MEASURING RESISTANCE TO THE COLORADO POTATO BEETLE (COLEOPTERA: CHRYSOMELIDAE) IN POTATO

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ABSTRACT

A sensitive test was sought that could be used to detect small differences in resistance to the Colorado potato beetle (CPB) in *Solanum* species. Three tests were evaluated and compared. One test compared adult CPB foliage consumption of leaf disks from a susceptible potato cv, *S. tuberosum,* with disks from two *S. chacoense* clones. The second test compared weight gain on foliage from the three plants by 4th instar larvae and the third test compared larval development rate and mortality. With sufficient replication all three of the tests could detect significant differences between each of the test clones. The most sensitive test measured the stage of development of neonate larvae after feeding on test plants four days. This test required only four replicates to detect a 50% difference from the overall mean assuming an alpha level of 0.05 and a beta level of 0.10.

Key Words: Bioassay, *Leptinotarsa decemlineata,* host plant resistance, plant resistance, *Solanum tuberosum, Solanum chacoense.*

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INTRODUCTION

The identification and selection of plants with resistance to an insect and the incorporation of this resistance into a horticulturally desirable plant is a lengthy procedure often taking many years. This is due in part to the large number of replications necessary to detect differences between plants, which reduces the amount of germplasm that can be screened at a time. This paper describes and compares a series of tests with the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), using *Solanum tuberosum* and *S. chacoense* as the test species. The objective was to find the most efficient and sensitive test for screening plant material for resistance to the Colorado potato beetle (CPB). *Solanum chacoense* accessions were used because some are highly resistant to the CPB (Torka 1950) due to their content of leptines, glycoalkaloids known only from *S. chacoense* (Sinden et al. 1986a, 1986b). Leptines were of particular interest because of their high toxicity to the CPB (Sturckow and Low 1961) and their absence in potato tubers (Kuhn and Low 1961).

MATERIALS AND METHODS

Test Clones

Three potato clones were compared in the tests: the cv Kennebec was used as the susceptible standard and two clones from the *S. chacoense* accession PI 320287,

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numbered 320287-1, and 320287-3. Each accession had similar quantities of total glycoalkaloids, but accession #320287-1 contained leptines whereas accession #320287-3 did not (Sinden et al. 1986b). The plants were grown under natural light in a greenhouse following conventional growing practices. Foliage samples were taken from the plant in the late prebloom stage.

Disk Test with Adults

From each clone three leaf disks, 1.4 cm in diameter, were cut, using a cork borer, from potato leaflets that had recently fully expanded. The area and weight of the three disks were measured. Then the disks from two of the clones were placed alternately in a circle on moist filter paper in 9 cm diameter petri dishes. Subsequently six recently emerged adult CPB from a laboratory colony, all of one sex either male or female, were placed in each dish and allowed to feed until at least half the area of the disks from one clone was consumed. The uneaten disk area was measured using an area meter and the area converted to weight. The three possible pairings of disks from the three clones were tested and each was replicated 6 times in 1984 and 10 times in 1985. Disk weights rather than disk areas were used to determine preference because of differences that occur between clones in leaf thickness and possibly density. The testing was done in a controlled environment chamber at 24°C in complete darkness. Foliage consumption data of each pair combination were tested using the RCB ANOVA procedure.

Larval Weight Gain and Consumption

One 4th instar larva was placed for 48 hours in a 9 cm petri dish containing a leaf from a test plant. The petiole was in a vial of water. Fourth instars were used because they have the longest stadium and also would be expected to show larger weight differences because of their larger size. The bouquet area and weight, and the larval weight were measured at the beginning and end of the test. Two tests were conducted with each clone replicated 8 times in 1984 and 20 times in 1985. The dishes in this and the following test were held at 24°C with an 18 h photoperiod. Weight change and foliage consumed were analyzed by CRD ANOVA.

Larval Development Rate

Ten neonate larvae that had completed feeding on the egg chorion were placed in a 9 cm petri dish with a leaf bouquet from a test plant. Neonate larvae were used because this stage is probably the most sensitive to plant allelochemicals. Insects were inspected daily and removed if they had died or molted. Each clone was tested six times in 1984. In 1985 this test was repeated with 19 replicates and instead of removing larvae after they molted, they were held for 4 days with mortality and instar being noted on day 3 and 4. Univariate ANOVA tests were applied to the arcsin transformation of the percent molted and percent dead by a given day. Multivariate analysis was used to help identify the developmental period of the larvae that best differentiated among clones. A Hotelling-Lawley Trace Test (SAS Institute 1982) was used to determine which day resulted in the most significant differences between clones using, in 1984, the percent molted and percent dead for each day and in 1985 the percent dead and percent in each instar. Where significance was found, Fisher's Exact Test (SAS Institute 1982) was

used to distinguish between clones in 1984 while Duncan's multiple range test was used in 1985.

RESULTS AND DISCUSSION

Disk Test with Adults

When adult beetles were given a choice between disks from Kennebec or a *S. chacoense* clone, nearly all the feeding was on Kennebec disks (Table 1).

When only the two PI accessions were offered, consumption differences were not statistically significant in 1984 but were in 1985 when the difference was greater and more replicates were used. There was a significant interaction between clone and year for each combination of pairs; however, this seems to be of little importance as the % difference in feeding between clones was negligible.

Larval Weight Gain and Consumption

When foliage weight consumption by the 4th instar larva was used as a criterion for determining plant acceptance or antibiosis, Kennebec could be distinguished from clones 320287-3 and 320287-1 in 1985 and in the combined data, but the latter could not be distinguished from each other (Table 2). Using larval weight gain as a criterion, differences between all three clones could be detected only by using the combined 2-yr data. The Pearson correlation coefficient for larval weight gain and foliage weight consumed was + 0.65 ($P = 0.0001$).

Larval Development Rate

Development of first instar larvae in 1984 on the different clones was best measured for molting on days 3 - 5 and for mortality on day 4. Day 3 had a higher F value than day 4 for molting (93 vs 63) although both were significant at the 0.0001 level (Table 3). The percentages did not increase daily because different populations were sampled. The Hotelling-Lawley Trace Test had an F value of 41 for day 3 and 29 for day 4, both being significant at the 0.0001 level, suggesting that observing the larvae for three days may be the optimum period to detect different development rates. An analysis of the neonate larval development in 1985 showed that by recording each instar, additional useful data were obtained (Table 4). Only data for days 3 and 4 were collected because the 1984 test showed these to be the more sensitive periods. Data for either day could be used satisfactorily to distinguish among clones. Day 4 may be preferred as all three categories (% dead, 1st instar, 2nd instar) had significant differences and also there is the possibility of 3rd instars being present. The Hotelling-Lawley Tract Test for both days was highly significant ($P = 0.0001$) with day 3 having an F value of 19 and day 4 having an F value of 11 which would suggest that differences between clones was easier to detect on day 3.

Another consideration in selecting a test method is the amount of replication required to achieve the sensitivity desired. Estimates of the replications needed were calculated (Appendix 4 of Anderson and McLean 1974) assuming a 5% probability of type I error, a 10% probability of a type II error, for true differences of 50, 100, and 200%. The number of first instars that had molted after three or four days stood out as the measurement requiring the fewest replicates, requiring only 6 or 4 replicates, respectively, to detect a difference between any two means equal to 50% of the overall mean (Table 5). The use of neonate larvae in

Table 1. Comparative feeding rates by six adult CPB on leaf disks from pairs of test clones.

Solanum pair	Year	Foliage consumed (g)			Wt. difference (g)†	% difference
		Kennebec	320287-1	320287-3		
Kennebec vs 320287-1	1984	0.072	0.000		0.072***	99.38
	1985	0.040	0.001		0.039**	98.13
	1984+1985	0.052	0.001		0.052***	98.78
Kennebec vs. 320287-3	1984	0.062		0.002	0.060***	97.15
	1985	0.046		0.004	0.043***	96.23
	1984+1985	0.052		0.003	0.049***	94.10
320287-1 vs 320287-3	1984		0.003	0.019	0.016ns	84.19
	1985		0.005	0.036	0.031***	85.26
	1984+1985		0.004	0.030	0.025***	85.01

† Differences significant at P = 0.0001 (***) or P = 0.001 (**) according to ANOVA, ns = not significant at P = 0.05.

Table 2. Effect on CPB 4th instar larval weight by feeding for 48 hours on three *Solanum* clones.

Year	Clone	Foliage wt. consumed (g)*	Larval wt. (g)			
			Initial	Final	Gain*	% gain
1984	Kennebec	0.374a	0.100	0.137	0.037a	36.69
	320287-3	0.297a	0.089	0.124	0.035a	39.56
	320287-1	0.207a	0.091	0.090	-0.001b	- 0.94
1985	Kennebec	0.372a	0.066	0.107	0.042a	63.70
	320287-3	0.093b	0.062	0.073	0.011b	17.91
	320287-1	0.114b	0.078	0.084	0.006b	7.69
1984+1985	Kennebec	0.372a	0.075	0.115	0.040a	53.77
	320287-3	0.149b	0.069	0.087	0.018b	25.60
	320287-1	0.139b	0.081	0.086	0.004c	5.08

* Means for year(s) followed by same letter are not significantly different according to Duncan's multiple range test (P = 0.05).

Table 3. Daily molting and mortality of neonate CPB larvae placed on leaves of test clones.

Day	Molted (%)*				Mortality (%)*			
	Kennebec	320287-3	320287-1	Significance†	Kennebec	320287-3	320287-1	Significance†
1	0	0	0	ns	0	0	0	ns
2	58.3	9.2	0	ns	0	0	9.2	ns
3	83.9a	56.5b	3.1c	0.0001	3.1	6.1	3.1	ns
4	86.9a	72.0ab	28.8b	0.0001	3.1a	14.7b	17.7b	0.05
5	86.9a	73.6ab	38.4b	0.0001	3.1	16.4	17.5	ns
6		56.8	40.9	ns		26.6	24.7	ns
7		56.8	38.5	ns		33.2	36.1	ns
8			38.1				51.9	
10			39.2				34.6	
11			39.2				18.4	

* Differences between clones within a day are not significant (P = 0.05) if followed by same letter, using Fisher's Exact Test.
† ANOVA test for clones within each day. Data are back-transformed from arcsin-square root.

Table 4. Stage of development of neonate CPB larvae after three and four days on potato foliage of test clones (1985).

Solanum clone	Day 3*			Day 4*		
	% dead	% 1st instar	% 2nd instar	% dead	% 1st instar	% 2nd instar
Kennebec	0.57a	4.46c	93.15a	2.21b	1.48b	94.87a
320287-1	2.41a	91.78a	2.14c	19.62a	53.07a	17.04c
320287-3	0.85a	70.70b	22.34b	5.12b	35.51a	47.40b

* Means in a column followed by the same letter are not significantly different according to Duncan's multiple range test (P = 0.05). Data are back-transformed from arcsin-square root.

Table 5. Number of samples necessary to detect indicated differences between any two clone means as a % of the overall mean of each test based on an alpha value of 0.05 and a beta value of 0.10.

Test	% diff. = 50	% diff. = 100	% diff. = 200
Adult feeding preference	22	6	3
Larval consumption and wt. gain			
Foliage eaten	40	11	4
Weight gain	102	27	8
Larval mortality and development (1984)			
Day = 3			
Dead	>200	86	23
Molted	6	3	3
Day = 4			
Dead	58	16	5
Molted	4	3	3
Larval mortality and development (1985)			
Day = 3			
Dead	~200	57	15
Instar 1	13	5	3
Instar 2	23	7	3
Day = 4			
Dead	75	20	7
Instar 1	24	7	3
Instar 2	21	7	3

evaluations has the practical advantage that they require less time, foliage, and effort to produce than 4th instars or adults and also they are available in greater numbers.

All three of the tests evaluated could be used satisfactorily to detect differences in resistance to the Colorado potato beetle between potato clones. Depending on the circumstances, any one could be preferred. It would appear, however, that the stage of development of neonate larvae after feeding on test plants for three or four days is the most sensitive criterion for detecting differences in resistance.

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LITERATURE CITED

Anderson, V. L., and R. A. McLean. 1974. Design of Experiments. Marcel Dekker Inc., New York. p. 418.

Kuhn, R., and I. Low. 1961. The structure of leptines. Chem. Ber. 94: 1088-95.

SAS Institute. 1982. SAS User's Guide: Statistics. SAS Institute Inc., Cary, NC.

Sinden, S. L., L. L. Sanford, and K. L. Deahl. 1986a. Segregation of leptine glycoalkaloids in *Solanum chacoense* Bitter. J. Agric. Food Chem. 34: 372-77.

Sinden, S. L., L. L. Sanford, W. W. Cantelo, and K. L. Deahl. 1986b. Leptine gylcoalkaloids and resistance to the Colorado potato beetle (Coleoptera: Chrysomelidae) in *Solanum chacoense*. Environ. Entomol. 15: 1057-62.

Sturckow, B., and I. Low. 1961. The effects of some *Solanum* glycoalkaloids on the potato beetle. Entomol. Expt. Appl. 4: 133-42.

Torka, M. 1950. Breeding potatoes with resistance to the Colorado potato beetle. Amer. Potato J. 27: 263-71.