

1. INTRODUCTION

Broad bean *Vicia faba* L. is one of the oldest cultivated field crop around the whole universe, where it constitutes a cheap source of protein for large proportion of the population (FAO, 1995).

The total area planted of green bean in Jordan was 12,240 dunoms in Jordan Valley and high lands, producing 16891 tons. In addition, the total area planted of dry bean was 3,265 dunoms in the high lands. Producing 262 tons (Statistical Year Book, 1996). The worldwide production of this crop is reduced by a number of factors, of which pest infestation is the most limiting one.

Black bean aphid *Aphis fabae* Scopli (Aphididae: Homoptera) is recognized as polyphagous insect pest feeding upon secondary host plants. In Jordan it is a primary insect on *Vicia faba* (Mustafa and Qasem, 1984), on sugar beet and other legumes in most European countries (Blackman and Eastop, 1985).

Over the long term plans, pesticides used in reducing the aphid damage become ineffective, due to the hazardous effect of pesticides on humans, other living organisms, environment pollution and development of resistant strains after an extensive use of insecticides. This necessitates the need for finding other control measures associated with the integrated pest management programme, which include the usage of resistant cultivars, potential bioagents and monitoring the flight activity of the alate forms.

Since little information are available on aphid resistant cultivars, this study was conducted to fulfill the following goals:

1. To determine the susceptibility of different vegetable and legume cultivars to *A. fabae* infestation,

2. To investigate some aspects of the aphid reproductive biology on different broad bean cultivars.
3. To study the effect of temperature on aphid reproduction and,
4. To monitor the flight activity of the aphid by using suction trap in the Central Jordan Valley.

LITERATURE

REVIEW

2. LITERATURE REVIEW

2.1. Distribution And Host Range In The World

Black bean aphid, *Aphis fabae* Scopoli is widely spread in temperate regions of the Northern Hemisphere, North and South America, Africa, middle East and Asia, but never in Australia (Blackman and Eastop, 1985; Cammell and Way, 1983). It is a key pest in temperate and Mediterranean climates (Cammell and Way, 1983). It has been recorded as a major insect on several plants belonging to various families in many countries (Bodenheimer and Swirski, 1957; Leonard, 1967; Blackman and Eastop, 1985; Younis *et al.*, 1985; Mustafa, 1988; El-Jassani and El-Adel, 1991).

2.2. Morphology

The classification of *A. fabae* has been the subject of considerable review because it is one of several very closely related black aphids (Cammell and Way, 1983). An early confusion arose because it depended on specific aphid/host-plant relationships which failed to recognize the polyphagous nature of *A. fabae* and this led to complex synonymy. The bodies of both winged and wingless forms of black bean aphid are black or brownish-black (Cammell and Way, 1983).

2.2.1. Apterous morph

Body colour varies from brown to black with irregular darker pigmented areas over the abdomen (Avidov and Harpaz, 1969). Antennae are 5-6 jointed, forms of the aptera are without secondary rhinaria (Bodenheimer and Swirski, 1957).

The antennal tubercles are little developed (Blackman and Eastop, 1985). Dorsal abdomen mainly unsclerotized, while parts of the head and thorax, stigmal plates, rows of small reticulated areas, siphunculi, cauda, anal and subgenital plates are sclerotized (Bodenheimer and Swirski, 1957). Cauda are dark long shaped (Avidov and Harpaz, 1969; Blackman and Eastop, 1985; Bodenheimer and Swirski, 1957), longer than its basal width, with more than 10 hairs (Blackman and Eastop, 1985). The longest hairs on the dorsum of hind femora about 0.8- 1.3 of basal diameter of this joint, 2- 3.5 times as long as median diameter of III antennae (63-87 μ). Apical joint of rostrum shorter than or equal to second joint of hind tarsi. Siphunculi 0.09-0.16 of body length, cauda 0.6- 1 of the length of siphunculi. Antennae are pale, with I, II, VI and some times transverse bars on abdominal segments VIII, VI or VIII (Bodenheimer and Swirski, 1957). Body length is 1.7-2.9 mm (Avidov and Harpaz, 1969) [Plate 1].

2.2.2. Alate morph

Head and thorax are black, abdomen varying from brownish black to dark olive green, usually with five irregular pigmented areas along the sides of the back and irregular transverse areas segmentally arranged. Body length is 1.7-2.7 mm (Avidov and Harpaz, 1969) [Plate 2].

2.2.3. Different instars

The aphid has four nymphal instars. The differences between the instars usually recognized by different-sized nymphs were last instars are larger, with longer appendages, bears more hairs and the number of segments in the antennae varied from 4 or 5 in 1st- instar, 5 in 2nd- instar, 5 or 6 in 3rd- instar and 6 in 4th- instar and adult (van Edmen, 1972).

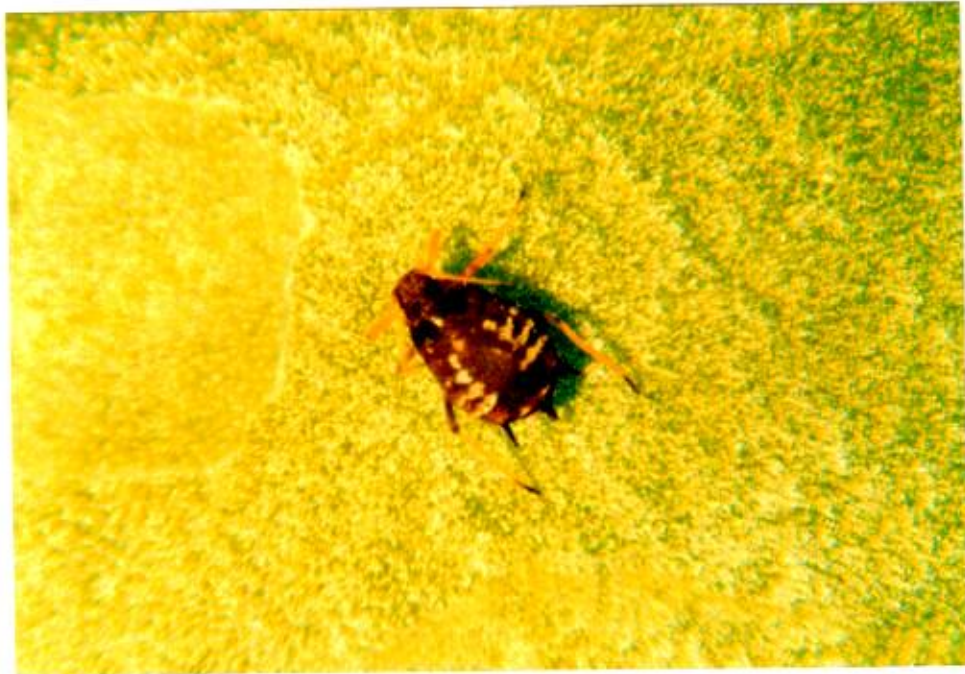


Plate 1 : Apterous morph



Plate 2: Alate morph

2.3. Aphid- Host Plant Relationship

2.3.1. Host preference

The principle of aphid location of fresh host plants, is governed by windborne dispersal of alate adults, physiological and behavioral mechanisms of aphid (Cammell and Way, 1983), including light, wind, temperature, relative humidity, pressure and wound. Along with color, odor and surface textures of host plant are also important (Dixon, 1985). Specific substances particularly phenols, oils, or alkaloids are known to influence probing, feeding and possibly guidance (Harris and Maramorosch, 1977). The growth and reproduction of aphids are dependent upon the state of growth or level of soluble nitrogen in their host plants (Dixon and Dharma, 1980 b). Usually phloem is rich in sugar but relatively poor in amino acids; thus aphid has to ingest large amount of sap to acquire sufficient protein (Dixon, 1985). *A. fabae* while feeding ingests large quantities of saliva into plant, such saliva contains many substances that affect the metabolism of plants (Dixon, 1985). Thus, improvement in quality of food available which results in the development of large and highly fecund aphids (Dixon, 1985; Dixon and Wratten, 1971). The degree of sensitivity against aphid infestation increases with the increase of the percentage of protein and nitrogen (Bond and lowe, 1975). However, aphid host preference could be due to the differences in the chemical composition and/or the morphological features (Banks and Macculary, 1964; Younis *et al.*, 1985).

2.3.2. Resistant varieties

A plant may be tolerant or able to produce well despite infestation that damage susceptible plants, or it may have antibiosis that contains materials injurious to the insect feed on it, or the part fed on may lack

some necessary nutrient (Pianter, 1951). Some amino acids may depress aphid performance (Cammell and Way, 1983). Fewer alate *A. fabae* settled on resistant plants and aphids multiplied more slowly (Banks and Macculay, 1964; Bond and Lowe, 1975; Cammell and Way, 1983). High percentage of nitrogen and protein increase the susceptibility of the host plant to aphid infestation (Bond and Lowe, 1975; Younis *et al.*, 1985). Secondary plant substances are also involved in aphid host selection and subsequent development. These may act as attractants or repellents (Cammell and Way, 1983).

Plant that does not react adversely by callous formation will be considered tolerant to the aphid infestation. Usage of plant physical and morphological characters in aphid control, e.g. hairs, cuticular wax could be used in breeding more resistant plant (Harris and Maramorosch, 1977; Holt and Birch, 1984).

2.4. Biology And Ecology

Over much of Europe *A. fabae* is heteroecious holocyclic, alternating between *Euonymus europaeus*, or sometimes *Viburnum opulus* and a wide range of secondary host plants (Blackman and Eastop, 1985; Cammell and Way, 1983). In Europe, eggs laid from October to December on the primary winter host (Blackman and Eastop, 1985). Eggs hatch in late February to April into parthenogenetic apterous females on *E. europaeus*. Winged migrants increased in mid-May to early June, where they colonize on herbaceous secondary hosts e.g. *V. faba*, sugar beet and many species of weeds, especially *Chenopodium album*. Through the summer they produce apterous individuals then produce alate virginoparae, late in September. Winged female gynoparae return to *E. europaeus*, where apterous oviparae are produced and then mate with winged males

migrating from the summer hosts (Cammell and Way, 1983; Dunning *et al.*, 1979). The rate at which colonies develop and increase the infestation depends on several factors; weather is one important factor; in warm sunny weather development is rapid and young may become mature in 8 days, but in cold and wet conditions maturity occur after 18 days (Dunning *et al.*, 1979).

2.4.1. Fecundity and longevity

All studies assumed that fecundity is an increasing function of reproductive effort which reduces survival. Therefore, the correlation between survival and fecundity is negative (Ward *et al.*, 1983).

Twelve ovarioles are typical for *A. fabae* and no difference could be detected between alatiforms and apteriforms (Tsitsipis and Mittler, 1976 a, b&c). The variation in ovariole number between individuals in each generation in the first few generations are not related to their weights nor to variation among clones (Dixon and Dharma, 1980 a; Dixon and Wratten, 1971).

Dixon and Dharma (1980 a) found that there was no significant relationship between the number of ovarioles in aphid's ovaries, its weight and the number of ovarioles in its mother, nor the individuals in the second, third or fourth generations.

Quality of the food available to aphids could influence ovariole number (Dixon and Dharma, 1980 a) and growth efficiency of aphids (Llewellyn and Leckstein, 1978). Total embryo growth and fecundity depend on nymphal investment which is related to the nutrition available to the adult (Ward *et al.*, 1983). Records showed that alate aphids contain fewer embryos than aptera of the same weight (Dixon and Wratten, 1971), also large apterae contained twice as many embryos than small apterae

(Dixon and Dharma, 1980 b). Born nymphs of alatae are lighter in weight than those born of apterae (Dixon and Wratten, 1971).

The rate of mortality increased and post-starvation longevity declined significantly with the duration of the starvation period. This is due to the inability of severely stressed animals to resetttle and begin feeding. The percentage of aphids surviving more than 3 days on plant declines significantly with the duration of starvation period. Starvation reduces life-time fecundity but increases the reproductive rate immediately after nutrition improves (Leather *et al.*, 1983). When aphid develops on a poor food supply, aphids with a high ovariole number possibly suffer a higher mortality than those with fewer one (Dixon and Dharma, 1980 a).

Aphid longevity differs with the differences of the chemical composition and/ or the morphological features of host plant (Younis *et al.*, 1985). Aphid longevity measured on different broad bean cultivars in Iraq; was the longest on Quwadaige cultivar about 23.5 days; while on 121FAO cultivar was 17.7 days (Younis *et al.*, 1985).

2.4.2. Population dynamics

Aphids could become very abundant on agricultural crops. A hectare of field beans could produce 4000 million alatae of *A. fabae* (Dixon, 1985). The size of aphid population on *V. faba* varied from one year to another. This depends on the sequence of events in the non-crop environment (Way, 1967). The greater the number of aphids hatching in spring, the greater the number of summer migrants (Dixon, 1985). Weather through its effect on the aphid rate of increase and colonization on plants, could modify the activity of natural enemies (Way and Banks, 1964, 1967 & 1968).

In years when large populations occur on crops and other summer hosts, natural enemies multiply on the abundant aphids and prevent the establishment of colonies on new hosts (Banks, 1958, 1962 & 1968). In years following an outbreak, natural enemies decline because aphids are scarce on crops early in the season (Cammell and Way, 1983).

2.4.3. Effect of temperature on reproduction

The number of alate *A. fabae* examined showed that the switch from ovipara to virginopara production by the alate occurred gradually from 22.5°- 25.5°C (Tsitsipis and Mittler, 1976 a). The production of alatae and ovipara decreased above 15.5°C (Tsitsipis and Mittler, 1976 a).

A. fabae produces sexual, egg-laying females ovipara only at low temperature below 20°C under short-day conditions from 8 to 12h light per day that induce the development of sexual females (Tsitsipis and Mittler, 1976 c). Generally, growth rate increase in successive instars, but at 23.5°C it declines as a result of poor growth in the last instar due to deterioration of leafdiscs at higher temperatures (Tsitsipis and Mittler, 1976 b). At 20°C the apterae produced more nymphs than the alatae from the fourth day onwards. As the apterae were 22% heavier than alatae, the differences in fecundity reflect the difference in size of the two morphs (Dixon and Wratten, 1971). At 10°C there was an increase in fecundity with increase in adult weight. Results showed that no difference between fecundity of apterae and alatae in the first 10 days at 10°C (Dixon and Wratten, 1971).

2.4.4. Dispersal and flight activity

Some alatae of *A. fabae* are strong migrants that have well-developed wings and does not deposit nymphs before flight. Others that

are less migratory, called fliers, produce few nymphs before flight, and some never fly known by non-fliers (Cammell and Way, 1983). Aphid colonies on *V. faba* tend first to produce 'non fliers' and 'fliers' and then a greater proportion of 'migrants' but by the end of the life of the colony the proportion of 'fliers' and 'non fliers' increases (Cammell and Way, 1983).

Flight is prevented only by low temperature and darkness and may be delayed by strong winds (Cammell and Way, 1983).

Alate *A. fabae* fly for a maximum of 2 hr although they have an average 7-12 h of fuel reserves as glycogen and fat (Cammell and Way, 1983). Two peaks of alate *A. fabae* occur daily in the air, one in mid-morning that consists of alate adults that matured overnight but prevented from flying by cold and darkness. While the other peak is in early afternoon consists of individuals formed during the morning and matured at the afternoon (Cammell and Way, 1983).

MATERIALS AND

METHODS

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3.1. Aphid Slide Preparation

Individuals of *A. fabae* were collected from black nightshade *Solanum nigrum* L. from the Central Jordan Valley in early spring 1997.

For permanent storage of the aphids, a permanent microscopic slide was prepared for both apterous and alate one. Aphid clearing and macerating techniques was accomplished according to that proposed by van Edmen (1972). Adults of aphid were transferred to 75 × 15 mm tube; 75% ethanol was added and plugged with cotton wool, then boiled in a water bath for 5 min. The alcohol was decanted and replaced with 3-4 ml of 10% KOH. The mixture was gently boiled over naked flame for 1-2 min. The decanted 75% alcohol was then added to the tube to dilute KOH solution. The mixture of alcohol and KOH was decanted carefully. Finally, a few clean 75% alcohol was poured to rinse the aphids. A well-cleaned slide was placed in position and small amount of mountant placed in the center of the slide by a dropper pipette carefully. By camel hair brush aphid were transferred to the slide, legs and antennae were then spread out before put the cover slide. Afterward the mounted slides were placed in the oven for 24h to dry. Finally aphid slides were examined under the microscope.

3.2. Glasshouse And Laboratory Experiments

Adults of *A. fabae* Scopl. used in glasshouse and laboratory experiments were collected from nightshade *S. nigrum* and broad bean *V. faba* in the University Farm Station in Central Jordan Valley in late January to early February 1997. These individuals were reared on broad

cage (1×1×1 m) covered with orchanza at the glasshouses in the Faculty of Agriculture, University of Jordan [Plate 3]. Plants were irrigated every two days, fertilizer (20: 20: 20 NPK) was mixed with irrigation water weekly. The temperature ranged from 16-30 °C, and relative humidity from 46- 80%.

3.2.1. Reproduction of *A. fabae* on different vegetable crops

Seedlings of tomato cv. Dual pride F1, squash cv. hybrid, sweet pepper cv. Calextra, cowpea cv. California black eye, kidney bean cv. Wonder brown, broad bean cv. Giza 4 and common pea cv. Onward; were transplanted in pots with 9 cm diameter and 14 cm deep filled with 2: 1: 1 mixture of peatmoss, sand and clay, respectively. The pots were replicated seven times in a completely randomized design inside a wooden cheese cloth growth chamber in the Faculty of Agriculture labs at the University of Jordan.

When the tomato, squash and sweet pepper plants were six weeks old and the cowpea, common pea, broad bean and kidney bean plants were three weeks old, a single fourth instar nymph from the stock culture (see 3.2) was placed on the lower surface of the second or third leaf from the tip of each plant and left to select their own feeding sights. Then the nymphs were caged with small clip-on cages [Plate 4] and left to develop and produce for 14 days. The newly nymphs produced were counted every other day and weighed weekly using Sartorius microbalance (Model 4503) sensitive to 0.002 mg [Plate 5] . Average daily temperature ranged from 22-33 °C, and 47-70% relative humidity with 16 h photoperiod, using two 15 W day light fluorescent lamp per 20 plants.



Plate 3: Wooden cheese cloth cage



Plate 4: Clip-on cage



Plate 5: The Sartorius microbalance

The clip-on cages were, 2 cm in diameter and 1 cm deep, made from transparent plastic rings covered with cheese cloth (Orchansa) at one end. Spongy circular rubber gasket was fixed to the ring rim at the other end to minimize leaf injury. These cages were specifically used to restrict the aphids to a circular section of the leaf. This type of caging was used by several workers (van Edmen, 1972; Masha'l, 1990; Samhan, 1990) to study aphid biology.

3.2.2. Reproductive biology of *A. fabae* on different broad bean cultivars

Four weeks broad bean seedlings cultivars Syrian Local Large, Syrian Local Medium, Aquadulce and Reyba, brought from ICARDA in Aleppo, Syria were transplanted to 7 cm wide cube plastic pots filled with a mixture of peatmoss, sand and clay 2:1:1, receptively sited randomly inside a wooden cheese cloth growth chamber in the Faculty of Agriculture labs at an average of 22-33°C temperature, 46-80% relative humidity and 16h photoperiod. To minimize the plants and aphids disturbance, irrigation method was made by pouring water in the tray containing the pots. The water moved upwards through the holes in the bottom of the pots. Complete fertilizer (20:20:20 NPK) was added to irrigation water weekly.

Single fourth-instar nymph taken from stock culture (see 3.2) was placed on the lower surface of the 2nd or 3rd leaf from the plant tip. The nymph was clipped by a clip-on cage (see 3.2.1) The nymph remained in the cages until they became adults and started to produce. Later, the adult and all of its progeny were removed, except a newly born nymph that was the closest to the mother, was retained in each cage and reared to maturity.

Aphids were handled smoothly using a camel hair brush to avoid injuring aphids while they were probing, touching them gently with the tip of the brush to induce stylet withdraw before they were picked-up.

Fourteen first instar nymphs caged on 14 plants of each cultivar in a completely randomized design. The mother and the new born nymphs on seven replicates were counted and weighed 9 days after the production of the first offspring using Sartorius microbalance (see 3.2.1), to find the effect of broad bean cultivars on fresh weight gained by *A. fabae* nymphs and mother. After being weighed, the mothers and nymphs were dissected to count the number of developed embryos inside each. Produced nymphs on the other seven replicates were counted and removed every other day, until the mother died naturally to determine the longevity and fecundity of the aphid on four broad bean cultivars.

3.2.3. Effect of temperature on the reproduction and weight of *A. fabae* reared on different broad bean cultivars

Three weeks old seedlings of broad bean cultivars (see 3.2.2) were transplanted to 9 cm diameter and 14 cm deep filled with 2: 1: 1 mixture of peatmoss, sand and clay, respectively.

Single fourth instar nymph of *A. fabae* from the stock culture (see 3.2) was placed on the lower surface of the 2nd or 3rd leaf from the tip of the plants. Nymphs were clipped in clip-on cage (see 3.2.1). Six plants of each cultivar were kept at $19\pm 1^{\circ}\text{C}$, $23\pm 1^{\circ}\text{C}$, $25\pm 1^{\circ}\text{C}$, $28\pm 1^{\circ}\text{C}$ and $33\pm 1^{\circ}\text{C}$ in a completely randomized design in diurnal growth chamber at photoperiod regime of 16:8 L:D hours with relative humidity of approximately 85%. After 10 days, the mother and the new nymphs on the incubated plants were counted and weighed using the Sartorius microbalance (see 3.2.1).

3.3. Broad Bean Mineral Content Analysis

Five plants of four weeks old for each of the four cultivars namely: Syrian local large, Syrian local medium, Aquadulce and Reyba; were cut at the soil surface, washed with tap water then with distilled water, air dried, weighed then put in an oven for 48h at 67°C. Dried plants were then weighed again to measure water content in the plant. They were grinded and then taken for determination of nitrogen, phosphorus and protein (Ryan *et al.*, 1996) in the Department of Agricultural Resources and Environment, Faculty of Agriculture, University of Jordan.

3.4. Monitoring The Flight Activity Of *A. fabae*

Flight activity of black bean aphid was monitored using suction trap located in the University Farm Station in the Central Jordan Valley, from 1995 to the end of October 1997. The trap was 12 meter high developed by Taylor, 1960. It consists of 9.14 m high tube and internal diameter of 940 mm, on top of a 3.00 m high box standing on a concrete. The air in the box is exhausted by electrical centrifugal fan causing the air with the insects to rush down into the pipe, then slowed in a conical expansion chamber. The insects were concentrated into a bottle containing preservative fluid. The fluid composed of 45% methyl alcohol (95%), 22% glycerol and 33% water. *Samples were collected weekly and examined under a compound microscope in a plastic petri dish to count the numbers of *A. fabae* and other aphids. Samples were preserved in labeled tubes containing the preservative fluid.

Aphids were identified according to Avidov and Harpaz (1969), Blackman and Eastop (1984) and confirmed by Dr. T. Mustafa.

* Suction trap samples tested in this research were collected by employee in the University Farm Station for Agricultural Research in the Central Jordan Valley