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# Preliminary investigations into the significance of floral applications of calcium, boron and polyphenols for increased seed set in confection sunflowers (*Helianthus annuus* L.)

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**Abstract**

One of the major problems of confection sunflowers is low seed set. We studied the relationship between seed set and the origin of pollen, either self or cross, and the effects of stigmatic sprays of calcium nitrate, boric acid and polyphenols on seed set. In a screen-house experiment, seed set was analyzed for three predetermined regions in the sunflower capitulum: distal, median and proximal. Cross-pollination combined with calcium nitrate or boric acid spray significantly improved seed set in the distal region compared with cross-pollination alone or with cross-pollination combined with self-pollination. Neither spray had a significant effect on seed set in the median or proximal regions. Cross-pollination of the proximal region only, with either fresh pollen or 24-h-old pollen, did not improve seed set compared with cross-pollination of the whole capitulum. We found no direct link between pollen germination on the stigma and seed set. However, each region of the capitulum responded differently to self- or cross-pollination. In two open-field experiments, stigmatic applications of calcium nitrate significantly increased seed set by approximately 9% compared with open pollination alone, while stigmatic applications of polyphenols significantly increased seed set by approximately 9% only once, suggesting that commercial yields may be increased by using similar applications.


*Keywords:* Pollen germination; Pollination; Polyphenols; Seed set; Sunflowers

## 1. Introduction

The cultivated sunflower (*Helianthus annuus* L.) is grown commercially worldwide as an oil crop, as an ornamental for cut flowers and for confectionary purposes, either in-shell or dehulled. Confection sunflowers are grown in Israel both for domestic use and for export, primarily to Spain, and occupy about 10,000 ha of land. Many Israeli varieties have relatively long seeds (~20 mm) with an oil content of <40%. Israeli confection sunflowers are sporophytically self-incompatible, having a genetic mechanism by which the plant identifies self-pollen, with which it shares alleles, on the stigmatic surface and avoids selfing. It is considered that this mechanism evolved to preserve the genetic variability of the species (Newbigin, 1996).

Sunflower pollination is best performed by insects, and in commercial fields honeybees are reported to be the most suitable pollen vectors (Free, 1970), mostly seeking nectar (Lior and Dag, 1994). Even under optimal honeybee activity, a relatively large proportion (15–50%) of seeds remains empty, primarily in the proximal (central) and distal (peripheral) regions of the capitulum (Xanthopoulos, 1991; Dag et al., 2002). The same problem was also found in oil sunflowers but on a smaller scale (Morozov, 1958). It has been suggested that the empty seeds in the distal and proximal regions of the capitulum resulted from several unrelated factors, and that the same factors affected the distal and proximal regions differently.

So far, very few studies have addressed the pollination biology of confection sunflowers, and the factors causing low seed set are yet to be discovered. Ben-Porat and Massad (1995) reported that when cross-pollination was preceded by removal of ‘‘excess’’ self-pollen, seed set was increased. In a later study,

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the activity of honeybees in the field was reported to be sufficient, and that supplementing open-pollinated sunflowers with hand pollination did not enhance seed set (Dag et al., 2002). It was suggested that the empty seeds were the result of limiting physiological factors, yet to be identified.

The major objectives of the present study were the following: (1) to characterize the relationship between seed set, on the one hand, and, on the other hand, the age of the pollen, its origin (self or cross), and the number of germinating pollen grains on the stigma; (2) to test the effects on seed set, of the pollen germination enhancers calcium nitrate, boric acid (Brewbaker and Kwack, 1963; Stanley and Lichtenberg, 1963), and polyphenols (Sedgley, 1975; Derkensen et al., 1999).

## 2. Materials and methods

### 2.1. Screen-house experiments (2005)

*H. annuus* L. var. 'Ambar' plants were grown in a screen-house (50 mesh) in order to prevent pollination by insects. The plants were grown in 10-L pots and were drip-irrigated during the growth period at 1.3 L day$^{-1}$ per plant, and during the bloom period at 1.6 L day$^{-1}$ per plant.

We tested the effects on seed set of self- or cross-pollen, combined with pollen germination enhancers, sprayed prior to the pollination treatments:

(1) Cross-pollination + calcium nitrate 0.024 M (cross + calcium; $N = 15$).
(2) Cross-pollination + boric acid 0.016 M (cross + boron; $N = 15$).
(3) Cross-pollination (cross; $N = 20$).
(4) Cross-pollination + self-pollination (cross + selfing; $N = 15$).
(5) Self-pollination (selfing; $N = 15$).
(6) Spontaneous self-pollination (Spon' self; $N = 15$).

We tested the effects of pollen age on seed set, specifically in the center of the capitulum (proximal region):

(1) Cross-pollination of the whole capitulum (cross; $N = 15$).
(2) Cross-pollination of the proximal region with fresh pollen (center − fresh pollen; pollen collected from florets at their staminate stage; $N = 6$).
(3) Cross-pollination of the proximal region with 24-h-old pollen (center − 24-h pollen; pollen collected from florets at their pistillate stage; $N = 7$).

All pollination treatments were applied daily, during the bloom period of 7–8 days. Cross pollen was applied with a pollination device, which is essentially an elaborate pollen blower that was designed in collaboration between the Volcani Center and Tel Aviv University (Vaknin et al., 2001). Each capitulum was exposed to 0.05 g of pollen per application. Self-pollen was applied with a brush which was stroked against the open florets, within the capitulum, thus transferring pollen from the anthers to the stigmas. Spontaneous self-pollination was achieved by leaving the capitula undisturbed. Stigmas were sprayed with solutions of boric acid or calcium nitrate that were recommended for pollen germination in vitro (Vaknin et al., 2003).

Forty-eight hours after emergence of the stigmas, florets were sampled from five plants in each treatment, for microscopic analysis, and were preserved in 70% ethanol. Ten florets were collected from each of three predetermined regions of the capitulum: distal, median and proximal, a third of the radius each (Fig. 1). The microscopic analyses of pollen germination used the Aniline Blue Epifluorescence method (Martin, 1959). At the end of the season (July 2005) the capitula were harvested and dried. Both empty and full seeds were manually sorted and weighed for each of the three regions of the capitulum. The percentage seed set for each region was calculated by estimating the number of empty and full seeds after weighing a sample of 50 seeds of each type.

### 2.2. Open-field experiments 2006–2007

In 2006, the experiment was conducted in a commercial field of sunflowers, var. 'D.Y.3', a very closely related variety to 'Ambar', at Kibbutz Be'erot Itzhack. The experiment was arranged in random blocks: three blocks, each containing four treatments, with 10 plants per treatment. Bee hives were distributed at the periphery of the field at a density of one hive per 0.7 ha. The experiment commenced at peak bloom (62.5% blooming plants). All treatments were applied once daily for 7 days:

(1) Open pollination + calcium nitrate (calcium; $N = 27$).
(2) Open pollination + boric acid (boron; $N = 27$).
(3) Open pollination + polyphenols (polyphenols; $N = 28$).
(4) Open pollination + double-distilled water (DDW; $N = 30$).
(5) Open pollination (open; $N = 30$).

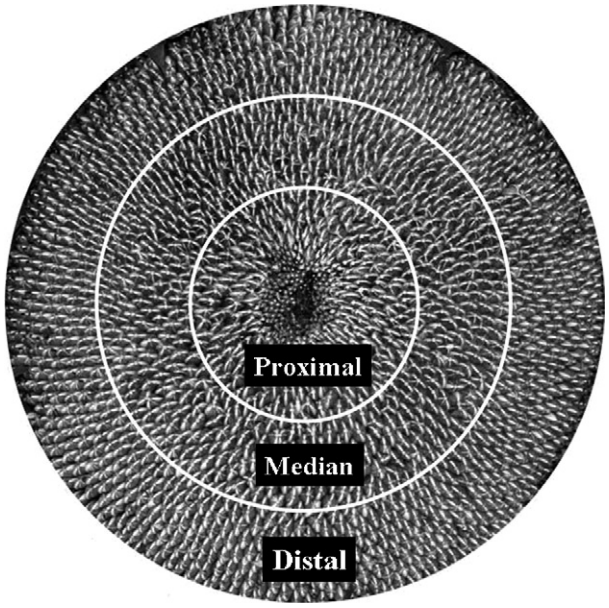


Fig. 1. The sunflower capitulum divided into three regions: distal (peripheral), median (intermediate) and proximal (central).