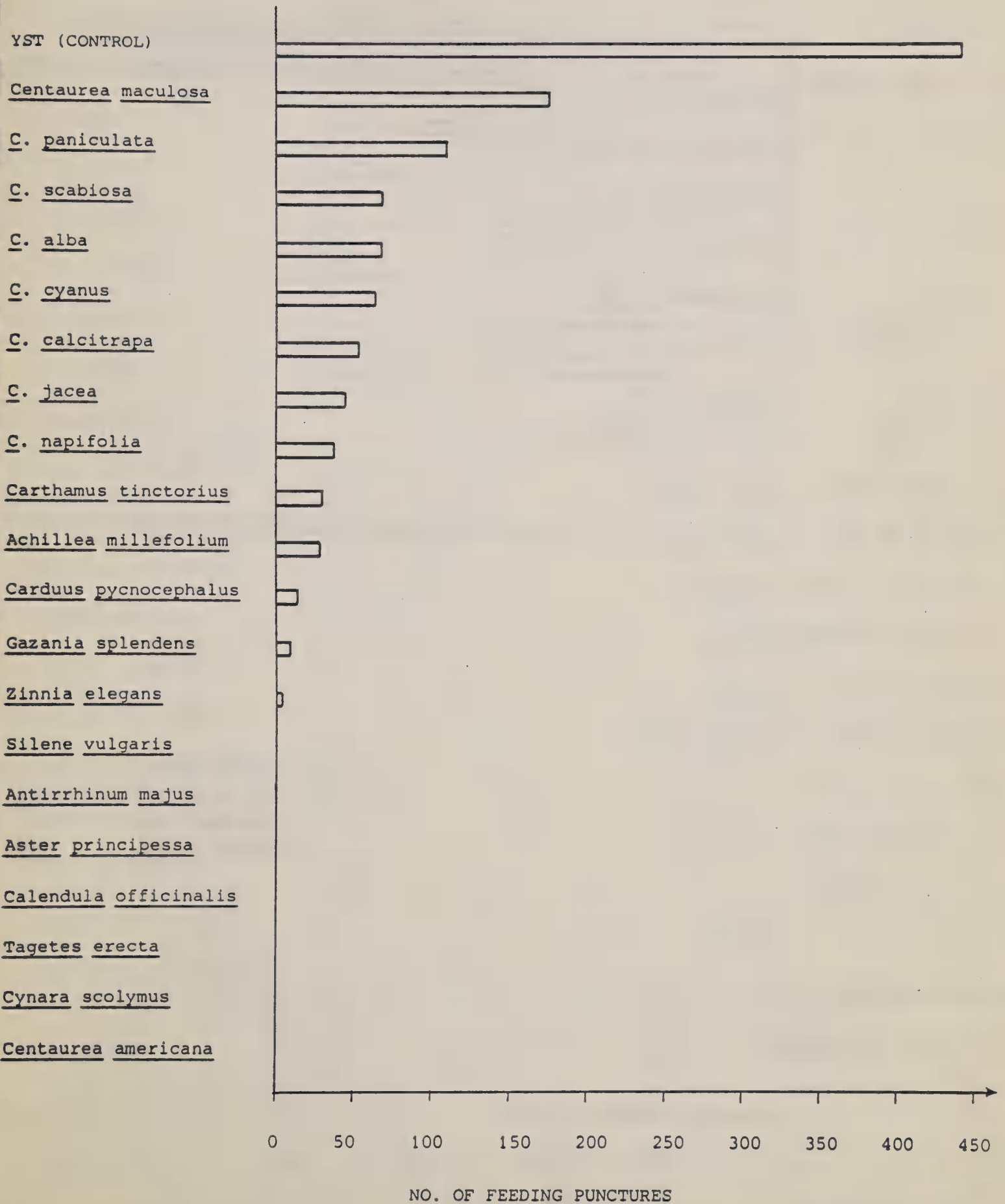


Fig. 2. Number and percentage of damaged buds in the no-choice test.

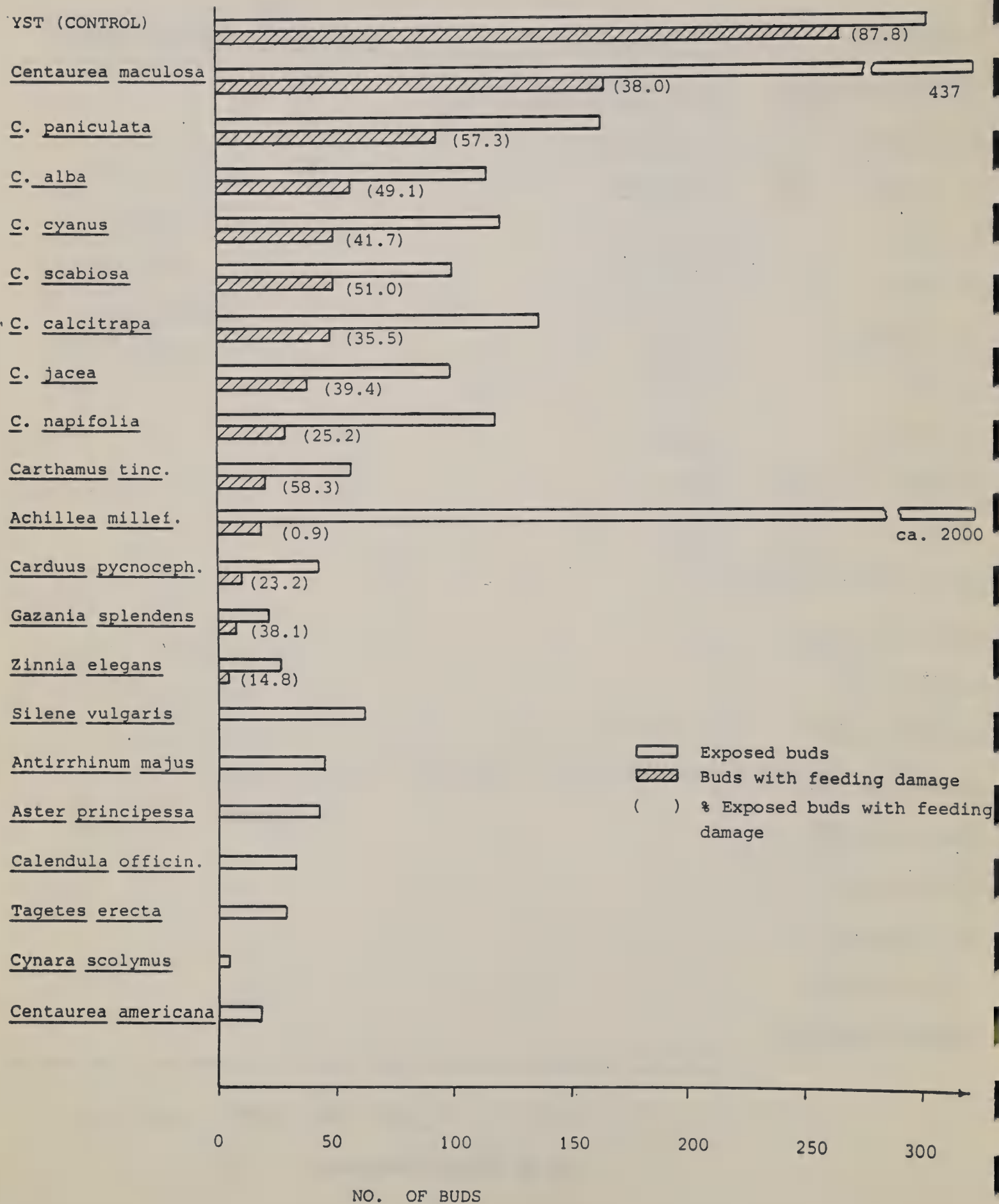
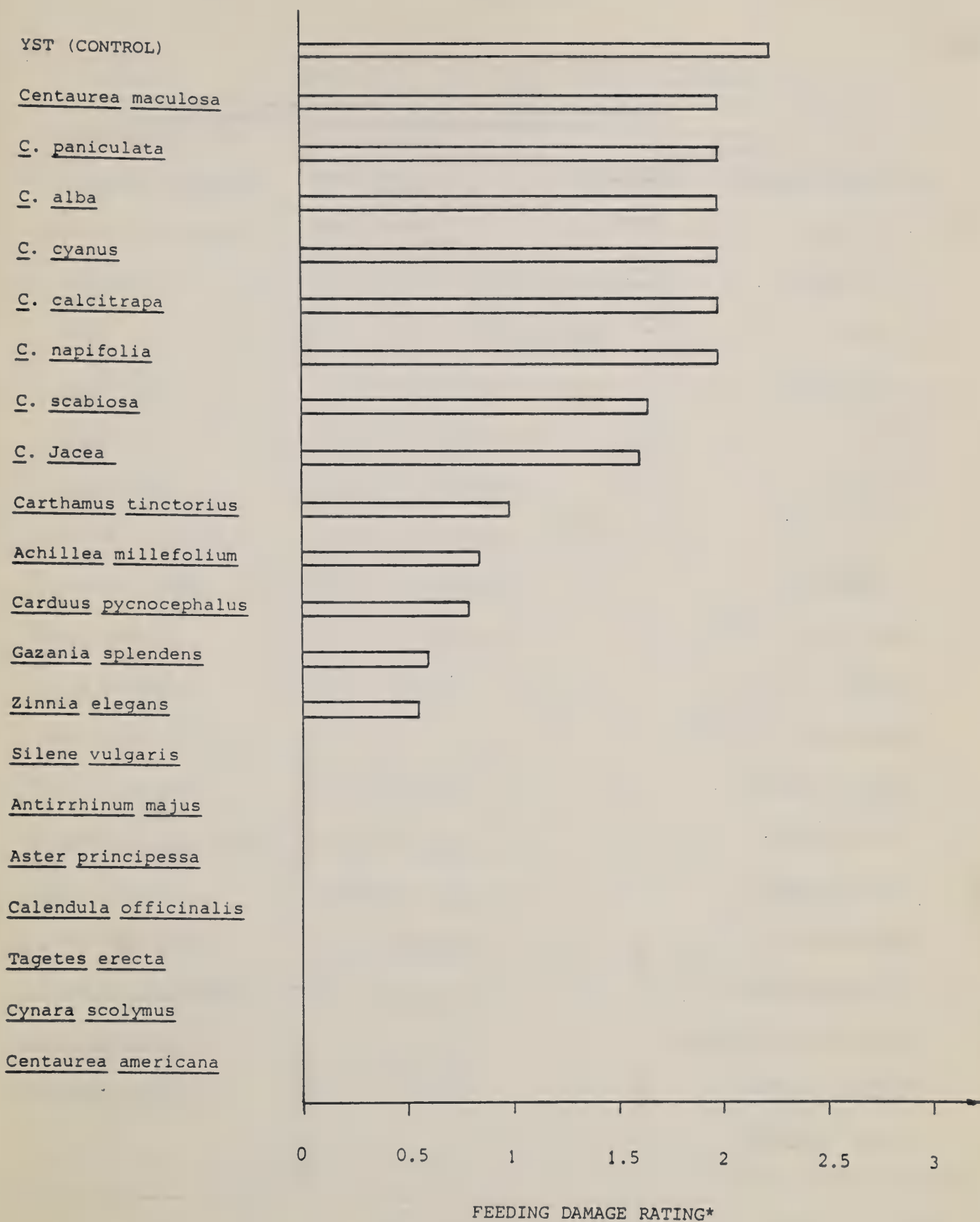


Fig. 3. Feeding damage rating in the no-choice test.



* Based on a scale of 0 to 3: 0= no feeding, no effect on bud development; 2= very little feeding on a well developed capitula, but considerable damage to young buds; 3= heavy damage, complete bud destruction.

Fig. 4. Number of eggs laid on the test plants in the no-choice test.

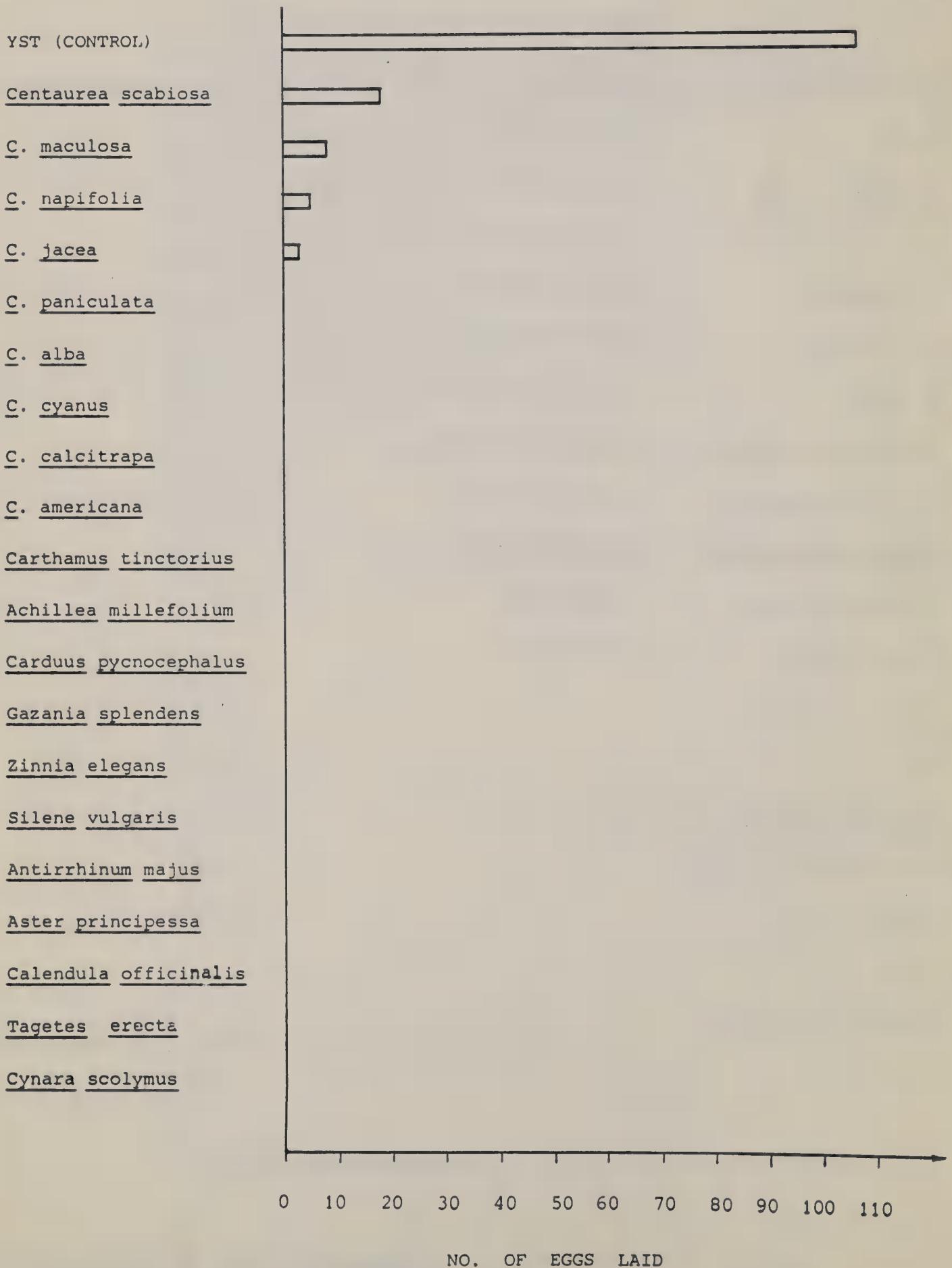
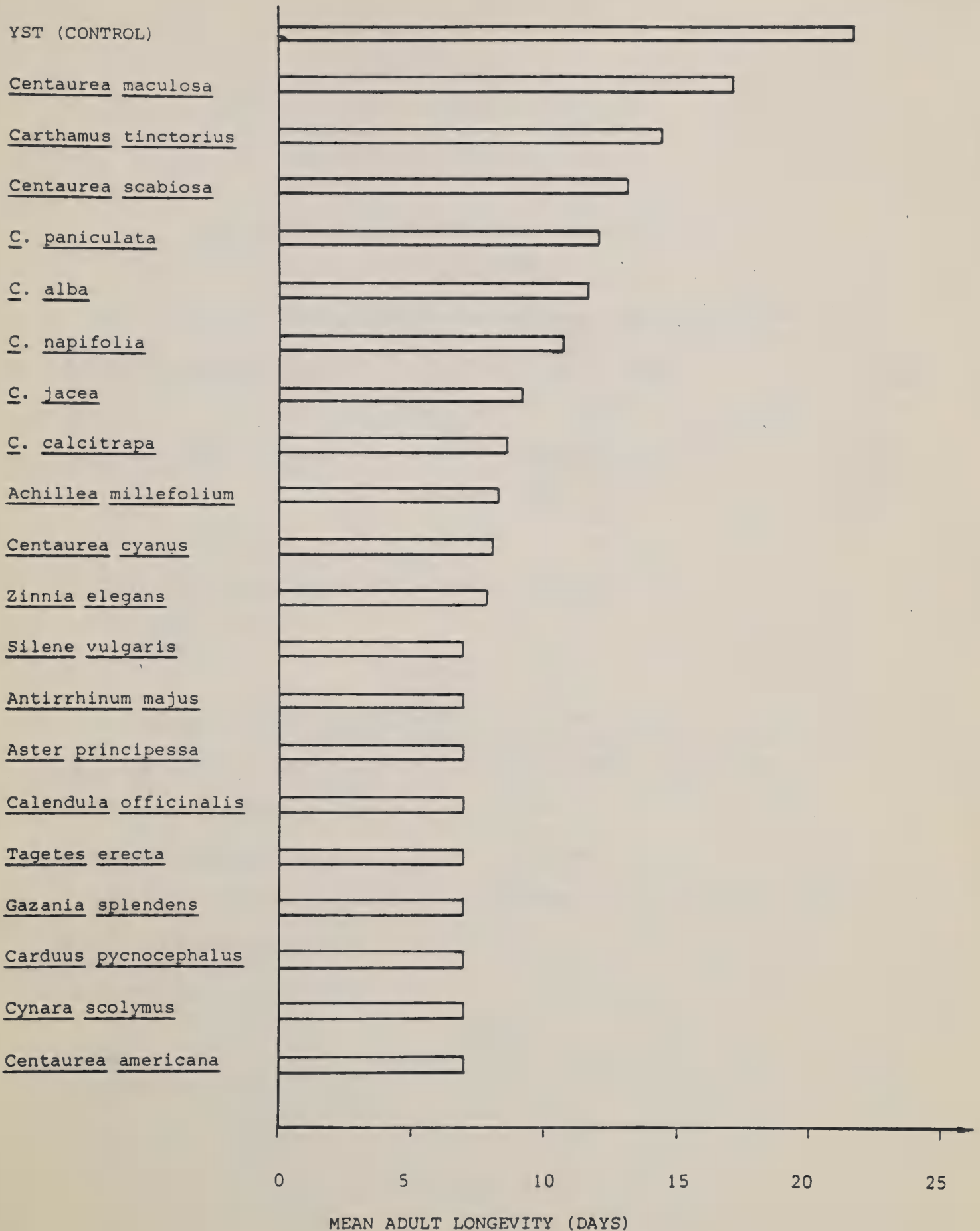


Fig. 5. Adult longevity in the no-choice test.



DIFFUSE KNAPWEED PROJECT 1987

Paul H. Dunn and G. Campobasso

Bangasternus fausti Reitter (Coleoptera: Curculionidae)

INTRODUCTION

For the fourth consecutive year we continued screening the seed-feeding weevil Bangasternus fausti (Col.: Curculionidae). Research objectives for the 1987 research season were: (1) to collect live adults of B. fausti in Greece to conduct tests at Rome, Italy, (2) to conduct oviposition, larval survival and development tests in the laboratory, (3) to continue study of the biology of the main species of diffuse knapweed in Greece.

Progress made under each objective is summarized below.

OBJECTIVE 1

MATERIALS AND METHODS

Living adults of B. fausti needed for conducting host specificity and biological studies were collected in northern Greece between May 18 and June 2. Emerging adults that had not started oviposition on the host Centaurea diffusa were hand-collected and brought back to the Thessaloniki laboratory where they were separated according to sex. Before sending the adults to Rome, dead and injured weevils found in the collection were discarded.

RESULTS

A total of 1,270 adults of B. fausti were packed and shipped to the Rome laboratory. Only N=30 (2%) of weevils were found dead or injured.

OBJECTIVE 2

EXPERIMENTS

1. Oviposition on single plant
2. Multiple choice test
3. Larval survival test

MATERIALS AND METHODS

Test plants: Plant spp. used for these laboratory trials were taken from the master list compiled in 1985-86. This year seventeen plant spp. in the genera Centaurea, Carduus, Carlina, Arctium, Silene, Calendula, Zinnia, Ranunculus, Malva, Tagetes, Tanacetum, Anthiorhynum, and Cirsium were tested with B. fausti.

OVIPOSITION ON SINGLE PLANTS

MATERIALS AND METHODS

Prior to setting up the experiment at Rome, adults of B. fausti shipped from the Thessaloniki laboratory were fed on plants of diffuse knapweed for about 4 days to allow them to recover from possible travel stress. On June 3, these adults were placed on potted plants covered with transparent plastic cylinder cages (20 cm. diam., height 70 cm.) with four holes (10 cm. dia.) in the walls covered with nylon organdy and an organdy top kept in place with a rubber band. On each potted plant 2 male and 2 female weevils were used. One potted plant comprised 1 replicate. Test plants were inspected every three days and adult feeding damage was quantified and recorded. The trial was set up on a quarantine greenhouse with fluctuating temperature and humidity (min. 15°- max.31°C; Rh min. 30% - max. 75%) with a photoperiod of ca. 16 hours. The test lasted until all adults died. Eggs found on test plants were counted and

recorded and left undisturbed to ascertain if hatching larvae were able to complete their life cycle on the test plant. A summary of the number of replications, average egg production, average of seedheads infested is shown in table 1.

RESULTS

Table 1 provides details and results of the no-choice oviposition tests. Oviposition occurred only on plants in the genus Centaurea. The most infested plant was control \bar{x} 158 \pm 53.6 eggs/rep, followed by C. pseudoalba \bar{x} 25.3 \pm 35.3 eggs/rep, C. alba \bar{x} 21.5 \pm 27.8 eggs/rep, and C. cineraria \bar{x} 3.3 \pm 4.5 eggs/rep. Besides the controls, no larval development occurred on the other Centaurea spp. even though flowerheads of these plants were superficially similar, morphologically, to those of the control. In our judgment, the most probable causes of larval development failure were: (1) presence of feeding deterrents, (2) hard tissue structure of seedhead involucre bracts, (3) seedhead growth (of non-host plants) not synchronized with occurrence of first instar larvae. Adult feeding damage was observed on Centaurea diffusa and sometimes on C. alba and C. pseudoalba but not on the ornamental plant spp. included in the test.

MULTIPLE CHOICE TEST

The object of this test was to determine if, in a cage situation, adults of B. fausti would select any of the exposed test plants as hosts. The test was conducted in a quarantine greenhouse with fluctuating temperature and humidity (min. 15°C-max. 31°C, Rh min. 30%-max. 75%) with a photoperiod of ca. 16 hours. On June 5, six males and six females of B. fausti adults were caged

in pots each containing 3 test plants and a control plant, (C. diffusa - Greek origin). Each pot was covered with a transparent plastic cylinder cage which differed only in size (33 cm. diam.; 80 cm. height) from those used in the previous experiment. There were 4 different combinations of test and control plants (treatments) and each treatment was replicated 6 times except for Centaurea panicula and Carduus thoermeri that were replicated 4 times. Plants and insects were randomly combined and each pot served as a replicate.

RESULTS

The data obtained in the multiple choice test are summarized in table 4. The failure of the insects to oviposit on any plants except the control and Centaurea pseudoalba despite the different plant combinations offered in this test, have confirmed B. fausti's high degree of specificity. Adult feeding occurred mainly on controls, but occasionally the adults were observed to feed on C. alba, C. cineraria, and C. napifolia, none of which are considered plants of particular economic value. C. cineraria is, however, a common ornamental.

FIRST INSTAR LARVAL SURVIVAL TEST

The following plant spp. were used for the 1st instar larval survival test: Centaurea diffusa (control), C. alba, C. pseudoalba, C. napifolia, C. cineraria, C. paniculata, Cirsium lanceolatum, C. eriophorum, Carduus thoermeri, Calendula officinalis, Zinna elegans, Tagetes erecta, Ranunculus auricomus, Anthrhythmum majus, Malva silvestris, Tanacetum vulgare, Carlina corimboza, Arctium lappa, Silene vulgaris. Two buds each of the 5 test plant replicates were infested with two fertile B. fausti eggs, (total of 20 eggs/test plant). The experiment was conducted in a quarantine greenhouse with fluctuating temperature and humidity (min. 15°C-max. 31°C, Rh min. 30%-max. 75%) with a photoperiod of ca. 16 hours. A fine camel brush was used

for inserting the fertile B. fausti eggs between the bracts of the buds on the test plants, and all the infested buds were labelled. The experiment started on June 10 and ended August 4.

RESULTS

Survival was seen in only one replicate. The validity of this experiment was compromised by two polyphagous insects (Lepidoptera, Diptera), which had oviposited into flower buds of test plants before the tested started, (test plants had been kept outdoors for 15 days before use), ruining the seed head content. Immature stages of both these unwanted insects were found while dissecting test plants. Both insects are known pests of several plant genera. The test will be repeated in 1988.

OBJECTIVE 3

Once we terminated B. fausti adult collection, three days were spent surveying in northern Greece to locate new populations of different knapweeds infested with B. fausti, Pterolonche inspersa, and Sphenoptera jugoslavica.

MATERIALS AND METHODS

On the road from Thessaloniki to Kavala several diffuse knapweed infestations were seen. At each site, a random sample (n=50-100) of diffuse knapweed plants were inspected, and dissected. The infestation rate of B. fausti, P. inspersa, and S. jugoslavica was recorded as well as the plant density/square meter at each site.

RESULTS

A total of five sites were investigated:

Site 1: South of Asprovolta, plant density 5-6 plants/sq. m. P. inspersa larvae infestation 37%. S. jugoslavica larvae and pupae infestation 24%. B. fausti adult infestation 19%.

Site 2: North of Asprovalta, plant density 10-12 plants/sq.m.

P. inspersa larvae infestation 3-4%, S. jugoslavica not present, and B. fausti adult infestation 42%.

Site 3: Southwest of Iraklitza, plant density 6-7 plants/sq.m.

P. inspersa and S. jugoslavica were not present. B. fausti adult infestation 12%.

Site 4: Enevtheropolis, plant density 1-2 plants/sq.m. P. inspersa larvae, and B. fausti adults were not present. S. jugoslavica larvae and pupae occurred in low percentage 2%.

Site 5: East of Kavala, plant density 10-15 plants/sq.m. P. inspersa larvae infestation 40%, S. jugoslavica not present, and B. fausti adult infestation 30%.

Figure 1 shows the collection sites discovered in Greece sofar.

OBJECTIVE 4

Examination of related thistles in the field still indicate that B. fausti has a narrow host-spectrum and is confined to plants in the genus Centaurea. At each visited site a random sample of 25-70 plants of Centaurea, Carduus, Cirsium, Notobosis, Tyrimnus, Cnicus, Sylibum, and Onopordum were examined and insects found were recorded. Results are presented in table 3A, 3B, 3C.

CONCLUSIONS

The following positive points provide justification for considering the seed weevil B. fausti as a safe and effective biological control agent for diffuse and spotted knapweed:

1. Literature search and personal contacts with European curculionid specialists did not provide any record of the weevil damaging plants of economic value.
2. Laboratory studies are continuing to produce satisfactory results. Weevil host spectrum is restricted to the genus Centaurea.
3. B. fausti is widely distributed in central and northern Greece, thus massive collections can be made without too much effort.
4. Endangered American plants in the genus Cirsium were not accepted as food by adults or larvae of B. fausti, nor were they accepted as an oviposition substrate.
5. A single larva of this weevil is able to destroy 100% of seeds in one seedhead.

Table 1. Summary of oviposition no-choice test of *Bangasternus fausti*, 1987.

TEST PLANTS	TOTAL NO. OF REPLICATES	TOTAL NO. OF INSECTS IN REPLICATES		NO. SEED HEADS EXPOSED/REP		NO. SEED HEADS INFESTED/REP		NO. EGGS OVIPOSITED/REP		SURVIVAL TIME OF OF ADULTS IN DAYS	
		♀♀	♂♂	\bar{X}	+ (SD)	\bar{X}	+ (SD)	\bar{X}	+ (SD)	\bar{X}	+ (SD)
<i>Centaurea diffusa</i> (Control)	6	12	12	239.5	(112.5)	113.6	(42.3)	158.8	(53.6)	34.6	(2.9)
<i>Centaurea pseudoalba</i>	6	12	12	15.0	(9.9)	9.02	(11.0)	25.3	(35.3)	29.3	(8.3)
<i>Centaurea alba</i>	6	12	12	15.5	(4.6)	6.6	(3.7)	21.5	(27.8)	23.2	(4.9)
<i>Centaurea cineraria</i>	6	12	12	13.3	(2.3)	1.8	(2.6)	3.3	(4.5)	21.1	(7.4)
<i>Centaurea napifolia</i>	6	12	12	13.0	(5.2)	0.0	(0.0)	0.0	(0.0)	13.5	(2.2)
<i>Centaurea paniculata</i>	4	8	8	131.2	(37.5)	0.0	(0.0)	0.0	(0.0)	31.5	(1.7)
<i>Cirsium lanceolatum</i>	6	12	12	2.5	(1.6)	0.0	(0.0)	0.0	(0.0)	10.5	(2.0)
<i>Cirsium eriophorum</i>	6	12	12	2.0	(1.2)	0.0	(0.0)	0.0	(0.0)	11.6	(2.3)
<i>Carduus thoermeri</i>	4	8	8	6.7	(3.0)	0.0	(0.0)	0.0	(0.0)	11.2	(2.2)
<i>Calendula officinalis</i>	6	12	12	3.8	(2.7)	0.0	(0.0)	0.0	(0.0)	11.3	(2.2)
<i>Zinnia elegans</i>	6	12	12	2.8	(0.4)	0.0	(0.0)	0.0	(0.0)	12.0	(2.0)
<i>Tagetes erecta</i>	6	12	12	3.1	(2.4)	0.0	(0.0)	0.0	(0.0)	10.6	(2.4)
<i>Ranunculus auricomus</i>	6	12	12	1.0	(2.4)	0.0	(0.0)	0.0	(0.0)	12.5	(2.0)
<i>Antirrhinum majus</i>	6	12	12	57.8	(17.6)	0.0	(0.0)	0.0	(0.0)	11.6	(2.2)
<i>Malva silvestris</i>	6	12	12	7.8	(1.3)	0.0	(0.0)	0.0	(0.0)	11.8	(2.3)
<i>Tanacetum vulgare</i>	6	12	12	4.1	(2.1)	0.0	(0.0)	0.0	(0.0)	13.0	(2.6)
<i>Carlina corimbosa</i>	6	12	12	3.1	(0.7)	0.0	(0.0)	0.0	(0.0)	10.5	(2.5)
<i>Arctium lappa</i>	6	12	12	2.3	(1.0)	0.0	(0.0)	0.0	(0.0)	16.6	(2.5)
<i>Silene vulgaris</i>	6	12	12	4.6	(1.0)	0.0	(0.0)	0.0	(0.0)	12.1	(2.1)

Table 2. Summary of multiple choice test of Bangasternus fausti, 1987.

TEST PLANTS	TOTAL NO. OF	TOTAL NO. OF	NO. SEED HEADS		NO. SEED HEADS		NO. EGGS	
	REPLICATES	INSECTS IN	EXPOSED/REP		INFESTED/REP		OVIPOSITED/REP	
		REPLICATES	\bar{X}	+ (SD)	\bar{X}	+ (SD)	\bar{X}	+ (SD)
<u>Centaurea diffusa</u> (Control))			248.3	(111.8)	208.6	(95.7)	285.6	(94.3)
<u>Centaurea pseudoalba</u>)			10.3	(2.5)	3.0	(3.2)	5.6	(7.3)
<u>Zinnia elegans</u>)	3	18	2.6	(0.5)	0.0	(0.0)	0.0	(0.0)
<u>Calendula officinalis</u>)			8.6	(1.5)	0.0	(0.0)	0.0	(0.0)
.....								
<u>Centaurea diffusa</u> (Control))			331.0	(103.5)	187.0	(125.6)	265.6	(227.2)
<u>Centaurea alba</u>)			15.0	(4.0)	0.0	(0.0)	0.0	(0.0)
<u>Arctium lappa</u>)	3	18	3.0	(0.4)	0.0	(0.0)	0.0	(0.0)
<u>Malva silvestris</u>)			6.0	(2.0)	0.0	(0.0)	0.0	(0.0)
.....								
<u>Centaurea diffusa</u> (Control))			483.6	(94.1)	313.0	(168.5)	432.0	(239.1)
<u>Centaurea cineraria</u>)			11.0	(7.9)	0.0	(0.0)	0.0	(0.0)
<u>Cirsium lanceolatum</u>)	3	18	2.6	(1.1)	0.0	(0.0)	0.0	(0.0)
<u>Carlina corimbosa</u>)			2.6	(1.1)	0.0	(0.0)	0.0	(0.0)
.....								
<u>Centaurea diffusa</u> (Control))			261.0	(149.9)	161.6	(89.7)	213.3	(130.2)
<u>Centaurea napifolia</u>)			14.6	(5.8)	0.0	(0.0)	0.0	(0.0)
<u>Silene vulgaris</u>)	3	18	4.6	(0.5)	0.0	(0.0)	0.0	(0.0)
<u>Antirrhinum majus</u>)			114.6	(35.7)	0.0	(0.0)	0.0	(0.0)
.....								
<u>Centaurea diffusa</u> (Control))			273.3	(90.1)	237.0	(76.6)	352.3	(127.6)
<u>Cirsium eriphorum</u>)	3	18	1.3	(0.5)	0.0	(0.0)	0.0	(0.0)
<u>Tagetes erecta</u>)			1.6	(0.5)	0.0	(0.0)	0.0	(0.0)
.....								

Table 3A. Plant species associated with Diffuse Knapweed which were examined for presence of Bangasternus fausti in Greece, June 1987.

LOCALITY	PLANT SPECIES	NO. PLANTS EXAMINED	% PLANTS INFESTED BY <u>B. fausti</u>	NO. ADULTS PRESENT <u>B. fausti</u>
Asprovolta	<u>Centaurea diffusa</u> (Control)	65	(27) 42%	38
"	<u>maculosa</u>	49	(15) 31%	22
"	<u>salonitana</u>	36	0	0
"	<u>macedonica</u>	23	0	0
"	<u>rupestris</u>	28	0	0
"	<u>calcitrapa</u>	41	0	0
"	<u>solstitialis</u>	23	0	0
"	<u>alba</u>	41	0	0
"	<u>jacea</u>	37	0	0
"	<u>cyanus</u>	45	0	0
<u>Carduus</u>	<u>thoeneri</u>	67	0	0
"	<u>acanthoides</u>	39	0	0
"	<u>pycnocephalus</u>	54	0	0
<u>Cirsium</u>	<u>eriphorum</u>	32	0	0
"	<u>lanceolatum</u>	40	0	0
"	<u>candelabrum</u>	32	0	0
"	<u>arvense</u>	40	0	0
<u>Notobasis</u>	<u>syriaca</u>	32	0	0
<u>Tyrimnus</u>	<u>leucographus</u>	27	0	0
<u>Cnicus</u>	<u>benedictus</u>	35	0	0
<u>Sylibum</u>	<u>marianum</u>	45	0	0
<u>Onopordum</u>	<u>acanthium</u>	57	0	0
"	<u>illyricum</u>	49	0	0

Table 3B. Plant species associated with Diffuse Knapweed which were examined for presence of Bangasternus fausti in Greece, June 1987.

LOCALITY	PLANT SPECIES	NO. PLANTS	% PLANTS	NO. INSECTS PRESENT
		EXAMINED	INFESTED	<u>B. fausti</u>
Iraklitsaa	<u>Centaurea diffusa</u> (Control)	49	(29) 59%	32
"	<u>maculosa</u>	37	(19) 51%	24
"	<u>calcitrapa</u>	31	0	0
	<u>Carduus nutans</u>	27	0	0
"	<u>pycnocephalus</u>	30	0	0
"	<u>candicans</u>	25	0	0
	<u>Cirsium arvense</u>	31	0	0
	<u>Carthamus lanatus</u>	28	0	0
	<u>Galactites tomentosa</u>	50	0	0
	<u>Scolymus hispanicus</u>	39	0	0
"	<u>maculatus</u>	47	0	0
	<u>Onopordum illyricum</u>	41	0	0
	<u>Carlina corymbosa</u>	42	0	0
"	<u>acaulis</u>	39	0	0
	<u>Sylibum marianum</u>	41	0	0
	<u>Echinops sphoerocephalus</u>	27	0	0
"	<u>microcephalus</u>	28	0	0
	<u>Cichorium intybus</u>	45	0	0
	<u>Lactuca virosa</u>	25	0	0
	<u>Cynara cardunculus</u>	44	0	0

Table 3C. Plant species associated with Diffuse Knapweed which were examined for presence of Bangasternus fausti in Greece, June 1987.

LOCALITY	PLANT SPECIES	NO. PLANTS	% PLANTS	NO. INSECTS PRESENT
		EXAMINED	INFESTED	<u>B. fausti</u>
Palio'	<u>Centaurea diffusa</u> (Control)	47	(14) 30%	22
"	<u>alba</u>	33	0	0
"	<u>salonitana</u>	21	0	0
"	<u>rupestris</u>	15	0	0
<u>Carduus</u>	<u>thoermeri</u>	29	0	0
"	<u>pycnocephalus</u>	31	0	0
<u>Cirsium</u>	<u>lanceolatum</u>	44	0	0
"	<u>arvense</u>	56	0	0
"	<u>eriphorum</u>	21	0	0
<u>Carthamus</u>	<u>lanatus</u>	31	0	0
"	<u>dentatus</u>	22	0	0
<u>Scolymus</u>	<u>maculatus</u>	40	0	0
<u>Onopordum</u>	<u>acanthium</u>	17	0	0
"	<u>illyricum</u>	19	0	0
<u>Carlina</u>	<u>corymbosa</u>	32	0	0
"	<u>acaulis</u>	16	0	0
<u>Sylibum</u>	<u>marianum</u>	33	0	0
<u>Cichorium</u>	<u>intybus</u>	29	0	0
<u>Cynara</u>	<u>cardunculus</u>	47	0	0

Aceria centaureae Nal. (Acarina: Eriophyidae)

Petition to Import the Eriophid Mite Aceria centaureae Nal.,
(a natural enemy of diffuse and spotted knapweeds)
into Quarantine in the United States for In-depth Testing.

Prepared by

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INTRODUCTION

Aceria centaureae Nal. is a central European species of eriophyid mite which causes severe leaf galling and sometimes death of rosettes of Centaurea diffusa and some other Centaurea spp. In 1984 a colony of this mite was found at Geroplatanos (Arnea), Greece by one of our staff scientists (Sobhian).

Preliminary out-of-doors trials showed that this mite would accept U.S. biotypes of Centaurea diffusa as a host plant, and that it could be moved and re-colonized in Thermi, where Dr. Sobhian's research facilities are located. As a follow-up of the favorable results from these trials, a literature search was made in 1986.

LITERATURE SURVEY

Reports of this mite are not common in the literature. There is no information available on the biology but the information is adequate to indicate its distribution and host preferences.

Schroeder (1977), quoting Buhr 1964 mentions the pan-European distribution of Aceria centaureae and gives Buhr's host records as Centaurea maculosa, C. diffusa, C. micanthros and C. sadleriana. Schroeder (1977) also notes that in surveys made by Commonwealth Institute of Biological Control (CIBC) workers, the mite was found on C. maculosa in the Swiss Valais and on C. diffusa near Lake Skutari in southern Yugoslavia. The mite has also been reported in Italy by Nalepa (1898) and by Trotter and Cecconi (1903 ca.) on Centaurea nigrescens and Centaurea scabiosa in the province of Treviso, in Northern Italy. However, some of the host plants mentioned in the older references are based on the presence of typical galls rather than on examination of the mite. While they are probably correct, there remains some question about the actual species of eriophyid concerned.

Henrik (1966) noted that the mite is a Central European species and that it can be found throughout Hungary on Centaurea amara, C. maculosa, C. scabiosa, C. sadleriana and C. pannonica. Houard (1908) adds Centaurea (Staelina) fructosa, C. alba, C. jacea, C. nigrescens, C. cineraria, C. (Psephellus) delabata, C. rehenana, C. solstitialis, C. calcitrapa, and C. aspera, C. aspera var. brevisetosus to the list of host plants as well as the description of the galls on several of these plants.

Despite its wide distribution, the mite was not reported from plants other than Centaurea spp. by the workers cited above. In the Review of Applied Entomology (60 volumes 1913-1973) the mite was not mentioned. No reference to the mite was found in "Insetti Dannosi all'Agricoltura" (della Beffa, 1961) or in "Mites Injurious to Economic Plants (Jeppson et al., 1975). Since our survey of the literature and the CIBC field survey showed the mite is not a pest of cultivated plants or plants of ornamental importance, further testing was in order.

FIELD TRIAL

In 1986 an open field trial was made in Greece using 6 native U.S. Cirsium spp., 1 Greek Cirsium sp., and the oil-crop plant safflower (Carthamus tinctorius) as test plants. This field trial was financed in part by an extramural grant from the USDA-ARS Biological Control of Weeds Laboratory, Rome, Italy, and was conducted by Dr. Byron Katsoyannos, Department of Entomology, University of Thessaloniki, Thessaloniki, Greece.

The trial was set up in a randomized complete block design with 10 treatments (species of plants) replicated 10 times. The plant treatments in the trial were:

- | | |
|---|--------------|
| 1. <u>Centaurea diffusa</u> (control) | Greek origin |
| 2. <u>Centaurea solstitialis</u> (yellow starthistle) | Greek origin |
| 3. <u>Carthamus tinctorius</u> (safflower) | U.S. origin |
| 4. <u>Cirsium creticum</u> | Greek origin |
| 5. <u>C. andersonii</u> | U.S. origin |
| 6. <u>C. brevistylum</u> | U.S. origin |
| 7. <u>C. cymosum</u> | U.S. origin |
| 8. <u>C. occidentale</u> | U.S. origin |
| 9. <u>C. pastoris</u> | U.S. origin |
| 10. <u>C. undulatum</u> | U.S. origin |

The plants were infested by putting Centaurea diffusa galls containing mites on the rosettes or growing points of the test plants.

RESULTS

At 6, 15, 24, and 32 days after the mites were placed on the test plants, 100% of the diffuse knapweed and yellow starthistle plants in the trial had galls. The yellow starthistle was less severely galled than the diffuse knapweed control. None of the other plants in the test had galls or other evidence of mite damage.

CONCLUSIONS

From the information available to date, we can say that Aceria centaurea is stenophagous to the genus Centaurea, poses no apparent danger to safflower or other cultivated crop or ornamental plants, nor to native North American Cirsium spp. Further, there is no evidence that would make it suspect as a pest of non-tested species of economic importance. The two native U.S. species Centaurea rothrockii and C. americana were not available for testing.

Considering all these factors, I feel the mite does not pose a serious threat to U.S. agriculture and is safe enough to introduce into quarantine in the United States for an in-depth testing program to confirm its limited host range and safety. Also, its possible impact on yellow starthistle and purple starthistle will be explored. Permission is requested from the Technical Advisory Group for the Biological Control of Weeds (APHIS) to introduce the candidate mite into quarantine at Albany, California for further host specificity testing.

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GREECE (R. Sobhian)

LEAFY SPURGE (Euphorbia virgata spp. complex)

Simyra dentinosa

A population of this insect was found feeding on Euphorbia seguieriana, near Volvi Lake (ca. 40 km. east of Thessaloniki) in 1981. In 1983 preliminary studies in Greece showed that the female moth will oviposit on a U.S. biotype of E. esula, and larvae can complete development on leafy spurge plants of North American origin. Preliminary host specificity tests showed that the host range of the insect is probably restricted to the genus Euphorbia (see annual report 1983), but since the insect was found on Euphorbia seguieriana in Greece there was some feeling that it would not be a promising candidate for biological control of E. esula in the United States, and thus no more attention was paid to the study of the insect for 2 years. I suggested that we do a host specificity test of Simyra dentinosa in Rome in 1986 and provided them with Simyra eggs for the tests. The results were promising so we decided to continue the host specificity testing and study the biology of the insect in 1987. Over 2000 Simyra eggs and 400 E. seguieriana plants (for control plants in the tests) were sent to Rome for the host specificity testing.

The biology of the insect had been studied some in the previous years, but it was not known where the larvae pupate or how they overwinter in the field. In captivity they pupated on cage walls, among old food plants, paper towels, etc. Extensive search for Simyra pupae around the host plants (root neck and soil) or among various shrubs in the area was unsuccessful in 1983 and 1986. Also, there was a paucity of information about natural larval mortality of the insect.

Pupation Site

In order to find out where the larvae pupate in field, June 3 and 4 from 14:00 to 18:00 hrs, June 5 from 12:30 to 20:30 hrs and June 6 from 8:00 to 19:00 hrs were spent in the field watching mature larvae and trying to find out what they do when they finish feeding, are ready for pupation and start the rapid dispersal movement. The time spent in the field with the larvae paid off. On June 5, at 18:30 I watched a mature larva as it fell from a Euphorbia branch to the ground and crawled for about 1.5 meters in grass and other weeds. It climbed a grass stem and stopped there for 35 minutes, after which it crawled to another non-host plant (Capsella bursa pastoris Medic.) and stopped there resting on a stem. The larva was observed for about an hour while it stayed in the same spot, doing nothing. It was getting dark and a cold wind was blowing, leaving the impression that the larva would stay at the same spot overnight. Hoping to find it the next day the observation was abandoned at 20:00 hrs. Next day, on June 6, at 08:00 observation at the same location commenced. The larva was still resting on the same stem. The larva had been emptying its stomach without feeding, and its body was more or less translucent. At 09:05, when the temperature warmed up the larva crawled down the stem to the ground and started moving rapidly towards a large Carduus candicans W. et K. plant. It was moving at an unbelievable speed about 90 meters (0.9 kms.) per hour. After searching for about 10 minutes among the old dry Carduus leaves, it left the plant and crawled to an Onopordum plant, 6 meters away from the Carduus plant, and started searching there again among the old, dry, and twisted rosette leaves. At times the larva would stop for a few minutes inside the curled leaves. It was obviously searching for a suitable location for pupation. After a while, it left the Onopordum plant and crawled to a blackberry bush, where it stayed and searched for about 15 minutes.

Leaving this bush it crawled into a grainfield where I could not follow it without disturbing it, so I returned the larva to the place where it had started the journey in the morning. Again, it crawled toward the same Carduus plant, as it had done previously and started searching among old dry rosette leaves. It was difficult to follow the larva without disturbing it. Finally, it became lost among the old Carduus leaves and other weeds, which formed a dense mat of vegetation. Giving up the observations of this particular larva, I started looking for another migrating larva. Fortunately one was found and it was followed until it reached an Onopordum plant, where it started searching among the old, dry, twisted, and curled leaves of the plant. Searching for about 10 minutes, it found a half closed tube, formed by a curled dry Onopordum rosette leaf. The larva entered the tube turning around slowly, examining the hole. Finally it started to make a web and closed itself in the tube by making a leathery silken cocoon inside the tube that was not visible from outside, because it was covered with a coat of dry leaf material. Searching among dry and twisted Onopordum leaves in the area for 10 minutes I found another cocoon and the question of the pupation site of Simyra was definitely answered.

Larval mortality: On May 18, 50 larvae of each stage (L_1 , L_2 , L_3 , L_4 , L_5) were field collected and caged with fresh food, which was changed every 3-4 days, until June 7, when they had all pupated. At the same time (every 3-4 days) the cages were checked for cocoons of parasites which may have exited from the the larvae. From May 24 to June 1, Apanteles wasp larvae emerged only from the L_3 and L_4 larvae, and formed clusters of cocoons. The percentage of parasitism was low, only 7 (14%) of the L_3 and 2 L_4 larvae were parasitized.

On May 28, 47 mature larvae were field collected and caged with fresh food. All of them had pupated by June 3rd except for one larva which was parasitized by Apanteles sp. A tachinid fly emerged from each of 3 Simyra pupae on June 19. The rest of the pupae were kept for rearing adults.

On June 3 a large tachinid fly was observed ovipositing on a mature Simyra larvae, in the field near Volvi Lake. The fly sat on a branch of the host plant near a Simyra larva, and extruding her ovipositor laid one egg at a time on an adjacent, accessible larva. There were 2 colonies of Simyra larvae on separate Euphorbia plants, about 2 meters apart. Each time the fly was disturbed she left one of the colonies and flew directly to the other and started to oviposit on larvae in that colony. Twenty mature Simyra larvae were collected in the field and checked for tachinid eggs, which were visible to the naked eye. The white eggs were similar to chicken eggs, in shape. Five of the larvae (25%) in this group had been parasitized with the tachnid eggs. Altogether, thirteen parasitized larvae were collected and checked under the microscope for number of eggs and the position of the eggs on the larvae. Nine larvae were parasitized with one egg/larva and these were situated thusly: one with one egg on the head capsule, one with one egg on the dorsal side, and 7 with one egg on the ventral side. Two larvae had 2 eggs/larva, both on the ventral side. One larva had 3 eggs (all ventral) and one larva had 4 eggs (all ventral). Most of the eggs were laid on the anterior half of the larvae.

A group of 20 parasitized Simyra larvae were kept in a cage with fresh food. They all made cocoons and pupated within 4 days. Seven adult tachinid flies emerged from the cocoons between June 19 and 25 and were sent for identification. Twelve cocoons found in the container were opened on July 3.

These are the results:

1	cocoon(s)	contained a	<u>Simyra</u> pupae
5	"	"	1 dead fly/cocoon
4	"	"	1 pupal exuvium of the fly in each
1	"	"	2 dead flies
1	"	"	4 dead flies

Two pupal exuviae of the tachinid fly were found outside of the cocoons (the larvae left the cocoons and pupated in the container).

Centaurea solstitialis Project (YST)

Bangasternus orientalis.

Adult weevils (more than 1,000 each year), were collected in the field in Greece, and were released in the United States in 1985 and 1986. In 1987, over 2,000 weevils were collected and sent to Albany, California for release. The weevil became established during 1985-1986 in all release sites. At this writing no information is available from the 1987 release. Due to the successful establishment it was decided to make no further releases and wait for the natural build-up of the population at the release sites.

Larinus curtus:

Adult Rearing: Since this insect is scarce and difficult to collect in large numbers for screening tests, it was decided to rear the insect on caged YST plants. Twelve YST plants were grown in a plot 2x2 m. On May 28 the plants were caged in a 2x2x2 m. screen cage. At this time only a few of the

plants were budding so these buds were removed in order to be sure that no other seedhead feeding insects were present in the cage to compete with L. curtus. On June 24, 50 L. curtus adults were released in the cage. At this time the plants were flowering. In the following 2 weeks the weevils were mainly feeding or resting on the flowers and we were hoping to rear large numbers of adults for our experiments in 1988. At the end of June the caged YST plants were found to be infested with black aphids and all the plants had dessicated by mid-July. Obviously the cage had protected the aphids from their parasites and predators and allowed the aphids to build up a damaging population. Six seed head samples of 20-150 heads each sample were collected and examined for the rate of infestation from July 21 to September 22 and only 2 larvae and 11 weevils were alive or had emerged. The results of the dissection and examination are given in Table 1.

Lack of pollination and early death of the plants were probably the reasons why the number of adults produced was so low. It seems that pollination and the subsequent development of achenes is essential for the development of the larvae, because many dead larvae were found in the seed heads with infertile and undeveloped seeds.

Host Preference: When L. curtus adults, labelled with nail polish, were released in an experimental field plot in Thermi, some adults were found on safflower as well as on YST plants^{1/}. Following the above observation, a preliminary host preference and oviposition test was carried out in Thermi.

^{1/} B.I. Katsoyannos, 1986 "Open field host selection test in Greece using three indigenous arthropod species and several North American species of Cirsium thistles". USDA-ARS grant No. 59-32U4-6-86.

Table 1. Results of attempt to rear Larinus curtus on YST in a large field cage.

DATE	No. seedheads examined	Larvae			Pupae		Adults		
		alive	dead	frass only	alive	dead	alive	dead	emerged
July 21	20	-	3	-	2	-	-	-	-
August 23	50	-	8	-	-	-	1	-	-
August 14	30	-	13	-	-	-	2	1	-
August 27	50	-	2 ^{1/2}	3	-	1	-	1	1
September 7	99	-	21 ^{1/2}	3	-	-	2	-	4
September 22	50	-	5 ^{1/2}	2	-	-	-	-	1
TOTAL	299	-	52	8	2	1	6	2	5

1/2 Sterile undeveloped achenes in these heads.

On July 16, 1 male and 1 female weevils were caged on YST (no choice treatment 1), on safflower (no choice treatment 2) and on YST-safflower (two choice treatment 3) for host preference trials. There were 2 flowers of each test plant in each cage, and each treatment was replicated 4 times. The flowers were kept as bouquets in vials with water. The bouquets were checked, 5 times on the first day (at one-hour intervals), 3 times on the second day and once on the third day. The number of adults seen on the flowers was recorded at each observation. The results are shown in Table 2.

The adults preferred the YST flowers, but they also were found on safflower flowers, even in 2-choice experiments. The adults fed on both YST and safflower flowers. The amount of feeding was not quantified.

On July 20 and 21, all the flowers exposed to the adults were dissected under a stereomicroscope and checked for presence or absence of L. curtus eggs. The results are shown in Table 3.

No eggs were found in safflower flowers in either the no-choice or two-choice trials, but 26 eggs (16 no-choice and 10 two-choice) were found on YST flowers. Up to 5 eggs were laid in one YST flower head, and by July 21 all the eggs had hatched and the larvae were feeding on the achenes in the YST flowerhead.

On the YST plants the ovipositing females insert their abdomen or sometimes nearly their whole body into the YST flowers and deposit their eggs inside the florets, near the developing achenes. On safflower flowers the bracts keep the entrance to the capitulum so tightly closed at the apex that the weevil cannot enter the florets with her abdomen or body to find a suitable oviposition site, thus physical structure of the safflower flowers does not permit access to the florets, which is necessary for oviposition.

Table 2. Number of L. curtus adults observed on safflower and YST in a no-choice and 2-choice host preference (A) oviposition (B).

<u>No. replicates</u>	NO CHOICE		TWO CHOICE	
	<u>YST</u>	<u>SF</u>	<u>SF</u>	<u>YST</u>
	No. of adults observed on the flowers		No. of adults observed on the flowers	
	<u>in each replicate:</u>		<u>in each replicate:</u>	
1	8	7	0	11
2	12	5	4	7
3	14	7	3	11
4	<u>17</u>	<u>9</u>	<u>1</u>	<u>8</u>
TOTAL	51	28	8	37

Table 3. Number of L. curtus eggs found in YST and safflower in a no-choice and 2-choice host preference-oviposition trial.

<u>No. replicates</u>	NO CHOICE		TWO CHOICE	
	<u>YST</u>	<u>SF</u>	<u>SF</u>	<u>YST</u>
	No. of eggs found in each replicate		No. of eggs found in each replicate	
1	1	0	0	1
2	5	0	0	4
3	8	0	0	1
4	<u>2</u>	<u>0</u>	<u>0</u>	<u>4</u>
TOTAL	16	0	0	10

Field Trial (Safflower):

On July 18, 25 L. curtus adults were labelled with nail polish and released on a small plot of safflower (10x3 m) at the University Farm at Thessaloniki. Most of the flower heads were in the post-flowering stage, however, some flowers were present. On July 19 the flowers on the edge of the plot were checked and 3 of the labelled L. curtus adults were found on them.

Collections for Albany and Rome: Two hundred L. curtus were field collected and sent to Albany for host specificity tests, and another 200 were collected by Paul Dunn and Luca Fornasari and me for experiments in Rome.

Eustenopus villosus:

Rearing adults on caged YST: The objective of the study was to rear large numbers of E. villosus adults for various tests and future releases.

Nine YST plants were grown close together in a plot 1x1 m. The plants were caged on June 9 in a black screen cage placed on a metal frame. All buds were removed in order to eliminate any other seed feeders which may have been present as larvae in the buds. On June 25, 30 E. villosus adults were released in the cage. At some point, the cage wall was damaged so parasites and tephritid flies entered the cage and nullifying the rearing experiment.

One hundred seed heads were collected from the caged plants on September 8, examined externally for oviposition sites, and dissected under a stereomicroscope to determine the rate of infestation with the following results.

The Eustenopus data are:

11	seed head(s)	with living adults
1	" "	with dead adults
35	" "	from which probably adults emerged
8	" "	with dead pupae
16	" "	feeding signs only (larvae missing)
14	" "	dead larvae
15	" "	parasitized larvae

Other findings:

Five seed heads with Isocolus galls, 3 seed heads with tephritid pupae, 15 parasitized Eustenopus larvae, and 10 seed heads with Lasioderma sp. (Colep.)

To find out where the adults overwinter, the cage was removed and the plants and soil in the cage were searched for adults. One adult was found on a twisted paper towel which had been put in the cage 2 weeks previously and 6 adults were found in the soft soil within the cage. The adults were motionless but alive.

It is now clear why the adults that appear on plants in early spring are very often covered with mud.

Field Collections:

On September 9, 100 YST seed heads with at least one oviposition/feeding scar/head were collected in the field near Thermi. The seed heads were examined externally for number of oviposition/feeding scars and dissected under a stereomicroscope to determine the fate of the larvae. Ninety nine seed heads had one oviposition site each and 1 seed head had 2. The following are the results:

2 seed heads with living adults

15	"	"	from which adults probably emerged
4	"	"	with parasitized pupae
22	"	"	with parasitized larvae
1	"	"	dead larvae
56	"	"	missing larvae

To determine if some adults overwinter in the seed heads, a sample of 100 seed heads was collected and examined on September 28. Eight adults were found in 8 seed heads, empty pupal cells were found in 44 seed heads, and 48 heads were uninfested. Adults (440) Eustenopus villosus were sent to Albany, California for their studies and another (670) were collected by L. Fornasari, Paul Dunn and me near Thessaloniki.

Chaetorellia hexachaeta:

Three thousand five hundred Centaurea cyanus seed heads infested with C. hexachaeta were collected and sent to Albany, California on June 2. The emerging adults were used to complete host specificity tests. Since no negative results have been obtained, C. hexachaeta will probably be released in the United States, against YST, in 1988 (C. Turner pers. comm.)

Field Trial (Multiple Candidate)

Preparation of a field experiment to determine host specificity of Larinus curtus, Urophora sirunaseva (candidates for control of yellow starthistle), and Larinus minutus (a candidate for biological control of Centaurea maculosa and C. diffusa). The latter species, common in northern Greece, has been under study by CIBC, Delémont for the past 3 years. Clive Stinson, the scientist in charge of the CIBC project agreed to provide a technician to help carry out the experiments in 1988.

Objectives: (1) To determine the dispersal, attraction and oviposition behavior of 3 insect species (L. curtus, L. minutus and U. sirunaseva) when exposed equally to 7 selected plant species in a field experiment. (2) To demonstrate the host specificity of each of the 3 insect species by recording the number of adults which emerge from the harvested seed heads of each plant species in the test.

Procedure:

1. A "Randomized Complete Block Design" experimental plot was established at the University Farm, Thessaloniki, consisting of 7 treatments replicated 7 times. (see design figure 1).

2. The size of each treatment block was 2x2 m.

3. Each treatment block will contain 3 individual plants of the species designated for that treatment block, planted equidistant (40 cm. between plants) in a triangular pattern in the center of each block.

4. The plant species to be used in the experiment were selected on the basis of (a) taxonomic relationship, (b) native plant consideration, (c) observed or recorded affinity of one of the test insects, (d) economic importance of the test plant.

5. Plants designated in the design as "A" safflower (cultivar Hartman) and "D" artichoke (var. Green Globe) were provided by the USDA-ARS laboratory in Albany, California; all others were supplied by the USDA Thessaloniki Laboratory from local sources.

6. All plants to be maintained in good condition during the time that the experiment is being conducted.

7. L. curtus adults will be collected on YST, males and females will be separated and labelled with different colors. Two beetles (1 male, 1 female) will be released in the center of each block (total of 98 weevils to be released).

In early spring, before U. sirunaseva emerges, 100 YST seed heads, infested with U. sirunaseva will be placed in the center of each block, so that the emerging flies will have free choice of host plants from the available test and control plants.

L. minutus adults will be collected on C. diffusa and C. maculosa, sexed and labelled with different colors according to host plant from which they were collected, allowing the investigators to keep track of the two biotypes and find out if there are any differences in their dispersal and host selection. Two beetles of each biotype (2 males, and 2 females) will be released in the center of each block (total of 196 weevils to be returned to the plot).

8. The day after release, weevils in each block will be examined for (a) presence or absence of weevil (by color) on the test plants; (b) the presence or absence of oviposition and/or feeding behavior (by color). Observations will be made again 3 days after the release, then once weekly during the oviposition period of the beetles.

9. Behavior of U. sirunaseva and presence or absence on the test plants will be recorded at each observation.

10. As soon as mature flower heads (post-flowering stage) appear on the test plants they will be collected and all the heads from each plant bagged together to capture the insects as they emerge. This procedure will be repeated once weekly until the end of the season when no more flower heads are produced by the test plants.

Fig. 1. Plot Plan of Randomized Complete Block Design For 1988 Field Trial of Larinus curtus, Urophora sirunaseva and Larinus minutus

Block size 2 x 2 meters.

TREATMENTS	REPLICATES						
	1	2	3	4	5	6	7
I	<u>B</u> ^{1/}	F	<u>D</u> ^{1/}	C	<u>G</u> ^{1/}	E	A
II	A	F	C	B	G	E	D
III	D	G	F	C	B	A	E
IV	E	A	C	B	G	F	D
V	D	G	F	C	A	B	E
VI	E	D	A	C	B	G	F
VII	C	A	D	F	E	B	G

^{1/} Centaurea diffusa (B), Centaurea maculosa (G), and Cynara scolymus (D): These species have been grown in their corresponding blocks on October 12, 1987.

1-7 - Replications

I-VII - Treatments

A - Carthamus tinctorius (cultivar 4440)

B - Centaurea diffusa (Greek biotype)

C - Centaurea solstitialis (Greek biotype)

D - Cynara scolymus (U.S. Green Globe)

E - Cirsium creticum (Greek biotype)

F - Helianthus annuus (sunflower, Greek variety)

G - Centaurea maculosa (Greek biotype)

11. All weevils and all Urophora flies will be pinned and labelled with (a) replicate number, (b) treatment and (c) date of seed head collection, (d) locality, then sent to specialists for identification. Since U. sirunaseva overwinters as mature larvae, all flower heads collected from July to September must be kept in a protected place until the adults emerge in the spring of 1989.

12. Analyze and interpret data when determination of insects is received. Plant species to be tested in design:

- A. Carthamus tinctorus (Cultivar Hartman)
- B. Centaurea diffusa (Greek biotype)
- C. Centaurea solstitialis (Greek biotype)
- D. Cynara scolymus (U.S. Green Globe)
- E. Cirsium creticum (Greek biotype)
- F. Heliathus annuus (Greek variety) sunflower
- G. Centaurea maculosa (Greek biotype)

DIFFUSE KNAPWEED PROJECT - Centaurea diffusa

INTRODUCTION

Bangasternus fausti

Assistance was given to Gaetano Campobasso, who came to Thermi to collect B. fausti weevils to complete the host specificity testing in Rome.

EXPERIMENTAL OBJECTIVES

1. To determine if the eggs of B. fausti are parasitized.
2. To study (a) the degree of natural mortality and (b) the seed consumption (destruction) by the weevil.

MATERIAL AND METHODS

1a. About 200 B. fausti eggs were collected in Thermi on June 10 and kept on moist paper towels in a petri dish in our laboratory in order to capture emerging parasites.

1b. Another sample of about 500 eggs collected with Paul Dunn and Luca Fornasari were taken to Rome to check again for egg parasites.

2. Five seed head samples (100-105 mature seed heads infested with at least one B. fausti egg per sample) were collected from August 2 to September 20 in Thermi and dissected under a stereomicroscope. Table 4 shows the results of the dissections.

RESULTS

In trial 1(a) with 200 eggs, some of the eggs hatched, but no parasites emerged from them. In trial 1(b) with 500 eggs about 60 % of the eggs hatched but no parasites emerged.

Table 4. Natural mortality rate of *B. fausti* at various stadia and rate of seed destruction.

Date	NO. OF HEADS			EGGS		LARVAE		PUPAE		ADULTS				
	Collected	Infested	Not Infested ^{1/}	1/Head	2/Head	No.	No. Not Alive	Parasit. or Dead	Missing	Alive Parasit.	Emerged In Heads			
August 2	103	85	18(135) ^{1/}	95	8	77	34	10(*)	24(18)	27	3(*)	9(*)	0	2(*)
			$\bar{x} = 7.5$											$\bar{x} = 0.75$
August 14	99	78	21(180)	98	1	53	48	1(*)	13(*)	11	0	23(*)	3(*)	1(*)
			$\bar{x} = 8.5$											
August 26	100	72	28(175)	100	0	60	40	0	14(*)	19	0	22(*)	2(*)	1(*)
			$\bar{x} = 6.2$											
September 11	100	100	0	94	6	48	57	0	19(4)	16	0	12(*)	0	0
														$\bar{x} = 0.2$
September 20	100	99	0	94	5 ^{2/}	53	55	0	24(5)	10	0	16(*)	0	0
														$\bar{x} = 0.2$

() = No. of seeds/heads in that category

^{1/} \bar{x} No. seeds/head

^{2/} 1 seed head had 3 eggs on it.

In 97 seed heads, where an adult or a pupa developed, no seeds were found. The developing larvae fed on the seeds, pappus hairs, and receptacles, leaving only the bracts, inside of which the larvae made pupal cells.

In 105 seed heads, in which larvae were found (alive, parasitized, dead), only 27 seeds were found ($\bar{x} = 0.25$ seeds/head). In 67 uninfested seed heads 490 seeds were found ($\bar{x} = 7.3$ seeds/head).

Pterolonche inspersa

P. inspersa eggs were collected for shipment to Albany, California to infest C. diffusa rosettes in the U.S. and start a colony of the insect in California. On July 29 and 30, 260 C. diffusa roots infested with P. inspersa were collected around Kardia (near Thessaloniki). About 30% of the adults had already emerged, 30% were pupae and 40% were still in larval stages. The infested roots were placed in moist soil under 3 screen cages (1x1x1 m.) in the field at the University Farm at Thessaloniki. Several potted C. diffusa rosettes were placed under the cages to serve as an oviposition substrate for the emerging adults. Adults started to emerge on August 2 and oviposition started on August 3 and 4. Most of the eggs were laid on cage walls, and were difficult to collect. Only 160 eggs were collected on August 5, 100 eggs on August 6, and 25 eggs on August 10. Fortunately, a good population of C. diffusa was found around Kardia, on which P. inspersa eggs were common, so over 6000 eggs were collected from this location between August 7 and August 17. These were sent to Albany along with the eggs collected from the cages (total 6,635). On August 10, 50 of the field collected eggs were examined and 15 (30%) had hatched.

Aceria centaurea

We were requested to send a sample of the diffuse knapweed gall mite (Aceria centaurea) to Albany to start host specificity tests.

Also, Dott.ssa. Marisa Castagnoli, an Italian eriophid specialist in Florence, has agreed to collaborate with us and wanted a colony of the mite to study its biology. The mite colony near Arnea where it has existed for the past few years was visited on May 13 and 25, June 8, 22, and 27, and July 17 and 30, and no diffuse knapweed infested with the gall mite could be found in the area. When collecting Bangasternus fausti around Thermi and collecting Pterolonche inspersa larvae and eggs around Kardia no gall mite infestation on C. diffusa was seen. However, another Aceria mite was discovered attacking meristem tissue of C. diffusa plants, turning the growing points into "witches'-brooms", which produced very small flower heads with no seed or just a few deformed or poorly developed seeds per seed head. This mite was first found on May 25, 1987 near Arnea at the junction to Riza. In that report it was called a bud mite because it was common on the bracts of the young flower buds or on axillary buds throughout the season. The mite was also common in a dense population of C. diffusa along the main Thessaloniki-New Mudania road, near Kardia. Two samples of this mite were collected on August 11 and 17 for shipment to Albany, California. Samples of the mite were collected for identification, and given to Dott.ssa Castagnoli.

Some of the infested plants with the witches'-broom dry out and die during the summer and fall, while others give rise to secondary growth from the root neck. A sample of 8 "witches'-broom" plants and 25 rosettes were collected from the location near Kardia on August 19 and checked for the mite. All the plants were infested except 3 rosettes. Older rosettes with many dry leaves were more heavily infested. Very small rosettes with just a few leaves also were infested. On rosettes, the mites were found between the leaf petioles around the root neck, and among the small axillary buds and young flower buds of the old plants. A sample of 8 old plants with secondary growth and a

sample of 12 rosettes were collected on August 28 near Arnea and examined for the presence of the "bud" mite. All the plants were infested, except one rosette. On September 14 a sample of 16 old plants and 12 rosettes were collected near Kardia and examined. All the plants were infested with the mite.

The same day a one-hour search for the gall mite was made at the location near Kardia. Only 4 infested C. diffusa rosettes could be found. On September 17, the location near Geroplatanos was visited, and in the fifty-minute search, only 6 rosettes infested with the gall mite could be found. Toward the junction to Riza a fallow wheat field was found, in which many C. diffusa rosettes were found to be infested with the gall mite.

On October 8, when Dott.ssa. Castagnoli visited our laboratory we went to the field and collected samples of the gall mite as well as the bud mite. She examined the samples in the laboratory and was very interested in getting living material of both species for her studies. Next day, on October 9, I collected 50 C. diffuse rosettes infested with the gall mite and 50 rosettes infested with the bud mite at the location near Kardia. The material was hand-carried by Dott.ssa. Castagnoli to Florence to study the biology of both species as well as to confirm their identity.

The bud mite is more common and more effective than the gall mite. In 50 seed heads collected from infested witches' broom C. diffusa plants, at the junction to Riza, only 37 seeds could be found ($\bar{x} = 0.7$ seeds/head). An infested plant has many seed heads, most of which are so small that no seeds develop in them. At the same time these plants bear a few larger heads in which some seeds are found. The mite generally alters the plants' morphology, the distance between the axillary buds on infested plants becoming reduced and the whole plant being considerably deformed.

EXPLORATION: Large infestations of both Eustenopus villosus and Larinus curtus are difficult to find in northern Greece, therefore, it was decided to search for new locations where larger numbers of adults could be collected in the future. A three-day trip to eastern Greece, (Alexandropoli and Ardanio) from July 7 to 9 and 4 half-day trips around Thessaloniki, in mid-June revealed a few good locations. On the trip to eastern Greece 25 locations were checked for presence of the two insects. L. curtus, found on 11 locations, was rare in 9 locations (a 30-minute search yielded 1-4 adults) and common in 2 locations (in 10-minute and 30-minute searches, 21 and 13 adults were found). The two good locations are 16 km. east of Kavala (800 meters west of the junction to Nea Komi and 44 km. east of Kavala, junction to Thalasias).

E. villosus was found in 7 locations. One location was 44 kms. east of Kavala and all other locations were around the village Ardanio (12 km west of the Turkish border). The weevil was common only at one location, where 41 adults were collected in 30 minutes searching. In the other locations 1-10 adults were found in the 30 minutes spent searching each location. Over 30 new locations were examined around Thessaloniki. Only in one location were good populations of both insects found. This location is on the Thessaloniki-Oreokastro road (north-east of Thessaloniki). L. curtus was present in nearly all locations around Thessaloniki but was rare (1-5 adults per half hour search). E. villosus also was more or less common along the same road to Oreokastro.

MISCELLANEOUS:

1 - U.S. Cirsium in vitro:

A shipment of U.S. Cirsium plants, grown by tissue culture and still in vitro, were shipped from the Rome laboratory to Thessaloniki. It was an

attempt to provide plants for transplanting in soil to be used in various experiments in the future. The shipment arrived in Thessaloniki with 2 days delay (sent air freight by Olympic Airline). Following the delay it was a weekend and we could not get the plants because the customs office was closed. Finally on Monday, May 18 the shipment was retrieved from the airport, but by this time the plants were yellowish and some were dead. On May 19, 1986 60 of the best looking small plants were transplanted into small pots and kept in transparent plastic bags in a shady room. Preparation of soil, pots, plastic bags, shady place, etc. were according to instructions but all plants died within 3-4 weeks. A few plants produced new small leaves but they died later. Most probably failure of the attempt was due to the long time en route.

Cooperation with CIBC, Delémont: A sample of several hundred mature C. cyanus seed heads infested with Chaetorellia hexachaeta collected from Agios Prodromos and 500 mature seed heads of Mantisalka salmantica collected near Thermi, were sent to Delémont on June 1, 1987. Also, 600 Larinus minutus adults were collected near Thermi on Centaurea diffusa and near Triadi on C. maculosa, and were sent to Delémont on June 23, 1987.

Cooperation with CSIRO, Montpellier: Four samples of Chondrilla juncea seeds for use in isoenzyme studies were collected, (30 kms south, 30 kms north, 30 kms east of Thessaloniki and one sample near Thessaloniki) in September and October 1987. Over 20 seeds per individual plant were collected from 20 to 28 separate plants at each location and were mailed to CSIRO on October 13, 1987. In the course of the seed collection the larvae of a tephritid fly was found feeding in the seed capsules of the C. juncea plants.

About 15 adults of the fly were reared out. They are probably known to CSIRO researchers. If not it might be worthwhile to identify the species and see if it is specific to C. juncea. We plan to send a few pinned specimens of the fly to Montpellier

A Lepidopteran larva also was found feeding inside the seed capsules. After the larva cleans out a seed capsule it leaves it and penetrates into another one. In this way each larva destroys several seed capsules. About 20 larvae were collected and provided with fresh food in the laboratory. Four larvae pupated in the sand placed in the cages with the food plants.

C. juncea populations were examined for rust infection in 9 locations along the road from Kavala to Ardanio and Suflí (eastern Greece) and at least 15 locations around Thessaloniki, Drama, Halkidiki etc. Only individually infested plants were found in several locations, but these were not collected.

Cooperation with the University of Thessaloniki: Necessary assistance was provided to Professor Katsoyannos in his field experiment with the host specificity test of L. curtus in Thessaloniki (collecting weevils, taking care of plants, taking data etc.). He will present the results of the trial in his final report. Assistance was also provided to I. Pittara for finishing her doctoral thesis on the behavior and biology of C. hexachaeta.

A prefabricated building to serve as a laboratory is being donated to the Department of Entomology by the U.S. Department of Agriculture, ARS, in appreciation for housing, rent-free, for 7 years the Rome laboratory's program in Greece. The building will become the property of the University of Thessaloniki as soon as it is built but ARS has guaranteed use of the building for 5 years after its completion with an optional 3 years' use if necessary. Space for one Greek scientist is provided in the building from the day construction is completed.

Shipments - Insects, plants and plants parts:

1. March 30 Euphorbia seguieriana-400 plants to Rome
2. April 22 Simyra dentinosa-2,000 eggs to Rome
3. May 27 Bangasternus fausti-1,500 adults to Rome
4. June 1 Samples of Centaurea cyanus and Mantisalka salmantica seed heads to CIBC, Delémont
5. June 2 Bangasternus orientalis-2,050 adults to Albany, California
6. June 2 Centaurea cyanus-3,500 seed heads to Albany, CA.
7. June 8 a sample of C. diffusa infested with Aceria sp. to Rome
8. June 23 Larinus minutus-600 adults to CIBC, Delémont
9. June 24 A sample of C. diffusa plants infested with Aceria sp. to Albany, California
10. June 24 Eustenopus villosus-440 adults to Albany, California
11. June 29 Eustenopus villosus-550 adults to Rome
12. June 30 Larinus curtus-200 adults to Albany, California
13. July 20 Eustenopus villosus-170 adults to Rome
14. August 11 Pterolonche inspersa-2,285 eggs to Albany, California
15. August 11 A sample of C. diffusa plants infested with Aceria sp. to Albany, California
16. August 18 P. inspersa-4,350 eggs to Albany, California
17. August 18 A sample of C. diffusa infested with Aceria sp. to Albany, California
18. August 18 tephritid flies-114 specimens YST to I. White, British Museum, London

19. October 9 C. diffusa-50 plants infested with Aceria sp. (the gall mite)
and 50 plants infested with the Aceria bud mite to Dott.ssa.
Castagnoli, Florence.
20. October 13 C. juncea-4 seed samples to CSIRO, Montpellier.

PUBLICATIONS

GREECE

1. D. M. Maddox and R. Sobhian. 1987. Field experiment to determine host specificity and oviposition behavior of Bangasternus orientalis and Bangasternus fausti (Coleoptera: Curculionidae), biological control candidates for yellow starthistle and diffuse knapweed. *Env. Entomol.* 16 (3):645-648
- 2/ R. Sobhian and I.S. Pittara. A contribution to the biology, phenology and host specificity of Chaetorellia hexachaeta Loew (Dipt. Tephro.), a possible candidate for the biological control of yellow starthistle (Centuarea solstitialis L.). *Z. Ang. Entomol.*, in press.

TRAVEL (ROME)

March 2-3	P. H. Dunn, G. Campobasso, L. Fornasari, T. Mimocchi, M. Stazi, M. Cristofaro, P. Pecora to Antibes to attend the joint CIBC/CSIRO/BCWLE-ARS-USDA/IPP-University Zagreb meeting.
April 21-24	G. Campobasso - Bari
April 22-23	M. Cristofaro - Perugia/Pisa
May 11-12	M. Cristofaro and P. Pecora - Pisa
May 18-28	G. Campobasso - Greece (Thessaloniki & vicinity)
May 22-23	M. Cristofaro and M. Stazi - Pisa
May 29-30	M. Cristofaro and M. Stazi - Pisa
June 3-5	M. Cristofaro and M. Stazi - Pisa
June 8-10	P. Pecora - Perugia/Pisa/Bologna
June 15-18	M. Cristofaro and M. Stazi - Pisa
June 15-21	L. Fornasari - Palermo
June 17-23	P. Pecora and A. Laregina - Vienna, Austria
June 22-30	M. Cristofaro and M. Stazi - Lucca/Piacenza/Pisa
June 25-30	P. H. Dunn and L. Fornasari - Greece (Thessaloniki & vicinity)
June 29-July 7	G. Campobasso - Bari
June 30-July 16	P. Pecora and A. Laregina - Vienna, Austria and Budapest, Hungary
July 7-9	M. Cristofaro and M. Stazi - Lucca/Pisa
July 7-10	R. Sobhian - Fanari, Alexandroupolis, Greece
July 13-15	M. Cristofaro and M. Stazi - Pisa
August 19-20	M. Cristofaro and M. Stazi - L'Aquila

September 21-22 M. Cristofaro and M. Stazi - Pisa

October 4-31 P. H. Dunn - U.S. (Home leave and consultation at
Beltsville and Albany)

October 8-12 G. Campobasso - Bari

October 11-22 M. Cristofaro and P. Pecora - Romania, Austria,
Czechoslovakia

November 4-5 M. Cristofaro and M. Stazi - Pisa

SHIPMENTS

ROME LABORATORY

DATE	N° SHIPPED	SPECIES SHIPPED	DESTINATION

INSECTS			
March 6	700	<u>Eteobalea serratella</u> (in roots)	Delèmont, CH
March 11	10,000	<u>Dasineura capsulae</u> (larvae)	Albany, CA
May 13	140	<u>Bayeria capitigena</u> (galls)	Albany, CA
May 25	317	<u>Bayeria capitigena</u> (galls)	Albany, CA
June 1	350	<u>Bayeria capitigena</u> (galls)	Albany, CA
June 6	250	<u>Bayeria capitigena</u> (galls)	Albany, CA
June 12	42	<u>Tyta luctosa</u>	Albany, CA
June 19	210	<u>Oberea erythrocephala</u>	Albany, CA
June 24	35	<u>Aphthona czwalinae</u>	Albany, CA
July 1	3,100	<u>Aphthona flava</u>	Albany, CA
July 6	2,402	<u>Aphthona cyparissiae</u>	Albany, CA
July 9	40	<u>Oberea erythrocephala</u>	Albany, CA
July 13	190	<u>Aphthona cyparissiae</u>	Albany, CA
July 15	1,580	<u>Aphthona flava</u>	Albany, CA
BOTANICAL MATERIAL			
April 27	many	<u>Uromyces scutellatus</u> (spore)	Ft. Dietrich, MD
May 7	ca 150	<u>Cirsium douglasii</u> (in vitro)	Delèmont, CH
May 13	ca 150	<u>Cirsium douglasii</u> (in vitro)	Thessaloniki, GR
May 13	many	<u>Uromyces scutellatus</u> (spore)	Ft. Dietrich, MD
July 8	5,000	<u>Centaurea alba</u> (heads)	Delèmont, CH
Sept. 2	ca 150	<u>Cirsium andrewsii</u> (in vitro)	Delèmont, CH

VISITORS

(in order of visit)

ROME LABORATORY

1. Dr. R. D. Perkins, USDA, ARS, EPL, Paris.
2. Dr. Antonio Quacquarelli, Phytopathology Institute, Ministry of Agriculture and Forestry, Rome.
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INSECTS SENT FOR IDENTIFICATION

ORIGIN	SHIPMENT N°	IDENTIFICATION
Parasites from <u>Simyra dentinosa</u>	87-1	Braconidae: <u>Cotesia</u> sp. possibly <u>vanessae</u> (Remhard). Det. P. M. Marsh
Cecidomyiids from leafy spurge	87-2	Cecidomyiidae: <u>Dasineura</u> sp. not <u>capsulae</u> . Det. R. J. Gagné
<u>Cheilosia</u> sp. (larvae from <u>Cirsium palustre</u> and <u>C. oleraceum</u> collected by Zwolfer in Germany	87-3	Not yet determined
Tachinid parasite of <u>Simyra dentinosa</u>	87-4	Not yet determined

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Handwritten notes or markings in the left margin, including what appears to be a date and some illegible text.

