Effects of Previous Leaf Injuries to Spring Canola on Western Black Flea Beetle (*Phyllotreta pusilla*) Feeding

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Two trials were conducted in 2002 with 14-day-old spring canola (*Brassica napus* L.) seedlings to evaluate the effects of previous leaf injuries of different cultivars on subsequent injury by western black flea beetle (WBFB), *Phyllotreta pusilla* Horn (Coleoptera: Chrysomelidae), under greenhouse conditions in Colorado (USA). Previous leaf cutting was found to have only minor effects on subsequent infestation and injury by WBFB, and such effects may be cultivar-specific. Only IMC205 showed significant differences by more than one parameter during these studies; cultivars Excel and 46A65 showed no responses in injury or infestation between previously cut and uncut plants.

KEY WORDS: Spring canola; *Phyllotreta pusilla* Horn; leaf injuries; cut and uncut plants.

INTRODUCTION

Flea beetles of the genus *Phyllotreta* (Coleoptera: Chrysomelidae) are serious pests of cruciferous crops in North America (3,9,10). At least four species are important to oilseed cultivars: *P. cruciferae* (Goeze), *P. striolata* (F.), *P. undulata* Kutsch. (9), and *P. pusilla* Horn (3). The last mentioned, known as the western black flea beetle (WBFB), is particularly damaging in the Rocky Mountain States, including Colorado (USA) (3; M.A. Al-Doghairi (2000) and N. Demirel (2003), Ph.D. dissertations, Colorado State Univ., Ft. Collins, CO, USA). Primary feeding injury is caused by adults, which chew small pits ('shotholes') into leaves (3). Seedlings are frequently killed or severely stunted by these injuries (3) and very high populations of the WBFB can also defoliate established plants (N. Demirel, 2003).

Plants produce a range of semiochemicals that serve as attractants, arrestants, excitants, and feeding stimulants to many insect species (16). Plants of the Brassicaceae are characteristically recognized by the distinctive tastes and odors imparted by the presence of glucosinolates (4,6). Allyl isothiocyanate, a product of the breakdown of a glucosinolate, has been shown to be a powerful attractant for adults of *P. cruciferae*, *P. striolata* (5,13) and *P. pusilla* in trapping trials (M.A. Al-Doghairi, 2000).

During the early stages of colonization, many *Phyllotreta* spp. associated with cruciferous plants feed on cotyledons of emerging seedlings in which the concentrations of glucosinolates are relatively high (6). All currently grown varieties of canola and oilseed rape, *Brassica napus* and *B. rapa*, are susceptible to attack by flea beetles, although to varying extent (7). Release of allyl isothiocyanate, a breakdown product of sinigrin (allyl glucosinolate), is particularly rapid after damage of plant tissues (1,4).

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Mechanical wounding or feeding by *P. cruciferae* caused concentration of indole glucosinolate (3-indolymethyl and 4-hydroxy-3-indolyl-methyl) to increase as much as threefold in the cotyledons of one-week-old seedlings of *B. napus* and *B. rapa* and the mustard *Brassica juncea* (2). These previous studies suggested a relationship between plant injury and production of plant compounds known to be attractive to *Phyllotreta* spp. The purpose of the current study was to evaluate the effects of previous leaf injuries of different spring canola cultivars on subsequent feeding injury by WBFB.

MATERIALS AND METHODS

Trials were conducted during 2002 with 14-day-old spring canola (*Brassica napus* L.) seedlings in a greenhouse located on the campus of Colorado State University, Fort Collins, CO. Two separate runs of the trial were made: the first was sown on 9 May and initiated on 23 May; the second was sown on 12 May and initiated on 26 May. In each, there were six different cultivars: Apollo, Excel, IMC205, 46A65, Hyola and IMC204.

The plants were tested at the two true-leaf stage. Treatment involved removal by cutting one-half of a leaf within 1 h of initiating the trial. An untreated control consisted of undamaged plants. Trials were conducted within cages (61 cm high × 61 cm long × 37 cm wide) into which 12 plants were introduced – six treated by cutting, six left uncut. Five replications (cages) were used in each trial run. One hundred field-collected WBFB beetle adults were introduced into each of the cages.

Evaluations were done at 24-h intervals following introduction of the beetles by counting the number of leaf pits and leaf holes on each plant. Leaf pits were defined as chewing injuries that did not penetrate the leaf; leaf holes completely penetrated the leaf. In the first and the second run, damage was rated on a scale of 0 – 3: 0 = no injury; 1 = slight injury (1–15 pits or holes); 2 = moderate injury (16–30 pits or holes); 3 = severe injury (more than 31 pits or holes). Two people did all the evaluations and the data were averaged. Numbers of flea beetles per plant were also determined, in experiments replicated two times. Evaluations for the first trial were made over a 3-day period from 24 to 26 May and for the second trial over the 2-day period 27 to 28 May. An ANOVA test and t-test were performed to test for significant differences among leaf pits, holes and damage rate (ANOVA), and number of WBFB (t-test) (14).

RESULTS AND DISCUSSION

There were few significant differences in any of the measured parameters between plants damaged by cutting and uncut plants (Table 1). Differences that were detected during the first run almost entirely involved cv. IMC205. For this cultivar there were significantly greater numbers of pits and holes in cut leaves at the 24-h evaluation (F=7.85, df=8, P=0.0487; F=9.85, df=8, P=0.0349, respectively), and a significantly greater number of flea beetles at 48 h (F=14.71, df=8, P=0.0185). The same cultivar had also a significantly greater number of flea beetles and a higher leaf damage rating in cut leaves at 72 h (F=8.26, df=8, P=0.0453; F=10.76, df=8, P=0.0305, respectively). The only other significant difference occurred at 72 h with cv. IMC204, which showed a reverse trend, *i.e.*, greater number of pits on undamaged plants compared to previously cut plants (F=15.89, df=8, P=0.0163).

Furthermore, the effects of leaf cutting indicated some other reverse effects during the second trial run. At this time, IMC205 had higher numbers of pits and holes chewed on

TABLE 1. Effect of leaf cutting on (i) subsequent damage (mean ± S.E.) by leaf pits and holes, (ii) mean number (±S.E.) of western black flea beetles, *Phyllotreta pusilla*, and (iii) damage rating, on different spring canola cultivars in the first run of the trial

Cultivars	24 May		25 May		26 May	
	Cut	Uncut	Cut	Uncut	Cut	Uncut
			(i) Pits per Plant[z]			
Apollo	7.4±2.1a	4.2±1.9a	15.2±3.2a	9.0±1.3a	15.8±1.4a	18.6±5.8a
Excel	5.0±1.5a	4.2±1.1a	10.4±1.8a	18.2±6.3a	17.4±3.0a	17.4±4.5a
IMC205	4.2±0.7a	2.2±0.9b	16.6±2.1a	10.6±5.3a	14.2±2.1a	15.0±4.2a
46A65	2.6±0.9a	2.6±0.9a	10.6±5.9a	12.8±3.6a	20.6±4.2a	30.4±4.5a
Hyola	2.0±0.7a	1.6±0.5a	15.4±3.8a	11.6±5.4a	28.8±5.6a	26.6±7.3a
IMC204	1.4±0.7a	4.0±1.3a	10.2±5.1a	17.8±3.1a	14.2±2.4b	29.4±3.5a
			(i) Holes per Plant[z]			
Apollo	3.8±0.2a	3.2±1.4a	12.4±2.2a	18.2±7.6a	35.8±9.6a	48.0±7.8a
Excel	3.6±1.3a	3.4±1.0a	8.2±2.1a	10.6±3.1a	39.2±5.9a	44.2±10.9a
IMC205	3.6±0.8a	2.0±0.5b	12.2±3.1a	7.8±1.8a	56.4±11.4a	31.6±5.7a
46A65	4.6±1.5a	1.8±0.5a	16.2±6.6a	8.6±2.5a	43.0±6.0a	40.2±9.4a
Hyola	2.8±1.5a	2.0±0.4a	7.4±2.1a	9.4±4.2a	31.2±8.9a	47.2±9.6a
IMC204	1.8±0.7a	2.4±0.5a	10.4±7.2a	7.0±1.5a	34.0±12.4a	34.4±9.11a
			(ii) Beetles per Plant[z]			
Apollo	3.0±0.9a	2.4±1.4a	4.2±0.7a	5.8±2.1a	4.8±1.0a	7.6±2.2a
Excel	2.6±1.2a	2.8±1.7a	2.6±0.7a	5.4±2.5a	7.4±2.2a	7.8±2.0a
IMC205	2.2±0.7a	1.6±0.8a	6.8±1.8a	1.8±1.4b	18.8±4.1a	6.4±1.0b
46A65	1.2±0.6a	2.2±1.1a	5.0±1.5a	3.8±1.4a	9.6±0.7a	12.6±3.8a
Hyola	1.0±0.5a	2.6±1.3a	3.6±1.3a	3.4±0.9a	11.2±2.8a	10.8±2.7a
IMC204	1.0±0.5a	1.6±0.9a	4.2±2.0a	3.4±1.2a	6.4±1.5a	4.4±1.7a
			(iii) Leaf Damage (± S.E.) Rating[z,y]			
Apollo					2.2±0.4a	2.3±0.4a
Excel					2.8±0.1a	2.1±0.6a
IMC205					2.7±0.2a	1.7±0.3b
46A65					1.9±0.5a	2.0±0.3a
Hyola					1.6±0.4a	2.3±0.3a
IMC204					1.4±0.6a	1.8±0.3a

[z] Data were analyzed separately for each cultivar on each date. Within rows, for the same sample date, means that are followed by the same letter do not differ significantly different ($P<0.05$, t-test).

[y] Rating on a scale of 0–3, with 0 = no damage; 1 = slight feeding damage; 2 = moderate feeding damage; 3 = severe feeding damage.

non-damaged plants compared with those cut prior to WBFB exposure (F=1.37, df=8, P=0.03074; F=8.46, df=8, P=0.0437, respectively) (Table 2). There were a greater number of holes in cut leaves compared with undamaged leaves of Apollo on 27 May (F=12.64, df=8, P=0.0237). The cultivar Hyola also had a significantly greater number of flea beetles on non-damaged plants on 28 May (F=57.04, df=8, P=0.0016).

There have been varied reports on interaction of plant wounding and flea beetle infestation of brassicas. The greatest differences in number of feeding holes were observed between injured and uninjured leaves, but canola also had fewer feeding holes than did collard or kale, regardless of injury (15). However, mechanical wounding of the cotyledons of two crucifer species did not influence flea beetle response or feeding (12). Moreover, mechanically wounded (*i.e.,* 6, 24 or 96 punctures per cotyledon) *B. napus* cv. Westar showed no significant reduction in damage by *P. cruciferae*, although there was a trend for the wounded seedlings to be less damaged than the unwounded ones (11).

This absence of effects may be related to the nature of the injury inflicted in these studies: a single cut across one of two existing true leaves. Under natural conditions

TABLE 2. Effect of leaf cutting on (i) subsequent damage (mean ±S.E.) by leaf pits and holes, and (ii) mean number (±S.E.) of western black flea beetles, *Phyllotreta pusilla*, and (iii) damage rating, on different spring canola cultivars in the second run of the trial

Cultivars	27 May		28 May	
	Cut	Uncut	Cut	Uncut
		(i) Pits per Plant [z]		
Apollo	5.6±1.6a	2.4±1.3a	10.2±3.9a	7.4±2.3a
Excel	8.4±1.8a	7.4±2.9a	8.6±3.1a	8.8±2.6a
IMC205	5.6±3.2a	2.8±0.9b	10.4±3.1a	9.2±3.8a
46A65	3.0±0.6a	3.6±1.4a	7.2±1.5a	11.6±3.7a
Hyola	5.0±1.3a	9.4±2.6a	7.6±1.2a	13.2±3.4a
IMC204	5.4±0.6a	5.4±1.5a	9.2±3.3a	7.8±3.1a
		(i) Holes per Plant [z]		
Apollo	9.4±1.4a	4.2±1.5b	33.0±6.8a	23.0±5.5a
Excel	40.2±6.9a	38.8±14.5a	46.0±8.6a	53.4±13.7a
IMC205	10.0±2.8b	29.6±10.9a	31.8±10.9b	49.6±9.4a
46A65	17.6±7.5a	20.4±10.4a	22.2±6.5a	40.6±12.7a
Hyola	46.4±16.6a	33.2±17.9a	60.0±4.7a	65.0±14.8a
IMC204	18.4±3.8a	25.6±7.9a	59.0±14.0a	54.6±4.2a
		(ii) Flea Beetles per Plant [z]		
Apollo	4.8±1.2a	3.6±1.0a	7.8±2.2a	4.4±1.5a
Excel	12.8±3.8a	14.8±5.5a	6.4±1.6a	12.3±5.5a
IMC205	9.2±2.6a	6.6±1.7a	11.8±4.3a	7.4±0.5a
46A65	6.0±1.9a	7.8±3.2a	3.2±1.6a	8.4±3.4a
Hyola	17.4±7.1a	19.2±7.2a	11.6±3.0b	19.0±2.9a
IMC204	9.4±1.9a	14.4±5.1a	7.8±3.1a	9.2±2.0a
		(iii) Mean Leaf Damage (± S.E.) Rating [z,y]		
Apollo			2.5±0.3a	1.6±0.5a
Excel			2.9±0.1a	2.3±0.4a
IMC205			2.2±0.3a	2.6±0.2a
46A65			1.1±0.3a	1.7±0.4a
Hyola			2.6±0.3a	2.4±0.2a
IMC204			2.0±0.4a	2.3±0.2a

[z] Data were analyzed separately for each cultivar on each date. Within rows, for the same sample date, means that are followed by the same letter do not differ significantly ($P<0.05$, t-test).
[y] Rating on a scale of 0–3, with 0 = no damage; 1 = slight feeding damage; 2 = moderate feeding damage; 3 = severe feeding damage.

feeding by WBFB is almost continuous, producing fresh wounding. Ethylene production from onion plants was significantly lower following mechanical damage when compared with plants infested by onion thrips, *Thrips tabaci* (Lindemann), which produced sustained injury (8). Similarly, the increased production of glucosinolates following injury to crucifers that was reported to follow mechanical injury or WBFB feeding (2) may differ due to the nature of the leaf injury. However, overall the results of this study do not suggest a strong relationship between previous injury and *Phyllotreta* infestation, consistent with other reports (11,12).

In conclusion, this study showed only minor effects from previous leaf cutting on subsequent infestation and injury by WBFB to spring canola. Only cv. IMC205 showed significant differences in more than one parameter during these studies. Two cultivars, Excel and 46A65, showed no responses in injury or infestation between previously cut and uncut plants.

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