
The Influence of Nectar Sugar Production on Insect Visitors to Flowers of *Brassica rapa* L.

M.A. Taylor and A.R. Davis

University of Saskatchewan, Department of Biology, 112 Science Place, Saskatoon, SK, S7N 5E2

Key Words: *Brassica rapa*, nectar, nectaries, insect visitation

Abstract

The purpose of this study was to determine if differing amounts of floral nectar sugar could affect the insect pollination of *Brassica rapa* L., a self-incompatible species. This knowledge could be directly applicable to crop-breeding programs interested in maintaining or enhancing the attractiveness of flowers to nectar-foraging visitors (possible pollinators). Several lines of rapid-cycling *B. rapa* were assayed (microcapillary nectar collection plus refractometry) for floral nectar-sugar production, and seed increases were made from plants selected on that basis. Both high and low nectar-producing plants were crossed reciprocally (high by high and low by low) in attempts to establish progenies distinct from each other. It appeared that reliably high and low nectar-yielding lines could be established. Outdoor insect visitation trials using high, intermediate, and low nectar-yielding lines of rapid-cycling plants of *B. rapa* in small plots resulted in more insects (especially flies and bees) visiting the high nectar-producing line, which in turn produced more seeds per silique.

Introduction

Several field studies of *B. rapa* have demonstrated the benefits of pollinating insects such as honey bees (Langridge and Goodman 1975, Fries and Stark 1983, Singh and Singh 1992) and hover flies (Ohsawa and Namai 1987) to increases in silique and seed yields. Fries and Stark (1983) have even connected increases in seed oil content in *B. rapa* to bee pollination, whereas Kevan and Eisikowitch (1990) attributed increases in seed germination of *B. napus* to bee visitation.

Leung *et al.* (1983) and Deng and Hu (1995) performed studies of nectary structure of *B. rapa* flowers. However, few publications exist where the relationship between nectary structure and nectar production has been investigated (Davis *et al.* 1994, 1996, 1998). One field study investigated hybrid-seed production in male-sterile and fertile lines of *B. napus*, where frequent nectary absence was noted (Mesquida and Renard 1979). Also, Leung *et al.* (1983) found an absence of nectaries in CMS *B. rapa* and that, through selective breeding, restoration of normal glands was achievable. Few studies have been conducted to record nectary presence and structure and then related these data to floral nectar-sugar production, insect visitation to the flowers in field plots, and quantified the resulting silique and seed set.

This study was initiated to determine the effect of floral nectar-sugar production of rapid-cycling *B. rapa* on the number of flower visitors, visitor duration, silique production, the number of

seeds per silique, and number of seeds per plant. Three lines were established to consistently produce different amounts of nectar sugar (none or very little, intermediate, and high), corresponding to apparent differences in nectary structure (absence, split, lobed, or normal). The hypothesis being tested was that insects will visit flowers of low nectar-sugar producing lines less frequently or for a shorter duration, which may have an adverse effect on the silique and seed set.

Materials and methods

Rapid-cycling plants of *B. rapa* (line C1-33 developed by the Crucifer Genetics Cooperative at the University of Wisconsin, Madison, Wisconsin, U.S.A., for uniform height and 15-day flowering period) were sown in Sunshine Mix 1 and reared at approximately 22±C/16 h days and 16±C/8 h nights in a growth chamber. The plants were evaluated based on their average nectar-sugar production per flower.

High and low nectar-sugar producing plants were selected as parents for reciprocal crosses with the intention of establishing high and low producing lines. Two trials (summers of 2002 and 2003) of F₁ plants were started in a growth chamber and screened for nectar sugar production. The F₁ plants were then placed into three groups (low, intermediate and high) based on nectar-sugar production per flower. In Trial 2, emergence of the high nectar-producing plants was unexplainably very low, which resulted in insufficient numbers of plants to repeat the Trial 1 experiment, exactly. Therefore, the highest nectar-producing plants in the intermediate group were included as high plants (instead of being discarded as they were in Trial 1). As a result, the high group in Trial 2 produced less nectar sugar per flower, on average, than the high plants in Trial 1. After nectar extraction, representative flowers of some lines were fixed in FAA for eventual preparation for scanning electron microscopy (SEM) to investigate nectary abnormalities (absence, splitting, and lobing).

Four days after the commencement of flowering, twenty plants of each group (low, intermediate, and high) were transplanted into the Biology plot at the University of Saskatchewan in each year. The plants were placed into five replicates for each nectar-sugar classification and organized into a random complete block design. Each replicate contained four plants that occupied approximately 12 x 12 cm, and replicates were placed 40 cm apart. Observations were made to record insect visitation to flowers (the number of insects per replicate and the visit duration of major pollinators). Each replicate of four plants was specifically watched for five minutes for visitors, but when large pollinators such as bumble bees were observed entering the plot, the insect was tracked through the plot and the duration per replicate was determined. Plants were harvested and the number of siliques per plant and the average number of seeds per pod were determined. Some plants were lost due to insect damage in both trials. The number of siliques per plant, seeds per silique, and seeds per plant were recorded.

Results

Plants were classified into three groups based on average nectar-sugar production per flower. Nectar-sugar production in the low and intermediate groups was very similar (approximately 5 µg and 50 µg of sugar per flower, respectively) among the two trials. The high nectar-producing

plants of Trial 2 had a lesser amount of nectar sugar per flower (approximately 160 μg) than the high nectar plants in Trial 1 (approximately 220 μg per flower). The differences were due to the poor emergence from the seed of high nectar plants and the necessary inclusion of relatively high-yielding intermediate plants in Trial 2.

Differences in nectary structure were observed by SEM throughout these trials. There were flowers of the low group with no or very little nectary tissue. Other flowers of the low group had different degrees of split lateral nectaries. The flowers with the very rudimentary nectary tissue were occasionally morphologically different, with the flower parts appearing to be more splayed (spread out) and they contained four to seven stamens usually of the same length, and sometimes fused (unlike the four long stamens plus two short stamens in normal flowers). Some flowers from the intermediate group of nectar-producing plants also possessed split lateral nectaries, indicating that this nectary morphology did not always lead to a great loss in carbohydrate-secreting function and some had lobed lateral nectaries. Flowers which were high nectar-sugar producers had normal nectaries.

Insect flower visitors were divided into seven different taxonomic categories: the total number of insects, flies, bees, wasps, crucifer flea beetles, ants, and other. Flies comprised the majority of flower visitors in both trials, whereas bees represented 15 to 35% of all flower visitors. Crucifer flea beetles were consistently present in Trial 1. There were few flea beetles present in Trial 2, during the flowering period, but the numbers started to increase later into the observation period as the flea beetles moved into one corner of the plot. At the time of harvest in Trial 2, the number of flea beetles had increased significantly (the plants appeared black with the large number of beetles present), resulting in the early commencement of harvest to prevent additional damage caused by the flea beetles. There were numerous lesions observed on the leaves, stems, and siliques of the plants in Trial 2.

When the data for insect visitation were analyzed by SAS, significantly more fly and bee visitors were found on the high nectar-sugar producing plants in both trials ($p < 0.05$). Observations of the large pollinators for their visit duration within a replicate showed that bees appeared to visit flowers of the high nectar-sugar producing plants for longer period of times. For example in Trial 2, a bumble bee visited a high nectar-sugar replicate for 38 s then moved to an intermediate replicate for 2 s before leaving the plot, but due to low sample size numbers, no significance in visit duration was found. When plants were harvested for seed, it was determined that high nectar-sugar producing plants produced a significantly larger quantity of seeds per silique in both trials.

Discussion

Approximately 70% of the F_1 generation from the low cross had rudimentary or split lateral nectaries, which was probably the cause of the extremely low nectar-sugar production. The F_1 generation of the high cross had normal lateral nectaries, and produced a normal to high amount of nectar sugar per flower. The selection of high and low nectar-producing lines may have been the result of inadvertently breeding for nectary structure. Leung *et al.* (1983) demonstrated that CMS lines of *B. rapa* lacking or having rudimentary nectaries could be partially restored with intensive selection and breeding.

Increases in the proportion of flower-visiting bees from Trial 1 to Trial 2 (approximately 15 and 35%, respectively) might be the result of less competition from the dominating bumble bees, which decreased in numbers between trials. Conner and Neumeier (1995) made a similar suggestion, that honey bees may have influenced the small bee numbers by out-competing them in *B. nigra*. In our study, the number of bumble bees may have decreased because the neighbouring plots of higher nectar-sugar producing flowers of *B. napus*, of another research plot located approximately 20 m away from the *B. rapa* plot, were not in full bloom in Trial 2 (unlike Trial 1), which may have originally attracted the bumble bees to the area.

The replicates with the higher floral nectar-sugar production had significantly more insect visitors to the flowers in both trials. Kubisova and Haslbachova (1984) and Mishra and Sharma (1988) also found a greater number of foraging insects on *Brassica* plants that secreted a larger amount of nectar sugar, which corresponds with the data obtained in this study. In *B. napus*, the absence of nectar caused honey bees to investigate three or four flowers in total, before flying some distance away from the nectar-less flowers (Eisikowitch 1981).

Large bees (including bumble bees) were the least troublesome of the foraging insects to time the duration spent on replicates and to track through the field plot. They appeared to spend longer visits on the flowers produced by the high nectar-sugar replicates. The small size, high alertness, and quick movements of the small bees and flies made it very difficult to track and time them easily. The grasshopper bee fly, *Systoechus vulgaris*, was an exception; it would sit on a single flower for minutes, then land on the ground for another extended period of time before moving to another flower with no apparent preference to the nectar-sugar levels. The wasps were also quite alert to movements around them and spent little time in the field plots. Mesquida *et al.* (1988) found that bumble bees foraged at a faster rate than honey bees, while Ohsawa and Namai (1988) and Langridge and Goodman (1975) determined that hover flies were less efficient pollinators with their slower movements between flowers than bees.

Cresswell (1999) determined that the bee's length of visit to *B. napus* flowers was affected by the amount of nectar present, but not by the amount of pollen. Mohr and Jay (1990) also suggested that bees spent more time per flower on *B. napus* than *B. rapa* flowers, because of the greater quantities of nectar present. Longer visits by insects on the flowers resulted in increased pollen deposition on the stigma of *Brassica* species (Ohsawa and Namai 1988, Cresswell 2000), so apparently an extended duration of visit can be important to increase the opportunity for pollination to occur in *Brassica*.

Both trials had similar results in the production of the number of siliques and seeds per plant, but the number of seeds per silique seemed slightly larger in Trial 1, which could be due to the large infestation of crucifer flea beetles causing damage to the plants and siliques in Trial 2. In both trials, the high nectar-sugar producing plants appeared to have fewer siliques per plant and more seeds per plant, but only the number of seeds per silique was significantly higher for the high nectar-producing plants. The plants that produced more nectar sugar per flower attracted a greater number of insects, which appeared to spend more time on these plants. This activity most likely resulted in greater pollen transfer and enhanced pollination. Increased pollination would explain the greater seed set per plant; combined with fewer siliques per plant, the number

of seeds per silique became significantly higher for the high nectar-producing plants. If pollination was indeed increased on the flowers of the high nectar-sugar plants, the resources would be allocated to the maturing siliques and seeds, instead of initiating more flowers. Lerin (1982) determined that bees foraging on *B. napus* caused a non-significant increase in seed yield but a reduction in the number of siliques on the main shoot when compared to plants that were caged to exclude insect pollinators. Mesquida and Renard (1979) and Mesquida *et al.* (1988) found in *B. napus* that there were more flowers and the flowering period lasted longer when insects were prevented from visiting the plants. This result led the authors to suggest that plants compensate by producing extra flowering shoots to make up the potential loss of seed production in the absence of insects for pollination.

It is evident from the data obtained in this study, that flowers of *B. rapa* plants with vestigial nectaries and poor nectar-sugar production, will still be visited by insects that results in seed production. However, plants secreting more nectar sugar attracted a greater number of insects that possibly spent more time foraging on the flowers. The increased visitation resulted in enhanced pollination and the high nectar-sugar plants produced more seeds per silique. Many researchers have already confirmed that more pollinators will have a positive effect on various yield components in *Brassica*, like seed yield, seed weight, siliques per plant, and seeds per silique (Langridge and Goodman 1975, Eisikowitch 1981, Lerin 1982, Fries and Stark 1983, Kubisova and Haslbachova 1984, Ohsawa and Namai 1987, Mishra and Sharma 1988, Singh and Singh 1992). This information shows the importance and possibility of breeding plants for increased floral nectar-sugar production to attract more pollinators, which would enhance pollination and could have a positive effect on seed production, especially in the self-incompatible species, *B. rapa*. Other situations may call for a decrease in pollen transfer (e.g. transgenic plants), so selecting lines with low nectar-sugar production to decrease the attractiveness of the plant, may be feasible in larger-scale settings.

Literature Cited

- Conner, JK; and Neumeier, R. 1995. Effects of black mustard population size on the taxonomic composition of pollinators. *Oecologia* 104: 218-224.
- Cresswell, JE. 1999. The influence of nectar and pollen availability on pollen transfer by individual flowers of oil-seed rape (*Brassica napus*) when pollinated by bumblebees (*Bombus lapidarius*). *J. Ecol.* 87: 670-677.
- Cresswell, JE. 2000. Manipulation of female architecture in flowers reveals a narrow optimum for pollen deposition. *Ecology* 81: 3244-3249.
- Davis, AR; Pylatuik, JD; Paradis, JC; and Low, NH. 1998. Nectar-carbohydrate production and composition in relation to nectary anatomy and location within individual flowers of several species of Brassicaceae. *Planta* 205: 305-318.
- Davis, AR; Fowke, LC; Sawhney, VK; and Low, NH. 1994. Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. *Apidologie* 25: 602-614. Erratum 1995 26: 534.
- Davis, AR; Sawhney, VK; Fowke, LC; and Low, NH. 1996. Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). II. Quantified variability of nectary structure and function in rapid-cycling lines. *Ann. Bot.* 77: 223-234.
- Deng, Y-b; and Hu, Z-h. 1995. Development process and histochemical changes of floral nectaries in *Brassica campestris*. *Acta Biol. Boreal. -Occident. Sin.* 15: 14-18.

- Eisikowitch, D. 1981. Some aspects of pollination of oil-seed rape (*Brassica napus* L.). *J. Agri. Sci. Camb.* 96(2): 321-326.
- Fries, I, and Stark, J. 1983. Measuring the importance of honeybees in rape seed production. *J. Apic. Res.* 22: 272-276.
- Kevan, PG; and Eisikowitch, D. 1990. The effects of insect pollination on canola (*Brassica napus* L. cv. O.A.C. Triton) seed germination. *Euphytica* 45: 39-41.
- Kubisova, S; and Haslbachova, H. 1984. Pollination by bees of self-incompatible cabbage lines (*Brassica oleracea* var. *capitata* L.) grown under cover. *Proc. Vth Int. Symp. on Pollination, Versailles, France, 1983.* 357-366.
- Langridge, DF; and Goodman, RD. 1975. A study on pollination of oilseed rape (*Brassica campestris*). *Aust. J. Exp. Agr. Anim. Husb.* 15: 285-288.
- Lerin, J. 1982. Effects de la pollinisation entomophile sur le colza dans une experience en cage. *Agronomie* 2: 249-256.
- Leung, H; X-K, N; Erickson, EH; and Williams, PH. 1983. Selection and genetics of nectary development in cytoplasmic male sterile *Brassica campestris*. *J. Amer. Soc. Hort. Sci.* 108: 702-706.
- Mesquida, J; and Renard, M. 1979. Entomophilous pollination of male-sterile strains of winter rapeseed (*Brassica napus* L. Metzger var. *oleifera*) and a preliminary study of alternating devices. *Proc. IVth Int. Symp. on Pollination.* 49-57.
- Mesquida, J; Renard, M; and Pierre, JS. 1988. Rapeseed (*Brassica napus* L.) productivity: the effect of honeybees (*Apis mellifera* L.) and different pollination conditions in cage and field tests. *Apidologie* 19: 51-72.
- Mishra, PC; and Sharma, SK. 1988. Growth regulators affect nectar-pollen production and insect foraging in *Brassica* seed crops. *Current Science* 57: 1297-1299.
- Mohr, NA; and Jay, SC. 1990. Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. *J. Apic. Res.* 29(2): 95-100.
- Ohsawa, R; and Namai, H. 1987. The effect of insect pollinators on pollination and seed setting in *Brassica campestris* cv. Nozawana and *Brassica juncea* cv. Kikarashina. *Japan. J. Breed.* 37: 453-463 (English summary on p. 461).
- Ohsawa, R; and Namai, H. 1988. Cross-pollination efficiency of insect pollinators (Shimahanaabu, *Eristalis cerealis*) in rapeseed, *Brassica napus* L. *Japan. J. Breed.* 38: 91-102 (English summary on p. 100).
- Singh, RP; and Singh PN. 1992. Impact of bee pollination on seed yield, carbohydrate composition, and lipid composition of mustard seed. *J. Apic. Res.* 31: 128-133.