

THE IMPORTANCE OF HONEY BEES (*APIS MELLIFERA* L.) AS POLLINATORS OF BORAGE (*BORAGO OFFICINALIS* L.) IN SASKATCHEWAN

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Introduction

Seed of borage (*Borago officinalis* L.) is a relatively rich source of gamma-linolenic acid (GLA) that serves as a medicinal drug for treatment of disorders ranging from elevated cholesterol levels to premenstrual syndrome (Galwey and Shirlin 1990). This pharmaceutical plant is being grown commercially in various parts of Europe and Canada, where large acreages (40-160 acres) have been sown in Saskatchewan for over ten years (A.E. Slinkard, pers. comm.). GLA content in borage seed is highest when seeds mature at cooler temperatures (A.E. Slinkard, pers. comm.). Accordingly, much of the commercial production of borage in the province occurs north of Saskatoon, where seed is planted very late in May to early June. Flowering begins in mid-late July and lasts until killing frost. Seed, as much as four (very rarely, six) nutlets per flower, is harvested from late August to mid-September.

Two recognized agronomic problems faced by borage producers are the loss of early-maturing seed and weed control. A third potential problem, inadequate pollination of borage flowers, is of direct relevance to seed production and remained to be investigated. Each year, many commercial growers of borage engage local beekeepers for the introduction of their colonies of honey bees (*Apis mellifera* L.) for pollination purposes. This practice has continued without scientific evidence to support the value of honey bees as pollinators of borage. Three of our project's main objectives were 1) to determine the relative attractiveness of borage to honey bee colonies, 2) to determine the pollination requirements of borage flowers, and 3) to evaluate honey bees and other insect visitors of borage flowers, as actual pollinators of the crop.

Materials and Methods

These three objectives were investigated during two consecutive field seasons (July-September 1993 and 1994). Operations were carried out both at commercial field sites and at the University.

Field Sites

Four different commercial borage fields were investigated each season; all fields had apiaries nearby, usually bordering them. Each apiary contained approximately 40 populous honey bee colonies operated by commercial beekeepers, such that the fields were stocked with approx. 0.5-1 hive per acre of borage. Field sites were situated just north of Saskatoon; in 1993, three sites were west of the South Saskatchewan River, and the next summer, three east. The Clarkboro ferry across the river permitted a regular, triangular route between sites. Two field sites (Aberdeen, Langham) were utilized both years.

Pollen collection by honey bees was assayed at each apiary in order to provide some measure of borage's attractiveness. For this purpose, the entrances of each of three bee colonies per apiary was fitted with a pollen trap (Ontario Agricultural College) that dislodged the leg-borne pollen pellets from incoming bee foragers into a hidden tray below. During apiary visits on Fridays throughout the blooming period, the pollen traps were activated. On Mondays, traps were deactivated and pollen pellets that had accumulated in them over the weekend carefully emptied into labelled plastic bags and stored in a freezer for future analysis. Because individual honey bees show a remarkably high fidelity to an individual plant species per foraging trip (see Davis 1991), pellets are monochromatic and could be sorted accordingly; pellets of borage pollen are light tan to off-white. Pollen of melliferous flora was then microscopically identifiable to species (Crompton and Wojtas 1993).

When weather conditions were suitable, individual insects and their foraging activities (nectar collection only, or collection of both nectar and pollen) on borage flowers were identified and recorded.

University Plot

In mid-May of each year, twenty borage plants were sown in the Department of Biology plot on campus. No efforts were made to introduce potential pollinators near the plot, so that insect visitors to these plants were natural to the local area. It was here that the most detailed observations and experiments on pollination were conducted.

The results of preliminary, careful hand-pollination experiments determined that borage flowers were self-fertile, setting seed as a result of both cross- and self-pollination. Pollen tubes (1 per germinated pollen grain) grew from the stigma to the ovules (style approx. 1 cm) where fertilization occurred.

Most experiments dealt with "virgin flowers". These are individual flowers that, a day earlier, had been labelled with coloured tape around the pedicel and surrounded in mesh bags (approx. 2.5 mesh/mm; Davis 1992) when they were still closed, mature buds, and so had not been visited previously by bees. After 24 h, these buds had opened inside the bags and were now available for experimentation. The following five treatments were performed using virgin flowers:

Treatment A - Emasculated, never unbagged - At the time of labelling but just before bagging, the petals of the mature bud were reflexed gently and the five indehiscent anthers removed using fine forceps.

Treatment B - Intact, never unbagged - Buds were labelled (no emasculaton) and bagged for the flower's entire lifetime.

Treatment C - Intact, unbagged but immediately rebagged - Buds were labelled (no emasculaton) and bagged; 24 h later, bags were carefully removed from around the fully-open "virgin" flowers, which were carefully rebagged immediately before receiving any insect visits.

Treatment D - Intact, unbagged left exposed for multiple visits - Buds were labelled (no emasculaton) and bagged; 24 h later, bags were carefully removed from around the fully-open "virgin" flowers, which were then available to multiple (unknown number) insect visits during their lifetime.

Treatment E - Intact, unbagged, left exposed for single visit - Buds were labelled

(no emasculation) and bagged; 24 h later, bags were carefully removed from around the fully-open “virgin” flowers which were watched vigilantly for the initial insect visitor, which was identified. Immediately upon insect departure, the visited flower was carefully rebagged, thus preventing any further insect visits.

Styles from treated flowers were collected and processed for pollen-tube counts (Davis 1992). Briefly, at the end of the treatment period (usually at 50% senescence), the style from each treated flower was gently teased from its junction with the four ovules and fixed (FAA) in a labelled vial. They were later stored in 70% ethanol. Thereafter, styles were softened (10% Na_2SO_3 overnight at 50°C), stained in 0.1% aniline blue (in 0.1 M Na_3PO_4), gently squashed with a coverslip and then examined by fluorescence microscopy for pollen tubes at their bases.

Results to date

Analysis of Pollen-Trap Collections

Much of the pollen analysis remains to be completed. However, it is clear that there is a marked difference in the percentage of borage pollen (as expressed by dry weight) collected by different honey bee colonies, even within the same apiary. In 1994 at Vonda, Hive 1P averaged approx. 40% borage pollen (peak 80%), whereas Hive 3P averaged < 6% (peak < 12%) throughout the borage flowering period. In 1994 at Ferry, 35% of Hive 1P's pollen consisted of borage (peak 86%), while Hive 2P averaged less than 5% borage. Therefore, considerable inter-colonial variation exists in pollen collection by honey bees from borage.

Observations of Foraging Honey Bees at the Field Sites and at the University Plot

At the field sites in 1994, of 1,753 recorded insect visits to borage flowers, 97% of these were made by honey bees. It is likely that most came from the apiaries nearby. Even at the University, *Apis mellifera* represented 40% of total insect visitors (n=498).

From 1,703 observations of honey bees in the commercial borage fields, it became evident that only a minority of bees collected borage pollen while foraging. For every foraging honey bee that collected both nectar and pollen, 3.1 more bees gathered nectar alone (as judged by their lack of borage pollen pellets on their hind legs). These data indicate that, generally, the pollen-trap data (above) considerably underestimate the foraging intensity of honey bees on borage. That is, the many returning bees which gathered nectar alone from the borage fields were not recorded in the pollen-trap collections, because of their absence of pollen pellets.

Controlled Pollination Experiments Involving Virgin Flowers

The results available at this time (n = 32 to 105 styles) are given in a table at the top of the next page.

Discussions and Conclusions

Field observations of honey bee foraging activity demonstrated that most bees (on average, 75%) visited borage flowers for nectar alone. Therefore, pollen-trap analyses by themselves did not fully represent the attractiveness of *Borago* to honey bees, because only pollen-laden foragers were recorded by them. Honey bees comprised 97% (average)

<u>Treatment</u>	<u>Treatment description</u>	<u>Average no. pollen tubes per style</u>
A	Emasculated, never unbagged	0
B	Intact, never unbagged	<1
C	Intact, unbagged but immediately rebagged	<1
D	Intact, unbagged, left exposed for <i>multiple</i> visits	>9
E	Intact, unbagged, left exposed for a <i>single visit</i> by	
	- a honey bee (<i>Apis mellifera</i>)	>8
	- a bumble bee (<i>Bombus</i> spp.)	>8
	- a hover fly (Syrphidae)	<1

of all flower visitors observed at the field sites. Trap analyses did show large differences in % borage-pollen collection between neighbouring colonies within an apiary, however, a phenomenon reported for other plant species (Shimanuki et al. 1967, Schmidt and Buchmann 1986).

Air currents are not important for pollination of borage. Not a single pollen tube has been detected in 66 styles taken from emasculated, permanently-bagged flowers. Note that the bag mesh would not have intercepted significantly any airborne pollen grains. Also, the stigma of the borage flower always appears dry and has no elaborations (e.g., plumose hairs) that would suggest a filtering mechanism for airborne pollen.

Instead, the experiments with virgin flowers have demonstrated clearly the need for insects, and in particular, bees, for the pollination of borage flowers. When insects were prevented access to flowers, the number of pollen tubes per style in those flowers was low. However, *single visits* by either honey bees or bumble bees to receptive flowers normally resulted in more than adequate pollination, the number of pollen tubes per style base averaging over twice the number of ovules (four) available to be fertilized per flower. Hover flies were infrequent visitors and generally ineffective as borage pollinators.

Honey bees were found to be effective pollinators of borage and introduction of honey bee colonies for the pollination of commercial fields is recommended. The large foraging populations of honey bees that are imported likely make a major difference in seed yields for the grower. It is anticipated that when all the data can be considered, accurate estimates of the number of honey bee colonies required per acre can be determined. It is interesting to note that although their population levels experience natural fluctuations from year to year and never approach a tenth the size of honey bee colonies, native bumble bees also eagerly visit borage flowers and can make a contribution as pollinators.

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