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Biometry and Responses of Faba Bean Varieties to Black Bean Aphid, *Aphis fabae* Scopoli

Hail K. Shannag and Ja'far A. Ababneh

Department of Plant Production, Faculty of Agriculture,
Jordan University of Science and Technology, Irbid 22110, Jordan

Abstract: Influence of the black bean aphid, *Aphis fabae* Scopoli, on the growth of different faba bean varieties, namely; 79S4, S82408-1-2-3, Aquadulce, FLIP87-26FB, *Vicia faba major* and Vicia faba *minor* was investigated under semiarid field conditions. Three late-nymphal instars of aphid were used to infest individual plant at 28 days after plant emergence. Results showed that aphid-infested plants were reduced in all growth parameters tested and the magnitude of damage relied on the variety. An enormous decrease in the shoot fresh and dry weights, leaf area and plant height were recorded for *V. faba major* and Aquadulce, while *V. faba minor* variety tolerated the aphid attack. The number of aphids increased exponentially at an early growth stage of *V. faba major* and Aquadulce, causing ultimately plants to die and thus aphid populations crashed. On other varieties, aphids propagated incessantly, reaching a peak at days 56 days after artificial infestation, but the infestation rates were variable with reliance on variety. Subsequently aphid populations declined steadily until the end of the growing season. Aphid-free varieties fluctuated in their growth rates during the study. S82408-1-2-3, 79S4 and FLIP87-26FB varieties produced overall plants with maximum sum of plant height, shoot fresh and dry weights, as well as leaf area, whereas *V. faba minor* was at least.

Key words: *Aphis fabae* · faba bean · plant growth

INTRODUCTION

Faba bean, *Vicia faba* L., is one of the most important legume crops around the globe [1]. In the Mediterranean region, faba bean is a stable food and cheap source of high quality protein for most population [2]. It is considered also as a great prolific animal resource as feed to all types of livestock [3] and used to make a silage of high quality in some countries [4]. Faba bean is capable to fix atmospheric nitrogen through the symbiotic relationship with *Rhizobium*-bacteria and so improves the nitrogen status in soil [5].

In Jordan, faba bean is the most common and widely used legume after lentil. The area planted to this crop under both rainfed and irrigation conditions compromises approximately 14% of the total area seeded to legumes [6]. However, the total production of faba bean is still low and far below the country's needs. In spite of the increasing demand for the faba bean in the country, the area designated to this crop and the annual production are decreasing due to low and erratic rainfalls, planting traditional low yielding cultivars, poor cultural practices and pest infestation [7]. Moreover, the black bean aphid, *Aphis fabae* Scopoli, is a major constraint of faba bean production, which inflicts a destructive damage to faba bean throughout the world. In addition to direct plant injury, aphid infestation harms extensively faba bean by honeydew excretion, which stimulates the growth of sooty mold. Honeydew deposited on the leaves interferes with some physiological processes in the host plant [8].

The high damage potential and unpredictability of *A. fabae* infestation usually lead to an extensive pesticide application based often on a fixed schedule. However, there are significant economical, environmental and health cost associated with this approach, which result in an increasing awareness of usefulness of integrated pest management schemes in which host plant resistance must have a central role. Several authors have recognized the potential value of plant resistance for controlling *A. fabae* and therefore some partially resistant faba bean cultivars were identified against this aphids [9-13]. However, high levels of resistance were detected only in landraces, progenies and wild relatives of *V. faba* [14].

Corresponding Author: Hail K. Shannag, Department of Plant Production, Faculty of Agriculture, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan

The present study was conducted to assess the responses of different faba bean varieties to the infestation by *A. fabae* under semiarid field condition, as indicated by the measurements of shoot fresh and dry weight, leaf area and plant height.

MATERIALS AND METHODS

Stock of black bean aphid, *A. fabae,* was collected from infested fields of faba beans in the Jordan Valley, Jordan. Aphids were reared on potted faba bean plant, *V. faba major* under organdy screened cages (80×60×60 cm), in an insectary at a temperature of 20±3°C, 46-80% relative humidity and 16L:8D photoperiod. New faba bean plants grown under greenhouse conditions were added when old plants senesce as a result of high feeding pressure of aphids. In order to infest the experimental plants with similar aged aphids, a synchronized colony of *A. faba* was established. Apterous adults were transferred from stock colony onto two-week old *V. faba* plants placed in a new cage. Cages were covered in sides with organdy screen and the top with transparent plastic sheet. Aphids retained on the plants for 4-5 h to produce progeny. Then, adult aphids were removed and the offspring were allowed to develop until they reach late-nymphal instars (8-days).

Seeds of faba bean varieties; 79S4, S82408-1-2-3, Aquadulce and FLIP 87-26-FB, provided by International Center for Agricultural Research in Dry Area (ICARDA) and two wide cultivated varieties in the region, *V. faba major* and *V. faba minor*, were grown in the field on Jordan University of Science and Technology campus, Irbid, Jordan. Seeds were hand planted in three rows per plot with 30 inter row space and 20 cm intra row space. Plants were watered by a drip irrigation system and fertilized by diammonium phosphate (18N-46P-0K) at rate of 30 kg ha^{-1} prior to seeding. Weeds were removed manually as needed.

Split plot design with four replicates was used in this experiment. Each block was divided into main plots with six units (subplots) of 1 m^2 with protection spacing of one meter between the units. Faba bean varieties were distributed randomly in each unit, each one contained 12 plants. At the time of aphid infestation, plots were randomly arranged into two groups, control and infested. Each experimental plant in infested group was infested at 28 days after plant emergence by three fourth nymphal instars (8 days old) obtained from a synchronized colony. Control plants remained aphid-free. Immediately after aphid release, all treatments including control were covered with organdy-screen cages, each measuring 1L×1W×1H m.

Three plants from each replicate were randomly sampled at 21, 42, 63 and 84 days after the artificial infestation. Sampled plants were cut direct above ground, placed individually in plastic bags and, thereafter, the plant height and shoot fresh weight were measured in the laboratory. Plants were then dried separately in drying oven at 68°C for 48 hrs and shoot dry weight was weighed. Leaf area of each plant was determined using a leaf area meter type LI-3000 area meter (Li-Cor. Inc., Lincoln, NE). Number of aphids was estimated at two-week intervals during the study. Data were subjected to analyses of variance (Two way ANOVA) using MSTATC software (Michigan State University, 1988). Means were compared using Fisher's least significant differences (LSD) test at a 0.05 probability level.

RESULTS

Aphid populations on different faba bean varieties: *A. fabae* populations on different faba bean varieties are illustrated in Table 1. Results indicated that there were differences in the development of aphid populations between faba bean varieties. Aphids started to increase

Table 1: Population growth of *Aphis fabae* on six faba bean varieties under semiarid field conditions

	Number of aphids after					
Varieties	14 days	28 days	42 days	56 days	70 days	84 days
79S4	163.3a	450.0a	2417.0ac	5267.0a	3067.0a	701.0a
S82408-1-2-3	155.0b	445.0a	2217.0a	6000.0b	3867.0b	504.0b
Aquadulce	193.3c	983.3b	5400.0b	9800.0c	-	-
FLIP87-26FB	180.0b	600.0c	2983.0c	5350.0a	3400.0c	633.3a
Vicia faba major	205.0c	1200.0d	7917.0d	-	-	-
Vicia faba minor	175.0b	1033.0b	4283.0e	6650.0d	4750.0d	833.3c
LSD	5.99	62.84	597.9	266.5	192.4	68.74

Means followed by same letter(s) within each are not significantly different at p = 0.05

Table 2: Average plant height (cm) of different faba bean varieties infested by *Aphis fabae* for different periods of time

	Days after infestation											
	21			42			63			84		
Varieties	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)
79S4	54.0a	40.5*	25.0	63.7a	60.9	4.4	76.5a	68.9*	9.9	82.5a	69.9*	15.2
S82408-1-2-3	54.7a	44.5*	18.7	61.5a	52.1*	15.3	75.9a	68.6*	9.6	78.6a	70.0*	10.9
Aquadulce	60.2a	39.2*	34.9	64.2a	46.2*	28.0	78.1a	48.7*	37.6	-	-	-
FLIP87-26FB	56.0a	47.8*	14.7	59.7a	49.3*	17.4	68.8b	60.2*	12.5	73.1b	62.1*	15.0
Vicia faba major	56.7a	41.7*	26.5	58.8a	49.0*	16.7	-	-	-	-	-	-
Vicia faba minor	56.0a	47.3*	15.5	60.5a	49.0*	19.0	63.6b	54.3*	14.6	67.7c	58.7*	13.3
LSD	7.381			7.094			5.312			5.045		

Means followed by same letter (s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

obviously in the number at 28 days followed aphid release, reaching a peak of day 56. *A. fabae* population was mainly abundant on *V. faba major* during the first six weeks and it's number exceeded significantly the aphid populations on the other varieties, apart from Aquadulce on 14 and 28 days. Aphid quantity on Aquadulce ranked in the second place, increasing significantly at 28, 42 and 56 days with respect to other treatments, excluding *V. faba minor* over 28 days. The growth of these tremendous aphid populations at an early stage of *V. faba major* and Aquadulce development caused these both varieties to die prematurely and the aphid populations on them to collapse at 42 and 56 days respectively. However, aphids achieved a maximum number on *V. faba minor*, FLIP87-26FB, 79S4 and S82408-1-2-3 varieties at 56 days which later dropped steadily until the end of growing season. Among these still alive varieties, aphids developed significantly a greater number on *V. faba minor* than the individuals on 79S4, S82408-1-2-3 and FLIP87-26FB during all monitoring dates, apart from days 14. On day 42, there were no significant differences in aphid numeral between 79S4 and S82408-1-2-3 varieties. However, aphid densities on S82408-1-2-3 variety exceeded significantly those on FLIP87-26FB and 79S4 at days 56 and 70, but decreased to a minimum on day 84.

Biometry of aphid-infested *V. faba* varieties

Effect of *A. fabae* on plant height: Results indicated that aphid-free varieties showed clear differences in the plant height during the growing season (Table 2). After 42 days, aphid-free faba bean varieties did not differ significantly among each other. However, FLIP87-26FB, *V. faba minor* and *V. faba major* varieties were more reduced in the plant height than other varieties on day 63. At the end of growing season (84 days), all tested varieties fluctuated significantly in the plant height among each other where 79S4 variety produced the tallest plants and *V. faba minor* was the shortest one.

Aphid attack harmed considerably the plant height on all sampling dates with respect to the relevant controls, except for 79S4 variety at 42 days (Table 2). This reduction ranged between 4-38% depending on variety and infestation interval. Aquadulace variety was most impaired by aphid feeding, showing a 28-38% decrease in the plant height in comparison with respective control.

Responses of shoot fresh and dry weights to aphid infestation: Aphid-free faba bean varieties varied remarkably in the shoot fresh weight among each other during the experiment (Table 3). After 21 days, 79S4, Aquadulce and FLIP87-26FB varieties produced the greatest shoot fresh weight, while *V. faba minor* was as a minimum. Three weeks later (42 days), however, the shoot fresh weights were about equal by all varieties, except for minor weight of *V. faba minor* variety. By day 63, the average shoot fresh weight of 79S4 and S82408-1-2-3 was greater than those of other varieties. However, at the last sampling date still alive faba bean varieties did not show significant differences among each other.

In all treatments, aphid infestation induced a 9-61% decline in the shoot fresh weight with reliance on variety and infestation period. Aphid feeding induced significant reductions in this parameter on faba bean varieties at 21 and 63 days. *V. faba major* variety suffered actually from aphid attack more than other varieties showing evidence of 62% and 44% decline in the fresh weight on 21 and 42 days, respectively (Table 3). In general, injury levels were

Table 3: Effect of *Aphis fabae* on the shot fresh weight of different faba bean varieties at different infestation times

	Days after infestation											
	21			42			63			84		
Varieties	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)	Control	Infested	Red. (%)
79S4	194.9a	68.2*	65.0	210.2a	192.0	8.7	222.3a	170.1*	23.5	243.8a	191.8*	21.3
S82408-1-2-3	151.2bc	64.9*	57.1	217.1a	193.3	11.0	241.3a	180.4*	25.3	249.5a	177.4*	28.9
Aquadulce	192.5a	65.6*	65.9	202.8ab	154.6*	23.8	207.4ab	145.7*	29.7	-	-	-
FLIP87-26FB	187.0ab	136.1*	27.2	208.1ab	184.4	11.4	219.0a	140.1*	36.0	232.3a	203.9	12.2
Vicia faba major	153.6b	59.0*	61.6	176.6bc	98.5*	44.2	-	-	-	-	-	-
Vicia faba minor	116.7c	55.7*	47.7	156.2c	112.5*	28.0	176.1b	103.7*	41.1	212.7a	193.5	9.0
LSD	36.06			32.51			36.71			44.07		

Means followed by same letter(s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

Table 4: Average shoot dry weight (g) of aphid-free and *A. fabae*-infested faba bean varieties at different times after aphid infestation

	Days after infestation											
	21			42			63			84		
Varieties	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%
79S4	26.42a	8.72*	67.0	32.07ab	27.87*	13.1	33.80a	28.67*	15.2	35.40a	31.03*	12.3
S82408-1-2-3	22.99b	9.60*	58.2	33.82a	27.87*	17.6	34.80a	29.67*	14.7	36.37b	31.77*	12.6
Aquadulce	26.32a	9.14*	65.3	29.87b	24.33*	18.5	30.87b	26.67*	13.6	-	-	-
FLIP87-26FB	21.97b	11.44*	47.9	30.95ab	24.87*	19.6	31.60b	26.53*	16.0	32.60c	28.93*	11.3
Vicia faba major	21.34b	6.26*	70.7	29.60b	14.20*	52.0	-	-	-	-	-	-
Vicia faba minor	18.95c	6.27*	66.9	26.42c	17.97*	32.0	27.97c	20.00*	28.5	30.23d	25.38*	16.0
LSD	1.911	3.158	1.300	0.619								

Means followed by same letter(s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

more prominent on 21 days after aphid release, which ranged from 27.2 to 65.9% depending on variety.

Variations in the shoot dry weight were also apparent amongst aphid-free varieties during the whole plant growth period (Table 4). Shoot dry weight of *V. faba minor* was significantly lesser than other varieties throughout the experimental period, while S82408-1-2-3 and 79S4 varieties produced generally the highest dry weight. When aphids were confined to the plants, all varieties decreased obviously in the mean shoot dry weight (Table 4). However, damage level turn down commonly with the progressive plant growth. After 21 days, the relative dry weight of infested plants varied between 47.8-70.6% of the respective controls with reliance on varieties. 10-11.3% decrease in the dry weight was only recorded between still alive aphid-infested plants on 84 days. Sever damage was apparent on *V. faba major* prior to its death due to heavily aphid infestation, followed by *V. faba minor* for the rest of experimental period. Other varieties, 79S4, S82408-1-2-3, Aquadulce and FLIP 87-26-FB, showed variable responses to aphid injury within all sampling dates.

Impact of aphids on leaf area: Aphid-free *V. faba* varieties demonstrated apparent differences in the leaf area among each other (Table 5). FLIP87-26FB variety generated significantly a greater leaf area than other varieties overall the experimental period, excluding at days 63. Minimum leaf area was produced by *V. faba major* in the first and the second sampling dates and then by *V. faba minor* for the rest of the growing season.

Also, aphid infestation impaired obviously the leaf area of fabae bean varieties. Significant differences in the leaf area were recorded between aphid-infested varieties and their respective controls in all sampling date, except for 79S4 on day 42 and FLIP87-26FB

Table 5: Mean leaf area (cm^2) of different faba bean varieties infested with *A. fabae* after different infestation periods

	Days after infestation											
	21			42			63			84		
Varieties	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%	Control	Infested	Red.%
79S4	981a	798*	18.7	1285a	1163	9.5	2246 a	1976*	12.0	2323a	2131*	8.3
S82408-1-2-3	1549b	1250*	19.3	1883b	1520*	19.3	2197 a	1969*	10.4	2288a	2129*	6.9
Aquadulce	1561b	1446	7.4	1733c	1082*	34.7	1866 b	1218*	34.7	-	-	-
FLIP87-26FB	2124c	1915*	9.8	2242d	2032*	9.4	2396 ac	2142*	10.6	2465b	2389	3.1
Vicia faba major	901 da	602*	33.2	990e	481*	51.4	-	-	-	-	-	-
Vicia faba minor	1072 ea	858*	20.0	1224a	975*	20.3	1622 d	1021*	37.1	2106c	1900*	9.8
LSD	119.37			131.53			106.98			78.62		

Means followed by same letter(s) within each date are not significantly different at p = 0.05, Numbers joined with (*) are significantly different from the respective control at p = 0.05

on day 84 (Table 5). In general, leaf area of *V. faba major* was harshly injured, with moderate damage on 79S4, S82408-1-2-3 and Aquadulce varieties. FLIP87-26FB variety was more tolerable to aphid attack than other varieties during the whole experimental period.

DISCUSSION

Aphid-free faba bean varieties fluctuated widely in the plant height, shoot fresh and dry weights, in addition to the leaf area under semiarid field conditions. S82408-1-2-3, 79S4 and FLlP87-26FB varieties showed in general the greatest vegetative growth rates, whereas *V. faba minor* was as a minimum. Substantial differences in the yield components were also recorded by Ishang [15] using other faba bean varieties and genotypes. These variations in the growth rates of faba been varieties could be attributed to the different adaptation talents of crop variety for the environmental conditions prevailing during the experiments [16], as well as to the erratic genetic complements of varieties.

All the six tested varieties responded to heavy aphid infestation through reducing the plant biomass. With respect to the vegetative components examined thus far, *V. faba major* and Aquadulce varieties appear to response more sensitive to the reduction in shoot fresh and dry weights, leaf area and plant height, while *V. faba minor* is more tolerant to aphid attack. Changes in these growth parameters were more evident at days 21 after aphid infestation, which concurs, to a large extent, with finding of Prüter and Zebitz [13] using a combination of *A. fabae* with other faba bean varieties. In contrast, Hawkins *et al.* [17] ascertained the greatest reduction in *V. faba* growth rate on the first week as a result of infestation by *A. craccivora*. These contrary results may indicate that the responses are specific to the plant-aphid combinations investigated

Sever damage to *V. faba major* and Aquadulce observed in the present study can be caused by the exponential increase in aphid populations at an early stage of plant development, which may exceeds the carrying capacity of aphid injury resulting ultimately in prematurely death of those both varieties. The other four varieties can be classified as the less attractive nourishment for *A. fabae*, since they delayed the development of aphid populations and therefore become more capable to overcome the sensitive growth stage at the beginning of infestation. In this case, the ratio of removed to produced assimilates during the further course of infestation is probably more advantageous for the growth of old plants [13].

Less favored plant varieties by aphids, sometimes referred as resistant or partially resistant varieties, have been reported to have deleterious effects on the reproductive rate, nymphal survival, longevity of original adults and development rate of aphids, including *A. fabae,* compared to the susceptible ones [9, 11, 18-20]. Changes in the host vulnerability to the black bean aphid have been partially referred to the chemical composition in the plant tissues, particularly the total free amino acids [9, 21-23] and/or morphological traits of the plant [24-26]. A low tolerance of *V. faba,* cv. Diana to *A. fabae* attack has been attributed to a prior high production potential of this cultivars, which does not allow any considerable increase to compensate for occurring injury, compared to resistant *V. faba,* cv. Bolero [13]. However, the resistance to different pests on one host might not be the same basis [18].

The mechanisms underlying the reduction of growth components of faba bean by aphid might include the removal of assimilates and adjusting the sink-source ratio to the benefit of aphids [27, 28]. The absolute decline of photosynthetic surface area of plants [29], the excretion, with aphid saliva, of toxic or phytohormone-analogue compounds [30, 31] and/or a combination of these factors [32] may be also accountable for the reduction in plant biomass. Besides these reasons, both honeydew deposited on the leaves and the growth of sooty molds can hamper photosynthesis, transpiration and respiration of host plant [33].

Moreover, aphid populations did not increased incessantly during the whole experiment, but a decrease in aphid numbers on less sensitive varieties started after 59 days. This reduction could be caused by altering host plant to an interior food source for aphids under heavy infestation [34] and/or by obligating aphid individuals to compete with each other on available food source or to feed on less nutrient parts of the plant, which affect adversely the fecundity and reproductive rate of aphids [35].

In summary, this study showed that the vegetative growth of aphid-free faba bean varieties varied considerably. Aphid infestation induced an obvious injury to *V. faba* plants. There was no immune variety among test faba bean varieties, but the magnitude of damage was greatest on *V. faba major* followed by Aquadulce, whereas other varieties proved a moderate tolerance to aphid attack. Therefore, none of these varieties could be recommended to introduce into a breeding program for plant resistance towards the black bean aphid. However, introduction more tolerant variety into agro-ecosystem leads often to a reduction in the pesticide application frequency and, therefore, the risk of pesticide use is minimized. Although, the basic information clarified in this study indicated that a further screening for *A. fabae* resistance among other genotypes, varieties and lines is worthwhile.

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