

Comparative study of nectar secretion and attractivity to bees of two lines of spring-type faba bean (*Vicia faba* L var *equina* Steudel)

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Summary — Two spring-type faba beans (D-27 self-fertile, D-23 non self-fertile) were observed in open field conditions for their nectar amount and sugar composition and their attractiveness to bees. Nectar amounts fluctuated according to the state of the flower, and in some cases also, the date of sampling and time of day. Nectar secretion of the D-27 line was four to six times higher than that of the D-23 line. High performance liquid chromatography analysis showed that the nectar was sucrose rich. There were substantial variations in nectar composition between plants and genotypes. D-23 was as attractive as D-27 to honeybees; in contrast, *Bombus terrestris* preferred D-27. Nevertheless, they paid a nearly equivalent proportion of robbing (non pollinating) visits to the two lines (D-23: 55%; D-27: 50%). In these conditions, the hole *B terrestris* made in the corolla to rob nectar, was used infrequently by honeybees. Most honeybees (68.6–76.2%) behaved 'legitimately' to probe nectar. The fact that the self-fertile (insect-independent) line is more attractive to bumblebees (because of its nectar production) is discussed.

Apis mellifera / *Bombus* / *Vicia faba* / nectar secretion / attractivity

INTRODUCTION

Investigations of the pollination of spring-type faba bean (*Vicia faba* L var *equina* Steudel) have been undertaken at Rennes since 1988. In a continuation of these stud-

ies, two male-fertile lines were chosen for comparison. Although they are genetically closely related, they differ in several aspects. For example, they can be distinguished morphogenetically at the seed coat level and differ in their enzymatic patterns (Carré et al, 1991).

Their flower morphology is similar. The most important difference concerns their floral biology: one line is spontaneously self-pollinated because of its natural self-fertility, whereas the second line is non self-fertile. The proficiency of various pollinating insects (Hymenoptera, Apidae: *Apis mellifera* L and *Bombus* spp) under insect-proof cages, has been compared to spontaneous self-pollination, to hand tripping and to open field free pollination on these lines. The data showed that the self-fertile line is independent of pollinators to produce seeds but the non self-fertile line requires insect visitation to be fully fertilized (Mesquida et al, 1990).

The importance of nectar secretions in the foraging behavior of pollinating insects is well established. This fact has been demonstrated by different authors working on various plant species (Vansel, 1934; Beutler, 1953; Baker and Baker, 1975; Harborne, 1982; Fonta et al, 1985; Mesquida et al, 1988a, 1988b). Very few data are currently available in the literature with regard to nectar secretions in faba bean. Only one preliminary study, made on 15 plants, indicated that the self-fertile line produced significantly more nectar than the non self-fertile line (Mesquida et al, 1990).

The present study was performed to investigate the comparative attractivity of these self-fertile and non self-fertile lines to the pollinating insects involved in faba bean pollination, specifically honeybees and bumblebees.

MATERIALS AND METHODS

Two lines of spring-type faba bean were used, one fully self-fertile (D-27) and the other not (D-23). Both were issued from the Plant Breeding Station at Dijon. Trials using this material were conducted at the INRA Research Center of Le Rheu in 1989 (nectar) and 1994 (nectar and attractivity).

In 1994, the experimental design consisted of microplots of five rows per genotype (1.80 x

6 m) because of the limited number of available seeds (50 plants/m²). The two lines were contiguous and their left-right position was randomized in three microplots (ie, three replicates).

Three parameters were considered at different dates of the flowering period: i) the number of fully flowering flowers; ii) the nectar secretion; and iii) the number of insects on the flowers and their foraging behavior.

Flower counting

During the flowering period, the number of opened flowers, ie, those capable of being probed by insects for pollen and nectar, was counted per m², on the same day as insect countings.

Nectar production and sugar composition

The first objective was to compare nectar secretion in the genotypes. It is known that nectar secretion depends on several factors, ie, the weather conditions, the time of day, the position of the flower on the plant, the state of the flower and insect visitations.

Twenty-four hours before sampling, the main stem was bagged with a transparent microperforated paper to prevent insect visitation, while ensuring a nearly normal evapo-transpiration. Nectar was collected on all the flowers of the highest inflorescence on the main stem using a 5 µL micropipette.

Samplings were made at three dates (07/06, 10/06, 14/06) at four times each day (8, 10, 12 and 16 h GMT) on five random plants from each of the three replicates (corresponding to the right or left position). For each collected plant, the following parameters were noted: i) the number of nodes on the main stem; ii) the level of the collected inflorescence on the main stem; iii) the number of flowers on the inflorescence; and iv) the state of the flower. The latter was defined according to the following categories (see fig 3): closed flower (cl): the flower still in bud (white colored); half-closed flower (h-cl): period of the flower-bud opening; the wing petals becoming visible under the standard petal; half-opened flower (h-o): the wing petals well dissociated from the standard petal; open flower (o): the standard petal com-

pletely erect and the wing petals well visible; and wilted flower (w). A total of 464 and 424 flowers were observed respectively on D-27 and D-23.

The analysis of the sugar contents in the nectar was performed by high performance liquid chromatography (HPLC). This technique was initially developed for nutritional analysis (Thean and Fundersburk, 1977; Black and Bagley, 1978) and then efficiently applied to the analysis of the sugars of flower nectars (Erickson et al, 1979; Severson and Erickson, 1983; Mesquida et al, 1991).

Immediately after sampling, the micropipettes were closed, frozen and stored at 20 °C. Results were expressed as sugar concentrations in mg per 100 µL nectar after being converted following the counting frame proposed by Weast et al (1988).

Statistical analyses of variance (Anova) of nectar volumes were performed using the mean values of nectar volume per flower over the five plants of each replicate. STAT ITCF software (Gouet et al, 1985) was used for statistical interpretations according to a factorial design (fixed model) with two uncontrolled factors (genotype, date of sampling or time of sampling) and replicates.

Attractivity and foraging behavior

Pollinators (*Apis mellifera* and *Bombus* spp) were counted on a 3 m² surface (0.50 x 6 m) on two dates (09/06 and 14/06) at full flowering. To allow comparison, the countings between the genotypes were made consecutively. The number of insects per 1 000 flowers was used in the statistical analysis because of its interest in entomophilous pollination.

Some aspects of the foraging behavior were observed, such as the presence of pollen pellets on the corbiculae, to determine if the insects were collecting nectar or pollen. Moreover, the type of visit as it pertained to pollination efficiency was noted. It is well known that *Bombus terrestris* can collect nectar 'legitimately' with a positive effect on pollination by tripping the blossom, but they are also able to perforate the corolla tube to rob nectar (Newton and Hill, 1983) and are thus called 'robbers' (Inouye, 1980). Holes made by *B terrestris* can be subsequently used by honeybees.

RESULTS

Flower counting

The total number of nodes on the main stem was significantly superior in the D-27 line from 7–14 June (D-27: 14.6–17.1 nodes versus D-23 13.2–15.8). The number of flowers produced by the upper inflorescence was also higher in D-27, except at the last date where the lines exhibited no difference (D-27, three dates respectively 6.8, 9.15 and 7.35 flowers; D-23: 5.2, 7.75 and 8.05 flowers), indicating that D-27 is slightly earlier than D-23. Therefore, on 14 June the rate of flowering was beginning to decline for D-27, while D-23 line was fully blooming. This is confirmed by the data for the opened flower density at each date (table I).

The distribution of the relative proportion of opened flowers (figs 1 and 2) was different between D-27 and D-23; the latter exhibited a higher proportion of opened flowers. Because the observations of the lines were made at the same time all day long, we can suppose that D-27 bloomed and wilted more quickly during the course of the day than D-23.

The flowers were not bitten when still closed, but a large percentage of flowers with a hole were found among half-opened and opened flowers. This proportion was significantly higher in D-27.

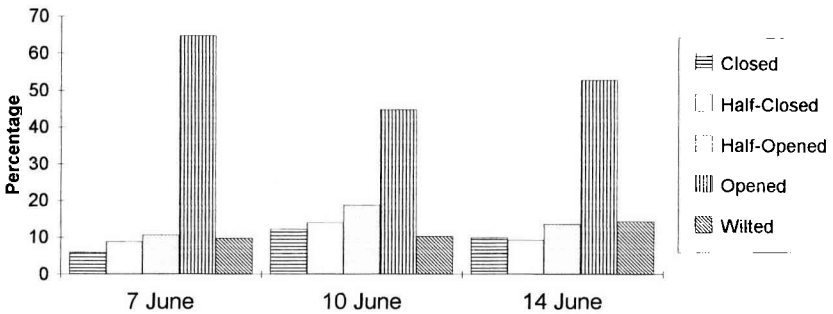
Nectar production and sugar composition

Nectar amounts in relation to the stage of the flower were studied on line D-27. Figure 3 shows that nectar secretion (expressed for one flower/plant on average) is detectable when the flower is half-closed, increases when it is half-opened, reaches

Table I. Number of opened flowers/m² and proportion of perforated flowers at different flowering dates and stages.

		Date	Faba bean line		Significance
			D-23	D-27	
Number of opened flowers/m ²		09 June	262	328	S
		13 June	229	182	S
		Mean	246	255	NS
Percentage of perforated flowers at each state	Half-closed	07 June	00.0	20.0	
		10 June	00.0	03.0	
		14 June	00.0	18.0	
	Half-opened	07 June	17.2	31.3	
		10 June	12.9	45.7	
		14 June	13.6	55.6	
	Opened	07 June	59.1	82.3	
		10 June	24.4	83.0	
		14 June	60.0	58.2	
	Mean	37.3	54.3	S	

S: significant; NS: not significant.

**Fig 1.** D-23 Line – Distribution of the flower different stages on one inflorescence.

a peak when the flower is opened and slumps when it wilts.

Nectar contents were independent of perforation, except when the flower was half opened. In that case, nectar amounts of the bitten flowers were superior (table II).

The comparison of the opened flowers between the lines showed that D-27 secreted significantly more than D-23 (mean nectar volumes of 1.17 μ L versus 0.24 μ L; table III).

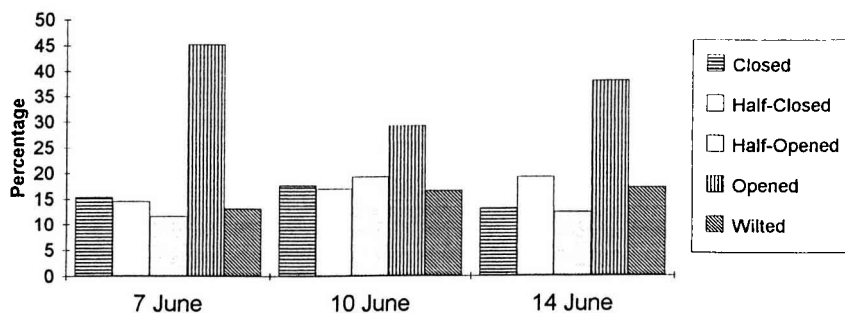


Fig 2. D-27 Line – Distribution of the flower different stages on one inflorescence.

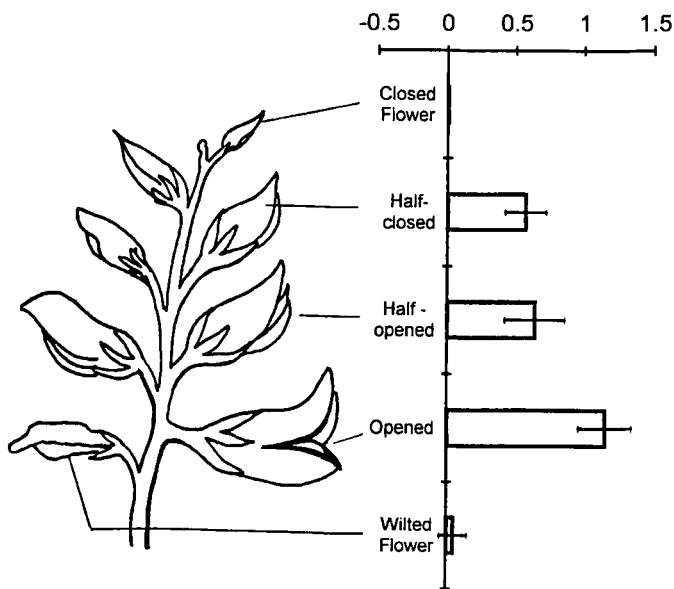


Fig 3. D-27 Line – Nectar volume per flower (µL).

Table II. D-27 Line: nectar volume (µL) at different flower stages: comparison between perforated and non-perforated flowers.

Flower state	Perforated	Non-perforated	Significance
Half-closed	0.57 (n = 69)	0.60 (n = 10)	NS
Half-opened	0.64 (n = 40)	0.98 (n = 30)	p = 0.039
Opened	1.15 (n = 43)	1.18 (n = 127)	NS

Table III. Nectar volume (μL) in opened flowers.

	<i>D-23</i>	<i>D-27</i>
Perforated flower	0.29 ± 0.03 ($n = 108$)	1.18 ± 0.06 ($n = 127$)
Non-perforated flower	0.19 ± 0.03 ($n = 112$)	1.15 ± 0.09 ($n = 43$)

\pm standard error.

Table IV. D-27 Line: nectar production in opened flowers at different dates and times of day.

	<i>07 June</i>	<i>10 June</i>	<i>14 June</i>	<i>Mean</i>
08 h GMT	1.36	1.92	1.59	1.62 ^a
10 h GMT	0.98	1.53	1.15	1.22 ^{ab}
12 h GMT	1.15	1.10	0.91	1.05 ^b
16 h GMT	1.27	0.77	1.43	1.16 ^{ab}
Mean	1.19	1.33	1.27	
Day time effect	NS	$p = 0.002$	NS	

Means followed by the same subscript are not significantly different at the 5% probability level (Newmann–Keuls test).

Table V. Qualitative aspects of nectar of the D-23 and D-27 lines: concentration of glucidic components ($\text{mg} / 100 \mu\text{L}$) and relative proportions of these components (%).

		<i>D-23</i>	<i>D-27</i>	<i>Significance</i>
Concentration ($\text{mg} / 100 \mu\text{L}$)	Sucrose	22.0 ± 3.9	24.1 ± 2.11	NS
	Glucose	4.82 ± 0.81	5.0 ± 0.31	NS
	Fructose	9.39 ± 0.96	9.3 ± 0.62	NS
	Total	36.2 ± 3.14	35.5 ± 2.18	
Percentage	Sucrose	52.8 ± 7.21	56.1 ± 3.16	NS
	Glucose	14.4 ± 2.36	14.3 ± 0.97	NS
	Fructose	32.8 ± 5.98	29.6 ± 2.53	NS

\pm standard error.

Table VI. Foraging insects density (number per 1 000 flowers) on D-23 and D-27.

		D-23	D-27	Line effect
<i>Apis mellifera</i>	09 June	1.60	1.87	NS
	13 June	4.66	4.23	NS
	Date effect	$p = 0.004$	$p = 0.000$	
	Mean	4.67	4.23	NS
<i>Bombus terrestris</i>	09 June	0.17	1.49	$p = 0.000$
	13 June	0.91	1.81	$p = 0.037$
	Date effect	$p = 0.009$	NS	
	Mean	0.48	1.62	$p = 0.000$

The effect of the sampling date and time were studied on the opened, non-perforated flowers of line D-27. The secretions fluctuated between plants and between flowers on the same plant, and the analysis of variance did not allow the detection of a date effect. However generally the mean values showed that the amount was higher early in the morning (8 h GMT) on June 10 which was the only day to have a time effect (table IV).

The analysis of sugar composition was performed on fewer samples than the nectar production because a 1 μ L minimum volume was necessary for the method (D-23, 17 plants; D-27, 54 plants). The mean proportion of sucrose (54.4%) was higher than that

of glucose (14.4%) and fructose (31.2%) and there was no difference between the lines (table V). The composition was similar for the two types and varied greatly between plants. No date, time of day or flower-state effects were detected. The total sugar concentration was 36 mg/ 100 μ L on average (individual readings from 13–75).

Attractivity and foraging behavior

A total of 302 pollinating insects were counted during 51 observations. Most of them were *A mellifera* (69.2%) and *B terrestris* (27.5%). Solitary bees and *Bombus hortorum* were less frequent (3 and 0.3% respectively). The number of insects per 1 000 flowers was higher on the later date (table VI) and *Bombus terrestris* showed a preference for the D-27 line even when the flowers were less abundant.

The foraging behavior of 63 and 70 honeybees and 9 and 44 bumblebees were observed on D-23 and D-27 respectively. Considering both nectar and pollen gathering on perforated and non-perforated flowers, honeybees exhibited more pollinating visits than *Bombus terrestris* (table VII). The percentage of honeybees entering a flower specifically only for nectar gathering was

Table VII. Proportion of pollinating visits on D-23 and D-27 by honeybees and bumblebees (*Bombus terrestris*) on flowers.

	D-23	D-27
<i>Apis mellifera</i>	84% ($n = 63$)	71% ($n = 70$)
<i>Bombus terrestris</i>	44% ($n = 9$)	50% ($n = 44$)

n = number of observed insects.

high on both lines (D-23: 76.2%; D-27: 68.6%) compared with values of 33.3% and 47.7% for *Bombus*. Honeybees with pellets never behaved as nectar robbers, in contrast to *Bombus terrestris* (11.1–15.9%). However, we watched 7.9 and 2.9% of honeybees with pellets versus 22.2 and 13.2% *Bombus* on D-23 and D-27 respectively.

DISCUSSION

It is generally agreed that nectar production and composition are predominant factors determining insect visitation.

Considering nectar production, our results confirm earlier unpublished data showing a clear difference between the lines (D-27 nectar production being four to six times higher than D-23). Nectar volumes were generally small and never exceeded 3.5 μL per flower; but they may be underestimated because nectar was not collected from the flowers for 24 h prior to the measurements and, in such a situation, a resorption process may occur. However, this phenomenon was described in extrafloral nectaries (Baker et al, 1978) and not demonstrated in floral nectaries of *Vicia faba*. The perforation of the calyx does not damage the secretory tissues and the flower bagging reduces evaporation, so practically no difference was observed between perforated and non-perforated flowers. Moreover, our data showed that the amount of nectar can fluctuate throughout the day, with a maximum at 8 h GMT.

The nectar composition analyzed by HPLC showed that D-23 and D-27 nectars were sucrose-dominant and could be classified as SFG types according to Percival (1961). Consistent with our results, this author described three species of *Vicia* (*V. cracca*, *V. sativa* and *V. sepium*) as being sucrose-rich. However the variability of the data in the 1994 experiment should be noted: for instance, the proportion of sucrose

fluctuated between plants from 3.26–81.61% in both lines and no relation was found with date or day time, nor with flower stage. This contrasts with a previous experiment performed under an insect-proof cage, which showed less variation and a significant difference between the genotypes ($p = 0.03$). In that case sucrose represented 80% (standard error 0.9, $n = 10$) of the three sugars in D-27, whereas the current proportion was only 66% in D-23 (see 4.7, $n = 10$) (Mesquida, unpublished data). HPLC revealed in several cases the presence of sugars other than the three main ones. These observations may be related to those of Figier (1971) who suggested that extrafloral nectaries of *V. faba* possess areas for sucrose conversion, since its nectar contains some monosaccharides. This may also be related to information provided in the review of Baker and Baker (1983) who reported that 'families such as Fabaceae are less conservative than others and showed marked differences in the sugar ratio between closely related species'. Further investigation is needed to explain the variation of this character between lines, considering that they are at the same level of homozygosity.

From these results it appears that it is better to collect opened flowers to compare lines, because this stage corresponds to the maximum nectar production and allows insect visitation. However, because flowers of this type are not numerous on a fully flowering inflorescence (two or three), it is necessary to sample several flowering inflorescences on the main stem, to use more than five plants per replicate and to collect at 8 am (GMT) on several days. By this means it is possible to obtain an accurate estimate of the nectar production and to obtain a sufficient volume to analyze the sugar composition even when a line has very low secretions.

Honeybees are known to prefer sucrose (Waller, 1972; Bachman and Waller, 1977) and not to need a large amount of nectar (Dafni et al, 1988). This is confirmed in our

experiment, since the foraging honeybee density is high on the two lines although the nectar volume collected on D-23 is low. Its nectar volume, concentration and composition make it attractive enough. Additionally, the percentage of pollinating visits during nectar gathering is high in both lines, indicating that honeybees behave as secondary robbers less often than could be supposed according to observations made by Free (1968) on runner-bean plants. In these lines, the flower morphology (depth, and tripping mechanism) is not a dissuasive factor and honeybees may be used as open field pollinators.

Secondly, *B terrestris* exhibited a marked preference for the line with higher quantities of nectar, showing that they are able to choose clearly between two lines that are spatially clumped in a small area and they perforate the flower only when it secretes nectar. Our results may corroborate the observations made in laboratory and natural contexts by several authors (Pleasants, 1981; Real et al, 1982; Cartar, 1991) that *Bombus* are risk-averse and appear to be sensitive to the mean energy reward offered by flowers. Honeybees are said to exhibit the same behavior (Waddington, 1980; Fischer et al, 1993), but perhaps no difference was found because the reward was sufficient in both lines.

If we refer to the coevolution theory, it could be supposed that a genetic link may exist between the self-fertility of a plant, ie, its degree of insect dependence, and its ability to produce a large amount of reward such as nectar and pollen. The pollen availability was not considered in this study, but considering these genotypes, the absence of a linkage between the self-fertility level and the nectar production may be because in this case nectar is not a limiting factor for honeybees.

Résumé — Étude comparative de la sécrétion nectarifère et de l'attractivité vis-à-vis des abeilles de deux lignées de féverolle de printemps (*Vicia faba* L. var *equina* Steudel). Deux lignées de féverolle de printemps très proches génétiquement, l'une autofertile (D-27), l'autre peu autofertile (D-23), ont été étudiées en conditions naturelles au champs pour leur nectar (production et composition) selon l'état de la fleur et pour leur attractivité vis-à-vis de l'abeille mellifère (*Apis mellifera* L) et des bourdons (*Bombus* sp). Les deux lignées étudiées ont une production de nectar très différente. La sécrétion de nectar de la lignée D-27 est en moyenne de 4 à 6 fois plus importante que celle de la lignée D-23 (tableau III) et c'est lorsque la fleur est ouverte que la production est optimale (fig 3). Les quantités recueillies après 24 heures d'ensilage à 8, 10, 12, 16 h GMT sont de l'ordre de 1 à 2 µL par fleur. Certains jours, on détecte un effet heure et, dans ce cas, c'est le matin que la sécrétion est la plus abondante (8 h GMT). Les analyses réalisées par la technique HPLC (chromatographie liquide haute performance) montrent que, parmi les sucres présents dans le nectar, le saccharose est nettement plus abondant que le glucose et le fructose. Les données présentent une grande variabilité tant entre plantes qu'entre génotypes (tableau V). Compte tenu de ces résultats (faible production entravant les analyses qualitatives et forte variabilité), une nouvelle méthodologie est proposée pour comparer de manière fiable les sécrétions entre les lignées. En ce qui concerne les abeilles mellifères, qui sont en majorité des butineuses de nectar, les deux lignées sont également attractives. En revanche, *Bombus terrestris* montre une nette préférence pour D-27 (tableau VI), et il y a pratique des trous de manière à prélever le nectar sans pénétrer dans la fleur dès que celle-ci en sécrète

(stade fleur demi-fermée). Ces morsures ne modifient pas la sécrétion (tableau II). Que la fleur soit percée ou non, la proportion de visites négatives par ce bourdon est la même sur les deux lignées (50 à 55 %). Dans nos conditions, les abeilles mellifères butineuses de nectar semblent utiliser assez peu les trous faits par les bourdons et 68,6 à 76,2 % d'entre elles effectuent des visites positives. Ceci implique qu'elle peuvent être utilisées comme agent pollinisateur sur ces lignées. On peut penser que les faibles quantités de nectar produite par D-23 sont suffisantes pour attirer les abeilles. Cependant le fait que D-27, qui est autofertile (indépendante de la pollinisation entomophile) est la plus attractive pour les bourdons (tableau VI) est discuté.

***Apis mellifera* / *Bombus* / *Vicia faba* / sécrétion nectarifère / attractivité**

Zusammenfassung — Nektarsekretion und Bienenattraktivität von zwei Linien frühblühender Saubohnen (*Vicia faba* L var *equina* Steudel). Nektarproduktion und Zuckerszusammensetzung in Abhängigkeit vom Blütenstadium sowie die Attraktivität für Honigbienen und Hummen wurde an zwei Linien frühblühender Saubohnen (D-27, selbstbefruchtend; D-23, nicht selbstbefruchtend) unter Freilandbedingungen untersucht. Die Nektarmengen änderten sich je nach Blütenstadium, Tag der Probenahme und Tageszeit. Sie waren bei der Linie D-27 4 bis 6 mal höher als bei der Linie D-23 (Tabelle III) und bei geöffneten Blüten am größten (Abb 3). Die um 8, 10, 12 und 16 h GMT von 24 Stunden zuvor umhüllten Blüten gewonnenen Nektarmengen betragen 1 bis 2 µg pro Blüte. An manchen Tagen trat eine Tageszeitabhängigkeit auf, die Nektarsekretion war dann um 8 h GMT am höchsten. Mit Hochdruckflüssigkeitschromatographie (HPLC) durchgeführte Analysen zeigten eine hohen Saccharosegehalt des Nektars. Zwischen den

einzelnen Pflanzen und den Zuchtlinien gab es erhebliche Unterschiede der Nektarszusammensetzung (Tabelle V). Auf Grund der durch geringe Mengen und hohe Variabilität gekennzeichneten Ergebnissen wird eine geeignete Methode zum Vergleich der Nektarsekretion verschiedener Linien vorgeschlagen. In Hinblick auf die Sammelaktivität waren beide Linien gleich attraktiv für Honigbienen. Im Gegensatz hierzu bevorzugte *Bombus terrestris* die Linie D-27 (Tabelle VI). Sobald die Blüten mit der Nektarsekretion begannen (halbgeschlossenes Blütenstadium) bissen sie die Blüten auf, um Nektar zu stehlen. Die Nektarproduktion der Blüten wurde hierdurch nicht beeinflusst (Tabelle III). Der Prozentsatz räuberischer Blütenbesuche ohne Befruchtung durch die Hummeln war gegenüber beiden Linien gleich hoch (50 bzw 55%). Das von ihnen verursachte Loch wurde nur selten von den Honigbienen genutzt und die meisten Nektarentnahmen waren "legitim" (68,6 bzw 76,2%). Honigbienen können daher zur Bestäubung dieser Linien genutzt werden. Offensichtlich stellen die Nektarmengen der nicht selbstbefruchtenden Linie D-23 eine ausreichende Belohnung für Honigbienen dar. Die durch ihre Nektarproduktion höhere Attraktivität der von der Insektenbestäubung unabhängige selbstbefruchtenden Linie D-27 für Hummeln (Tabelle VI) wird diskutiert.

***Apis mellifera* / *Bombus* / *Vicia faba* / Nektarsekretion / Attraktivität**

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