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Entomophilous pollination in rape (*Brassica napus* L var *oleifera*) in Brazil

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Summary — The present experiment was performed to study the development of rape flowers (*Brassica napus* var *oleifera*, cv CTC 4), volume and concentration of nectar produced, insects involved in the pollination and their behaviour in the flower (nectar or pollen collection), and the effects of this pollination on the production of pods and seeds, and germination and oil content in the seeds. The most frequent insect visitors to the flower were: *Apis mellifera*, 80.6%; *Trigona spinipes*, 12.8% and *Dialictus* sp, 6.6%. The sugar content averaged of 16.3 µg, and the percentage of sugar in the nectar collected from workers (*Apis mellifera*) was 37 ± 18%. Total protein content and ether extract in the pollen were 17.5 and 6.7%, respectively. The visits of *A mellifera* collecting nectar and pollen contributed to pollination and to an increased production of pods per square meter and individual seed weight. No influence was registered on total number of seeds per pod, normal seeds (fully formed) or abnormal seeds (shrivelled and undersized) per pod, percentage of seed germination and oil content.

***Brassica napus* var *oleifera* / pollination / nectar / foraging behaviour / *Apis* / *Trigona* / *Dialictus* / seed production**

INTRODUCTION

Rape (*Brassica napus*) was cultivated in Japan 2 000 years ago with seeds which had come from China. Rahman (1940), studying *B napus* in India, concluded that bees *Andrena ilderda* Cam, *Halictus* sp, *Apis florea* F and *Eristalis tenax* (L) were the main pollinating insects.

According to Jenkinson and Jones (1953), the presence of bees in the fields resulted in a production increase to 8.8

seeds per pod against 5.3 seeds per pod in bee-free fields, even though the connection between the anthers and the stigma in individual flowers favors self-pollination.

Free and Nuttall (1968) noticed that the great majority of bees visiting the flowers collect nectar although some of them, mainly in the morning, collect pollen.

Williams (1985) reviewed the effects of honeybees and others insects on *B napus* flowers and their foraging behaviour and

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concluded that although swede rape is self-fertile, an external agent is important in transferring pollen from the anthers onto the stigma and the economic importance of the effect of bees on crop quality still needs to be established.

Having in view its recent introduction to Brazil as a possible fodder crop source or green manure (Granja, 1981), we conducted experiments and observations to study the development of rape flowers, the volume and concentration of its nectar, the insects involved in its pollination and their behaviour in the flowers (nectar or pollen collection), and the effects of pollination on the production of pods and seeds, germination, and content of oil in the seeds.

MATERIAL AND METHODS

The present work was performed at the Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal, UNESP (Brazil), from June to October 1987, on 9 2.5 x 5.0 m plots of rape (*B napus* L var *oleifera*, cv CTC 4), receiving 3 different treatments: T₁ covered with a nylon net with a colony of *Apis mellifera*; T₂ only covered; and T₃ not covered. The experiment were carried out in a dark red median texture latosol (Aloisi and Dematté, 1974).

The main chemical characteristics of this soil were: pH (H₂O) = 5.8; C = 1.14%; Al³⁺, Ca²⁺, Mg²⁺ e H⁺; 0.0; 3.6; 0.5; and 3.6 e mg/100 ml, respectively; K⁺ and P (H₂SO₄ 0.05 N) and S-SO₄²⁻ in the order, 158 µg/ml; 10 µg/ml; and 7 ppm. The crop was established in lines 0.45 m apart using 7 seeds per linear meter.

The experimental site is located at an altitude of 595 m and the mean temperature of the region is 21 °C. The climate is subtropical with an average annual precipitation of 1 431 mm.

Throughout the whole flowering period, the cultures were kept under observation and the following aspects were studied:

- time of flower opening, time period up to wilting, and formation of mature pods (5 replications with 20 flowers per sampling);

- visiting insects in T₃. The most frequent visiting insects were collected and stored in alcohol for further identification;

- activities performed by visiting insect (pollen or nectar) and their frequency;

- frequency of visits per hour (7:00 to 18:00 h) determined weekly (5 weeks with 62 samplings). Numbers of pollen and nectar foragers were recorded on the plots uncaged and open pollinated for 10 min in each interval;

- time spent for pollen or nectar collection (121 replications);

- concentration of sugars in the nectar collected from *A mellifera* workers by dissecting out honey-stomachs at the following times: 8:00, 10:00, 12:00, 14:00 and 16:00 h (4 replications);

- quantity of total soluble carbohydrates (average values—µg/flower) at 8:00, 10:00, 12:00, 14:00 and 18:00 h with 3 replications, according to the methodology described by Roberts (1977);

- number of seeds per pod for flowers visited only by *A mellifera* but visited by different insects;

- number of pods originated from 50 flowers perforated by *T spinipes*;

- total protein content and ethereal extract of pollen collected by *A mellifera* during the foliate peak (AOAC, 1965), with 3 replicates.

The number of pods as well as the number of seeds per pod were also determined (100 replicates per treatment). The germination test, according to the rules for seed analysis (Brasil, 1976) was also performed in 4 replications, using 50 seeds/replication along with oil content determination in the seeds following the AOAC procedure (1965) with 3 replications, using 1 g of seeds/replication.

The data were analysed statistically by *F* tests, and the means were compared by the Tukey test (*P* < 0.05).

RESULTS AND DISCUSSION

The flowering period extended for 35 days and the flowers remained open from 6:30 to 17:30 h. *B napus* flower wilting started after an average time period of 32.5 ± 8.6 h after the opening of the flowers.

Insect visits to *B napus* flowers reduced the duration of flower life and flowering. The absence of insects may induce some compensatory effect which improves production as reported by Mesquida *et al* (1988).

During the flowering period, the flowers were visited by *A mellifera*, *Dialictus* sp, *Dialictus phaedrus* (Schorotky), *Megachile* (Ptilosarus) (Schorotky), *Thectochlora alaris* (Vachal), and *Trigona spinipes* (Fabricius).

The species with the highest visit frequency was *A mellifera* (80.6%), followed by *T spinipes* (12.8%) and *Dialictus* sp (6.6%).

The highest daily visit frequency was around 09:00 h for *A mellifera* and 10:00 h for *T spinipes* and *Dialictus* sp (fig 1). Free (1983) found that more pollen is collected in *B napus* by *A mellifera* between 08:00–09:00 and 09:00–10:00 h on spring and winter rape, respectively. Free and Nuttall (1968) showed that the number of flowers visited per minute by honeybees ranges from 6.8–14.5.

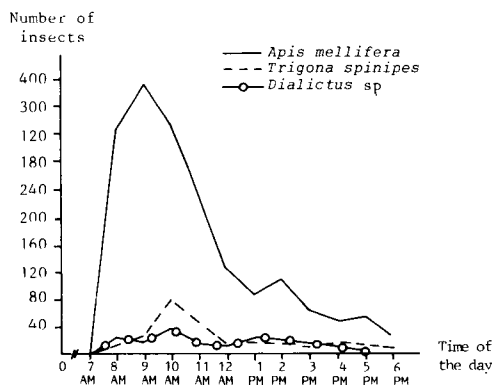


Fig 1. Frequency of *Apis mellifera*, *Trigona spinipes* and *Dialictus* sp in rape flowers during the day.

Regarding behaviour, it was observed that *A mellifera* collected pollen and nectar in T_1 and T_3 . The pollen collection was from 7:00 to 11:00 h and lasted an average time of 20 ± 13 s for T_1 and 21 ± 14 s in T_3 .

Nectar collection was performed throughout the day, with bees staying on the flowers for an average of 3.2 ± 0.2 s and 3.5 ± 0.4 s for T_1 and T_3 respectively.

T spinipes collected pollen exclusively, staying on the flower for an average of 52 ± 42 s. This longer time was due to the fact that *T spinipes* perforated the flower to collect pollen, reducing pod production by 86.0%.

Dialictus sp collected pollen and nectar with an average visit time of 63 ± 44 s and 8 ± 10 s, respectively.

The average time spent for pollen collection was 5–6 times longer than for the collection of nectar, consistent with the findings of Almeida (1985).

The concentration of sugars (4 repetitions per hour) in the nectar collected from *A mellifera* workers at 8:00, 10:00, 12:00, 14:00 and 16:00 h was 36, 37, 34, 33 and 45%, respectively.

The average concentration of sugars sampled from *A mellifera* during foraging activity was $37 \pm 18\%$, showing no significant differences ($P > 0.05$) throughout the day ($P > 0.05$). The quantity of sugars produced by the flower during the day averaged $16.3 \mu\text{g}/\text{flower}$. Davis *et al* (1986) studied *Brassica napus* L and found that the floral nectaries consist of 2 pairs of glands which are supplied by phloem only. The lateral pair produces most of the nectar, whereas the median pair produces relatively little nectar. The nectar of *B napus* (Regent) contained 38.7% sugar, with a sugar value (mg/flower/day) of 0.177 (Szabo, 1982).

Total protein and ether extract in the pollen sampled from confined bee colonies was determined as 17.5% and 6.7%, respectively.

The average number of pods per square meter, average weight of seeds (g), average number of seeds per pod, average number of normal seeds (fully formed) per pod, average number of abnormal seeds (shriveled and undersized) per pod, and germination percentage are summarized in table I.

For the average number of pods, the results are the following: the production of pods in T_1 was higher when compared to T_2 . This was due to the pollination activity of the honeybees in the flowers. The increased production of pods by T_1 over T_3 was probably due to *T spinipes* perforating the flowers in T_3 .

Regarding the average weight of individual seeds, a significant difference among treatments was observed, since T_1 and T_3 produced heavier seeds than T_2

($P < 0.05$). It was also noticed that the average number of seeds per pod in T_2 was greater than in T_3 ($P < 0.05$).

Plots visited by bees (T_1 and T_3) contained fewer seeds/pods but heavier seeds than plots from which insects were excluded ($P < 0.05$). Jenkinson and Jones (1953) noted that when bees are present, plants produce fewer flowers and more seeds per pod.

Free and Nuttall (1968) found that although plots caged with bees produced more seeds, the seeds were smaller and weighed less than plots caged without bees.

Williams (1985) in a review on this subject verified that although caged plots have usually given lower or similar yields, sometimes yields have been greater. Williams *et al* (1987) explained the conflicting results regarding the effects of honeybees on seed yield of oil seed rape by the differences in growing conditions during seed production and concluded that plots

Table I. Number of pods, weight, and number of seeds with their percentage of germination. Average results in T_1 , T_2 , T_3 .

Treatments	Average No of pods /m ²	Average seed weight (mg)	Average No of seeds /pod	Average No of normal seeds (fully-formed) /pod	Average No of abnormal seeds (shriveled and undersized) /pod	Seed germination	
						Normal	Abnormal
T_1	798.84 ^a	2.6 ^a	4.28 ^{ab}	3.74 ^a	1.15 ^a	99.50 ^a	55.25 ^a
T_2	308.19 ^b	2.1 ^b	4.46 ^a	3.92 ^a	1.32 ^a	98.00 ^a	53.75 ^a
T_3	539.57 ^{ab}	2.6 ^a	4.19 ^b	3.66 ^a	1.36 ^a	97.75 ^a	61.50 ^a
F for treatment	11.31*	18.72*	5.10*	1.30 ^{NS}	0.68 ^{NS}	0.60 ^{NS}	1.98 ^{NS}

Averages followed by same letters are not statistically different according to Tukey. NS: not significant; * significant at the 5% level; T_1 : netted with honeybees; T_2 : netted without honeybees; T_3 : open-pollinated.

with honeybees produced pods containing more seed than those from plots without honeybees.

Barbier (1978) also reported that honeybee foraging activity produced greater numbers of seeds.

The average number of normal seeds per pod, the average number of abnormal seeds per pod and the percentage of germination showed no significant differences for T_1 , T_2 or T_3 ($P > 0.05$). The same was true for the oil content of seeds, which presented an overall average of $39.3\% \pm 0.6$ ($P > 0.05$).

We conclude that *A mellifera* was the main pollinating agent of rape (*Brassica napus*) during nectar and pollen collection. The second most frequent species on these flowers was *T spinipes*, although it perforated flowers, and reduced pod production by 86%. The *Apis* visits increased pod production and individual seed weight.

Moreover, in Brazil rape can be considered a high quality source of nectar and pollen for *A mellifera*.

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Résumé. — La pollinisation entomophile du colza (*Brassica napus* L var *oleifera*) au Brésil. L'expérimentation avait pour but d'étudier le développement des fleurs de colza (*Brassica napus* var *oleifera*, cv CTC 4), la production quantitative et qualitative de nectar, les insectes impliqués dans la pollinisation ainsi que leur comportement de butinage du pollen et du nectar et les effets de la pollinisation sur la production de siliques et de graines, la germi-

nation et la teneur en huile des graines. Elle a eu lieu à 595 m d'altitude dans une région où la température moyenne est de 21 °C. Le climat est subtropical, les précipitations annuelles moyennes atteignent 1 431 mm. Chacun des 3 traitements (T_1 , filet en nylon + une colonie d'abeilles; T_2 : filet en nylon seul, T_3 : ni filet ni colonie) a été appliqué à 9 parcelles (2,5 x 5,0 m) de colza. Les insectes les plus fréquents sur les fleurs ont été: *Apis mellifera* (80,6%), *Trigona spinipes* (12,8%) et *Dialictus* sp (6,6%). Le maximum de visites se situait à 9 h pour *A mellifera* et 10 h pour *T spinipes* et *Dialictus*. La figure 1 donne la fréquence de ces insectes au cours de la journée sur les fleurs de colza. *A mellifera* et *Dialictus* ont récolté du pollen et du nectar sur les parcelles T_1 et T_3 . *T spinipes* n'a récolté du pollen qu'en perforant les fleurs, ce qui a réduit la production de siliques de 86,0%. Le temps moyen passé était 5–6 fois plus long pour la récolte de pollen que pour la récolte de nectar. La concentration moyenne en sucres du nectar récolté par *A mellifera* était de $37 \pm 18\%$ et la quantité de sucres produite par fleur était en moyenne de 16,3 µg. Les pourcentages de protéines totales et d'extrait à l'éther des échantillons de pollen provenant des colonies d'abeilles confinées étaient respectivement de 17,5 et 6,7%. Le tableau I donne le nombre moyen de siliques au m², le poids moyen des graines (g), le nombre moyen de graines par silique, le nombre moyen de graines normales (bien formées) et de graines anormales (ridées ou de taille réduite) par silique et le taux de germination. La production de siliques a été plus élevée avec T_1 qu'avec T_2 . T_1 et T_3 ont produit des graines plus lourdes que T_2 . Les divers traitements n'ont eu aucune influence sur le nombre total de graines par silique, sur le nombre de graines normales et anormales par silique, sur le taux de germination et sur le teneur en huile des graines.

***Brassica napus* var *oleifera* / pollinisation / *Apis* / *Trigona* / *Dialictus* / comportement butinage / nectar / production de graines**

Zusammenfassung. — Insektenbestäubung von Raps (*Brassica napus* var *oleifera*). Die vorliegenden Experimente wurden durchgeführt, um die Entwicklung der Blüten des Raps (*Brassica napus*, var *oleifera*, cv CTC 4), Volumen und Konzentration des produzierten Nektars, die an der Bestäubung beteiligten Insekten und ihr Verhalten an den Blüten (Sammeln von Nektar und Pollen), den Effekt der Bestäubung auf die Erzeugung von Schoten und Samen, und die Keimung und den Ölgehalt der Samen zu studieren.

Das Versuchsfeld war in einer Höhe von 585 m gelegen, bei einer mittleren Jahrestemperatur von 21 °C. Das Klima ist subtropisch mit einer mittleren Niederschlagsmenge von 1 431 mm. Es wurden drei verschiedene Versuchsanordnungen angelegt, jede mit 9 Parzellen (2,5 x 5 m) mit Raps: T₁, bedeckt mit Nylonnetz und versehen mit einem Bienenvolk; T₂, nur bedeckt, ohne Bienen; T₃, unbedeckt. Die häufigsten Blütenbesucher unter den Insekten waren: *Apis mellifera* (80,6%), *Trigona spinipes* (12,8%) und *Dialictus* sp (6,8%). Für Honigbienen wurde die höchste Besuchsfrequenz um 9,00 h beobachtet, für *T spinipes* und *Dialictus* sp um 10,00 h. Abbildung 1 zeigt die Häufigkeit dieser Bienen auf den Rapsblüten während des Tages. Es wurde beobachtet, daß *A mellifera* und *Dialictus* sp Pollen und Nektar in T₁ und T₃ sammelten. *T spinipes* sammelte ausschließlich Pollen, wobei sie die Blüte durchbohrte; dadurch wurde die Erzeugung von Schoten um 86,0% reduziert. Die Zeit für das Pollensammeln war im Durchschnitt 5 bis 6 mal größer als für das Sammeln von Nektar.

Der Zuckergehalt der Blüten betrug im Durchschnitt 16,3 µg/Blüte, die Zuckerkonzentration des von Honigbienen während des Sammelns entnommenen Nektars 37 ± 18%. Der Prozentsatz des Gesamtproteins und des Ätherextrakts von Pollen von im Flugkäfig gehaltenen Bienen betrug 17,5% bzw 6,7%.

Mittlere Schotenzahl pro m², mittleres Samengewicht (g), mittlere Samenzahl pro Schote, mittlere Zahl normaler (voll ausgeformter) Samen pro Schote, mittlere Zahl abnormer (geschrumpfter/zu kleiner) Samenkörner pro Schote und Prozentsatz der Keimung sind in Tabelle I zusammengefaßt. Im Vergleich zu T₂, war die Produktion von Schoten in T₁ höher. Die Versuche T₁ und T₃ lieferten schwerere Samenkörner als T₂. Kein Unterschied zwischen den Versuchsanordnungen wurde hinsichtlich der Gesamtzahl der Samen pro Schote, der Zahl normaler (voll ausgeformter) und abnormaler (geschrumpfter/zu kleiner) Samenkörner pro Schote, des Prozentsatzes der Samenkeimung und des Ölgehaltes gefunden.

Raps / Nektar / Samenentwicklung / *Apis* / *Trigona* / *Dialictus*

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