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Effects of plant chemical extracts and physical characteristics of *Apium graveolens* and *Chenopodium murale* on host choice by *Spodoptera exigua* larvae

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Abstract

Choice tests with whole plants and leaf discs indicated that fourth instar *Spodoptera exigua* (Hübner) (Noctuidae: Amphypyrini) were found more frequently and ate significantly more of the weed *Chenopodium murale* than the associated crop plant *Apium graveolens*. In order to explain the preference, plant extracts, plant volatiles, soluble protein concentrations, water contents, and leaf toughness of the two plants were investigated. Bioassays of aqueous methanol (90%) and hexane extracts of leaves on cellulose discs indicated that neither attractants in *C. murale* nor repellents in *A. graveolens* could account for the observed preference. No significant difference could be found between the effects of plant volatiles from *C. murale*, *A. graveolens* and a control on larval dispersal by *S. exigua*. Selective feeding for higher levels of proteins also was not a factor, because *A. graveolens* had nearly twice the soluble protein of *C. murale*. Water content was approximately 6% higher (by weight) in *C. murale* than *A. graveolens* but most polyphagous larvae do not typically show compensatory feeding for water alone. However, the potentially related characteristic of leaf toughness was significantly different, with *A. graveolens* exhibiting 1.53 times the toughness of *C. murale*. Studies comparing five types of larval behavior on both plant species showed that the time spent in swallowing behavior was significantly greater on the tougher *A. graveolens* leaves relative to *C. murale*. To test the hypothesis that leaf toughness was affecting larval host choice, both plants were finely ground and incorporated into agar blocks. No differences in feeding behavior were detected. The implications of leaf toughness for larval diet and host choice are discussed.

Introduction

The beet armyworm, *Spodoptera exigua* (Hübner), is a polyphagous pest attacking a variety of agricultural crops including celery (*Apium graveolens* L.) and tomatoes (*Lycopersicon esculentum* Mill.) (Van Steenwyk & Toscano, 1981; Lange & Bronson, 1981; Trumble *et al.*, 1990) as well as a wide variety of non-agricultural species (Griswold & Trumble, 1982). Most of the efforts to control this insect have focused on strategies such as host plant resistance (Diawara *et al.*, 1992), chemical control (Brewer *et al.*, 1990), mating disruption (Wakamura *et al.*, 1989) and integrated pest management programs (Trumble, 1990). Behavior, particularly larval host selection behavior, has only rarely been considered in the development of control strategies (Weiss *et al.*, 1987).

The lack of attention to larval host selection is due principally to the concept that evolution of host choice operates largely through female oviposition behavior (Chew & Robins, 1989; Courtney & Kibota, 1990). Although this paradigm holds for lepidopterous insects that lack substantial mobility in the larval stage, host selection behavior could reasonably be expected to evolve on those larvae which are mobile. In general, between-plant foraging behavior by larvae is dependent on mobility; larger individuals tend to move faster and further (Reavey, 1993). Smits *et al.* (1987) reported that *S. exigua* larvae, dispersing from a single egg batch of 35 eggs, damaged 140 chrysanthemum (*Chrysanthemum morifolium* Ramatuelle) plants during their

development. Thus, for this insect species, the ability to disperse several meters per day gives the larvae the potential for substantial input into host selection.

Several plant characteristics have been shown to be important factors in host acceptance by lepidopterous larvae. Such characteristics include plant phenology (Scriber & Slansky, 1981), nitrogen content (White, 1984), water content (Scriber & Slansky, 1981), defensive chemicals (Ehrlich & Raven, 1964), and physical characteristics of the plant (Roden *et al.*, 1992). For example, leaf toughness has been shown to reduce the suitability of plant materials for herbivores in several ways including increased time and energy expenditure (Rausher, 1981) and dilution of nutritious plant parts with non-nutrient forms such as lignin and cellulose (Coley, 1983). Raupp (1985) reported that the leaf beetle *Plagiodera versicolora* Laich suffered increased mandibular wear, reduced developmental rate, and an increase in generation time on plants with tough leaves. Although these and similar studies have shown the effect of leaf toughness on herbivore development, the impact of leaf toughness on larval behavior has not, to our knowledge, been reported. However, the existence of any single mechanism that solely governs host selection and acceptance is not likely. Instead, particular systems are often influenced to a greater or lesser degree by one or several of these and other characteristics acting alone or in combination (Mauricio & Bowers, 1990; Ruehlmann *et al.*, 1988).

Preliminary observations of *S. exigua* larvae in *A. graveolens* fields suggested that they prefer to feed on *Chenopodium murale* L. Nettleleaf goose foot (J.T.T., unpubl.). *A. graveolens* L. (celery) is a member of the family Apiaceae. As with other members of this family, *A. graveolens* is an aromatic plant containing terpenoids and phenylpropanoids (Hegnauer, 1971). Some of the common chemical components of the Apiaceae include substituted coumarins which are more diverse and abundant in this taxon than in any other plant family (Berenbaum, 1990). Other common constituents include triterpenoid sapogenins, triterpenoid saponin, cyclitols, scyllitol, mannitol and umbelliferose (Hegnauer, 1971).

C. murale is a member of the family Chenopodiaceae. The genus *Chenopodium* is the principal genus in this family and comprises about 250 spp. Although information on the chemical composition of the genus *Chenopodium* is scarce, several members are reported to possess medicinal properties. *C. murale*, in particular, is known to possess anthelminthic activity (Verma & Agarwal, 1985). Reports on the chemical constituents of *Chenopodium* indicate the presence of flavonoids (Verma & Agarwal, 1985), flavonol glycosides (De Simone *et al.*, 1990; Jain *et al.*, 1990), phenolic amides (Horio *et al.*, 1993), coumarins (Verma & Agarwal, 1985), alkaloids (Verma & Agarwal, 1985), phytoecdysteroids (Dinan, 1992) and saponins (Risi & Galwey, 1984).

Our first objective in this study was to determine if the initial observed preference were repeatable and statistically significant under controlled conditions in the laboratory. If the preference proved significant, our second objective was to document the relative role(s) of chemical composition, soluble protein, leaf water content, and leaf toughness of *A. graveolens* and *C. murale* on host plant selection by *S. exigua* larvae.

Materials and methods

Insects. All experiments were conducted with fourth instar larvae (24 h after molt) obtained from a laboratory colony maintained on artificial diet (Shorey & Hale, 1965) at 28 ± 2 °C and L14:D10 photoperiod. The colony was originally collected from Orange Co., CA and had new genetic material added within 6 months prior to the study.

Plants. The commercial *A. graveolens* cultivar 'Tall Utah' was sown on August 20, 1993 and transplanted into 12.7 × 15.24 cm pots on 5 October 1993 in UC soil mix (Matkin & Chandler, 1957). Ten plants were placed outside the greenhouse on 20 October 1993, one month before the bioassay. Another commercial *A. graveolens* cultivar, 'Conquistador', was transplanted into field plots at the University of California's South Coast Research and Extension Center on 19 August 1993. Ten plants were transferred from the field into 15.24 × 17.78 cm pots on 30 November 1993. *C. murale* plants were transplanted from the field into 12.7 × 15.24 cm round pots and placed outside the greenhouse on 12 November 1993.

Fresh plant material bioassay. A test arena was built inside a greenhouse (15–23 °C) and consisted of a sleeve cage (96 cm long × 50 cm wide × 42 cm in height) placed on a soil-filled arena (1 × 0.62 m, UC Mix [Matkin & Chandler, 1957]). Four potted plants, two of *A. graveolens* (Tall Utah) and two of *C. murale*, were placed in the soil in a rectangular array (0.26 × 0.44 m) with plants of the same type in opposite corners. All plants were selected for approxi-

mately the same size and shape to minimize effects of variable leaf area to the extent possible. Soil and a top layer of sand were used to cover the pots completely in order to expose only the aerial part of the plants.

Ten larvae (starved for approximately 20 h) were placed in the center of the arena and a total of twelve observations on the position of each larva was made. The first eleven observations were on an hourly basis and the last observation was made 24 h after the first. Due to the possibility of lack of independence between one observation to the next, the experiment had a repeated measurements design with observations as the within subjects component and treatments as the between subjects component. This test was replicated four times on 23, 29 November, 14 and 15 December 1993.

Field grown plants also were used for whole leaf and leaf disc bioassays. Although selecting truly comparable leaves may be impossible, the leaves were standardized to the extent possible by selecting fully-expanded leaves of approximately the same size (± 15%), thickness and age class from the same location within the top third of the plant canopy in both plant species.

Disposable Petri dishes (150 × 25 mm) filled to a depth of 3 mm with 4% agar in order to minimize dehydration of the plant material (Diawara *et al.*, 1992) were used as test arenas. The initial area of the leaves was measured with a leaf area meter (LiCor 3000 Leaf Area Meter, LiCor Inc., Lincoln NE). Leaves from each test plant were placed opposite each other in each arena. One larva (starved for 20 h) was released in the center of the arena. Dishes then were held in an environmental chamber at 28 ± 2 °C and L14:D10 photoperiod. Twenty four hours later the final area of the leaves was measured with the leaf area meter. This test had 10 replicates.

In order to compare results from tests with different *A. graveolens* cultivars, a second test comparing 1.1 cm^2 leaf discs of 'Conquistador' and 'Tall Utah' was performed using the previously described disposable Petri dish arenas. Four discs (two per test plant) were arranged at the opposing cardinal points of the arena (leaf area = 2.20 cm^2 per treatment per dish). One larva, previously starved for 20 h, was placed in the center of the arena and the dish was covered. Dishes were held in the environmental conditions previously described. The experiment had 20 replicates. Each replicate was evaluated for leaf area eaten when approximately 50% of one of the treatments was consumed. Remaining leaf area was recorded with a leaf area meter.

Plant extract studies. Unless otherwise specified, all solvents used were Fisher Scientific Optima grade. Sixteen g of fresh leaves of each plant were cut immediately prior to extraction. The leaves were rinsed once in a 10% bleach solution and then four times in distilled water. The material then was allowed to dry for approximately one hour in the laboratory (approximately 21 ± 3 °C). Eight g of *C. murale* and *A. graveolens* var. Conquistador, respectively, were homogenized (Polytron® tissue homogenizer, Luzern, Switzerland) for 5 min with 40 ml of 90% aqueous methanol. The homogenized material was poured into 28 × 116 mm round bottom sample tubes, capped with aluminum foil and vortexed for 1.5 min. The resulting emulsions were centrifuged at 1500 rpm for 10 min. The upper solvent layer was then transferred by disposable Pasteur pipette into new sample tubes. If needed, the solvent (extract) was concentrated by rotary evaporator to a volume of 20 ml. Another set of leaf samples of 8 g from *C. murale* and *A. graveolens* var. Conquistador, respectively, were extracted under the same conditions with hexane.

Comparisons between pairs of fractions were made by applying a 50 µl volume of each fraction to a 1.32 cm^2 cellulose acetate disc (MSI® AcetatePlus Membranes, Westboro, Ma.) with a micropipette. The discs were left to dry under room conditions for about 4 h at which time two discs per fraction were placed in an agar-lined disposable Petri dish (150 × 25 mm) at the opposing cardinal points of the arena. A larva, starved for 20 h, was placed in the center of the Petri dish which was then covered with a lid. The dishes were placed inside an environmental chamber at 28 ± 2 °C, 75% RH and L14:D10 photoperiod. After 24 h the damaged surface area of each disc was measured with a reticle with 1 mm^2 subdivisions (10 mm^2 grid) and a dissecting microscope at 10 × magnification. In many cases the larvae only fed on the disc surface which contained the extract; hence percent damage (%D) was calculated with the formula %D = (damaged surface area per treatment/damaged surface area of both treatments) × 100. These tests were replicated 21 times.

Initially, a polar fraction (extracted with 90% methanol) was compared to a non-polar fraction (extracted with hexane) in order to identify the polarity of any potentially active component(s) in *C. murale* and *A. graveolens* (var. Conquistador). Comparisons were made by applying four *C. murale* leaf equiv-

alents (on a leaf area basis, i.e. 5.28 cm^2 leaf area to 1.32 cm^2 cellulose acetate discs) in hexane or methanol to the cellulose acetate discs as above. The same extract method was used in another pair-wise comparison between *A. graveolens* (var. Conquistador) and *C. murale* methanol extracts. Comparisons between discs containing the polar (90% methanol) and non-polar (hexane) fractions of *C. murale* and *A. graveolens* var. Conquistador were made by applying 2 leaf equivalents (25 μl of hexane extract + 25 μl of 90% methanol extract) of each plant per cellulose acetate disc.

To detect the possible presence of deterrents in *A. graveolens*, a fifth and sixth comparison were made with the polar and non-polar fractions of *A. graveolens* (25 μl of 90% methanol + 25 μl of hexane) versus 25 μl of *C. murale* 90% methanol extract and the polar and non-polar fractions of *A. graveolens* (25 μl of 90% methanol + 25 μl of hexane) versus the 90% methanol fraction of *A. graveolens* alone.

To determine whether plant volatiles had an effect on larval behavior, the following arena was constructed. A 2 cm diam. syntillation vial cap was placed upside-down in a filter paper-lined 150 × 25 mm plastic Petri dish. The cap contained either no leaf discs (control) or seven 0.95 cm^2 leaf discs of either *C. murale* or *A. graveolens*. A 150 mm fine brass screen (100 mm mesh) was placed resting on the cap so that direct contact with the plant material by a larva on the screen was prevented. One fourth instar larva was placed inside the arena and its position was recorded on the plastic disc placed on the lid with a marker every three minutes for one hour. The experiment was conducted in a dark room with photographic red light. At the end of the test, the distance traveled by the larva was measured with a ruler by connecting the position marks. This test had a randomized block design with sampling with three observation dates as the blocking factor and with 5 replicates for each of the treatments and the control.

Soluble protein, water content and leaf toughness measurements. Leaf samples (1 g) were taken from eight *C. murale* and nine *A. graveolens* plants and freeze dried. The material was then finely ground in a mortar, and 0.1 g samples were stored in polystyrene disposable test tubes (17 × 100 mm) in an ultracold freezer at −61 °C until used. The samples subsequently were extracted with 0.1 N NaOH, homogenized in a Virtis homogenizer (Gardiner, N.Y.), centrifuged and the protein content of the supernatant was estimated using a Bovine Serum Albumin (BSA) standard according to the methods of Jones *et al.* (1989).

Fifteen leaves from five *A. graveolens* var. Conquistador and *C. murale* plants were cut and immediately weighed. The area of each leaf then was determined with a leaf area meter (LiCor 3000, Lincoln, NE). The leaves of each treatment were then placed inside an electric oven at 60 °C. After three days the dry weight was recorded. Both fresh and dry weights were standardized to 1 cm^2 for both *A. graveolens* and *C. murale*. Percent water content (%W) was calculated by the formula: %W = (fresh weight-dry weight)/fresh weight × 100.

The toughness of *A. graveolens* var. Conquistador and *C. murale* leaves was measured with a University of California fruit penetrometer (Coggins *et al.*, 1965) consisting of a 1 mm dia cylindrical tip connected to a reversible electric motor traveling at a speed of 2.54 cm in 4.5 seconds. Each leaf was placed between two 4 mm thick rectangular plexiglass plates (11 cm × 10 cm) with a 6 mm hole in the middle in order to allow contact between the tip and the leaf. The leaf-plexiglass sandwich was placed on a Sartorius balance (Brinkman Instruments Co., Westbury, N.Y.). The rupture force of the leaf was obtained by observing the display in the balance for maximum number occurring immediately before the sudden decrease to zero. Twenty two leaves from five plants of each treatment were examined; each leaf was tested at three different points near the midrib, two at the base of the leaf and one at the tip. Values from each leaf were averaged.

Behavioral studies. Behavior of starved (20 h) one-day-old fourth instars was studied by observing the larvae individually on *A. graveolens* or *C. murale* (no choice tests) over periods of five minutes. A five minute threshold was chosen based on preliminary observations suggesting that most feeding started within the first minute of observation (MB, unpubl.). The observations were made using a computer-assisted monitoring device (Eigenbrode *et al.*, 1989). This device records time spent and number of events associated with specific behaviors observed at 10 × magnification with a dissecting microscope. The recorded behavior were: eating, defined as chewing with the mouthparts in contact with the plant surface; questing, when larvae walked or lifted their thoraces, and moved from side to side in a searching motion; swallowing, when larvae repeatedly contracted esophageal muscles and moved their mandibles as if chewing, but mandibles were not in contact with the plant surface; swallowing

and questing, when the larvae combined the activities of swallowing and questing; and lastly, resting, when no apparent activity was observed. This technique has been used successfully to document behavioral modification in other lepidopterous larvae associated with plant chemistry (Eigenbrode *et al.*, 1989; 1991).

For these studies, 1.1 cm^2 leaf discs from the penultimate position in the tallest branches of the plant for *A. graveolens* var. Conquistador and from fully expanded leaves from the top third portion of *C. murale* plants (2 plants/treatment) were used. Each disc was placed in the center of an agar-lined disposable Petri dish (150 × 25 mm) with the abaxial side facing up or down, alternately. A single larva was placed on the leaf disc, and the lid of the Petri dish immediately replaced. Observations began as soon as the larvae showed any activity; the experiment was repeated 21 times for both *C. murale* and *A. graveolens*.

To determine if potential differences in leaf toughness were affecting host selection, fresh *C. murale* and *A. graveolens* var. Conquistador leaf material (18.4 g each) was dried for 96 h at 60 °C in an electric oven. The material was then finely ground in a mortar and mixed with 205 ml of liquid agar, the mixture was then poured into a 1 cm thick plastic plate (15 × 9 cm). The plant-agar mixture was allowed to solidify at room conditions for 1 h. A 1.54 cm^2 cork borer was used as a mold to obtain 1.54 cm^3 cylinders (1.5 leaf equivalents/cylinder). Two cylinders of each treatment were arranged at opposite cardinal points inside an agar-lined 150 × 25 mm disposable Petri dish. A fourth instar was placed in the center of the Petri dish, and the dish was immediately covered. Ten discs of each treatment were placed as above in five Petri dishes without larvae to control for water loss. This experiment had 16 replicates. The 16 Petri dishes plus 5 controls were placed in an incubator at 28 ± 2 °C and L14:D10 photoperiod. Approximately 24 hrs later the cylinders were weighted to the nearest 0.0001 g.

Cylinders made as before were used as substrate for five minute observations of the five previously defined larval behaviors one-day-old fourth instars. These recordings were made by observing the larvae individually on *A. graveolens* or *C. murale* cylinders (no choice tests) using the methods mentioned subsequently for the behavioral studies. This test was repeated 19 times for *A. graveolens* and 20 times for *C. murale*.

Data analyses. Data on larval position from the whole plant choice test were analyzed with the REPEATED statement of the ANOVA procedure (SAS, 1990). Soluble protein content results were analyzed using the GLM procedure. Traveled distance results from the volatiles bioassay were analyzed with the ANOVA procedure of SAS (1990). Because the data of the feeding damage comparisons were not normally distributed they were analyzed using a Chi-square test (χ^2) with NPAR1WAY procedure (SAS, 1990). All other statistical comparisons were analyzed with TTEST and MEANS procedures (SAS, 1990).

Results

Fresh plant material bioassay. In whole plant choice tests (Fig. 1), ten out of twelve observations showed a significantly higher proportion of larvae on *C. murale* plants as compared with *A. graveolens* ($P<0.05$). In bioassays designed to evaluate feeding on individual leaves, *C. murale* had a significantly greater percentage (86.56 ± 12.09%; mean ± standard error) of the total leaf area eaten (*C. murale* plus *A. graveolens*) than did *A. graveolens* (13.44 ± 12.09%; $\chi^2 = 8.16$; $P<0.01$). These latter results, combined with those of the whole plant bioassay, confirmed our field observations that *S. exigua* larvae preferred *C. murale* over *A. graveolens*.

There was no difference ($P=0.5$) in final leaf disc area between the commercial celery varieties 'Conquistador' (1.27 ± 0.10 cm^2 and 'Tall Utah' (1.35 ± 0.11 cm^2). Because *S. exigua* larvae showed no preference for one variety over the other, results of tests using either variety were considered comparable on the basis of their preference by *S. exigua* larvae.

Plant extract studies. Initially, the polarities of the most active fractions were determined. Paired extract comparisons (Figs. 2A and 2B) indicated that the disc with the methanol aqueous fractions received more feeding than the discs with the hexane fractions in both *C. murale* and *A. graveolens* ($P<0.001$). Thus, the most biologically active compounds were moderately polar.

An initial hypothesis was that *C. murale* was preferred due to the presence of a chemical attractant/stimulant in *C. murale* or possibly a repellent/deterrent in *A. graveolens*. This hypothesis predicted that extracts from *C. murale* would be preferred to *A. graveolens* fractions. However, a comparison of the methanol extracts of both plants (Fig. 2C) showed that discs with *A. graveolens* extract suffered signifi-

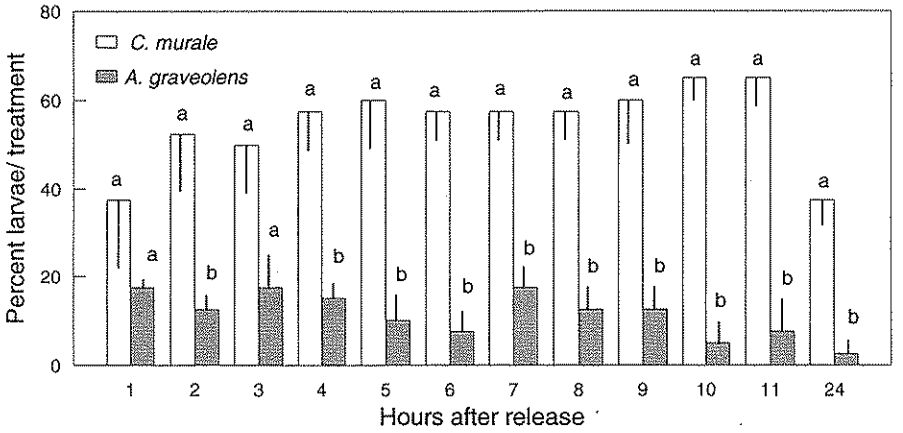

Fig. 1. Preference test using *C. murale* and *A. graveolens* var. Tall Utah plants. Bars indicate percent larvae on each plant. Lines within bars indicate standard errors from a mean of 4 replicates. Different letters above bars indicate significant difference at $P<0.05$ level (ANOVA).

cantly more damage than discs with *C. murale* extracts ($P<0.001$). Furthermore, the comparison between both plants (Fig. 2D) for the combination of both fractions (hexane + 90% methanol) showed that larvae caused significantly more damage on discs treated with *A. graveolens* extracts than they did on discs with *C. murale* extracts ($P<0.05$).

To discern if the apolar *A. graveolens* fraction contained a deterrent or repellent, the originally preferred *C. murale* methanol extract (Fig. 2A) was compared to a combination of both extracts (hexane + 90% methanol) from *A. graveolens* (Fig. 2E). Discs with the combination of both *A. graveolens* fractions again showed significantly more damage than discs with the methanol fraction of *C. murale* ($P<0.001$). In addition, a comparison between the combination of both extracts from *A. graveolens* versus only the preferred methanol fraction of *A. graveolens* (Fig. 2F) supported the conclusion that the hexane fraction did not contain substantial amounts of deterrents since discs with the combination treatment, which included the hexane fraction, suffered significantly more damage ($P<0.0001$).

No significant difference could be found between the effects of plant volatiles from *C. murale*, *A. graveolens* and a blank control on larval dispersal by *S. exigua* ($F=0.20$; df = 2,4; $P=0.83$). Hence, it's unlikely that volatiles from *C. murale* and *A. graveolens* affect larval dispersal of *S. exigua*.

Soluble protein, water content and leaf toughness measurements. *A. graveolens* leaves had a higher concentration of soluble proteins (618.4 ± 26.83 g/kg dry weight; $F=52.98$; df = 1,7; $P<0.001$), significantly less water content percentage ($80.4 \pm 0.38\%$; $P<0.001$) and were significantly tougher (16.27 ± 0.68 g; $P<0.001$) than *C. murale* leaves (328.17 ± 22.91 g/kg dry weight, $86.6 \pm 0.73\%$ and 10.64 ± 0.47 g, respectively).

Behavioral studies. Significant differences in swallowing time were documented for the five minute behavioral observations with fresh leaf material (Fig. 3; $P<0.01$). There were no significant differences ($P>0.05$) among the other four behaviors for time spent in a particular behavior (Fig. 3) or number of occurrences of each behavior and number of occurrences for swallowing (Data not shown). Larval preference tests with dried and ground leaf material in agar showed no behavioral differences in consumption between *C. murale* and *A. graveolens* ($P>0.05$).

Discussion

Other reports of selection between plants by highly mobile larvae include a study by Ampofo (1986), who reported that *Chilo partellus* (Swinhoe) larvae actively disperse from resistant to susceptible maize plants. Roome (1988) observed that larvae of *C. partellus* oviposited on a non-host plant readily migrated to acceptable plants. Waladde *et al.* (1990) reported that aqueous extracts of the resistant maize variety reduced consumption rates and weight gain in the larvae. They also showed that electrophysiological differences in dose-response curves suggested that the maxillary sensilla styloconica could distinguish between aqueous extracts of the resistant and susceptible varieties. Furthermore, chemical constituents such as fura-

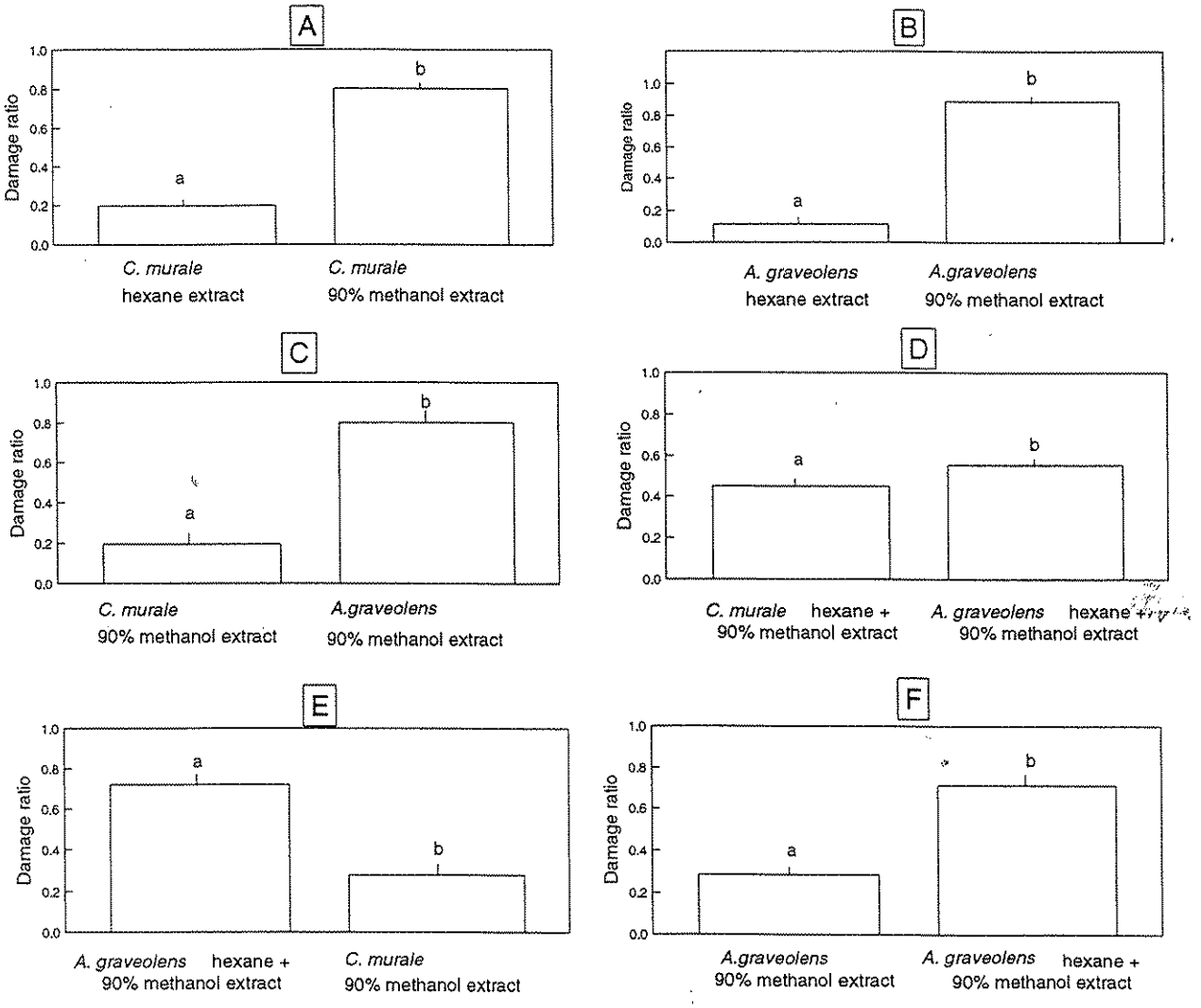

Fig. 2. Pair-wise comparisons of different *C. murale* and *A. graveolens* var. Conquistador extracts. Damage ratio = total damaged area/damaged area of either treatment. Different letters above bars indicate significant difference at $P<0.05$ level; T-test. Lines within bars indicate standard errors.

nocoumarins (Berenbaum, 1990), alkaloids (Wada & Munkata, 1968; Boer & Hanson, 1987), amino acids (Hedin *et al.*, 1993), and phenolic compounds (Boer & Hanson, 1987) have been shown to influence the behavior of lepidopterous insects. In trying to asses the importance of plant chemistry in the form of attractants/stimulants and repellents/deterrents on host selection by *S. exigua* larvae, our results (based on extracts from whole leaves) suggested that the observed preference of *C. murale* was not due to the existance of a strong attractant or stimulant in *C. murale* or a deterrent or repellent in *A. graveolens*.

The lack of difference on larval dispersal between plant volatiles from *C. murale*, *A. graveolens*, and the control suggested that neither repellents nor attractants were responsible for the observed preference of *C. murale*. These results were further confirmed by the fact that on the behavioral studies the larvae spent more than 80% of their time eating both, *A. graveolens* and *C. murale* (Fig. 3).

The existence of feeding deterrents in *A. graveolens* or feeding stimulants in *C. murale* cannot be conclusively excluded by our bioassays. However, the lack of difference in number of events and time spent eating, questing, swallowing and questing, and resting

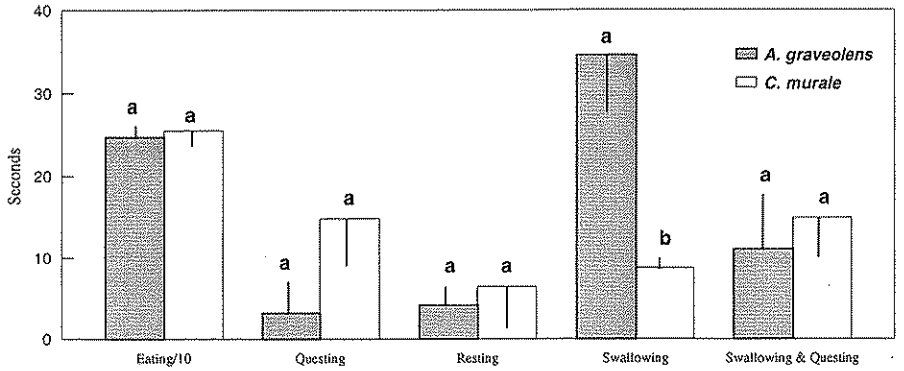

Fig. 3. Pair-wise comparisons of larval behavior (five events) on *A. graveolens* var. Conquistador and *C. murale*. Eating/10 represents total times(s) spent eating divided by 10. Different letters represent significant differences in that specific larval behavior between *A. graveolens* var. Conquistador and *C. murale* ($P<0.05$; T-test). Means based on 21 replicates. Lines within bars indicate standard errors.

by *S. exigua* larvae on *C. murale* and *A. graveolens* suggests that, at least within the first 5 min after initial contact with the plant material, feeding deterrents or stimulants are not playing a major role in larval behavior.

A. graveolens has almost twice the amount of soluble protein when compared to *C. murale*. This suggests that preference of *C. murale* is not based on soluble protein. Although the use of soluble protein analysis as an indicator of nitrogen content does not provide information on individual, potentially phagostimulatory free amino acids, soluble proteins are often the dominant form of foliar nitrogen (Jones *et al.*, 1989). Water content results also indicated a greater water content percentage in *C. murale* than in *A. graveolens* leaves. Therefore, higher leaf consumption of *C. murale* by *S. exigua* was not the result of compensatory feeding for low leaf water content. This, however, was not surprising since phytophagous caterpillars have commonly shown a lack of compensatory feeding for low water content (Slansky, 1993).

The greater toughness of *A. graveolens* leaves, plus the over 3 × greater amount of time spent swallowing *A. graveolens* as compared to *C. murale* leaf discs, suggests that leaf toughness was primarily responsible for the observed differences in feeding behavior. The larvae showed higher activity levels, moving their mandibles and contracting their esophageal muscles, when feeding on *A. graveolens* as compared with *C. murale*. Therefore, differences in swallowing time by *S. exigua* larvae between these two hosts could produce an improved feeding efficiency on *C. murale* and thus explain some of our earlier results.

The precise mechanism(s) by which *S. exigua* is able to discriminate between *C. murale* and *A. graveolens* is still unknown. However, our studies suggest that leaf toughness and perhaps water content, rather than plant volatiles, feeding stimulants or deterrents and nitrogen content play key roles in host plant selection behavior by the larvae. The literature contains several references on the effect of plant characteristics on the foraging patterns by caterpillars within a plant (Scriber & Slansky, 1981; Cockfield & Mahr, 1993). However, these reports do not include the effects of leaf toughness and water content on host selection behavior among plant species by lepidopterous larvae.

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