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Insect-plant relationships and behavioral observations of the stem-feeding beetle *Thamnurgus euphorbiae* Küster (Coleoptera: Scolytidae), a new biocontrol agent from Italy to control leafy spurge in the U.S.

M. CRISTOFARO ¹, F. LECCE ¹, G. CAMPOBASSO ², G. TERRAGITTI ², N. R. SPENCER ³, and K. D. MANN ³

¹ ENEA, C.R. Casaccia, INN BIOAG ECO, Rome, Italy. ² USDA-ARS EBCL, Rome, Italy. ³ USDA-ARS, Sidney, Montana 59270, USA

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M. CRISTOFARO¹, F. LECCE¹, G. CAMPOBASSO², G. TERRAGITTI²,
N. R. SPENCER³, and K. D. MANN³

¹ENEA, C.R. Casaccia, INN BIOAG ECO, Rome, Italy

²USDA-ARS EBCL, Rome, Italy

³USDA-ARS, Sidney, Montana 59270, USA

Abstract

Thamnurgus euphorbiae Küster (Coleoptera: Scolytidae) adults are found in early spring on flowering plants of *Euphorbia characias* in Italy. The hypothesis was that *T. euphorbiae* beetles recognize the host plant at the correct phenological stage by its specific odor. Behavioral bioassays were conducted under choice and no-choice conditions to verify the capability of the insect to detect the target weed leafy spurge (*E. esula*). A wind tunnel and two-way-olfactometer were used in no-choice bioassays to expose the insect to the odor of the host plant at different phenological stages. The results did not show any response of the beetle to the host plant volatile compounds. In choice tests, the beetle showed a preference for bud stage stems vs. flowering stems with the top covered by dark cotton net. This result can be explained only by a visual attraction of the insect to the shape of the stems, since green leaves covered the 20cm bud stage stems, while the flowering stems were almost leafless.

Keywords: Biological control, scolytid beetle, *Euphorbia esula*, host selection.

Introduction

Thamnurgus euphorbiae Küster (Coleoptera: Scolytidae) (Balachovsky, 1972; Wood, 1986), a univoltine stem feeder recorded from *Euphorbia characias* L. in Italy, was selected as a candidate for biological control of leafy spurge, *Euphorbia esula-virgata* complex in North America. Its potential host range was studied at the USDA-ARS-EBCL Rome laboratory from 1992 to 1998. Of 40 plant species or varieties in 13 families tested, the beetle oviposited on and completed its life cycle only on plants in the genus *Euphorbia*, subgenus except for 3 species, *E. stepposa*, *E. incisa*, and *E. robusta*, outside of the subgenus.

One of the key-questions in the evaluation of a new biocontrol candidate agent is to understand its mechanisms to recognize the target weed in order to synchronize the release of the insect to the suitable phenological stage of the plant. The aim of this work was to identify the system used by the newly emerged beetle to recognize the natural host plant *E. characias*, and the target weed *E. esula*. The hypothesis was that *T. euphorbiae* beetles recognize the host plant at the correct phenological stage by its specific odor (Mustaparta, H., 1979). Behavioral bioassays were conducted under choice and no-choice conditions to

verify the capability of the insect to detect the target weed leafy spurge in the release and establishment processes in North America (Dunn, 1979; Noble *et al.*, 1979).

Materials and Methods

Both unmated and mated individuals were used in the tests because the behavioral response to a specific odor is often related to the physiological stage of the insect. Since there is not sexual dimorphism in the adults, the behavioral tests were carried out using more than one individual (from 4 to 8) for the unmated beetles, and with single mated females recognized during the copula.

No-Choice Tests

A wind tunnel and two-way-olfactometer were used in no-choice bioassays to expose the insect to the odor of the host plant at different phenological stages (Payne *et al.*, 1976).

Wind tunnel. Adults emerged from dry cut stems of *E. characias* collected during April on M. Soratte (30 km North of Rome). In the wind tunnel, a transparent Plexiglas cylinder (1m long, 30cm diameter, with the two extremity holes covered by close organdy to permit ventilation), the insects were located on the center by a 1 cm diameter hole. The odor source (*E. characias*) was put at the end of the tube (fig. 1), covered by a dark container to avoid the visual perception. On the opposite part, a similar container was located, without any plants or odor. Air was periodically sucked from the central hole by a pump to have a laminar flow. The response was determined recording the position of the insect(s) every 5 minutes for 30 minutes. Six replications were made with unmated individuals, and 10 with mated females.

Olfactometer. Mated 5-day-old females were tested singularly by using a two-way olfactometer. The instrument had a typical y shape, with one of the two branches connected with the odor source (*E. characias*), while the insect was located on the opposite branch. The flow was sucked from the insect branch by an air pump (fig. 2). The response was determined recording the position of the insect every 5 minutes for 20 minutes. Ten replications were carried out with mated females.

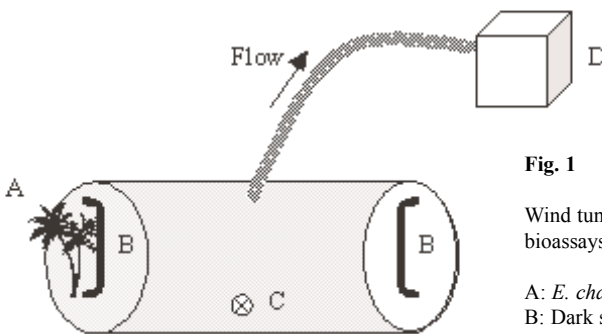
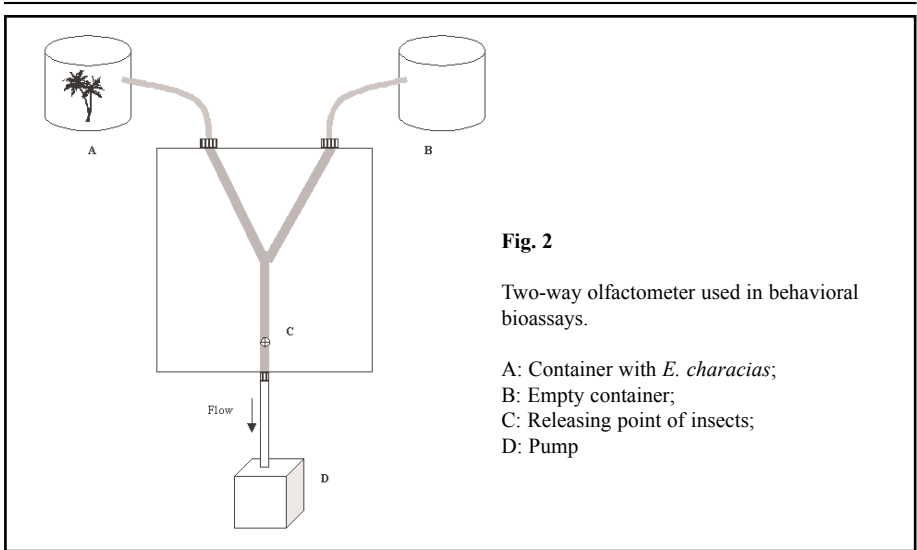


Fig. 1

Wind tunnel used in olfactometry bioassays.

- A: *E. characias*;
- B: Dark screens;
- C: Releasing point of insects;
- D: Pump

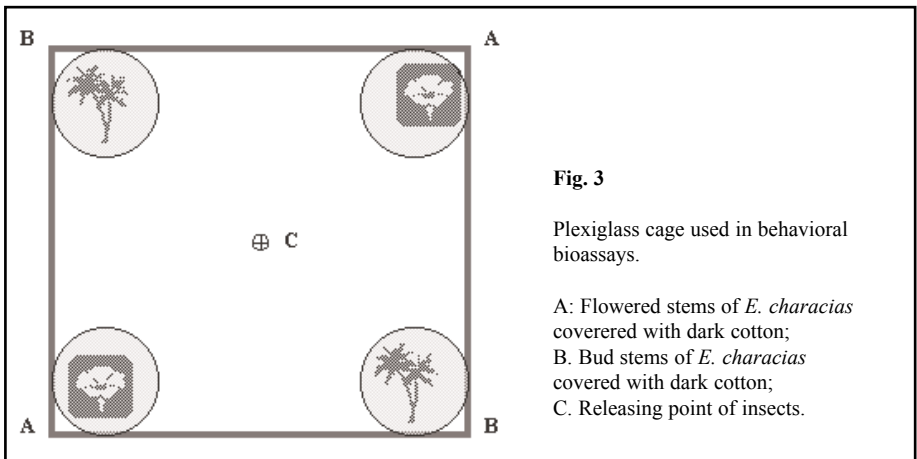


Choice Tests

Four bioassays were carried out in 40x40 cm Plexiglas cages, exposing six newly emerged females to the host plant, the target weed, and synthetic spurges at different phenological stages under choice conditions.

E. characias in different stages. In the first choice test, four 20 cm cut stems of *E. characias* (two in bud stage and two in flower) were exposed to the beetle females. The four tips of the stems were covered by a dark cotton net, to hide the phenological stage but to let the passing of odors (fig 3). The number of holes/stem was recorded after 3 days. Twenty replications were made.

E. characias vs. synthetic plant. Two 20cm cut stems of *E. characias* in flower and two synthetic *E. characias* stems were exposed to six adult females. *Euphorbia characias* tips were covered by dark cotton net, to hide the phenological stage but to let the passing



of the odors, while the synthetic stems were left uncovered. Entomological glue was applied on the bottom of the 4 exposed stems. The number of trapped beetles was recorded after 3 days. Ten replications were made.

Synthetic plants in different stages. Two 20cm synthetic stems of *E. characias* in flower and two synthetic leafless stems were exposed to six adult females. Entomological glue was applied on the bottom of the 4 exposed stems. The number of trapped beetles was recorded after 3 days. Ten replications were made.

E. characias vs. *E. esula*. One 20cm cut stem of *E. characias* in flower and one *E. esula* stem in flower were exposed to six adult females. *Euphorbia characias* tip was covered by dark cotton net, to hide the phenological stage but to let the passing of the odors, while leafy spurge stem was left uncovered. The number of holes/stem was recorded after 3 days. Ten replications were made.

Results

No-Choice Tests

All the tests showed a no-preference of the insects (unmated and mated) towards the odor of the target plant. The adults were always very active, moving and visiting very often all the sites of the arena (for the wind tunnel), and with a non significant difference in the choice of the two olfactometer branches (Table 1).

Choice Tests

In the first choice test, *T. euphorbiae* showed a significant preference toward the bud stage stems. In fact, the beetles penetrated the upper part of the stems in the bud stage in all the replications while no damage occurred on the flowering stems. This unexpected result can be explained only by a visual attraction of the insect to the shape of the stems, since green leaves covered the 20cm bud stage stems, while the flowering stems were almost leafless (during the flowering period the stem ears, keeping the leaves about 40 cm down). A similar response was already observed in other bark beetle species (Lanier, 1983).

Table 1.
Total number of beetles recorded in the wind-tunnel and the olfactometer
(the neutral zone in the olfactometer is the branch with the point of release).

Time (min.)	WIND TUNNEL				OLFACTOMETER		
	Unmated		Mated		Mated		
	Plant	Empty	Plant	Empty	Neutral	Plant	Empty
5	19	17	4	6	8	2	0
10	18	18	5	5	7	2	1
15	18	18	5	5	5	3	2
20	17	19	6	4	2	3	5
25	18	18	5	5	1	4	5
30	19	17	4	6	0	5	5

The second and the third choice-tests confirm the mechanism of attraction phytophagous-host plant for the complex *Thamnurgus-Euphorbia*. In fact, in the second choice test the beetles preferred the plastic flowering stems, which confirmed the importance of visual selection for finding the target host. Obviously, the beetles confirmed to prefer the flowering plants to the leafless stems, when we offered only synthetic plants. The fourth tests gave not reliable results, because it was carried out to late in the season, using insects already feeding into the stems of *E. characias*.

These results must be considered very useful for the detection of the correct timing of release of *Thamnurgus* for the biological control of the related species *E. virgata* in USA.

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