ENVIRONMENTAL ASSESSMENT

I. Cover Sheet

Title of Project: Host plant specificity testing of Eustenopus villosus (Boheman) (Coleoptera: Curculionidae) against plant species native to North America.

Proposed Action: Petition being submitted to obtain approval for the introduction of Eustenopus villosus into the United States to assist in the biological control of Yellow Starthistle, Centaurea solstitialis L., (Asteraceae: Cardueae).

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III. Abstract

We propose to release populations of adults of the weevil, Eustenopus villosus, from Greece in infestations of the noxious weed yellow starthistle, Centaurea solstitialis, in California. It is expected that the weevil will spread, over a period of many years, to other infestations of the weed. No-choice and two-choice tests, confirmed by field tests, against 34 plant species in the United States and Europe showed that the weevil is restricted to C. solstitialis (yellow starthistle), C. nicaeensis, and C. diffusa (diffuse knapweed), and thus has no potentially harmful environmental effects. Incipient populations could be destroyed within the first three years after release by treating the weeds with herbicides at the time when larvae are feeding in the flowerheads.

IV. Introduction

This petition is the result of studies and testing conducted at the USDA-ARS Biological Control of Weeds Laboratory - Europe in Rome, Italy, by Luca Fornasari, Stephen L. Clement, and Tiziana Mimmocchi; in Thessaloniki, Greece by Rouhollah Sobhian; and at the USDA-ARS Biological Control of Weeds Laboratory in Albany, California, by Charles E. Turner and Lloyd A. Andres.

Yellow starthistle (Centaurea solstitialis L.) (Asteraceae: Cardueae) is an herbaceous winter annual that occasionally exhibits a biennial habit. It is an adventive in the United States, and is believed to have been introduced after 1824 (Maddox and Mayfield, 1985). Yellow starthistle presumably arrived from the Middle East through numerous introductions, alfalfa seed being suspected as the principal carrier. It is a pioneering plant that is now widespread in the U.S., occurring in at least 208 counties in 23 states, primarily in the Northwest (Maddox et al., 1985) and especially in California, Idaho, Oregon, and Washington (Maddox, 1981; Maddox and Mayfield, 1985). In California yellow starthistle is estimated to have increased from 1.2 million gross acres in 1958 to 7.9 million gross acres in 1985, primarily in northern California (Maddox and Mayfield, 1985). According to Callihan et al. (1982), yellow starthistle has the potential to invade nearly all of the semiarid to subhumid rangeland in the western U.S. It is a persistent weed of considerable economic importance because it invades rangelands, grain fields, orchards, vineyards, cultivated crops, pastures, roadsides, and wastelands. Moreover, yellow starthistle seeds are an important contaminant in commercial seeds. The weed is also a contaminant in alfalfa hay and invades cereal grains in California. It is toxic to horses, in which it causes "chewing disease", a neurological disorder that leads to eventual death if ingested over an extended period of time (Cordy, 1954).

V. Purpose and Need

The objective of releasing Eustenopus villosus is to establish a fourth natural enemy to assist in the biological control of yellow starthistle, primarily in California and the northwestern USA.

POTENTIAL CONTROL VALUE

Rome test (1988)

A laboratory test was carried out in the quarantine greenhouse to evaluate the impact of E. villosus on yellow starthistle plants from Greece. Ten replicates of two adult males and two females each were caged on a potted yellow starthistle plant using black, nylon tulle sleeve cages. Dead weevils were replaced with fresh ones until food was available. Ten replicates were also used in the control (yellow starthistle without Eustenopus). The experiment was started on June 22 and was terminated on August 12, 1988, when all the plants were dead. Observations of the conditions of the plants were conducted during the experiment, and at the end of the trial all of the undeveloped buds, flowers, and seedheads were collected and counted and the number of seeds produced by each plant were counted.

No overall differences in plant size as indicated by their height (range 65-80 cm) were observed between test and control plants. On the contrary, the plants caged with weevils produced fewer seedheads, due to the weevil feeding on the buds. On control plants, 192 undeveloped buds and 117 flowers were collected. On test plants, 218 undeveloped buds and 53 flowers were collected. Control plants (n = 10) produced a total of 107 seedheads and 6,416 seeds, and test plants produced a total of 27 seedheads and 80 seeds. The number of seeds produced was extremely low on test plants, due to the overall damage by the insect. The overall reduction in seeds per plant has two components: the effect of external feeding by adults in reducing the number of seedheads per plant and the effect of internal feeding by larvae in reducing the number of seeds per seedhead. On test plants only 8.0 ± 9.3 seeds per plant (n = 10) and 3.0 ± 5.4 per seedhead (n = 27) on average were produced, while on the control plants a mean of 641.6 ± 416.9 seeds per plant (n = 10) and 59. 9 ± 21.8 seeds per seedhead (n = 107) were produced. The effect of adult Eustenopus feeding led to 75 per cent reduction in the total number of seedheads produced by ten plants, larval feeding reduced seeds per seedhead by 95 per cent, and the overall reduction of seeds per plant was 99 per cent. This trial was conducted under the following temperature and humidity conditions:

Mean Temp. (C ± S.D.)	Temp. Range (C)	Mean R.H. (% ± S.D.)	R.H. Range (%)
22.9 ± 4.9	15-35	59.1 ± 16.7	30-90

Evaluation scoring systems

The effectiveness of Eustenopous villosus for the biological control of yellow starthistle was evaluated using the scoring systems proposed by Harris (1973) and Goeden (1983). From the scores obtained we can place this weevil among the candidates that should be partially effective and should be complemented by other imported agents for successful control.

HARRIS SYSTEM		GOEDEN SYSTEM	
1 Host Specificity	3	INITIAL ASSESSMENT OF DESTRUCTIVENESS IN NATIVE RANGE	
2 Direct Damage Inflicted	5	1 Direct Damage Inflicted Under Field Conditions	6
3 Indirect Damage Inflicted	0	2 Indirect Damage Inflicted	0
4 Phenology of Attack	4	3 Phenology of Attack	6
5 Number of Generations	0	4 Number of Generations	0
6 Number of Progeny/Generation	0	5 Number of Progeny/Female/Generation	0
7 Extrinsic Mortality Factors	0	6 Extrinsic Mortality Factors	0
8 Feeding Behaviour	2	7 Feeding Behavior	3
9 Compatibility with Other Control Agents	2	8 Distribution	4
10 Distribution	4	SUITABILITY AS A BIOLOGICAL CONTROL AGENT	
11 Effectiveness	3	9 Host Plant Source of Insect	6
12 Size of agent	2	10 Ease of Culture	2
		11 Potential Safety	4
		12 Host Plant Specificity	0
		POTENTIAL EFFECTIVENESS IN AREA OF INTRODUCTION	
		13 Evidence of Effectiveness as a Control Agent	4
		14 Ecoclimatic Similarity	4
		15 Colonization History of Agent	0
	25		39

The benefits from release and establishment would be effective, environmentally sound, economic, and energy-efficient, non-chemically based control of this major weed.

A. Description of the program

The purpose of the proposed field release of E. villosus is for biological control of the weed yellow starthistle. Successful biological control control of yellow starthistle would be highly beneficial economically and ecologically: increased sustainable productivity on rangeland and pasture, reduced levels of "chewing disease in horses, increased sustainable profitability in croplands, reduced inputs of herbicides such as picloram into the environment, reduced interspecific competitive pressure from the weed on native plant species, and enhanced quality on recreational lands.

The high degree of host specificity of E. villosus is described in the next section (Description of Organism to be Released). If E. villosus populations are established from field releases, conventional techniques of population ecology will be utilized to monitor the populations of E. villosus, yellow starthistle, and associated vegetation (Harper, 1977; Southwood, 1978).

B. Description of the Organism to be Released

TAXONOMIC POSITION OF Eustenopus villosus

Csiki (1934) established a large number of subgenera in the genus Larinus, and placed the species villosus in the subgenus Eustenopus Petri. There is a consensus among curculionid taxonomists (D. R. Whitehead (U.S.A.), M. E. Ter-Minasyan (U.S.S.R.), E. Colonnelli (Italy), M. L. Fremuth (Czechoslovakia), and M. L. Cox (U.K.), that Eustenopus should be elevated to the generic level. In addition to E. villosus (= hirtus (Waltl, 1838), not hirtus (Gyllenhal, 1836)), Ter-Minasyan (1978) recognized two other species in the genus, E. lanuginosus (Faust) and E. abbreviatus (Faust).
The candidate insect being petitioned herein is Eustenopus villosus (Boheman), determined by the curculionid taxonomist Dr. E. Colonnelli, (Dipartimento di Biologia Animale e Dell'Uomo, Viale dell'Universita' 32, Roma, Italia) and confirmed by Dr. D. R. Whitehead (Systematic Entomology Laboratory, USDA-ARS, Washington, D.C.). In previous reports from this laboratory the weevil called E. abbreviatus and E. hirtus is in fact E. villosus. The classification is as follows:

Family	: Curculionidae
Subfamily	: Cleoninae
Tribe	: Lixini
Genus	: Eustenopus Petri
Species	: villosus (Boheman) (Larinus)

Voucher specimens from studies in Europe are deposited in Biological Control of Weeds Laboratory-Europe (BCWL-E) and Systematic Entomological Laboratory (SEL). Voucher specimens from populations to be released will be deposited in BCWL-E, SEL, and State Museums in the states were releases will be made.

GEOGRAPHIC DISTRIBUTION

Eustenopus villosus is known from Greece, Turkey, Syria, Iran, and the Caucasus region of U.S.S.R. (Clement et al., 1988; Sobhian and Zwölfer, 1985; Ter-Minasian, 1978).

HOST PLANTS

With regard to the insect-plant host literature, C. solstitialis is the only known breeding host of E. villosus (Clement et al., 1988; Sobhian and Zwölfer, 1985). The weevil is not recorded from any crop plant in Europe, the Middle East, or western Asia, the area in which E. villosus occurs (Clement et al., 1988).

LIFE HISTORY AND PARASITOIDS

Sobhian and Zwölfer (1985) described the life cycle, phenology, and parasitoids as follows. Eustenopus villosus (Fig. 1) produces only one

generation per year. The adult weevil hibernates during the winter outside the host plant, in the litter layer on the soil surface, and becomes active during late May or June. Mating adults have been observed during late June in northern Greece (Thessaloniki area). The female weevil chews a hole into the flower bud, where she lays a single egg (Fig. 2), then plugs the oviposition hole with frass. The eggs hatch within 3 days at 27 C. The larvae (Fig. 3) are capable of destroying almost all of the achenes in small heads of yellow starthistle. Their development is completed inside the flowerhead, and the adult weevil chews its way out of the flowerhead to emerge during the summer. In our studies, adult weevils feed on flowerhead buds (stages BU 1 - BU 4 of Maddox, 1981) by piercing through the sides in the involucral area with the rostrum. There is some preference for the early bud stages (BU 1 and BU 2). Adult weevils oviposit in relatively mature flowerhead buds (stages BU 3 - BU 4 of Maddox, 1981). An Ichneumonid wasp, Exeristes sp., and a chalcid wasp, Habrocytus sp., have been observed as larval parasitoids (Sobhian and Zwölfer, 1985).

HOST SPECIFICITY EXPERIMENTS

Material and Methods

LABORATORY TESTS - ROME, ITALY

Adult feeding and oviposition tests were conducted on weevils (Fig. 1) collected in Oreokastro, Thessaloniki, and Doirani, Greece and shipped to the Rome laboratory. They were allowed to feed on yellow starthistle buds, after which mating pairs were selected for no-choice and choice tests during 1985, 1987, and 1988. During 1986 adult weevils from two sources were used: 1) weevils collected in Greece that were allowed to overwinter in plant debris in two outdoor cages at Rome; and 2) weevils that were collected in Greece and shipped to Rome during the season. These weevils were allowed to feed initially on yellow starthistle as before, and then were used in "no-choice" and "choice" type tests. The procedures for each type of test were as follows:

No-choice tests (feeding and oviposition) - 1985 and 1986

These tests consisted of presenting a single test plant or control plant (yellow starthistle) caged in a nylon organdy sleeve cage (diameter 14-20 cm; length 30-42 cm). Plants tested are listed in Table 1. Each cage contained 2-9 weevils (1-4 females) and branches of mature buds of one test plant species. Each plant species was replicated 1-15 times. Feeding damage was classified in the following way: (-), no feeding or very slight nibbling on buds; (+), light to moderate feeding, some buds with two or more feeding punctures; (++), moderate to heavy feeding, less than 1/3 of buds riddled with feeding punctures; and (+++), heavy feeding, more than 1/3 of buds riddled with punctures. Observations were made for 15 days. Weevil mortality was also recorded, and dead females were examined for the condition of their ovaries.

No-choice tests (feeding and oviposition) - 1987 and 1988

These tests were conducted (in a quarantine greenhouse with natural lighting) on 25 plant species and on yellow starthistle plants from

Greece, California, and Washington, listed in Tables 4 and 5. Branches of each test plant were caged in black, nylon tulle sleeve cages (Fig. 4). Two males and two females were caged on each potted plant; there were 5 replicates of each test plant species in 1987 and ten replicates in 1988. Weevils were allowed the opportunity to feed and oviposit for 7-10 days, then caged onto another fresh plant and again left for 7-10 days. This procedure was repeated until all the beetles died. All of the exposed buds were dissected to record the feeding damage and count the number of eggs laid. During 1987 this test was made between July 5 and August 27, and in 1988 between June 17 and August 12.

Choice tests (feeding and oviposition) - 1986, 1987, and 1988

Field collected adult weevils (2 mating pairs per cage) were placed in black organdy sleeve cages, containing branches from a potted yellow starthistle plant from Greece and the test plant species were tied together. The test plant species used were:

1. Carthamus tinctorius L.
2. Cynara scolymus L.
3. Cirsium arvense Scopoli
4. Cichorium intybus L.
5. Centaurea nicaeensis Allioni
6. Centaurea americana Nuttall
7. Zinnia elegans Jacquin Nicolaus Joseph
8. Calendula officinalis L.

A choice of food and oviposition substrate was thus offered and the test was terminated when choice was no longer available, i.e. when yellow starthistle branches were completely destroyed by the weevils.

Field tests

These were conducted in Thermi, Greece in 1985, to measure diversity, abundance, and pattern of attack of the adult weevil as well as other parameters. The garden plot contained C. solstitialis from three souces (Greece, California, Idaho), Cirsium creticum (De Lamarck) Dumont'Urville, Cynara scolymus L., and Carthamus tinctorius L. in each of 6 rows, using a randomized block design for a total of 36 plants. The rows and plants were spaced about 2 m apart. Sampling was done by harvesting and holding the seedheads for emergence of weevils and parasitoids.

LABORATORY TESTS - ALBANY, CALIFORNIA

Adult feeding and oviposition tests were conducted during 1988 on weevils field-collected near Doirani, Greece and shipped to the Albany laboratory. The weevils were initially allowed to feed on bouquets of yellow starthistle flowerhead buds in sleeve cages. Apparent mixed-gender pairs were removed for no-choice cage and carton tests conducted in the quarantine greenhouse. Because the literature and the garden plot experiment in Greece indicated a very narrow host range, all test plant species were from the Asteraceae, and most were from the thistle tribe Cardueae.

No-choice cage tests

These tests consisted of multiple pairs of weevils placed inside a 1 m^3 screen cage enclosing multiple potted plants of one test plant species per cage. The test plant taxa, chosen on the basis of taxonomic affiliation, commercial significance, and place of origin, were as follows: Centaurea solstitialis L. (from California), Centaurea rothrockii Greenman (one of two closely related Centaurea species native to the southwestern U.S.), Carthamus tinctorius L. var. "4440" (safflower variety grown in California), C. tinctorius var. "S541" (safflower variety widely grown in the northcentral states), Cirsium douglasii De Candolle (native to California), and Helianthus annuus L. (sunflower). For each cage test, the number of Eustenopus pairs was equivalent to the number of plants per cage. Ten or 15 plants were used per cage (Tables 8, 9). Thus, for example, the test with C. tinctorius "S541" involved 15 plants of this safflower variety and 15 pairs (30 weevils total) of Eustenopus. The weevils could move freely on and between plants in each cage. All test plants possessed flowerhead buds at stages potentially suitable for Eustenopus adult feeding and oviposition. For each test, the plants were exposed to the weevils for 14 days, at which time the weevils were removed from the cages. The cage tests for all test plant species were completed between 23 June and 20 July. All flowerheads at a suitable stage during the 14-day exposure period were later examined between 15 August and 12 September for feeding scars and for oviposition holes and were dissected to inspect for evidence of larval feeding and development. To measure adult feeding, all flowerheads that had been in any bud stage (Bu 1 to Bu 4) were examined. To measure oviposition, all flowerheads that had been in the late bud stages (Bu 3 to Bu 4) were examined. Flowerheads containing living Eustenopus were set aside to allow development to proceed.

No-choice carton tests

In these tests, pairs of weevils were placed in pint (ca. 473 cm^3) cardboard cartons which enclosed one flowerhead bud of a potted test plant species: either Centaurea solstitialis or Carthamus tinctorius var. "4440". This test isolated the activities of the weevils onto specific flowerhead buds. The weevils used in this test originated from the sleeve cage containing bouquets of yellow starthistle flowerhead buds or from previous carton tests with either yellow starthistle or safflower. Closed flowerhead buds (stages Bu 1 - Bu 4) were inserted into the cartons via slits in the side of the cartons. Any gaps between the carton and the stem were filled with cotton. The cartons had clear plastic lids to allow light passage and facilitate observation of the activity of the weevils . The weevils were introduced into the cartons through holes in the sides; the holes were then plugged with cotton. All tests were conducted July 6- 22. Flowerhead buds were exposed to Eustenopus for 2-4 days, then the weevils were removed; any living weevils were used in subsequent carton tests. The exposed buds were held for possible larval development, then examined August 10-17 for feeding punctures and oviposition holes.

Results

LABORATORY TESTS - ROME, ITALY

No-choice tests - 1985 and 1986

Adult feeding was recorded to some degree on all test plant species and yellow starthistle plants (from Greece and U.S.) tested. There was considerable damage to the seed heads of yellow starthistle, Centaurea nicaeensis Allioni, C. diffusa De Lamarck, Cnicus benedictus L., and Cirsium spp. Although adult feeding occurred, it is important to stress that eggs were deposited only into the buds of two Centaurea species, C. nicaeensis, and C. diffusa in addition to C. solstitialis (from Greece and U.S.). The results of the no-choice tests, which include the mortality data, are given in Table 1. Only two larvae were found in C. diffusa buds and they died as first instars (Clement et al., 1988). Dissection of 50 females revealed rudimentary oocyte development in only two females, one each from Carthamus tinctorius and a Scolymus hispanicus L. plant (Clement et al., 1988).

No-choice tests - 1987 and 1988

These tests were conducted under the following temperature and humidity conditions:

	Mean Temp. (C ± S.D.)	Temp. Range (C)	Mean R.H. (% ± S.D.)	R.H. Range (%)
1987	22.5 ± 4.1	14-33	60.7 ± 17.8	28-88
1988	22.6 ± 5.0	14-35	59.4 ± 16.9	30-90

The results of these tests are given in Tables 2 and 3, showing the total number of exposed and damaged buds for each species. As in the previous tests, Greek and American ecotypes of yellow starthistle were very well accepted, with a high level of feeding and oviposition (Table 3). Adult feeding occurred to some degree on several plant species under no-choice conditions, but significant damage occurred only within the genus Centaurea. Oviposition occurred principally on yellow starthistle (107 eggs in 1987), but a few eggs also were laid on other Centaurea spp., i.e. Centaurea scabiosa L. (18 eggs), C. maculosa De Lamarck (8 eggs), C. napifolia L. (5 eggs), and C. jacea L. (3 eggs).

Choice tests - 1986, 1987, and 1988

The results of the preference tests showed that heavy feeding and oviposition only occurred on yellow starthistle and C. nicaeensis. Some feeding but no oviposition occurred on C. americana Nuttal (Table 6). Very little feeding and no oviposition took place on safflower (Table 4), Zinnia elegans Jacquin Nicolaus Joseph (Table 5), and Cirsium arvense, while no feeding occurred on Cichorium intybus, Cynara scolymus, and Calendula officinalis. Weevil survival was high and well developed ovaries were observed in dissected dead

females. It is important to note that only minor feeding was recorded on safflower (leaves only) when yellow starthistle buds were present.

Field tests

The number of adults that emerged from seed heads of the host plant (yellow starthistle from Greece and the U.S.) and non-host test plant species, grown together in the field plot at Thermi, Greece was as follows. A total of 303 adult weevils emerged from yellow starthistle, and no weevils emerged from any of the test species (safflower, artichoke, and Cirsium creticum). Furthermore, no eggs were laid in the heads of these three non-host species (Table 7). Collected yellow starthistle seedheads revealed extensive parasitization (45-50 per cent) of Eustenopus larvae.

LABORATORY TESTS - ALBANY, CALIFORNIA

No-choice cage tests

ADULT FEEDING - All buds that were at any bud stage (Bu 1 through Bu 4) during the 14-day test period were examined for feeding scars caused by adult weevils (Table 8). All test plant taxa in the thistle tribe were fed upon to some degree. The greatest amount of feeding was on yelllow starthistle (26 per cent of the flowerhead buds), with the next greatest amount of feeding on another Centaurea species (C. rothrockii, with 20 per cent of the flowerhead buds). There was a small extent of feeding on both safflower varieties, as we found feeding scars on 9.2 per cent of the buds in variety "4440" and on 3.6 per cent of the buds of variety "S541". There were 2.8 (variety "4440") to 7.2 (variety "S541") times as many yellow starthistle buds with feeding scars as safflower buds with feeding scars. The effect of adult feeding on yellow starthistle flowerhead buds was to cause considerable damage especially to the early stage buds, which were frequently destroyed by the adult feeding. Safflower produces two sets of flowerhead buds. The primary buds are larger and more vigorous than the secondary buds, which arise in the axils of the shoots bearing the primary buds. Compared to yellow starthistle, safflower flowerhead buds are more protected, as the involucral bracts are thicker than those of yellow starthistle, and the early stage primary buds are also surrounded by a layer of tough foliage leaves. It was difficult to ascertain the effect of adult feeding on safflower buds in our tests because the primary flowerhead buds were already at a late stage or were flowering at the time of exposure to Eustenopus. Most of the early stage buds exposed to Eustenopus were secondary flowerhead buds, and most of these secondary buds failed to develop further whether or not Eustenopus adults attempted to feed from them.

OVIPOSITION AND DEVELOPMENT - All flowerheads that were at the Bu 3 or Bu 4 bud stage during the 14-day test period were examined for oviposition holes (Table 9). Eustenopus oviposited only on yellow starthistle, with about 18 per cent of the flowerhead buds being attacked. At the time of dissection, 16 of the 23 flowerheads with oviposition holes contained immature Eustenopus (larvae or pupae) (Table 10). Each of these flowerheads contained a single immature

Eustenopus. Three of the five Eustenopus pupae ultimately developed into adults. Two of these emerged from their flowerheads by 12 September, and one was found alive but inside its re-opened flowerhead in early October.

No-choice carton tests

As shown in Table 11, adult feeding was much more extensive on yellow starthistle (96.6 per cent of the flowerhead buds with feeding scars) than on safflower (26.6 per cent of the flowerhead buds). There were 5.4 times as many yellow starthistle buds with feeding scars than safflower buds. Oviposition holes (on 51.5 per cent of the buds) and evidence of larval activity were present on yellow starthistle buds, while no oviposition occurred on safflower. Behavioral observations corresponded to these differences: Eustenopus adults were generally much more active on yellow starthistle flowerhead buds than on safflower buds.

C. Description of Organism Targeted for New Biocontrol Agents

Family : Asteraceae
Tribe : Cardueae
Subtribe: Centaureinae
Species : Centaurea solstitalis L.

Yellow starthistle (Centaurea solstitialis L.) is in the subtribe Centaureinae of the thistle tribe Cardueae in the family Asteraceae. The species is native to the eastern Mediterranean Basin. Yellow starthistle is an annual that reproduces only by seed production. Flowering and seed formation occurs during the summer months. Two types of seeds are produced, pappus-bearing seeds and seeds without a pappus. Seeds without a pappus simply fall from the spent heads at the end of the growing season. The pappus-bearing seeds have a pappus of short bristles, which may allow for some degree of wind-mediated dispersal or animal-mediated dispersal where the pappus gets lodged in the fur of passing animals. Human-mediated dispersal by yellow starthistle seed contamination of transported alfalfa or grain crop seed is probably an important mode of long distance dispersal, and is likely the means by which the weed arrived in North America (Maddox and Mayfield, 1985).

All of the insects including E. villosus being considered for biological control of yellow starthistle have a high degree of host specificity that does not extend beyond the genus Centaurea, and usually is confined to a small number of species within the genus. Plant taxonomy is an excellent predictor of relative likelihood of host use of nontarget plant species in stenophagous biological control insects (Turner, 1985). The nontarget relatives that would potentially most likely attacked by a yellow starthistle biocontrol insect are other plant taxa within the subtribe Centaureinae. The Centaureinae is essentially an Old World group of plants. The only native North American species in the Centaureinaem are one or possibly two species of Centaurea native to the southern United States. Centaurea americana Nutt. is native to the southcentral U.S. and northern Mexico, while the closely related and possibly conspecific Centaurea rothrockii Greenm. is native to mid-elevations in the southwestern U.S. (Kerney and Peebles, 1960; USDA-SCS, 1982). These

species, which are outside the primary geographic range of yellow starthistle, were included in the host specificity tests for E. villosus, and there is no evidence of host use of these native Centaurea species by the insect. The only cultivated plant species of importance in the Centaureinae in the oilseed crop safflower, Carthamus tinctorius L. This species was also included in the host specificity testing, and there is also no evidence of host use of safflower by E. villosus. A number of other, more distantly related plant species (e.g. native North American Cirsium species and artichoke, Cinara scolymus L.) were also included in the host specificity testing with the predictable result of no evidence of host use of them by the insect.

Voucher specimens of yellow starthistle are located in the herbarium of the USDA-ARS Biological Control of Weeds Laboratory in Albany, California (C. E. Turner, curator) and in the weed herbarium of the California Department of Food and Agriculture in Sacramento, California (D. Barbe, curator) as well as in the herbaria of all the land grant Universities in the states of California, Idaho, Oregon, and Washington.

D. Alternatives within Proposal

The purpose of the field releases is field establishment for biological control of the weed yellow starthistle. We will release E. villosus adults in areas infested by yellow starthistle, and plan ultimately to try to obtain field establishment in all areas with serious infestations of the weed. All lines of evidence indicate a high degree of host specificity to E. villosus. Accordingly, there is no need for restriction of releases inside field cages, in remote test sites, or to sites with a hostile climate.

E. Site-Specific Description

Potential release sites include any areas with a serious infestation of the weed yellow starthistle. The most serious infestations of the weed are in the western states of California, Idaho, Oregon and Washington (Maddox et al., 1985; Maddox and Mayfield, 1985; Roché et al., 1986; Roché and Roché, 1988; Callihan et al., 1989). Specific initial field release sites will be determined later. The purpose of this proposal is to ultimately obtain field establishment of E. villosus at all sites of serious infestations of the weed in order to effect biological control of the weed. This would occur through human-mediated redistributions as well as through dispersal of the winged adults from established field populations.

VI. Affected Environment and Envirnmental Consequences

A. General. As the result of extensive literature searches and the results of the biological studies and host specificity testing, no adverse or negative affect on the environment is anticipated.

B. Physical Environment

1. Air. There were no published or non-published records located of this species or any member of the genus contaminating any air or

altering the air quality in any way.
2. Water. There were no published or non-published records located of this species or any member of the genus contaminating water or altering any water quality in any way.
3. Land. There were no published or non-published records located of this species or any member of the genus contaminating land or negatively altering any land quality. It should be noted on the other hand, that this species should complement other natural enemies in reducing the population of yellow starthistle.

C. Human Health Risks. There is no health risk anticipated from the release of this weevil. No record has ever been made of any detrimental contamination, either in the literature or laboratory records.

D. Ecological Relationships.
1. Wildlife. This species is of no threat to wildlife.
2. Endangered and Threatened Species. Host specificity testing indicates that there will be no threat to endangered or threatened species of plants.
3. Non-target Invertebrates. Because of its size and life history and habit information, this species is of no threat to invertebrates.
4. Other Biocontrol Agents. Because of its size, location of larval feeding, and its delicate nature, this species is of no threat.
5. Pollinators. This species is of no threat to pollinators.

E. Cumulative Impacts.
If E. villosus and other insects (Bangasternus orientalis - Coleoptera: Curculionidae; Urophora sirunaseva - Diptera: Tephritidae; Chaetorellia australis - Diptera: Tephritidae) for which field release permits were previously issued are unsuccessful in biological control of yellow starthistle, the weed will continue to increase, and there will be a corresponding increase in the undesiderable impacts of the weed: competitive displacement of native plants in grassland and woodland habitats, reduced substainable productivity of rangelands and pastures, reduced profitability on grainfields and other croplands, higher levels of poisoning (encephalomalacia = "chewing disease") in horses,higher levels of applications of herbicides such as picloram into the environment for chemical control of the weed (Roché et al., 1986, Callihan et al., 1989), and reductions in the quality of recreational lands (because of the weed's spiny capitula).

If E. villosus and other insects for wich field release permits were previously issued are successful in biological control of yellow starthistle, there could be many indirect beneficial economic and ecological impacts: improved environments for native plants due to reduced competition from yellow starthistle, increased sustainable productivity on rangelands and pastures, reduced levels of encephalomalacia in horses, reduction in applications of herbicides such as picloram that are used for chemical control of the weed, increased profitability of croplands infested by the weed, and enhancement of recreational lands.

VII. Mitigative Measures.

Because biological control of yellow starthistle is environmentally and economically beneficial, and because of the high degree of host specificity of E. villosus to yellow starthistle, there are no planned mitigative measures. In the event of some unknown problem that arises shortly after field release and makes necessary to destroy the initial field colonization populations, this could be done by using pyrethroid or carbamate insecticides on the adults, and a systemic insecticide such as aldicarb or mechanical harvesting of yellow starthistle capitula for the larvae (which feed inside the capitula of the weed).

VIII. Conclusions

The results of laboratory studies showed a high level of larval host specificity of E. villosus to C. solstitialis. These studies also confirmed and completed the results of previous host specificity testing (Clement et al., 1988). In nature, C. solstitialis is the only known host of E. villosus. In the laboratory, under no-choice conditions, oviposition was restricted to some species within the genus Centaurea of those tested, with much higher amounts of oviposition on C. solstitialis. There was no oviposition on the Centaurea species native to North America, C. americana and C. rothrockii. In the laboratory, under choice conditions, oviposition occurred only on yellow starthistle. Sobhian and Zwölfer (1985), and Clement and Mimmocchi (unpublished data)[3/] reported larval development on safflower, following artificial larval transfer. However, oviposition never occurred on this species, and all tests, both in the laboratory and in the field, clearly demonstrated that safflower is not a host of E. villosus. The oviposition information is critical for determining the specificity and value of this insect as a biological control agent, since the adult is the only stage capable of dispersal from one plant to another. Also, E. villosus adults showed a low level of attraction for safflower, with reduced activity and feeding on this plant under laboratory conditions, even though it is known that under such conditions the feeding of a monophagous or oligophagous species could be broader than in the field (Force, 1966). Adult feeding on safflower should not be a problem because it is minimal, and safflower buds are well protected by thick, tough cauline leaves and involucral bracts. Because of the combined effects of adult and larval feeding, E. villosus appears to be a very promising candidate agent for biological control of yellow starthistle.

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XII. Appendices

A. List of Release Areas

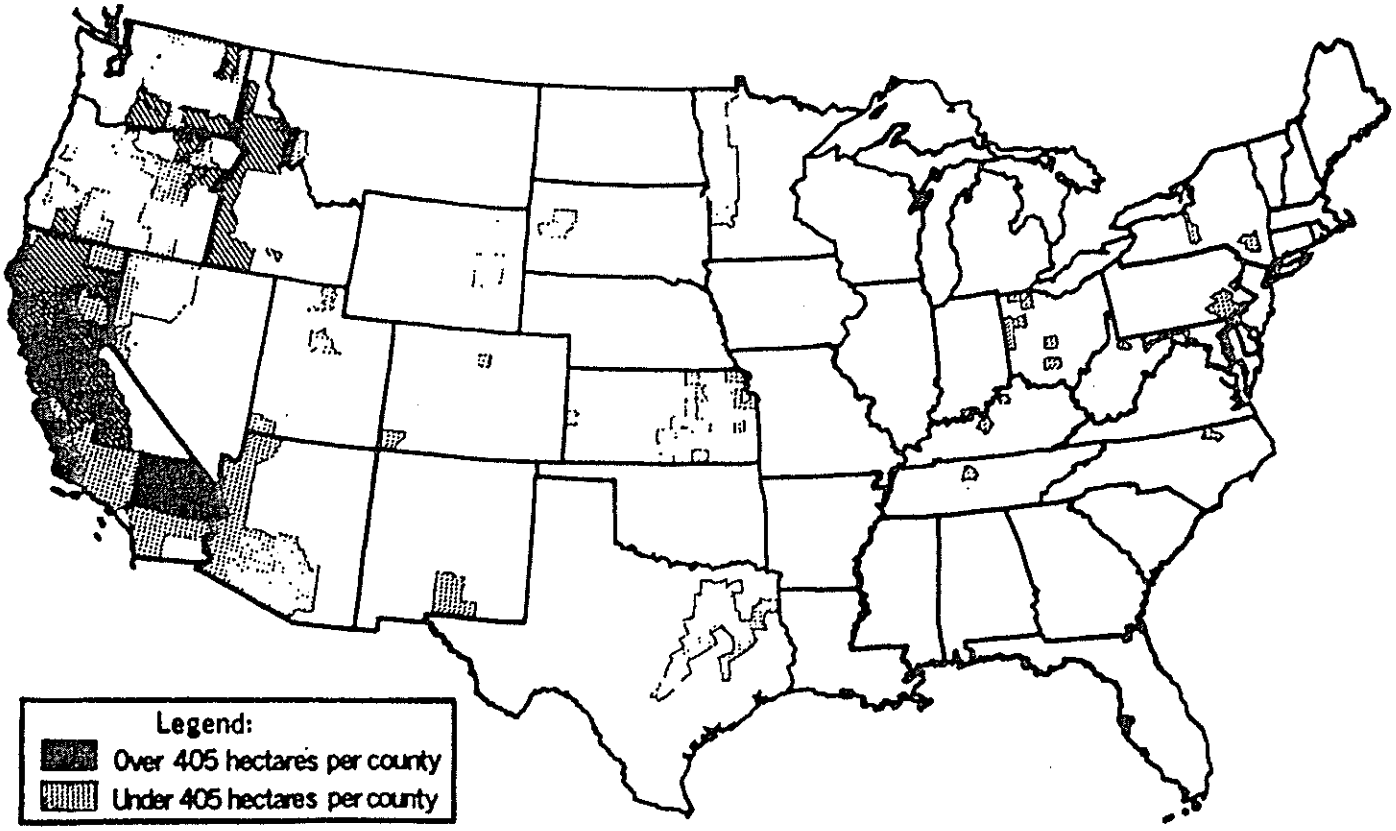
Field releases are planned in areas with severe infestations of yellow starthistle in the states of California, Idaho, Oregon, and Washigton (see map in B below). Specific initial release sites will be determined later.

B. Map

This map (ex Maddox et al., 1985) shows areas infested by yellow starthistle. There are no known sensitive areas.

C. PPQ Form 526

PPQ Form 526 has previously been submitted through the states of California, Idaho, Oregon, and Washigton.



1982 distribution of yellow starthistle (*Centaurea solstitialis*) in the United States.

APPENDIX B

Table 1. Synopsis of host specificity screening of Eustenopus villosus adults allowed contact with only one plant species, June-July, 1985-1986, Rome, Italy (modified, from Clement et al., 1988)

Plant Species	Test no.	Total No. Plants	Total No. Closed and flowering buds	Total No. Beetles[1/]	Amount of bud feeding[2/]	No. found in buds: Eggs	No. found in buds: Larvae	No. days beetles confined to plants	Beetle mortality (%) during test
Centaurea solstitialis Greece	1 & 2	12	105	36(18)	+++	54	14	6-10	16.67
Centaurea solstitialis Washington, U.S.A.	1	3	18	6(3)	+++	3	4	10-11	33.3
Centaurea nicaeensis	1 & 2	8	73	26(13)	++	12	12	4-10	11.54
Centaurea diffusa	1	5	203	10(5)	+++	0	2(dead)	7-8	60.0
Centaurea americana	2	5	23	20(10)	+++	0	0	9-10	0
Carthamus tinctorius var. Hartman	1 & 2	15	72	56(28)	++	0	0	3-10	76.79
Carthamus lanatus	1	5	57	19(8)	+	0	0	4-7	100.0
Carthamus dentatus	1	5	43	22(12)	-	0	0	4-6	100.0
Cirsium arvense	1	5	77	10(5)	++	0	0	4-8	60.0
Cirsium undulatum	1	2	15	12(6)	++	0	0	4	100.0
Cirsium douglasii	1	1	7	6(3)	+++	0	0	5	16.7
Cynara scolymus	1	1	1	9(4)	+	0	0	5	100.0
Cnicus benedictus	1	5	21	10(5)	+++	0	0	8	80.0
Helianthus annuus	1	3	10	6(3)	-	0	0	4-7	100.0
Scolymus hispanicus	2	5	56	20(10)	+	0	0	5-6	100.0
Lactuca sativa	1	3	141	6(3)	+	0	0	5	100.0

1/ Numbers of females in parentheses.
2/ Legend: (-) = no feeding or very slight nibbling; (+) = light to moderate feeding; (++) = moderate to heavy feeding; (+++) = heavy feeding (see text for more details).

ible 2. Eustenopus villosus adult feeding, oviposition, and longevity no-choice test in Rome, Italy, 1987

Plant Species	No. plants	Total no. eggs laid	Total no. feeding scars	Feeding* damage rating	Total no. exposed buds	Total no. buds with adult feeding scars (No.- %)	Longevity (days) X ± SD	Bud diameter range (mm)
entaurea solstitialis	5	107	440	3	304	267-87.8	21.8 ± 8.54	7 - 9
from Greece entaurea scabiosa	5	18	69	0 - 2	98	50-51.0	13.3 ± 6.54	10-13
entaurea paniculata	5	0	110	2	164	94-57.3	12.1 ± 4.93	5 - 7
entaurea maculosa	5	8	177	2	437	166-38.0	17.2 ±12.70	7 - 9
entaurea calcitrapa	5	0	54	2	138	49-35.5	8.7 ± 3.11	6 - 8
entaurea napifolia	5	5	37	2	119	30-25.2	10.8 ± 4.35	5 - 8
Centaurea alba	5	0	69	2	116	57-49.1	11.8 ± 5.98	8 -12
Centaurea jacea	5	3	46	0 - 2	99	39-39.4	9.2 ± 4.08	9 -11
Centaurea cyanus	5	0	64	2	120	50-41.7	8.1 ± 2.63	4 - 6
Carthamus tinctorius	5	0	32	1	36	21-58.3	14.6 ± 4.52	15-30
Carduus pycnocephalus	5	0	16	0 - 1	43	10-23.3	7	4 - 6
Cynara scolymus	5	0	0	0	5	0	7	60-80
Zinnia elegans	5	0	4	0 - 1	27	4-14.8	7.9 ± 2.39	10-12
Aster principessa	5	0	0	0	43	0	7	15-30
Calendula officinalis	5	0	0	0	33	0	7	12-17
Achillea millefolium	5	0	30	0 - 1	ca. 2,000 (27 corymbs)	19-0.95	8.3 ± 2.83 (20-50 corymbs)	2-2.5
Tagetes erecta	5	0	0	0	28	0	7	9 -12
Gazania splendens	5	0	12	0 - 1	21	8-38.1	7	14-17
Silene vulgaris	5	0	0	0	63	0	7	3 - 6

*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.

e 3. Eustenopus villosus adult feeding, oviposition, and longevity no-choice test in Rome , Italy, 1988

T SPECIES		No. plants	Total no.eggs laid	Total no. feeding scars	Feeding damage rating	Total no. exposed buds	Total no. buds with adult feeding scars (no.- %)	Longevity (days) X ± S.D.	Bud diameter range (mm
aurea solstitialis,	Greece	10	90	430	3	330	261-79.0	19.9±4.6	7-9
"	California	10	35	356	3	297	251-84.5	16.6±4.5	7-9
"	Washington	10	24	284	2-3	284	203-71.5	16.0±3.8	7-9
aurea cineraria		10	0	104	2-3	52	46-28.4	16.2±4.7	10-15
aurea americana		10	0	132	3	34	31-91.1	14.6±5.2	16-20
ra scolymus		10	0	0	0	11	0	7	60-80
ina corymbosa		10	0	5	0-1	94	3-3.2	7	25-40
anthus annuus		10	0	0	0	11	0	7	120-300
cio vulgaris		10	0	0	0	115	0	7	5-6
uca sativa		10	0	0	0	638	0	7	2-3
rrhinum majus		10	0	3	0	44	1-2.2	7	1-2

.ble 4. Results of plant feeding and oviposition two-choice test conducted with adults of Eustenopus villosus, laboratory study, June 29-July 7, 1986, Rome, Italy (modified, from Clement et al., 1988)

·st plant combinations 1/	Degree of feeding on 2/ C. solstitialis Greece	Test plant	No. eggs and larvae found on C. solstitialis	Test plant
Carthamus tinctorius	+++	+	35 eggs; 5 larvae	0
Cirsium arvense	+++	-	31 eggs; 4 larvae	0
Cichorium intybus	+++	-	21 eggs; 3 larvae	0
Centaurea nicaeensis	+++	+	29 eggs; 5 larvae	10 eggs

There were 5 replications per test plant combination. One replicate consisted of 2 pairs of beetles (2° °;2 o o) in an organdy sleeve cage placed over branches from potted plants (one branch of yellow starthistle and one from a test plant species).

Legend: (-) = no feeding or very slight nibbling; (+) = light to moderate feeding; (++) = moderate to heavy feeding; (+++) = heavy feeding (see text for more details).

Table 5. Eustenopus villosus adult feeding and oviposition two choice test in Rome, Italy, 1987

Plant Species	Total no. eggs laid		Total no. feeding punctures		Feeding damage rating 3/		Total no. exposed buds		Total no. attacked buds	
	YST1/	TP2/	YST	TP	YST	TP	YST	TP	YST (No.- %)	TP (No.- %)
Cynara scolymus	6	0	33	0	3	0	47	5	27-57.4	0
Zinnia elegans	16	0	95	4	3	0-1	149	27	75-50.3	3-11.1
Calendula officinalis	10	0	193	0	3	0	120	44	92-76.6	0

1/ YST = Yellow starthistle from Greece

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.

Table 6. Eustenopus villosus adult feeding and oviposition choice test in Rome, Italy, 1988

Plant species	Total no. eggs laid		Total no. feeding scars		Feeding damage rating		Total no. exposed buds		Total no. attacked buds	
	YST	TP	YST	TP	YST	TP	YST	TP	YST (No.- %)	TP (no.- %)
Centaurea americana	32	0	226	19	3	1-2	242	23	169-69.8	8-34.7
Cynara scolymus	51	0	276	0	3	0	195	11	172-88.2	0

le 7. Number of Eustenopus villosus that emerged from seedheads of host and non-host plants grown together in a field plot, Thermi, Greece, 1985

Number of adults emerging from					
taurea stitialis, Greece	Centaurea solstitialis, Idaho	Centaurea solstitialis, Calif.	Carthamus 1/ tinctorius	Cynara 1/ scolymus	Cirsium 1/ creticum
84	140	79	0	0	0

No eggs were laid in heads of these three non-host species.

Table 8. Feeding by Eustenopus villosus adults in no-choice cage tests in Albany, California, 1988

Test plant species	No. Test plants and weevil adult pairs	No. flowerhead buds[1/]	No. (%) flowerhead buds with feeding scars	Total no. feeding scars	No. feeding scars per flowerhead bud
Centaurea solstitialis	15	503	131 (26.0 %)	166	0.330
Centaurea rothrockii	10	85	17 (20.0 %)	19	0.223
Carthamus tinctorius "4440"	15	142	13 (9.2 %)	16	0.112
Carthamus tinctorius "S541"	15	83	3 (3.6 %)	4	0.048
Cirsium douglasii	10	593	44 (7.4 %)	66	0.111
Helianthus annuus	10	71	0	0	0

1/ Includes all stages (Bu 1 through Bu 4) of closed flowerhead buds.

Table 9. Oviposition by Eustenopus villosus adults in no-choice cage tests in Albany, California, 1988

Test plant species	No. test plants and weevil adult pairs	No. flowerhead buds[1/]	No. (%) flowerhead buds with oviposition holes	Total no. oviposition holes	No. oviposition holes per flowerhead bud
Centaurea solstitialis	15	128	23 (17.9 %)	30	0.234
Centaurea rothrockii	10	46	0	0	0
Carthamus tinctorius "4440"	15	63	0	0	0
Carthamus tinctorius "S541"	15	68	0	0	0
Cirsium douglasii	10	131	0	0	0
Helianthus annuus	10	70	0	0	0

1/ Includes later stages (Bu 3 through Bu 4) of closed flowerhead buds.

Table 10. Development of Eustenopus villosus in cage tests in Albany, California, 1988

Host plant species	No. flowerhead buds with oviposition holes	Total no. oviposition holes	No. immatures in flowerheads with oviposition holes at time of dissections			Total no. emerged adult weevils
			Living Larvae	Dead Larvae	Living Pupae	
Centaurea solstitialis	23	30	4	7	5	3

Table 11. Feeding and oviposition by Eustenopus villosus adults in no-choice carton tests in Albany, California, 1988

Test Plant Species	No. Flowerhead Buds	No. (%) Flowerhead Buds With Feeding Scars	No. (%) Flowerhead Buds With Oviposition Holes
Centaurea solstitialis	33	32 (96.9 %)	17 (51.5 %)
Carthamus tinctorius "4440"	56	10 (17.8 %)	0

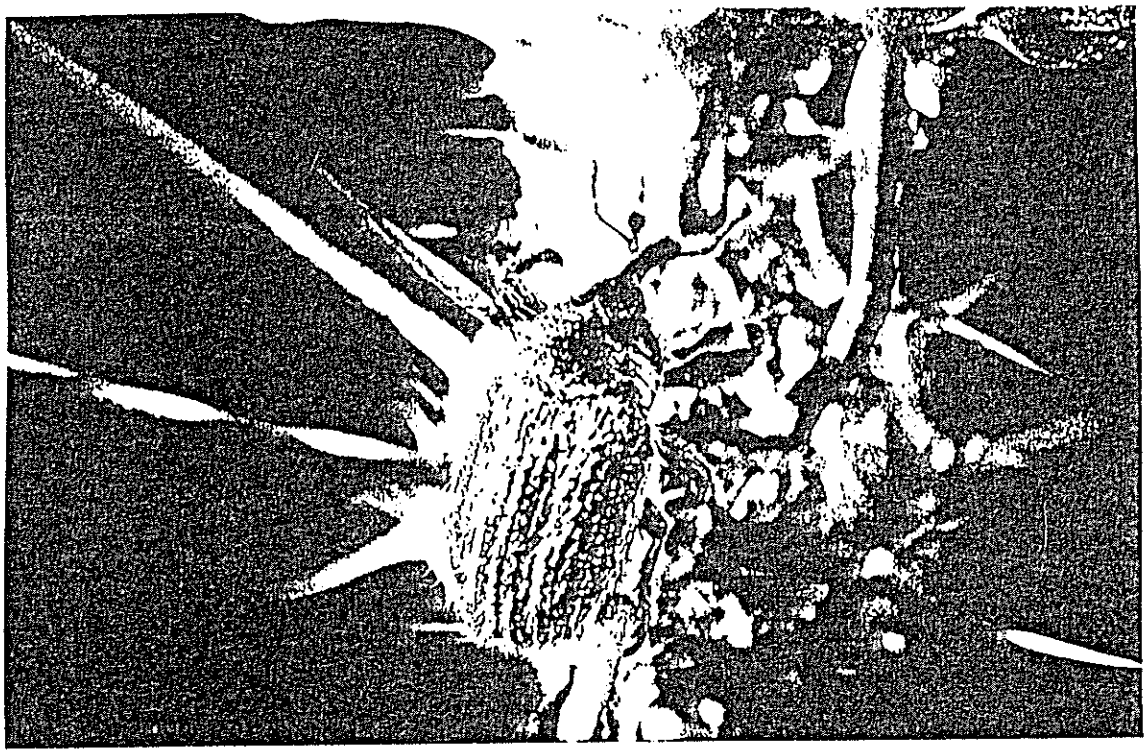


Fig. 1 - Eustenopus villosus adult on yellow starthistle flowerhead.

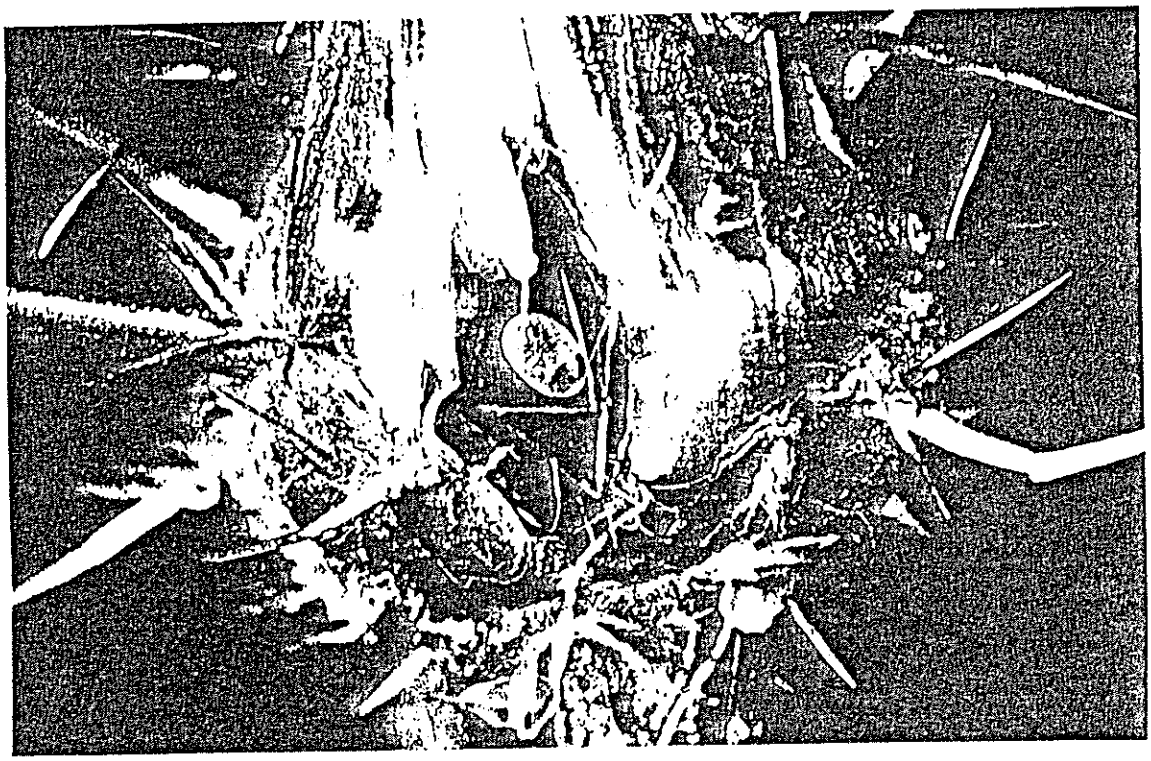


Fig. 2 - Eustenopus villosus egg in a dissected yellow starthistle bud. It is possible to see the larva under the chorion.

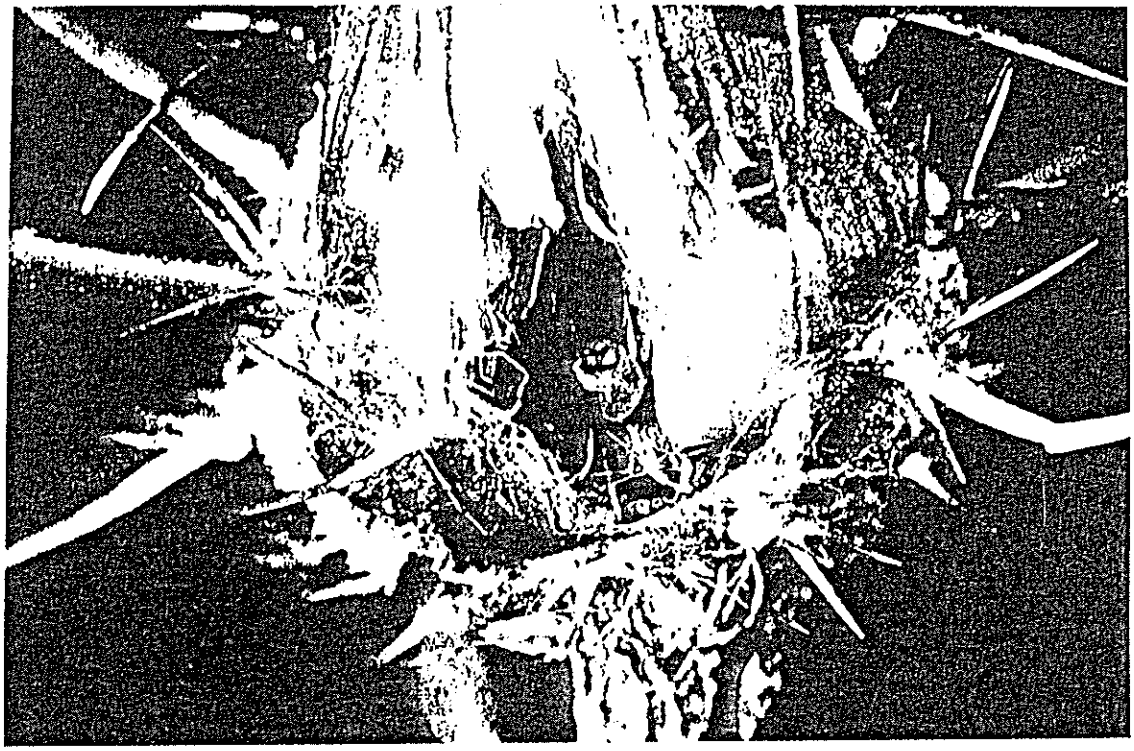


Fig. 3 - Eustenopus villosus first instar larva in a dissected yellow starthistle bud.

Fig. 4 - Test plant in a black nylon tulle sleeve cage.