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Evaluation of Relative Host Plant Preferences of Western Black Flea Beetle, *Phyllotreta pusilla* Horn (Coleoptera: Chrysomelidae), for Various Canolas and Mustards in Greenhouse and Field in Colorado

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Abstract: All trials were conducted in 2000, 2001 and 2002 with different canola (*Brassica napus* L.) and mustard (*Brassica juncea* (L.) plants to evaluate the relative host plant preferences of Western Black Flea Beetle (WBFB) *Phyllotreta pusilla* Horn (Coleoptera: Chrysomelidae) in greenhouse and field conditions in Colorado (USA). The spring mustard (ZEM1) and winter mustard (Debut) were significantly attractive and more susceptible for the WBFB. However, the spring mustard (W1-23) was less susceptibility to WBFB and suggests a possible source of reduced susceptibility in oilseed mustards. Most of currently registered varieties of canola were susceptible to WBFB feeding. The spring canola (CO1) was significantly more attractive and susceptible for the WBFB. In addition, spring canola (Helios) sustained relatively high plant injury with WBFB and plant had low population density and suggesting in tolerance to WBFB injury.

Key words: Western Black Flea Beetle (WBFB), *Phyllotreta pusilla* Horn (Coleoptera: Chrysomelidae), host preferences, canola, mustard

INTRODUCTION

The Western Black Flea Beetle (WBFB), *Phyllotreta pusilla* Horn (Coleoptera: Chrysomelidae), is one of the most important pests on cruciferous plants grown in the Rocky Mountain region in Colorado (Chittenden and Marsh 1920; Demirel and Cranshaw, 2005, 2006). WBFB overwinters as adults under clods of earth, or under heaps of weeds, dead leaves, or other rubbish. There are apparently three generations annually in Colorado. Egg lying begins as early as mid-April and continues into early September (Chittenden and Marsh, 1920). Primary feeding injury is done by adults, which chew small pits ("shotholes") into leaves (Chittenden and Marsh, 1920). Seedlings are frequently killed or severely stunted by these injuries (Chittenden and Narsh, 1920) and very high populations can also defoliate established plants (Demirel and Cranshaw, 2006).

Adult stages of *Phyllotreta* spp. are primarily damaging, causing feeding wounds in foliage, stems and pods of canola. The most important damage to the canola crop occurs within three weeks of germination (Bracken and Bucher, 1986; Gavloski *et al*., 2000; Lamb, 1984; Turnock, 1982). Surviving seedlings grow slowly and more susceptible to effects of further attack. Seedling damage is ultimately important in contributing to yield reduction (Bracken and Bucher, 1986; Lamb, 1984). All currently registered varieties of canola (*Brassica napus*) and oilseed rape (*B. rapa*) are susceptible to attack by *P. cruciferae*, although to varying degrees (Gavloski *et al*., 2000). Differences in cultivar susceptibility can be critical to stand and seedling establishment under WBFB feeding pressure common to Colorado.

The purposes of this study were to evaluate several oilseed brassicas for relative WBFB susceptibility so that this information could be incorporated into cultivar selection and possible breeding.

MATERIALS AND METHODS

Greenhouse host plant preference trial 2000: Two trials were conducted under greenhouse conditions at Colorado State University, Ft. Collins, CO (Table 1 and 2). For evaluation, plants were confined within screened cages, 61 cm high x 61 cm long x 37 cm wide, into which field collected approximately 100 WBFB adults were introduced. After introduction host plant preference was

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Table 1: Western black flea beetle adult numbers and damage rating to young brassica plants exposed to western cabbage flea beetle, *Phyllotreta pusilla*, in cage trials, trial 1, 2000

	Number of WBFB adults/plant[x]					
Cultivars	8-Aug	10-Aug	11-Aug	14-Aug	Avg. WBFB/Plant[y]	Leaf damage rating[z]
ZEM 1	8.5a	7.0a	10.0a	9.8a	8.8a	2.9ab
Debut	10.3a	8.8a	10.5a	5.3ab	8.7a	3.0a
CO1	8.8a	7.3a	4.0a	6.0ab	6.5ab	2.8ab
Casino	4.5a	8.0a	6.8a	5.5ab	6.2ab	2.0ab
IMCO1	7.0a	6.3a	5.3a	3.0b	5.4ab	2.1ab
Sterling	5.3a	5.5a	6.3a	4.3ab	5.3ab	1.9b
Helios	7.0a	4.5a	6.0a	3.0b	5.1b	1.9b
Springfield	3.3a	6.3a	5.8a	3.0b	4.6b	2.4ab
Westar	5.3a	3.8a	4.3a	2.3b	3.9b	2.4ab
Alto	2.5a	3.8a	5.0a	2.8b	3.5b	1.8b
W1-23	5.5a	3.8a	2.3a	1.5b	3.3b	1.8b

[x]Means within a column that are followed by the same letter are not significantly different ($p<0.05$) by SNK. [y]Average over four dates- 8, 10, 11 and 14 Aug. [z]Leaf damage rating (0-3): 0: indicated to plant 10% or less leaf area damage; 1: indicated slight injury in the range of 11 to 30% leaf area injury; 2: Indicated moderate injury of 31-60% leaf area injury; 3: indicated severe leaf injury exceeding 60% leaf area injury

Table 2: Western black flea beetle adult numbers and damage rating to young brassica plants exposed to western cabbage flea beetle, *Phyllotreta pusilla*, in cage trials, trial 2, 2000

	Number of WBFB adults/plant[x]				
Cultivars	10-Aug	11-Aug	14-Aug	Avg. WBFB/Plant[y]	Leaf damage rating[z]
ZEM 1	18.0a	21.5a	11.8a	17.1a	3.0a
Debut	13.5ab	16.0a	5.3a	11.6b	3.0a
CO1	8.5ab	11.0ab	6.0a	8.5bc	2.0a
Helios	13.0ab	5.8ab	2.3a	7.0bc	2.0a
Casino	7.3ab	6.5ab	7.0a	6.9bc	2.3a
Sterling	7.0ab	0.5ab	5.5a	6.7bc	2.0a
Springfield	6.0ab	6.0ab	2.8a	4.9bc	2.3a
W1-23	7.0ab	5.0ab	2.5a	4.8bc	2.0a
Westar	4.8ab	0.8ab	5.3a	4.6bc	2.0a
IMCO1	2.5b	0.8ab	2.8a	3.0bc	2.1a
Alto	2.0b	1.5b	1.5a	1.7c	1.3a

[x]Means within a column that are followed by the same letter(s) are not significantly different ($p<0.05$) by SNK. [y]Average over three sampling dates - 10, 11 and 14 August. [z]Leaf damage rating (0-3): 0: indicated to plant 10% or less leaf area damage; 1: indicated slight injury in the range of 11 to 30% leaf area injury; 2: Indicated moderate injury of 31-60% leaf area injury; 3: Indicated severe leaf injury exceeding 60% leaf area injury

repeatedly evaluated by various means, described below. In the course of these trials 11 different canola and mustard varieties were evaluated for relative WBFB preference. Seven spring canola (*Brassica napus*) cultivars were included: CO1, Helios, IMCO1, Westar, Sterling, Springfield, Alto and also one cultivar of winter canola (*Brassica napus* cv. Casino), one winter mustard (*Brassica juncea* cv. Debut) and two spring mustard cultivars (*Brassica juncea* cvs. ZEM 1, W1-23) were included (Table 1 and 2).

The first trial was set up on 7 August using potted plants seeded 23 June and consisted of four replications (individual plants). This was repeated 9 August, using potted plants seeded 25 June, during which five replications were included. Into each cage a single potted plant of each variety was placed, arranged in random order. Subsequently 100 WBFB adults, collected by sweeping winter canola, were introduced.

Evaluations were made beginning at 24 h. Two evaluations were made. The first was a visual count of all leaf beetles on plants. At the end of the evaluation period damage rating evaluation was made using a 0-3 scale. In this scale: 0: indicated to plots showing 10% or less leaf area damage; 1: indicated slight injury in the range of 11 to 30% leaf area injury; 2: indicated moderate injury of 31-60% leaf area injury; 3: indicated severe leaf injury exceeding 60%. During these evaluations two persons made the assessments and data from both were averaged for analysis. Data from trials were analyzed by analysis of variance (ANOVA) with using the SAS software and means were separated using Student Newman Keuls (SNK) Multiple Comparison Tests (SAS,1990).

Field host plant preference trials 2001: Plots were established at the Horticulture Research Center by direct seeding on 26 April to 6.1 m double-row beds at 76.2 cm row spacing. In the first trial seven different spring canola (*Brassica napus*) varieties were included: Hyola, Excel, Apollo, Helios, Alto, Defender and 46A65 (Table 3). The second trial repeated planting of five of these, Hyola, Excel, Apollo, Defender and 46A65, in a separate area of the field (Table 3). Plots consisted of two beds and both trials were arranged in randomized complete block design with 4 replications.

Table 3: Numbers of western black flea beetles/plant and leaf damage to canola cultivars. Horticulture Research Center, Ft. Collins, CO. June 6, 2001 evaluation

Cultivars	WBFB/ 5 plants[y]	Leaf damage rating[z]
Plot 1		
46A65	1.0b	1.1a
Apollo	6.5a	1.9a
Alto	2.0ab	1.8a
Defender	1.7ab	1.9a
Excel	4.8ab	2.0a
Helios	5.3ab	1.5a
Hyola	3.5ab	1.8a
Plot 2		
46A65	3.8a	1.6a
Apollo	2.3a	2.0a
Defender	4.8a	2.0a
Excel	3.8a	1.9a
Hyola	6.0a	2.0a

[y]Within each plot, means within a column that are followed by the same letter(s) are not significantly different ($p<0.05$) by SNK. [z]Leaf damage rating (0-3) scales. 0: Indicated to plant 10% or less leaf area damage; 1: Indicated slight injury in the range of 11 to 30% leaf area injury; 2: indicated moderate injury of 31-60% leaf area injury; 3: indicated severe leaf injury exceeding 60% of the leaf area damaged by flea beetle feeding

Two different evaluations were done in both plots. Flea beetle numbers on plants were measured by counting all beetles on five plants in the center of each plot. The second measurement was assessment of feeding damage using a 0-3 scale describing the percentage of leaf area damaged by flea beetles. In this scale: 0: indicated to plots showing 10% or less leaf area injury; 1: indicated slight injury in the range of 11 to 30% leaf area injury; 2: indicated moderate injury of 31-60% leaf area injury; 3: indicated severe leaf injury exceeding 60%. Evaluations were made simultaneously by two observers walking through the plot and estimating the leaf damage. Data from all trials were analyzed by analysis of variance (ANOVA) with using the SAS software and means were separated using Student Newman Keuls (SNK) Multiple Comparison Tests (SAS,1990).

Field host plant preference trials 2002: Three different trials were established by direct seeding 3 April at the Horticulture Research Center. Individual plots were 6.09 m long double-row beds at 76.2 cm row spacing. Seven spring canola cultivars were used in the first trial, Apollo, Excel, Defender, Hyola, Helios, IMC205 and 46A65 and five of these were repeated in a second trial, Apollo, Excel, Defender, Hyola, and 46A65. The third trial compared to two spring canola cultivars, IMC204 and IMC205 (Table 4). All of three trials were arranged as a randomized complete block design with four replications. Estimates of the number of flea beetles/plant and feeding damage assessments were made in a manner similar to that used the previous season. Data from all trials were analyzed by analysis of variance (ANOVA) with using the SAS software and means were separated using Student Newman Keuls (SNK) Multiple Comparison Tests (SAS,1990).

RESULTS AND DISCUSSION

Greenhouse host plant preference trials 2000: In the first three evaluations (1, 3, 4 DAT) no significant differences were observed in numbers of flea beetles on plants ($F = 2.08$, $p = 0.0591$; $F = 1.20$, $p = 0.3291$; $F = 1.35$, $p = 0.2513$, respectively) (Table 1). On August 14 (7 DAT) greatest numbers of flea beetles were present on the spring mustard ZEM 1 ($F = 2.88$, $p = 0.0121$). WBFB feeding occurred on all cultivars, but there was a 2.7X range in the total number of beetles on plants when all counts were combined. Interestingly the two spring mustards included the cultivars that supported the greatest number of WBFB (ZEM 1) and the lowest (W1-23) ($F = 4.64$, $p = 0.0001$). The winter mustard Debut had also the greater number of flea beetle comparing to spring canolas Helios, Springfield, Westar and Alto ($F = 4.64$, $p = 0.0001$). Plant damage was generally correlated with number of adult beetles present on plants. Greatest injury occurred to ZEM 1, the winter mustard Debut and the spring canola CO1 ($F = 3.69$, $p = 0.0026$). Least plant injury was observed on the spring canolas Helios, Sterling and Alto and with the spring mustard W1-23 ($F = 3.69$, $p = 0.0026$).

Results were similar to the repeat trial (Table 2). Lowest numbers of WBFB adults were present on Alto ($F = 4.82$, $p = 0.0001$). ZEM 1 (10.3X) and Debut (7.0X) again supported the greatest number of WBFB on plants and had the greatest associated plant injury compared with Alto ($F = 4.82$, $p = 0.0001$; $F = 1.49$, $p = 0.01928$, respectively). Similarly greater feeding injury by *P. cruciferae* found on *Brassica juncea* (L.) Czern. (mustards) compared to *Brassica campestris* L., *Brassica oleracea* L. (cabbage) and *Brassica napus* L. (canola) (Palaniswamy *et al.*, 1992). In addition, the turnip (*Brassica rapa* L.) and mustard were about equally attractive to the beetles and unprotected beds are frequently destroyed (Chittenden and Marsh, 1920). However, the relatively low preference and injury to the spring mustard W1-23 found in this study suggests that mustards are not uniformly susceptible to WBFB.

Field host plant preference trials 2001: Few differences were observed in the number of flea beetles/plant (Table 3) among the seven spring canola cultivars. The cultivars 46A65 had significantly lower numbers of flea beetles, while Apollo had high numbers of beetles on the first plot but these differences were not repeated on

Table 4: Numbers of western black flea beetles/plant and leaf damage to canola cultivars. Horticulture Research Center, Ft. Collins, CO, 2002

	WBFB/5 Plants[y]				
Cultivars	15 May	23 May	28 May	7 June	Leaf damage rating[z]
Plot 1					
46A65	0.5a	3.5a	20.8ab	46.3a	1.3c
Apollo	0.3a	1.5a	24.3ab	40.3a	1.4bc
Defender	0.8a	3.0a	13.3b	41.0a	2.1ab
Excel	0.5a	3.0a	35.3a	51.3a	1.0c
Helios	0.5a	1.0a	10.0b	38.8a	2.7a
Hyola	0.8a	7.3a	38.0a	66.3a	1.7bc
IMC205	0.8a	3.8a	20.8ab	54.0a	0.9c
Plot 2					
46A65	1.8a	9.5a	44.0a	90.5a	1.0b
Apollo	0.3a	7.3a	38.0a	58.3a	1.8a
Defender	1.8a	9.0a	33.0a	56.5a	1.8a
Excel	3.2a	7.3a	44.5a	92.8a	1.4ab
Hyola	4.8a	13.5a	54.0a	81.0a	1.7a
Plot 3					
IMC204	2.3a	6.0a	49.3a	64.8a	1.4a
IMC205	1.5a	7.0a	37.8a	62.0a	1.6a

[y]Within each plot, means within a column that are followed by the same letter(s) are not significantly different ($p<0.05$) by SNK.
[z]Leaf damage rating (0-3) scales. 0: indicated to plant 10% or less leaf area damage; 1: indicated slight injury in the range of 11 to 30% leaf area injury; 2: Indicated moderate injury of 31-60% leaf area injury; 3: Indicated severe leaf injury exceeding 60% of the leaf area damaged by flea beetle feeding

the second plot ($F = 3.72$, $p = 0.0140$). No differences in amount of leaf injury were observed in the two trials ($F = 2.30$, $p = 0.0799$; $F = 2.22$, $p = 0.1283$, respectively).

Field host plant preference trials 2002: Again, few significant differences were observed in the number of WBFB found on the various spring canola cultivars (Table 4). On the 28 May evaluation significantly higher WBFB numbers were observed on Hyola and Excel ($F = 4.35$, $p = 0.0070$); this trend was consistent with Hyola on the second plot ($F = 0.74$, $p = 0.5807$). Lower numbers of flea beetles on Helios and Defender were also observed on one observation (28 May) in the first plot, but these were not repeated ($F = 4.35$, $p = 0.0070$). The highest number of flea beetle occurred the varieties of Hyola, while the lowest number of flea beetle occurred on varieties of Helios (7 June) in first plots ($F = 0.60$, $p = 0.7259$). The variety of Excel had the highest preference by flea beetle in (7 June) in second plots ($F = 2.00$, $p = 0.01591$). There was no significant WBFB preference between IMC204 and IMC205 in the third plot ($F = 0.29$, $p = 0.6286$).

Observed differences in leaf damage on 23 May indicated that the greatest damage occurred on Helios at seven cultivars ($F = 8.23$, $p = 0.0002$). Lowest leaf injury occurred on 46A65, Excel and IMC205 ($F = 8.23$, $p = 0.0002$). Cultivars of Apollo, Defender and Hyola also had greatest damage at second plot ($F = 3.91$, $p = 0.0294$). There was no significant different leaf damage injury of third plot ($F = 2.00$, $p = 0.2522$). All three of these cultivars were heavily infested by WBFB and the relatively lower leaf injury is suggestive of some host plant resistance, perhaps in the form of tolerance. Conversely, Helios sustained relatively high plant injury with WBFB plant populations that were low, suggesting in tolerance to WBFB injury.

Although all tested cultivars in these trials were susceptible to WBFB feeding injury there was a range. Gavloski *et al.* (2000) similarly noted some range among brassica cultivar to feeding by the flea beetle *P. cruciferae*. *Brassica napus* cv. Cresor had the lowest damage rating canola cultivars, although it was not significantly different from the control cultivars. *Brassica napus* cv. Zephyr showed variable resistance between seasons, being more resistant to feeding by flea beetle in the second year than in the first year of testing. *Brassica rapa* cv. Horizon was particularly susceptible to *P. cruciferae* among *B. rapa* cultivars. Furthermore, within tested *Brassica juncea* the cultivar Cutlass was more susceptible to *P. cruciferae* than was Demo. Observations in this trial generally support those of Gavloski *et al.* (2000) that currently registered varieties of canola are susceptible to WBFB as well as to *P. cruciferae*.

In conclusion, the spring mustard (ZEM1) and winter mustard (Debut) were significantly attractive and more susceptible for the WBFB. Those might be used as traps crops to protect flea beetle injury on the canola. However, spring mustard (W1-23) was less susceptibility to WBFB and suggests a possible source of reduced susceptibility in oilseed mustards. Most of currently registered varieties of canola are susceptible to WBFB feeding. The spring canola (CO1) was significantly more attractive and susceptible for the WBFB and might be used as traps crops for protecting WBFB injury on the canola crops. In

addition, spring canola (Helios) sustained relatively high plant injury with WBFB and plant had low population density and suggesting in tolerance to WBFB injury.

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