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STIMULATION OF OVOGENESIS BY FLOWERS OF *LATHYRUS SYLVESTRIS* L. AND *LATHYRUS LATIFOLIUS* L. IN *BRUCHUS AFFINIS* FRÖLICH (COL. BRUCHIDAE)

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Introduction

Bruchus affinis Frölich (Col. Bruchidae) is a palearctic species that develops in Southwest of France on two leguminose plants: *Lathyrus sylvestris* L. and *Lathyrus latifolius* L. It is a monovoltine bruchid. The adults appear in nature at the time of flowerings of the *Lathyrus* species; the females lay their eggs on young green pods at the end of June and beginning of July. Larval development takes place in the seeds and the adults of new generation emerge in Autumn. They go to hibernation in winter time. No detailed reports are available stating the exact hibernating places during the unfavourable days in winter. But, Caillol (1954) pointed out that this bruchid hibernates in the faggots of pine trees and under the barks of plane tree (*Acer platanus*). Dead leaves, bark of trees, bark of chippings, splinters of fences posts, cracks in the soils are considered as the hiding places for other monovoltine bruchids during the winter season (Whithead, 1930; Walkland, 1934; Baranyovits, 1944; Leonide, 1962; Parnell, 1966; Al-Ravy and Kaddou, 1971). The females of *B. affinis* which had emerged from the seeds about ten months ago in the year before lay their eggs on young green pods of *L. sylvestris* and *L. latifolius* at the end of June and beginning of July. The adults pass through a so called 'Imaginal diapause' from the time of their emergence in autumn to the next spring when the *Lathyrus* species start flowering in the field. Ovogenesis in the females seems to be synchronized with the appearance of the pods and related with the presence of *Lathyrus* flowers (Leonide, 1962). There are some common reports that the univoltine bruchids need to have new or specific alimentation from their host plants at the time of their first arrival in nature after passing the long hibernation. This necessity of having to feed on specific alimentation arises in order to make the adults copulate and show other reproductive activities. (Vasilev, 1939; Leonide, 1962; Richart, 1964; De Luca, 1966; Pajni *et. al.*, 1975; Pesho and Houten, 1981).

The object of the present investigation was to examine as to how the *B. affinis* adults get or adapt their synchronization of egg-layings with the certain stage of the host-plant, i.e., young green pods of *L. sylvestris* and *L. latifolius*. How the induction of vitellogenesis is made just before the host's pods in the field? This selection of certain stage of the host plants is essential for further development of the next generation. Thus the induction of ovogenesis may be related with specific alimentation from a specific origin just before or during the flowering of the *Lathyrus* species. An attempt was made to examine the above hypotheses by following a series of experiments.

1. Direct observations of the reproductive relationships of *B. affinis* with the

host-flowers (*L. sylvestris* and *L. latifolius*) in the field.

2. Activities of *B. affinis* with the other flowers available before the flowering of the preferred host (*L. sylvestris* and *L. latifolius*).

3. Activities of *B. affinis* with the host-flowers (*L. sylvestris* and *L. latifolius*) during their flowering season.

Materials and Methods

1. Direct observations on reproductive relationships of *B. affinis* with the host-flowers (*L. sylvestris* and *L. latifolius*) in the field.

During high bloom of *L. sylvestris*, a random collection of *B. affinis* adults was made from ten different field stations in the region of Pau (France). They were preserved in 70% alcohol immediately after collection. The specimens were examined in the "Laboratoire de Phytomorphologie Generale et Comparée — Paris". Each of the collected adults was dissected under a binscular miscroscope. The cuticular parts of the mouth and anal region were very carefully removed without cansing any disturbance to the gut contents. The entire digestive tube was transferred to a small watch glass for further steps of acetolysis. For each of the experimental field stations a total of 15 digestive tubes were passed through the technique of acetolysis. Following the acetolytic analysis, the gut contents were made ready on glass slides for identification of the pollens eaten by the bruchids.

Available flowers other than *L. sylvestris* and *L. latifolius* growing in close proximity to the *Lathyrus* populations were also collected from the same stations and preserved. The same method was followed for the collections of the *L. sylvestris* and *L. latifolius* flowers.

Pollen analysis was made following the same acetolysis technique as in the case of *B. affinis* adults. Measurement of eqatoral and polar axis provided a good technique for identification of leguminose pollens (Planchais, 1964). Following Planchais (1964) measurements of pollens of the flowers collected directly in the field were compared with those eaten by the adults of *B. affinis*. This comparison of the measurements produced a good technique for identification as to which of the pollens were eaten by the bruchids in nature.

Relationship of reproductive maturity of *B. affinis* females and the abundance of *Lathyrus (L. sylvestris)* flowers in the field was compared. A *L. sylvestris* foot was selected in a field station and the number of flowers on that foot was recorded every two-days. On the other hand, weekly collections of the adult *B. affinis* were made from the same station. Females from the collected adults were dissected and number of mature ovocytes in retention per female was counted. This record was continued throughout the whole flowering season of *L. sylvestris*.

2. Activities of *B. affinis* with the other flowers available before the flowering of the preferred host (*L. sylvestris* and *L. latifolius*).

This experiment was conducted with a view to examine the reproductive activities of *B. affinis* adults with some of the spring flowers, and it started at the moment when the air temperature in nature was found to exceed 20°C. Both laboratory and field stations were utilized conducting the experiment.

(*a*) *In laboratory:* Adults for the experiments were used from stock source of the laboratory. The stock was maintained in the laboratory in a fridge at 3–4°C temper-

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ature. They were supplied with honey and water in the fridge. The adults were collected during their emergence from the infested seeds of *L. sylvestris* and *L. latifolius* the year before. Following materials were used in the laboratory experiments: (i) Large round plastic box 21 cm × 11 cm), (ii) Medium size glass jar (10 cm × 4.5 cm), (iii) Plastic plate (16 cm diameter × 3.0 cm height), (iv) cotton pads. Five to seven inflorescences were kept in a glass jar (10 × 4.5 cm) with water. The inflorescences were covered with a large plastic box (21 cm × 11 cm). The plastic plate was cut in the middle so that the glass jar containing the inflorescences could be inserted through it into the plastic box. The adult bruchids were introduced into the box so that they could move freely over the inflorescences.

(*b*) *In the field:* The adults were confined to some of the same inflorescences in the field also. They were confined in muslin bags (35 cm × 25 cm). An iron vire, coiled into loops, was placed inside each muslin bag. The muslin bags were put around the stem of the experimental plants. Inflorescences with a good number of young flowers were selected. Some cotton padding was provided for surrounding of the young stem plant, and an iron wire was placed at the point of entry inside the bags so that the young stems remained free from exerting any pressure from the weight of the iron wire. The adults were then confined in the bags with the inflorescences. A bamboo stick was fixed in the soil to support the bags. The adults dispersed inside the bag and had free access to the flowers. The bag were changed to new inflorescences when the flowers inside them became older.

3. Activities of *B. affinis* with the host-flowers (*L. sylvestris* and *L. latifolius*) during their flowering season.

This experiment was conducted with several lots of *B. affinis* adults confining them with:

(i) The inflorescences of *L. sylvestris* and *L. latifolius* both laboratory and in the field.

(ii) The nectar of *L. latifolius*.

(iii) The pollens of *L. sylvestris*.

(iv) The odour of *L. latifolius*.

(v) The castrated flowers of *L. latifolius* and *L. sylvestris*.

(vi) The pollens and young pods of *L. sylvestris*.

(vii) The castrated flowers and young pods of *L. sylvestris*.

(viii) The young pods of *L. sylvestris* and honey.

(i) *The inflorescences of L. sylvestris and L. latifolius:* The adults were confined with the inflorescences both in laboratory and in the field following the same methods as in the case of experiment 2 (*a*) and (*b*) respectively.

(ii) *The nectar of L. latifolius:* The nectar was collected from the flowers of *L. latifolius* in the field by using a micro-pipette (Hossaert *et. al.*, 1985). Two to three drops of nectar were put on the middle of a glass slide and, that was placed over a filter paper in a petridish. The petridish was provided with water soaked cotton in a small plastic cup. The nectar was changed once in every three days.

(iii) *The pollens of L. sylvestris:* The pollens were collected by taking the anthers from fresh flowers in the field. A small plastic cup (2.5 cm × 0.3 cm) was used as the pollen container. The cup containing pollens of 40–50 flowers was placed in a petridish. Filter paper and water was maintained as in the case of nectar. The pollens in the plastic cup were changed every alternative day.

(iv) *The odour of L. latifolius:* First *B. affinis* adults were confined in a small plastic cap (4 cm × 6 cm) provided with water and honey inside. The box was perforated with several holes and covered with muslin tissues. These perforations were made for allowing experimental bruchids to have sufficient odour form the *Lathyrus* flowers. The entire box was hanged in a large plastic box (21 cm × 11 cm) which was provided with the experimental flowers as in the case of experiment 2 (*a*).

(v) *The castrated flowers of L. latifolius and L. sylvestris:* The flowers were castrated by taking away the anthers from them at a young stage. The mode of confinement of the adults was the same as in the experiment 2 (*a*).

(vi) *The pollens and young pods of L. sylvestris:* The pollens were maintained as in the experiment 3 (iii), and arrangements of the young pods with their young stem was made as in the case of experiment 2 (*a*).

(vii) *The castrated flowers and young pods of L. sylvestris:* The flowers were castrated as in the experiment 3 (v), and the young pods were arranged as in the earlier experiments.

(viii) *The young pods of L. sylvestris and honey:* This lot of *B. affinis* were confined to young pods only. Honey was supplied to the adults in a small plastic cup. The young pods were maintained as in the earlier experiments inside a large plastic box (21 cm × 11 cm).

In all the above experiments, two couples of *B. affinis* were released in each of the plastic boxes (21 cm × 11 cm) or petridishes. Each of the box and petridish represented one replication. There were five replications with each of the experimental lots. In all the experiments the alimentation materials were changed every alternative day except in the case of the nectar. After a certain interval of days, the adults were taken out of the experimental boxes and petridishes, and dissected under a binocular microscope to examine their gut contents and reproductive development.

Results

1. Direct observations of the reproductive relationships of *B. affinis* with the host-flowers (*L. sylvestris* and *L. latifolius*) in the field:

During *L. sylvestris* and *L. latifolius* bloom no *B. affinis* adults were noticed visiting other flowers in the field. Random collection of the adults from ten different stations (St. Suzanne, Peyrouse, Coarraze, Baliros, Lescar, Bilhere, Laruns, Urdos and Lasque) and analyses of their gut-contents showed that the pollens eaten by the bruchids were exclusively of *L. sylvestris* and *L. latifolius* according to their presence in the respective field stations. The pollen grains corresponded in size with those of the *Lathyrus* flowers collected at the same time from the same field station. This observation is similar to that of Pajni (1981) who pointed out that *B. pisorum* fed only on the pollens of *Pisum sativum* in the field even when other flowers are available.

Fig. 1 (a + b) indicate a relationship between the maintenance of mature ovocytes in retention by the females of *B. affinis* and the abundance of *L. sylvestris* flowers in the field. The mature ovocytes in retention were found to occur in ovogenesis about one week following the availability of the host-flowers (*L. sylvestris*) in nature. The flowers appeared at mid July and ovogenesis in the females seemed to have started in the beginning of the fourth week of that month. The abundance of the flowers ended in the first week of September and the holding of mature ovocytes in

retention by the females was found to diminish to zero at the end of the second week of the month (Fig. 1 (a + b)).

2. Activities of *B. affinis* with the other flowers available before the flowering of the preferred host (*L. sylvestris* and *L. latifolius*).

Table 1 presents the activities of *B. affinis* with the inflorescences of spring flowers. Pollens of some of the spring flowers (*Salix, Lotus, Lathyrus linifolius, L. pratensis*) were found to be hardly eaten by the adults and, the others were not eaten at all (*Ulex, Corylus, Vicia* spp.). The females were sexually inactive on all of the experimental flowers. No qualititive change was noticed in the fat bodies. Absence of sperms in the spermatheca indicated that copulation did not occur in the presence of the inflorescences. On the other hand, in the beginning of July with the first appearance of *L. sylvestris* and *L. latifolius* flowers in the field

FIGURE 1 (*a*)

Seasonal abundance of flowers of *L. sylvestris* in a field station

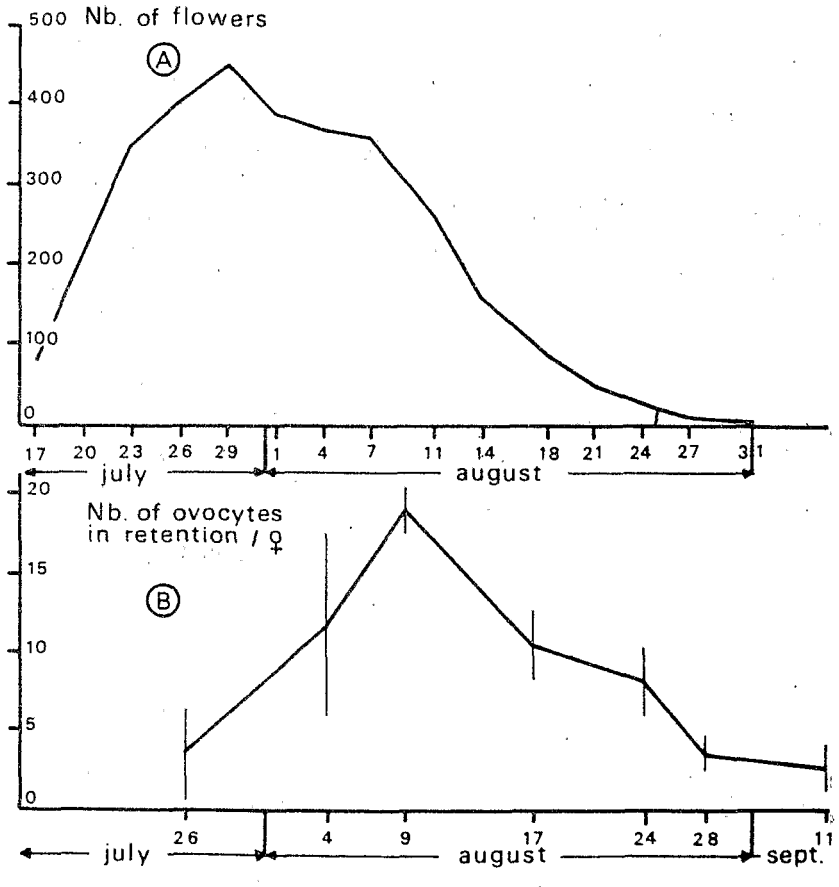

FIGURE 1 (*b*)

Mature ovocytes in retention in *B. affinis* females periodically collected from a field station on *L. sylvestris* flowers

TABLE 1

***Bruchus affinis* with the Flowers Available Before the Host-Flowerings (*L. sylvestris* and *L. latifolius*) in the Field**

Experiments	Inflorescences	Nos. of *B. a.* ♀	Duration in days	Presence of pollens in the digest tube	Fat body	Copulation	Ovogenesis	
							Retention	Vitellogenesis
Laboratory	*Ulex*	10	20–30	—	Whitish	—	—	—
Laboratory	*Corylus*	10	20–35	—	Whitish	—	—	—
Laboratory	*Salix*	10	20–32	+	Whitish	—	—	—
Laboratory	*Lotus cernicu*	10	20–25	+	Whitish	—	—	—
Field	*Lotus cernicu*	10	20	+	Whitish	—	—	—
Laboratory	*Vicia sativa*	10	20–35	—	Whitish	—	—	—
Laboratory	*Vicia craca*	10	20–35	—	Whitish	—	—	—
Field	*Vicia sativa*	10	20–35	—	Whitish	—	—	—
Field	*Vicia craca*	10	20–35	—	Whitish	—	—	—
Laboratory	*L. linifolius*	10	20–35	+	Whitish	—	—	—
Field	*L. linifolius*	10	20–25	+	Whitish	—	—	—
Laboratory	*L. pratensis*	10	20–25	+	Whitish	—	—	—
Field	*L. pratensis*	10	20–30	+	Whitish	—	—	—
Field*	*L. pratensis*	20	Unknown	+	Whitish	—	—	—

*Adults collected from nature on *L. pratensis* flowers.

Note: + = Rare. — = Absent. *B. a.* = *Bruchus affinis*.

(Fig. 2), both laboratory and field experiments showed induction of ovogenese 'in' the females in the presence of above two *Lathyrus* flowers. Successful population and change in the fat body quality were also observed (Table 2).

3. Activities of *B. affinis* with the host flowers (*L. sylvestris* and *L. latifolius*) during their flowering season.

Table 3 shows the induction of ovogenesis in the females of *B. affinis* by the host-flowers *L. sylvestris* and *L. latifolius* and the specific stimulative source contained by the flowers. The results indicated that the consumption of host-pollens alone initiated ovogenesis in the females and brought changes in the quality (colouration) of the fat bodies. Vitellogenesis was not initiated by the presence of other sources in the flowers (Nectar. Odour) or young pod. But rare population were observed in the presence of castrated flowers. The ovogenesis seemed to be highly influenced by the presence of inflorescences and pollens of *Lathyrus* species.

In all the experiments, the males were also examined. In the males, mature spermatozoa were seen even in the presence of only honey and water at 24 ± 2°C temperature in the beginning of May. When mature spermatozoa were noticed in the males, the accessory glands were found to be enlarged and the testes looked more yellowish than those of males having spermatozoa not developed. In all the above cases (Table 3) mature spermatozoa were noticed in the males, but copulation was found to occur only in the specific lots of the bruchids experimented.

Discussions

Bruchus affinis was practically very active and available on *L. sylvestris* and *L. latifolius* in the months of June and July in nature. This active part of its lifespan coincided with the availability of host-flowers and pods (*L. sylvestris* and *L. latifolius*). This synchronization is essential for the developmental needs of the insect's next generation.

Host-specific monovoltine bruchids were reported to leave hibernation when soil and air temperature exceeded 20°C in the spring before their host-flowers appeared in the field (Pesh and Houten, 1981). The present experiments showed (Table 1) that *B. affinis* adults did have some nutritional responses to the pollens of some of the spring flowers in confinement. However, no reproductive development in the females was noticed with any one of the experimental flowers. Among all the flowers experimented, the bruchids were found to visit only *L. pratensis* flowers in nature. *L. pratensis* flowers about one month earlier than *L. sylvestris* and *L. latifolius* flowering in the field, Adult *B. affinis* were not observed to visit *L. pratensis* flowers in the field where *L. sylvestris* and *L. latifolius* populations were absent in their close proximity. These observations may lead to two hypotheses: (*a*) Before the host-flowering (*L. sylvestris* and *L. latifolius*), in the late spring *B. affinis* adults became active because of climatic changes and passed their days in the so called 'waiting stage' on *L. pratensis* flowers for normal source of their alimentation awaiting the bloom of *L. sylvestris* and *L. latifolius*. (*b*) Adult *B. affinis* visit only the *L. pratensis* flowers growing in close proximity to the *L. sylvestris* and *L. latifolius* populations; and as soon as the preferred host plants started flowering they flock back to them from *L. pratensis* flowers. Other monovoltine bruchids were also reported to visit and eat pollens of some spring flowers before flowering of their preferred host-plants in the field, although eggs were not

laid on their pods at that time (Brindley, 1934; Southgate, 1979).

The present results (Table 1) showed that the females of *B. affinis* were sexually immature when they become reactivated after hibernation (collected females on *L. pratensis* flowers in the field). Table 3 detailed that feeding of the adults on *L. sylvestris* pollens stimulated ovogenesis in the females, but not on the other plants. This posed a question as to why consumption of the host-pollens (*L. sylvestris*) develop ovogenesis in the females and not in others. It is tentatively concluded that the cause is the presence of 'odd' chemical substances in the pollens of *L. sylvestris*, which the other plants differ. These 'odd' chemical substances render the plant able to induce the ovogenesis of the bruchids.

It was observed that the number of mature ovocytes in the females was higher when confined to the inflorescences com-

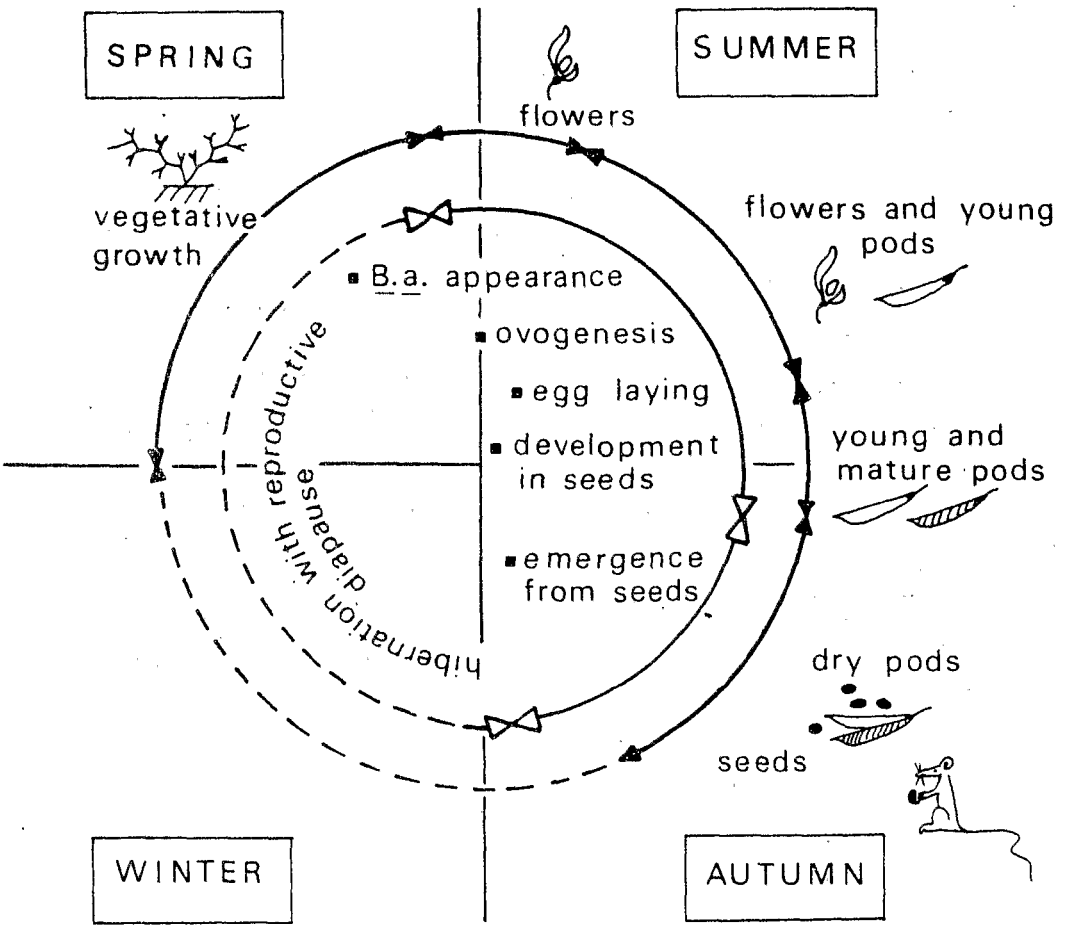

FIGURE 2

Schematic diagram showing life cycle of *B. affinis* in relation to its host-plants (*L. sylvestris* and *L. latifolius*) in the field

pared to the pollens or pollens and young pods. This result poses some complex questions as to why the consumption of pollens stimulated ovogenesis. It is because of the 'odd' chemical substances in the pollens, one may question that the petals and other flower parts may also contain similar biochemical components that increases the speed of ovogenesis. In fact, it was also observed that in nature the adult bruchid remained on the petals for a long time without any movement.

Actually (1981) Pesho and Houten observed in case of *B. pisorum* that not only feeding on host-pollens, but the other chemical contents of the flowers are associated with the reproductive activity (copulation) and stimulation of ovogenesis in the females. In the present investigation, the occurrence of copulation in the presence of emasculated flowers also indicates the above possibilities.

Qualitative change (coloration) in the fat body was also noticed in the induction of ovogenesis during the present experiments (Table 2 and 3). This supports the idea that the so called 'odd' chemical substances present in the pollens/flowers change fat body quality and stimulate ovogenesis at the same time or change in the fat body quality occurred first; and this changed quality in the fat body initiates ovogenesis. Such indication is rare in case of other bruchids, but Pranel (1966) suggested that the 'Change' in the fat body quality is a distinguishing character between new and old generation of *Bruchidius* in the field populations.

The adults emerging from the infested seeds in the antumn were found to be not as active as the post-hibernation ones on the host-flowers. The behaviour of the new generation adults may explained with the assumption that the neuroendocrine physiology (the neurouormones which act both on protein metabolism and on the follicular cells that surround the ovocytes) in the females is not stimulated by the chemical components of *L. sylvestris* and *L. latifolius* flowers/pollens. This cause may make the bruchids less attractive to the host-flowers during the autumn when the flowers are still present. At this time they may visit any other flowers or places without being selective. Actually they prepare for their forthcoming diapause. Eventually the bruchids fly to search hibernation places rather than to stay on the host-flowers during this time of the year.

TABLE 2

***Bruchus affinis* with the Host-Flowers (*L. latifolius* and *L. sylvestris*) During the Season of their Flowerings**

Experiments	Inflorescences	Nos. of *B. a.* ♀	Duration in days	Presence of pollens in the digest tube	Fat body	Copulation	Ovocytes per female	
							Retention	Vitellogenesis
Laboratory	*L. latifolius*	10	14	+++	Yellowish	+++	14.6 ± 3.8	18.1 ± 4.2
Field	*L. latifolius*	15	9	+++	Yellowish	+++	4.3 ± 2.0	10.0 ± 3.2
Laboratory	*L. sylvestris*	10	16–20	+++	Yellowish	+++	18.3 ± 4.2	15.0 ± 3.8

Note: +++ = Maximum. *B. a.* = *Bruchus affinis.*

TABLE 3

Stimulation of Ovogenesis in *Bruchus affinis* by Host-Flowers and the Examination of Source Specification in Laboratory

Experiments with	Nos. of *B. a.* ♀	Duration in days	Presence of pollens in the digest tube	Fat body	Copulation	Ovocytes per female	
						Retention	Vitellogenesis
Inflorescences *L. sylvestris* (Labo)	10	16–20	+++	Yellowish	+++	18.3 ± 4.2	15.0 ± 3.8
Inflorescences *L. sylvestris* (Field)	10	16	+++	Yellowish	+++	19.8 ± 3.5	17.3 ± 2.8
Inflorescences *L. latifolius* (Labo.)	10	16	+++	Yellowish	+++	16.5 ± 2.5	16.8 ± 3.0
Nectar *L. latifolius* (Labo.)	10	16	—	Whitish	—	—	—
Pollens *L. sylvestris* (Labo.)	10	16–20	+++	Yellowish	+++	8.8 ± 2.9	12.4 ± 3.5
Odour *L. latifolius* (Labo.)	10	16	—	Whitish	—	—	—
Cast. flowers *L. syl.* + *L. lati* (Labo.)	20	16	—	Whitish	+	—	—
Pollens + young pods *L. sylvestris* (Labo)	10	20	+++	Yellowish	+++	9.1 ± 3.0	20.8 ± 4.5
Young pods + Cast. flowers *L. syl.* (Labo)	10	20	—	Whitish	+	—	—
Young pods + honey *L. sylvestris* (Labo)	10	20	—		—	—	—

Note: +++ = Maximum. — = Absent. *B. a.* = *Bruchus affinis.*

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