Impact of bee pollinators on seed set and yield of *Vicia villosa* spp. *dasycarpa* (*Leguminosae*) grown under semiarid conditions

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ABSTRACT

A field experiment was conducted during 2005/2006 at Jordan University of Science and Technology campus (32°30” N, 35°59” E), Irbid, Jordan, to study the role of bee visitors on seed set and production of *Vicia villosa* spp. *dasycarpa* grown under semiarid conditions. Two treatments were imposed on *Vicia villosa* plants before flowering: 1) Plants were covered in cages (control) or 2) Plants were left uncovered to permit bee visiting. The results of this experiment showed that *V. villosa* flowers were very attractive to worker honeybees as well as to few numbers of wild bees. The most frequent visitor species were *A. mellifera* and *Anthophora albigena* of family Apidae. *V. villosa* flowers attracted most of the bee visitors in the early hours of the day. The duration of their visit on the flowers also peaked early in the day and decreased toward the end of the day. The percentage of pod set of the un-covered plants averaged 14% out of the total florets on the plants, which was significantly higher than the covered plants (2%). These results indicated that the percentage of flower abscission was high and averaged more than 86%. Plant covering significantly reduced seed yield by reducing seed and pod number per plant and seed number per pod, but had no effect on individual seed weight. In conclusion, preventing bees from visiting during flowering of *V. villosa* spp. *dasycarpa* decreased seed set, seed yield and yield components. Further studies are needed to understand the high flower abscission and failure of seed set in this species.

Key words: *Vicia villosa* spp. *dasycarpa*, Pollination, Seed set, Seed yield.

RIASSUNTO

IMPATTO DI API POLLINATRICI SULLA QUANTITÀ DI SEMI E SULLA PRODUTTIVITÀ DI VICIA VILLOSA SPP. DASYCARPA (*LEGUMINOSAE*) CRESCIUTA IN CONDIZIONI SEMIARIDE

Nel campus della Jordan University of Science and Technology (32°30” N, 35°59” E), Irbid, Giordania, è stata condotta nel 2005/2006 una prova sperimentale tesa a studiare il ruolo di api visitatrici sulla produzione di semi per fiore impollinato e sulla produttività di Vicia villosa spp. dasycarpa (*Leguminosae*) in
condizioni semiaride. Sono stati applicati due trattamenti alle piante di Vicia villosa prima della fioritura: 1) piante coperte ossia in gabbia (controllo), o 2) piante scoperte per consentire la visita delle api. I risultati hanno evidenziato l’attrattività dei fiori di V. villosa sulle api mellifere e anche verso i pochi esemplari di api selvatiche. Le specie di api visitatrici più frequenti sono state A. mellifera e Anthophora albigena della famiglia Apidae. I fiori di V. villosa sono stati attrattivi per la maggior parte delle api nelle prime ore del giorno. Anche la durata delle visite ai fiori è stata massima all’inizio del giorno, diminuendo verso la fine. La percentuale di produzione di baccelli delle piante non coperte è stata mediamente del 14% del totale dei fiorellini, significativamente più alta rispetto a quella delle piante coperte (2%). I risultati rivelano un’alta percentuale di escissione dei fiori, in media superiore all’86%. La copertura delle piante ha ridotto significativamente la produzione di semi, abbassando il numero di semi e di baccelli per pianta e il numero di semi per baccello, ma non ha avuto effetti sul peso del singolo seme. In conclusione, impedire la visita delle api nel corso della fioritura di V. villosa spp. Dasycarpa ha causato una diminuzione sia nel raccolto di semi sia nella produttività. Saranno necessari ulteriori studi per comprendere l’elevata escissione di fiori e l’insuccesso nella produzione di semi per fiore impollinato in questa specie.

Parole chiave: Vicia villosa spp. Dasycarpa, Impollinazione, Semifiore, Produzione di semi.

Introduction

The area of rangelands in Jordan is about 25 million acres located in seven major regions. These regions are Northern and Eastern deserts, Southeastern sand stone desert, Southwestern mountain desert, Wadi Araba and Dead Sea, brush vegetation, grasslands and forests (Meigs, 1962). Jordan is unique in its seven ecological regions which result in great botanical diversity in the rangelands. The Jordanian climatic condition is Mediterranean and is characterized by mild, humid winters and long hot, dry summers. As in other Mediterranean regions, the vegetation is dominated mainly by annual plants. The rangeland, which cover 90% of the Jordanian land area, is either open grasslands, mixed grass-brush lands, or mixed grass-brush-savannahs with 200-600 mm of annual rainfall. The main growth season is during November to April.

The semiarid lands which receive 250-400 mm annual rainfall are dominated by cereal and food legume crops. The main forage legume species are Vicia sativa, Vicia ervilia, Vicia monantha and Vicia villosa. Wild Vicia species are naturally grown in the Mediterranean and Sahara Arabian regions. These two regions are characterized by cold winters. The main flowering season begins in January with a sequence of species (both cultivated and uncultivated plants) flowering until May. The flowering period of the wild plants covers the rest of the year.

The natural vegetation of the rangelands is deteriorating due to overgrazing, cultivation of the rangelands, and restriction of the nomadic movement. Therefore, re-vegetating of the rangeland is an important step to return the productivity of the area. The initial requirement for the plant establishment in the rangeland should be determined before starting the re-vegetation process. The knowledge of insects and insect pollinators in Jordan is very limited. Pollination is an essential stage in the reproduction of flowering plants. It is the transfer of pollen from the male to the female part of a flower (Mcgregor, 1976). Yield instability is a common problem in domestic Vicia species cultivated under semiarid conditions. As in many other crops, the number of both flowers and ovules that are formed almost always greatly exceeds the resulting number of pods and seeds that are produced. In the literature the re-productive system of Vicia plants is self-pollination with some extent of cross pollination (Mecgregor, 1976). Insects play a significant role in flower fertilization but the indiscri-
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The use of pesticides has reduced the number of various pollinators (Free, 1993). Pollination is an important link in seed fertility of flowering crops.

In general, there are low fruit and yield production by *V. villosa* under natural conditions in Jordan. Such patterns of low fruit set within inflorescences could be attributed to insufficient pollen receipt (Lee, 1988; Berry and Calvo, 1991; Brunet and Chalesworth, 1995), competition among ovaries of the same inflorescence for a limited source of nutrients (Bawa and Webb, 1984; Guitián, 1994; Medrano et al., 2000), and finally it may be attributed to intrinsic limitations on the reproductive success of flowers in different positions within the inflorescence (Lee, 1988; Berry and Calvo, 1991; Diggle, 1995). Variations in fruit-set among flowers within inflorescences is influenced not only by pollination success but also by post-pollination processes such as variation in the quality of pollen arriving to flowers, pollen germination activity, pollen tube growth, and development of fertilized seeds (Stephenson, 1981; Lee, 1988). The variation in the quality of pollen received by flowers has been related to directional movement of pollinators, the order of flower development and to the degree of within-inflorescence dichogamy (temporal overlap of male and female function) (Wyatt, 1982; Nilsson, 1983; Berry and Calvo, 1991; Brunet and Chalesworth, 1995; Brunet, 1996). Fruit set is also influenced by the time of flowering during the different months of the year. Completion of reproductive events during the warm period of the season may be advantageous to assure the dispersal of pollens from anthers and their arrival to the stigmas to increase seed production. The objective of this research was to study the impact of bee visitors on pollination; seed set and production of *Vicia villosa* spp. *dasycarpa* grown under semiarid conditions in Jordan.

### Material and methods

#### Study area

Field experiment was conducted during the growing season 2005/2006 in Northern Jordan (32°30” N, 35°59” E). The location has a Mediterranean climate with mild, rainy (250-300 mm) winters and dry, hot summers. The soils at the experimental planting site were shallow, rocky silt clays.

#### Planting

Seeds of *Vicia villosa* spp. *dasycarpa* were sown by hand on 15 February 2005. Distance between the rows was 100 cm and plants were spaced 40 cm within the row, resulting in a sowing density common in the area. The alleys between replicates were 1 m wide. To prevent moisture stress, plants were irrigated twice a week.

#### Insect collection and Identification

Bees were caught by a national park net by sweep netting the bees that had visited the flowers of *Vicia* in the transect area. Insect visitors were collected weekly on a sunny day between 09:00 and 14:00 during the entire flowering period. The collected specimens were kept in special insect boxes supplied with foam plates for pinning, and naphthalene galls to enable long storage without pest damage. The identifications were conducted by M. Schwarz (Ansfelden, Austria) [*Nomada, Sphecodes*], M. Kuhlmann (Münster, Germany) [*Colletes*], E. Scheuchl (Velden, Germany) [*Andrena*], A. Ebmer (Linz, Austria) [*Halictus, Lasioglossum*]. Voucher specimens of bees are kept in the Laboratory of beekeeping, Faculty of Agriculture at Jordan University of Science and Technology.

#### Pollination treatments

Pollination treatments were randomly assigned to plants before flowering in three replications. In each replicate, two treat-
ments were used: covered (control) (plants in a cage with no insect pollinators) and uncovered (open pollination) (plants were accessible to naturally occurring insect pollinators). In each replication, there were 12 plants in a double row. Number of florets, pods and seed per inflorescence and per plant, seed number per pod and seed weight per plant were recorded.

Statistical analysis
The experiment design was a randomised complete block design (RCBD) in three replications. Data were subjected to analysis of variance (ANOVA) according to Steel and Torrie (1980). Comparisons between means were made using Least Significant Differences (LSD) at 0.05 probability level.

Results

Insect visitors
A total of 340 bees were collected visiting the flowers of Vicia villosa spp. dasycarpa during a total of 46 census days as shown in Table 1. The 6 different species belonged to 6 genera of four families. The most frequent visitor species were A. mellifera with 45.9% (n=156) and Anthophora albigena with 22.4% (n=76) of family Apidae. Both made up more than 68.3% of the total number of the collected bees. The rest of flower visitors were shared in very low numbers except in the case of Polistes dominulus (Vispidae) which ranked as a third visitor in the abundance of 17.4% (n=59). P. dominulus visited the flowers for a considerable time period showing no interest in pollen, apparently stealing nectar.

While visiting Vicia villosa spp. dasycarpa flowers, most insect visitors collected pollen, nectar and both resources together at all census times. Figure 1 shows the average number of visits performed by different bee visitors to Vicia villosa spp. dasycarpa flowers at different times of the day. The greatest proportion of resource gathering by honey bees occurred during the early hours of the day between 08:00-09:00. Thereafter, the worker bees decreased their foraging activity on Vicia villosa flowers in the afternoon. A. albigena and P. dominulus showed similar behaviour in visiting patterns of Vicia villosa flowers during the day as recorded by honey bees. In the case of E. nigrescens, P. palaestanicum and H. quadricinctus showed one peak of visiting activity within the day. They began their foraging activity during the early hours of the day and continued to increase reaching their visits peak from 12:00-13:00. Thereafter, foraging activity on Vicia villosa flowers declined steadily and they seemed to cease their activity earlier than honey bees.

| Family     | Genus    | Species   | No. collected bees (n=340) | Species % |
|------------|----------|-----------|---------------------------|-----------|
| Apidae     | Apis     | mellifera | 156                       | 45.9      |
|            | Anthophora | albigena | 76                        | 22.4      |
|            | Eucera   | nigrescens | 24                      | 7.0       |
| Megachilidae | Pseudoanthidium | palaestanicum | 13                  | 3.8       |
| Halictidae | Halictus | quadricinctus | 12                   | 3.5       |
| Vispidae   | Polistes | dominulus   | 59                       | 17.4      |
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As respects their visit duration on *Vicia villosa* flowers, in general the insect visitors followed the same patterns spending more time on flowers during the early hours of the day and the duration gradually declined at the end of the day as shown in Figure 2.

**Pods and seed set**

Pod and seed set of *Vicia villosa* spp. *dasycarpa* plants grown under uncovered and covered conditions during flowering are shown in Table 2. The number of floret per inflorescence averaged 18 florets before the treatments were imposed. The uncovered plants had an average of 2.4 pod set per inflorescence. The pod of the uncovered plants contained an average of 8.8 seeds per inflorescence and had a higher number of seeds per pod than the pod of the covered plants. The percentage of pod and seed set of the uncovered plants averaged 14 and 51% out of the total florets on the plants, which was significantly higher than the covered plants. These results indicated that the percentage of flower abscission was high and averaged 86% for the uncovered plants. The results indicated that plant covering for short periods of time during flowering negatively affected the pod and seed set.

The impact of plant covering on seed yield and yield components of plants harvested at maturity is shown in Table 3. Plant covering significantly reduced seed yield by reducing all yield components except the individual seed weight. The covered plants had significantly lower seed and pod numbers per plant and seed number per pod (Table 2). Individual seed weight was not significantly different.
between the covered and uncovered plants, which might be due to filling the remaining seeds on plants to large size after the covering treatment was released. These results indicated that *Vicia villosa* spp. *dasycarpa* is very sensitive to covering at this critical stage of development, which negatively affected seed yield and yield components due to lack of pollinator access to plants.

**Discussion**

The hermaphrodite flowers of *Vicia villosa* spp. *dasycarpa* were unattractive to insect visitors. The results showed that only 6 different species visited *Vicia villosa* spp. *dasycarpa* flowers grown under semiarid conditions which is about 11.3% of the total species identified in Jordan (Al-Ghzawi et al., 2006). *A. mellifera* and *A. albigena* were the most frequent visitors to *Vicia villosa* spp. *dasycarpa* flowers to ensure satisfactory pollination. An important aspect used in many pollination studies is the number of visits made by a pollinator (Proctor et al., 1996). It is generally thought that the more visits made the more efficient the pollinator is (Primack and Silander, 1975; Herrera, 1989). The remaining visitor species also engaged in pollen and nectar collection as pollinators of *Vicia villosa* flowers with low frequency, while *Polistes dominulus* engaged in nectar collection only. The unattractiveness of *Vicia villosa* flowers to wild bees may be attributed to several factors such as the presence of other floral resources. During the ex-
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Experiments, *Vicia villosa* flowering coincided with that of other species such as *Centaurea syriaca* and *Sinapis arvensis* which are important for apiculture in Jordan due to their abundant nectar and the large floral patches throughout the area (Zaitoun and Vorwohl, 2003). Such competition for honeybees is also known in other crops. The attractiveness of any given species is a function of variables such as flavour, colour, nectar volume, sugar concentration and fragrance (Frisch, 1967) and the bees fly to plant species that yield the greatest nectar and pollen (Gary, 1979). Savchenyuk (1977) observed that buckwheat growing in the vicinity of red clover distracted the bees from pollination of the red clover. Dandelions detracted the bees from pollination of apple flowers (Free, 1970). Similar competitive effect was seen in pear (*Pyrus* spp.), plum (*Prunus* spp.) and almond (*Amygdalus communis*) orchards growing next to *Sinapis alba* (Vansell, 1952; Stephen, 1958). The major effect of pollination conditions was shown by the percentage of flowers that developed into young pods. Open pollination (uncovered treatment) increased this value suggesting that a low seed set could result, at least in part, either from insufficient pollinators or lack of compatible pollen. Seed-set success is influenced not only by pollination success but also by post-pollination processes such as pollen germination activity, pollen tube growth, and development of fertilized seeds (Stephenson, 1981; Lee, 1988). Flowers may be shed in response to internal genetic timing mechanisms as well as exter-

| Table 2. | Number of florets, pods and seeds per inflorescence, seed per pod, and percentage of formed pods and seeds for *Vicia villosa* spp. *dasycarpa* grown under uncovered and covered conditions during flowering stage of development. |
|----------|-------------------------------------------------------------------------------------------------|
| Treatments | No./inflorescence | Seed/pod | Percentage of total florets |
|           | Florets | Pods | Seeds | Florets | Pods | Seeds |
| Uncovered | 18<sup>a</sup> | 2.4<sup>a</sup> | 8.8<sup>a</sup> | 3.8<sup>a</sup> | 14.1<sup>a</sup> | 51.3<sup>a</sup> |
| Covered   | 17<sup>a</sup> | 0.3<sup>b</sup> | 1.1<sup>b</sup> | 0.3<sup>b</sup> | 1.7<sup>b</sup> | 6.4<sup>b</sup> |

<sup>a,b</sup>: P<0.05.

| Table 3. | Seed yield and number, pod number, seed number per pod and seed weight of *Vicia villosa* spp. *dasycarpa* plants grown under uncovered and covered conditions during flowering stage of development. |
|----------|-------------------------------------------------------------------------------------------------|
| Treatments | Seed yield | Seed no. | Pod no. | Seed no. | Seed weight |
|           | (g.plant<sup>-1</sup>) | (no. plant<sup>-1</sup>) | (no. pod<sup>-1</sup>) | (mg.seed<sup>-1</sup>) |
| Uncovered | 18.6<sup>a</sup> | 528<sup>a</sup> | 176<sup>a</sup> | 2.9<sup>a</sup> | 43<sup>a</sup> |
| Covered   | 0.23<sup>b</sup> | 5<sup>b</sup> | 4<sup>b</sup> | 1.4<sup>b</sup> | 35<sup>a</sup> |

<sup>a,b</sup>: P<0.05.
nal factors such as environmental stimuli and pathogen attacks (Ascough et al., 2005) and flower abscission is generally mediated by endogenous ethylene (Saxena et al., 2000; Doom, 2002).

Under arid conditions the worker bees synchronized their flying activities outside the hives with the availability of nectar and pollen grains in the morning in flowers and reduced their activity in mid and late hours of the day (Zaitoun and Vorwohl, 2003). McGregor et al. (1965) mentioned that bee activity begins on the flower shortly after it opens, reaches a peak at about 11:00, and ceases about 17:00. The flower is mainly attractive to bees for both pollen, because of their odour, and to visual nectar guides (Foster et al., 1965; Free, 1993); this attraction is important to ensure arrival of pollen to the stigma of pistilate flowers by bees during their collecting of nectar. Insect pollination contributed to an increase in the seed set since the insect omission treatment resulted in a reduction in the seed production compared with those plants exposed to insect visitors. These results tend to confirm the evidence given by McGregor (1976) that pollen of Vicia villosa has a better chance of reaching the stigma if carried by a vector. The high seed set obtained in the present study may, at least in part, be attributed to the mild spring conditions and suitable daily temperature, which favoured the activity of potential insect pollinators. Our findings are in agreement with those of other hermaphroditic flower species with flowers grouped in inflorescences, including for example, Lupinus luteus (Van Stevenick, 1957; Williams, 1987), Phaseolus vulgaris (Tamas et al., 1979), Catalpa speciosa (Stephenson, 1979, 1980), Asclepias tuberosa (Wyatt, 1980), Yucca whipplei (Udovic and Aker, 1981), Caesalpinia eriostachys (Bawa and Webb, 1984), Calochortus leichlinii (Holtsford, 1985), Solanum caroliniense (Solomon, 1988), Lavandula stoechas (Herrera, 1991), Lathyrus vernus (Ehrlén, 1992), Banksia spinulosa (Vaughton, 1993), Prunus mahaleb (Guitián, 1994), Petrocopitis grandiflora (Guitián and Navarro, 1996), and Aquilegia caerulea (Brunet, 1996).

Conclusions

Pollination, the transfer of pollen from the male to the female part of a flower, is an essential stage in the reproduction of flowering plants. Yield instability is a common problem in domestic Vicia species cultivated under semiarid conditions. Bees play an important role in crop pollination. Based on the current results, it can be concluded that the role of wild bees and honeybees must be enhanced to ensure more visitation rates to V. villosa flowers. Further studies are needed in order to gain a better understanding of the most effective pollination agents and their behaviour in relation to pollen transport.

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