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EFFECT OF SCREENING METHODS ON EXPRESSION OF ROMAINE LETTUCE RESISTANCE TO ADULT BANDED CUCUMBER BEETLE, *DIABROTICA BALTEATA* (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Resistance in lettuce, *Lactuca sativa* L., to feeding by adult banded cucumber beetle, *Diabrotica balteata* (LeConte), was evaluated using three screening methods: leaf disks, excised leaves and intact leaves attached to plants. Dual-choice and no-choice bioassays were used to evaluate each method based on leaf area consumption. Methods of testing had a significant effect on the level of feeding damage by *D. balteata* on two lettuce cultivars, Tall Guzmaine and Valmaine. Valmaine expressed a significant degree of resistance to *D. balteata* damage when intact leaf and excised leaf methods were used in dual-choice bioassays between Tall Guzmaine and Valmaine, but the latter failed to show resistant characteristics in no-choice tests when excised leaves were used. Furthermore, there was no significant difference in *D. balteata* feeding between Tall Guzmaine and Valmaine in the leaf disk tests. Therefore, whole plants are the best method to evaluate lettuce cultivars for resistance to *D. balteata*. Reduction or cessation of resistance characters in excised Valmaine whole leaves and disks are discussed with references to potential changes in concentration of feeding stimulants and deterrents and changes in latex pressure.

Key Words: *Lactuca sativa*, leaf disk, excised leaves, intact leaves

RESUMEN

La resistencia en la lechuga, *Lactuca sativa* L., hacia la alimentación de adultos del escarabajo rayado del pepino, *Diabrotica balteata* (LeConte), fué evaluada usando tres métodos de seleccionar: hojas cortadas en forma de disco, hojas cortadas, y hojas intactas pegadas a la planta. Se utilizaron un bioensayo de una prueba de doble opción y de una prueba sin opción para evaluar cada método basado en el consumo del área de la hoja. Los métodos de prueba tienen un efecto significativo sobre el nivel de daño causado por la alimentación de *D. balteata* en dos variedades cultivadas de lechuga, la "Tall Guzmaine" y la "Valmaine". La variedad Valmaine expresó un grado de resistencia significativo al daño de *D. balteata* cuando fueron usados los métodos de las hojas intactas y de las hojas cortadas en los bioensayos de doble opción entre las variedades Tall Guzmaine y Valmaine, pero la última no mostró características de resistencia en pruebas de una sola opción cuando se usaron hojas cortadas. Además, no habia una diferencia significativa entre la Tall Guzmaine y la Valmaine en cuanto de la alimentación de *D. balteata* en pruebas de hojas cortadas en forma de discos. Por lo tanto, las plantas enteras son el mejor método para evaluar las variedad de lechuga para su resistencia al *D. balteata*. Se discuten la reducción o el paro de las características resistentes en hojas cortadas de la Valmaine en hojas enteras y en de hojas cortadas en forma de discos con referencia a los cambios potenciales en la concentración de estimulantes y disuacivos de alimentación y cambios en la presión de latex.

Host plant resistance is recognized as an effective component of IPM (Panda & Khush 1995), because it has low impact on non-target organisms and the environment and is usually compatible with other control tactics. Reliable and efficient screening techniques are essential for accurately evaluating resistance levels. Excised leaves or leaf disks are often used for evaluating plants for resistance to leaf feeding insects. For example, Sams et al. (1975) found that the use of excised

leaflets was an efficient method for evaluating resistance to green peach aphid (*Myzus persicae* (Sulzer)) in tuber-bearing *Solanum* germplasm. Excised leaves were found to be quite reliable for screening bean cultivars for Mexican bean beetle (*Epilachna varivestis* Mulsant) feeding preference as long as large mature leaves were used (Raina et al. 1980). The leaf disk and whole leaf techniques worked equally well for screening resistance to two-spotted spider mites (*Tetranychus urticae* Koch) on muskmelon leaves (*Cucumis melo* L.) (East et al. 1992). However, some screening methods may have a significant impact on the test outcome by altering natural resistance mechanisms. Risch (1985) found that screening methods affected the expression of resistance and the order of feeding preference among corn (*Zea mays* L.), bean (*Phaseolus vulgaris* L.) and squash (*Cucurbita pepo* L.) to specialist and generalist chrysomelid beetles.

In this study, we evaluated the effect of three screening methods (leaf disks, excised leaves and intact leaves attached to plants) on the expression of resistance in romaine lettuce cultivars to feeding by adult banded cucumber beetles (*Diabrotica balteata*). Leaf area consumed by beetles was evaluated in dual-choice and no-choice bioassays to compare results for both leaf disks and detached leaves against intact leaves.

Materials and Methods

Plants and Insects

A previous study showed that Valmaine (Val) was the most resistant cultivar and Tall Guzmaine was the most susceptible one to *D. balteata* feeding among four lettuce cultivars (Huang et al. 2002). Therefore, Valmaine and Tall Guzmaine were used for this experiment. Seeds of each cultivar were kept overnight in the laboratory in separate petri dishes lined with wet filter paper for better germination. Germinated seeds were planted in a transplant tray filled with a commercial soil mix (MetroMix 220, Grace Sierra, Milpitas, CA) and grown for 2 wk in a greenhouse with natural light. Seedlings were transplanted to 10-cm diameter plastic pots filled with MetroMix 220. Each plant was watered daily and fertilized weekly with 10 ml of a 10 g/L solution of a soluble fertilizer (Peters 20-20-20, N-P-K, W. R. Grace, Fogelsville, PA) from transplantation until the end of the experiment. Fully expanded leaves from the seventh position (counting from the first true leaf) were used in all the assays and selected plants had seven to eight fully expanded leaves.

Adult *D. balteata* for feeding bioassays were obtained from a laboratory colony originally collected from the field in Belle Glade, Florida in June 1996. Adults were fed lima bean leaves and sweetpotato tubers and larvae were reared on corn seedling roots as previously described (Huang et al. 2002). Only unfed adults which had emerged within 48 h were used for the assays. All tests were conducted in a rearing room at 25 ± 1°C, 14:10 (L:D) h photoperiod.

Intact Leaves (Experiment 1)

Dual-choice tests were first conducted using intact leaves of Tall Guzmaine and Valmaine plant pairs. Feeding arenas made from plastic petri dishes (8.9 cm diameter) were attached using hair clips to the upper leaf surface of a pair of leaves from each cultivar. Two round holes (2.9 cm diameter) that were 65 mm apart provided access to the leaves, and a 5.8 cm diameter hole that was covered with gauze material at the top of the dish provided ventilation. One pair (female plus male) of beetles was placed in each feeding arena and allowed to feed for 48 h. Each test was replicated 11 times. The extent of feeding was evaluated by scanning the leaf material (JADE 2, Linotype-Hell, Taiwan) and importing the resulting images into an imaging program (ImagePC beta version 1, Scion Corporation, Frederick, Maryland) where leaf area consumed (mm^2) was determined. The difference in leaf area consumption between cultivars was analyzed by paired *t*-test using Proc MEANS (SAS Institute 1999).

Excised Versus Intact Leaves (Experiment 2)

Resistance to beetle feeding was next compared between excised and intact leaves. The petioles of individual leaves excised at their base were immediately immersed in separate beakers filled with tap water and maintained therein for the duration of testing. Dual-choice feeding arenas as described above were used to expose single pairs of adults (female plus male) for 48 h to pairs of either excised or intact Tall Guzmaine and Valmaine leaves. Each test was replicated 17 times in each bioassay. The difference in leaf area consumption between cultivars was estimated and analyzed as described above.

Since a significant difference was found in leaf area consumption between the two cultivars in dual-choice tests, the resistance level of excised and intact leaves was further evaluated using no-choice bioassays. Excised and intact leaves were chosen and prepared as above. One pair of female and male beetles was confined on individual excised or intact leaves of a single cultivar using a modified feeding area with only a single 4 cm diameter hole through which beetles accessed the upper leaf surface. The adults were allowed to feed for 48 h. This study was arranged as a randomized complete block design with excised and intact leaves from each cultivar in each block. Each block was replicated 18 times. Leaf area consumed was estimated as described above and

analyzed by Proc GLM (SAS Institute 1999). Means with significant ANOVA were separated using Tukey's HSD test with a significance level of $\alpha = 0.05$ (SAS Institute 1999).

Leaf Disks

Leaf disks for the bioassays were harvested from freshly excised Valmaine or Tall Guzmaine leaves. Two 380 mm^2 disks were punched out from non-midrib areas of each leaf using a No. 15 corkborer. The bioassay consisted of four leaf disks (two from Tall Guzmaine and two from Valmaine) placed an equal distance apart on two layers of moistened paper towel inside a 8.9 cm diameter plastic petri dish. A female and male beetle was placed in each petri dish and allowed to feed for 48 h. Bioassays were replicated 15 times. Leaf area consumed was estimated as described above, but the remaining leaf area was subtracted from the mean disk area of 10 disks not offered to beetles for two days in order to account for shrinkage during the assay. The difference in leaf area consumption between cultivars was analyzed by paired *t*-test using Proc MEANS (SAS Institute 1999).

Results

Valmaine was strongly resistant to beetle feeding compared with susceptible Tall Guzmaine when intact or excised leaves were presented in dual-choice tests (experiments 1 and 2, Table 1). However, beetles ate significantly more from excised leaves of both cultivars compared to intact leaves (experiment 2, $t = 3.88$, $df = 32$, $p < 0.0001$). Beetles on intact leaves consumed 12-fold less from Valmaine compared to Tall Guzmaine, while on excised leaves consumption was only 4-fold less. When leaf disks were used as test materials, there was no significant ($p \geq 0.05$) difference in feeding between Tall Guzmaine and Valmaine. Adults ate up to 23 times as much Valmaine on leaf disks as on intact leaves (experiment 1). Mean leaf area consumed on leaf disks and intact leaves of Tall Guzmaine was similar, and both were over 318 mm^2.

A significant difference among treatments in leaf area consumed per pair of adults in no-choice tests was also found ($F = 21.78$; $df = 3, 51$; $P = 0.0001$) (Fig. 1). Beetle pairs consumed significantly less (81%) Valmaine than Tall Guzmaine on intact leaves, but consumed similar leaf areas from excised leaves. Feeding was significantly increased on excised leaves compared to intact leaves, irrespective of cultivar. Intact Valmaine leaves were the least damaged by adult *D. balteata* with mean leaf area consumption of 66.3 mm^2, which was only 12% of that on excised Valmaine leaves.

Discussion

Although excised leaves or leaf disks are often used for evaluating plants for resistance to leaf feeding insects, biochemical and physiological changes in such plant tissue may affect the feeding of the test insects (Raina et al. 1980; Risch 1985; van Emden & Bashford 1976). In our case, Valmaine expressed a higher degree of resistance to adult feeding on intact leaves than on excised leaves in choice tests. Moreover, Valmaine failed to show significant resistance to *D. balteata* feeding in either the leaf disk choice test (Table 1) or the excised leaf no-choice test (Fig. 1). Therefore, methods of testing for resistance had a significant effect on relative leaf consumption of Tall Guzmaine and Valmaine by adult *D. balteata*. The intact leaf method was the most suitable and reliable of the tested methods used to evaluate lettuce cultivars for resistance to *D. balteata*.

Risch (1985) also reported that testing method (i.e., whole plants, excised leaves, and leaf disks) had a significant effect on preferences of chrysomelid beetles, including *D. balteata*, when corn, bean and squash were tested. In his tests, differences in resistance ratios between leaf disk and whole plant tests were much greater than those between excised leaves and whole plant tests.

TABLE 1. MEAN ±SEM LETTUCE LEAF AREA CONSUMED PER PAIR (FEMALE PLUS MALE) OF ADULT *D. BALTEATA* IN 48 H WHEN PRESENTED A CHOICE BETWEEN TWO CULTIVARS USING DIFFERENT SCREENING METHODS

Methods	Cultivar[a]	N	Leaf area (mm^2)	Pr > |t|
Intact leaf (expt 1)	TG	11	318.1 ± 29.3	0.0001
	Val	11	14.4 ± 2.1	
Intact leaf (expt 2)	TG	17	350.5 ± 28.7	0.0001
	Val	17	28.8 ± 4.8	
Excised leaf	TG	17	532.2 ± 57.8	0.0004
	Val	17	125.9 ± 53.8	
Leaf disk	TG	15	382.8 ± 36.3	0.0941
	Val	15	334.6 ± 37.4	

[a]TG = Tall Guzmaine, Val = Valmaine.

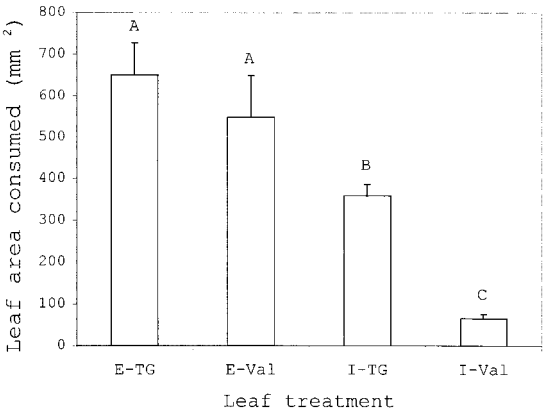

Fig. 1. Mean leaf area consumed per pair of adult *D. balteata* within 48 h during no-choice test using excised (E) and intact (I) leaves from susceptible Tall Guzmaine (E-TG, I-TG) and resistant Valmaine (E-Val, I-Val). Bars topped with the same letter are not significantly different by Tukey's HSD test at the 0.05 level. Vertical lines indicate + 1 SEM.

Furthermore, the feeding preferences of the two specialist species, *Acalymona thiemei* (Baly) and *Ceratoma ruficornis* (Olivier) were less affected by test method than were the more generalist species, *D. balteata* and *D. adelpha* (Harold). In another example of different results between intact and excised leaf tissue, lettuce cultivars normally resistant to the lettuce aphid, *Nasonovia ribisnigri* (Mosley), lost their resistance when leaf fragments were given in a leaf disk test (Schoonhoven et al. 1998).

Leaf disk size is another variable that could affect the outcome of insect feeding preferences. The ratio of cut edge to overall leaf disk surface area can influence the chance of encountering internal attractants and stimulants (Jones & Coleman 1988).

The fact that leaves of both Valmaine and Tall Guzmaine were consumed much more when excised from the plants suggests a change in the chemical profile inside leaves or a reduced capacity to deliver deterrents effectively after cutting. Latex in some laticiferous plants has been reported as a natural defense system against certain herbivores. In many laticiferous plants, including *L. sativa*, latex is stored under pressure within laticifers, which results in rapid release of latex upon cutting (Fahn 1979; Data et al. 1996; Dussourd 1995). The secretions often contain secondary metabolites known to be toxic or deterrent to animals (Farrell et al. 1991). Data et al. (1996) found that young vine material of sweet potato produced more latex and had fewer sweetpotato weevils, *Cylas formicarius* (F.), than older and more mature portions of the vine. Several insects have been observed immobilized in exudates, such as caterpillars (Dussourd 1993), ants (Dillon et al. 1983), aphids and whiteflies (Dussourd 1995). Many different organic compounds have been identified in latex of *Lactuca* sp., including organic acids, phenolics and a triterpene alcohol (Crosby 1963; Gonzalez 1977; Cole 1984). Like many plant secondary compounds these organic compounds may act as deterrents or toxins to potential herbivores. Both Valmaine and Tall Guzmaine produce latex upon cutting, but latex flows from Valmaine longer after cutting than from Tall Guzmaine (Huang et al. 2003). Beetles may have eaten more on excised than on intact leaves because latex flow from injured tissue on excised leaves placed in water may be decreased and diluted compared to intact leaves. No latex emission was observed from leaf disks which may be the major reason why no feeding difference was found between Valmaine and Tall Guzmaine. However, Tall Guzmaine was preferred over Valmaine by beetles when excised leaves were used in choice tests and when intact leaves were used in both choice and no-choice tests. Therefore, the observed feeding preferences may be due to differences in the composition or concentration of secondary compounds within the latex between Tall Guzmaine and Valmaine.

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