

## The pattern of foraging paths of the Honey bee (*Apis mellifera* L.) can also explain the appearance of located regions with incompletely developed fruits in the sunflower capitulum

Luis F. Hernández<sup>1,2</sup>

<sup>1</sup>Laboratorio de Morfología Vegetal. Depto. de Agronomía, UNSur. Bahía Blanca, 8000, Argentina

<sup>2</sup>Comisión de Investigaciones Científicas de la Pcia. de Buenos Aires (CIC). La Plata, 1900, Argentina,  
E-mail: lhernan@criba.edu.ar

### ABSTRACT

The occurrence of fruits with absent or poorly developed embryos, also defined as seedless fruits or incompletely developed fruits (IDF), respectively, significantly impacts sunflower yield. Failures in pollination, fertilization and physiological or morphological defects in the ovary and embryo, either genotypic or environment-induced, post-pollination or post-fertilization, are among the most common reasons for the generation of this kind of fruits. A detailed study of the foraging pattern of diurnal pollinators in the sunflower crop, mainly honey bees, showed that there was a significant inverse correlation between the percentage of areas covered by foraging paths (ACP) and the total IDFs counted per capitulum's concentric sector. Almost a complete limitation of visitations in these sectors (0-30% ACP) resulted in poor seed set and IDFs ranging from 9 to 17%. At that level of ACP, a significant inverse correlation ( $r^2=0.61$ ;  $p=0.05$ ) was found between the density of honeybee visitation and the percentage of IDFs. Partial limitation of the insect visitation (30 to 59% ACP) generated 5 to 9 % of IDFs. It is concluded that as much as 60% or more of the capitulum's area must be covered by pollinators to minimize the occurrence of IDFs.

**Keywords:** *Apis mellifera* - *Helianthus annuus* - pollen - seed set - sunflower.

### INTRODUCTION

At maturity, the capitulum of sunflower [*Helianthus annuus* L., var. *Macrocarpus* (D.C.) Cockerell] usually has fruits with a different degree of pericarp and embryo development. In most of them, the embryo reaches its full size filling the internal cavity of the ovary. These fruits are defined as fully developed (FDF) (Lindström et al., 2006; 2007). On the other hand, many fruits often contain ovules that did not fully develop into seeds. In those fruits, growth processes stop at different moments, leaving the fruits with an incompletely developed pericarp and/or seed so being defined as seedless or incompletely developed fruits (IDF; Alkio et al., 2002; Alkio and Grimm, 2003; Lindström et al., 2004). Generally, IDFs can be seen to be randomly distributed over the capitulum surface (Hernández et al., 2002; Lindström et al., 2004).

The causes of the origin of IDFs are unknown but several proximate mechanisms put forward to explain the low seed to ovule ratio in many species of the *Angiospermae* can be applied to the sunflower. Poor seed set occurs mainly due to inadequate pollination, the competition for resources between developing ovaries, or vascular deficiencies at the ovary-receptacle interface (Birch and van der Sandt, 1985; Durrieu et al., 1985; Hernández and Orioli, 1991; Hernández and Palmer, 1992; Connor and Hall, 1997; Alkio and Grimm, 2003; Cantagallo et al., 2004; Lindström et al., 2006). Several studies have shown that the foraging activity of the honey bee (*Apis mellifera* L.) can increase seed set and yield (Parker, 1981a; Birch and van der Sandt, 1985; Fell, 1986; Skinner, 1987; Medan et al., 2003; DeGrandi-Hoffman and Chambers, 2006). Nevertheless, the bee foraging pattern on the sunflower capitulum has not been deeply studied (Parker, 1981b) and its relationship with seed set has not been totally established.

The aim of the present work was to determine the relationship between the path of daily visits of pollinators on capitula of the cultivated sunflower and the pattern of IDFs.

### MATERIALS AND METHODS

The experiment was carried out at the Agronomy Department-UNSur, Bahía Blanca, Argentina (Lat. S. 38°45'; Long. W. 62°11') over one growing season. A low self-fertile experimental sunflower genotype, provided by Dow Agrosiences of Argentina, was sown starting the first week of October on three

successive dates, separated by 5 days, in order to obtain plants at the beginning of flowering (first anthesis [FA]; Schneiter and Miller, 1981) during several consecutive days and study them individually.

At seedling emergence, plant density was adjusted to 5.6 plants/m<sup>2</sup>. Weeds, pests and irrigation were adequately controlled. The experimental plot was near (300 m) 20 bee colonies. This ensured that visitation at flowering was highly intense. Daily records of temperature and solar radiation were obtained from a meteorological station located 800 m from the experimental field.

#### *Plant selection and pollinator visits observations*

Two plants displaced 4 to 5 days in time for each seeding date were randomly selected in the stand (n=6). Before FA, the selected plants were staked with the florets oriented eastwards. At FA, the capitulum diameter was measured and four landmarks were placed at the periphery using colored pearl head pins. The capitulum of one plant at a time was then continuously recorded using a digital camera. The recording process took 2 to 3 days, from FA until the first 6 to 7 rows of peripheral flowers finished opening. Bee foraging on a head was continuously watched from 8.00 a.m. to 5.00 p.m. Recording was interrupted when visitors were absent. Only honey bees and sporadically carpenter bees (*Xylocopa* sp.) were observed. At dusk, each capitulum was covered with a mesh bag to avoid the action of night pollinators. After the study was completed, the procedure was repeated with another plant that by that time was at FA. The observed capitula were covered during the night until harvest.

#### *Data processing*

Digital files for each observed plant (n=6) were processed using the software VideoPoint v.2.5 (Lenox Softworks, Lenox, MA) to define, in Cartesian coordinates, the pattern of foraging routes of the pollinators (Fig. 1). The bee's thorax was the reference point of movement to digitize the route followed by the insect during its visit (arrival-departure) to the capitulum. The landmarks on the capitulum allowed the correct location and correspondence of the recorded paths at anthesis and at maturity (Fig. 1). Each image of the capitulum was then fractionated in 60 sectors and the pixel density corresponding to the foraging routes in each sector was quantified with the software Object-Image v.2.21 (Vischer et al., 1994) in a Macintosh platform. After calculating the area of each sector of the capitulum, the average pixel density was estimated for each sector (pixels% per sector) for each capitulum for each one of the six observed plants (Fig. 2).

At harvest, IDFs were identified on each mature capitulum and its location per capitulum sector defined using the reference landmarks. The IDF proportion was calculated per each capitulum sector and then compared with the intensity of visitations (Fig. 1), defined in this work as area covered by paths or ACP (Fig. 3).

## RESULTS

The complete pattern in the capitulum generated by the routes followed during two consecutive visiting days for one of the six studied plants is shown in Fig. 1. Its corresponding density of visits per capitulum sector calculated according to the above methodology is presented in Fig. 2. The relationship between the % of areas covered by paths (ACP%) and the percentage of IDFs per sector in capitula of the six plants observed in this work is presented in Fig. 3.

The main floral visitors in all observations belonged to the order Hymenoptera (100% of total visits): *Apis mellifera* L., (98%), and *Xylocopa* sp. (2%). The path density was not homogeneous, showing zones with quite different densities (Figs. 1-2). There was an inverse relationship between the absence of visits or a low density of visits in a sector and the percentage of IDFs (Fig. 2). From Fig. 3 three intervals for the relationship between the ACP% and the IDF% can be defined. Thus, between 0 and 30% ACP an inverse relationship ( $r^2=0.61$ ;  $n=91$ ;  $p=0.05$ ) was observed (Fig. 3). Between 30 and 100% ACPs, IDF magnitudes were distributed in two levels of a broad fluctuation, ranging from 5.0 to 9.0 % IDFs between 30 to 100% ACPs and 0% IDF or 6.0 to 9.5 % IDFs between 56 and 100% ACPs (Fig. 3).

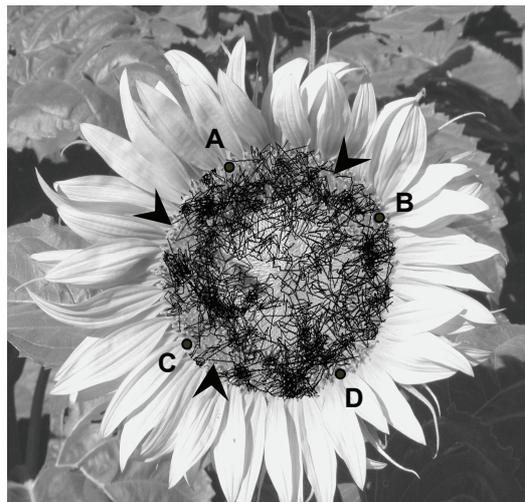
## DISCUSSION

Lack of sufficient pollen loads on the stigma to fertilize all the flowers (Zimmerman and Pyke, 1988) and physiological and/or anatomical alterations and source limitations to provide for seed development (Stephenson, 1981; Zimmerman and Pyke, 1988; Connor and Hall, 1997) have been most commonly attributed as causes for a low seed set.

According to the "non-uniform pollination hypothesis" (Thomson, 1989; Berry and Calvo, 1991) the

observed patterns of IDF's in the mature capitula may be attributable to variations in pollen receipt over the flowering period. Specifically, the relatively low seed set in central areas of the sunflower capitulum has usually been attributable to insufficient pollen quantity or pollinator visits.

The reason why some florets are left unvisited in the observed regions of the capitulum is not known. Recently, Giurfa (2004) demonstrated that the honey bee can discriminate color, and, regarding this, it has been noticed that some floret corollas in different capitulum locations of recently open florets, have a different color intensity compared with their neighbors (L.F. Hernández, unpublished). It is also known that honeybees avoid probing flowers that have been recently depleted by conspecifics, presumably repelled by foraging scent marks deposited by the previous visitor (Giurfa and Nuñez, 1992; Gawleta et al., 2005). Probably, if this is the case, the reason to leave some disc florets unvisited (Fig. 1) could be related with its proximity to already visited neighbor florets.



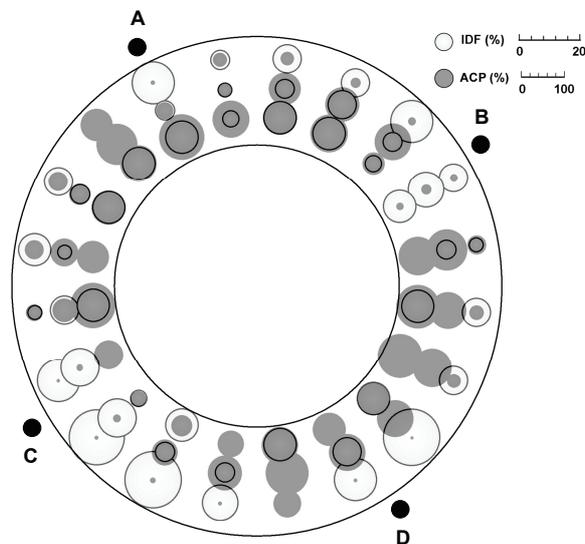
**Figure 1.** Pattern of routes of daily visits of honeybees (*Apis mellifera* L.) and, in a lesser proportion, carpenter bees (*Xylocopa* sp.) during two consecutive days after FA, on the capitulum of one plant studied in this work. The tracing of the image of 2 pixel width was accomplished after processing the digital images with the software VideoPoint. Arrows show some of the unvisited regions. Circles noted with letters A, B, C and D correspond to the landmarks defined with colored pearl head pins. The routes followed towards the central region of the capitulum were not considered in this study because these flowers were not open at the time of the analysis.

In this work, climate conditions during capitulum maturation were optimal. No rain occurred during the observation period, which could induce pollination failures by pollen lixiviation, and air temperature was always near or below 30° C, a thermal level known to affect sunflower pollination (DeGrandi-Hoffman and Chambers, 2006).

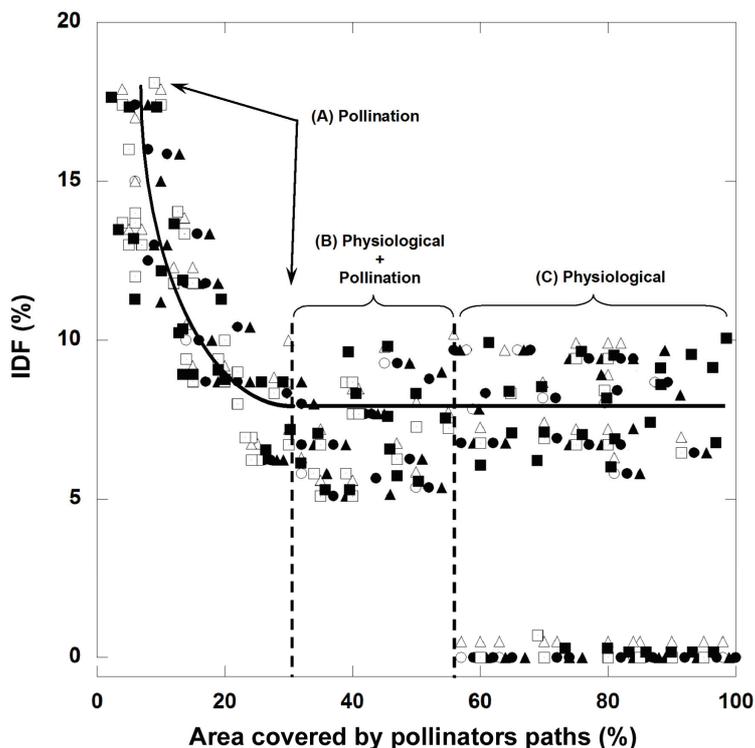
Sunflower genotypes vary in their attractiveness to the honey bee. Short corolla length, non pigmented stigmas, many nectaries, and high sucrose content of the nectar are preferred by bees. If the flower was never visited it could be indicating that perhaps the floret *per se* was responsible for the lack of attractiveness due to some intrinsic difference that made it special and “unvisitable”, compared with the surrounding ones. Sammataro et al. (1984; 1985) found intragenotypical differences in the quality, quantity and anatomy of nectaries. Perhaps some interplant differences could also exist.

The availability of resources can vary in both space and time for an individual flower, due to local competition for the resources (Stephenson, 1981). Hence, within a single plant, resources may be limited for some flowers but not for others. Nevertheless, perhaps this was not the case for the external flowers in the capitulum. It has been observed that at early anthesis, recently open flowers in the capitulum are not deprived of an assimilate supply (Hernández and Orioli, 1991; Alkio et al., 2002; Alkio and Grimm, 2003). Finally, according to the “architectural effects hypothesis”, the pattern of seed production can also be caused in some plants by intrinsic factors, biological or physical, limiting the ripening of ovules located in some inflorescence positions (Diggle, 1995). The proximate causes of these architectural effects are still unknown (Diggle, 1997), although accumulating evidence is showing that it can have an

important effect on the observed pattern of seed production (Medrano et al., 2000 and references therein).



**Figure 2.** Emerging relationship between the area covered by visit paths (○ ; ACP%) and the IDF% (●) produced in each sector for the capitulum of Fig. 1. The scales indicate the length of the diameter of each circle with the percentage magnitude for each variable. A,B,C and D, as in Fig. 1.



**Figure 3.** Relationship between the area covered by paths (ACP%) and the percentage of incompletely developed fruits (IDF%) observed in each sector (n=60) in which each capitulum of the 6 sunflower plants was divided for analysis. The sum of observations of IDF% are divided into three intervals ranging from 0% to 30%, 31% to 59 and 60% to 100% of ACP to discriminate the causes acting in the generation of IDF: (A), pollination failures, associated with the lack of pollinator visits:  $IDF\% = 31.48 \times ACP^{-0.43}$ ;  $r^2 = 0.61$ ;  $n=91$ ;  $p = 0.05$ , (B), physiological plus pollination failures and (C), mainly physiological causes. Different markers indicate individual plants.

Considering the conditions under which the experiment and the observations were conducted and assuming that no contribution from other pollinators occurred from anthesis to fertilization, the analysis of Fig. 3 revealed at first glance three intervals which can separate different causes for the generation of IDFs. From 0% to 30% of ACP, the negative correlation found between ACP% and IDF% ( $r^2= 0.61$ ) suggests that within this range, the lack of visits had a high incidence on fruit set (Fig. 3).

Approximately from 30% of ACP and above this value, the stable level of IDFs, fluctuating from 5 to 10% suggests that we would have to consider other variables. Probably there was a combination of a low occurrence of bee visits and factors related to the floral biology (physiological factors) of the tested genotype (Fig. 3).

Above 60% of ACP, the absence of IDFs (0%) in several sectors (Figs. 1 and 2) and the occurrence of sectors with a fluctuating level of IDF% ranging from 6 to 10% of the total value, would suggest that the IDFs generated in that region were produced by physiological causes, which were neither detected nor studied in this present work. Probably, they were associated with the low self-compatibility of the genotype used. Given the present information, it would be expected that in sunflower genotypes with high self-compatibility, the IDF fraction, although fluctuating, could descend to levels under 5% per sector.

The correlation between the percentage of IDFs per sector and the ACP (%) over 30% was then weak (Fig. 3), probably because the data was masked with other variables, which would act to generate IDFs.

Another weakness is the fact that the ACPs were sampled only after the bees settled during the day, without quantifying the behavior of other pollinators during the night. Nevertheless, the positive relationship between the density of foraging routes and the development of IDFs in several areas of the capitulum, confirms the important role of day-sheltering bees as sunflower pollinators. Unvisited areas are positively correlated with the presence of seedless or incompletely developed fruits at maturity. This suggests that some flowers from those areas are inclined to show an absence or delay in pollination with respect to the adjacent flowers. Due to night covering, we could assume that nocturnal pollinators would substitute the lack of visited sites by areas that bees did not visit during the day. Nevertheless if a deficiency in the number of nectaries or floret functionality occurred, these sites would not be visited at night either.

#### ACKNOWLEDGEMENTS

This work was funded by grants to L.F.H. of the Agencia Nacional de Promoción Científica y Tecnológica and the Argentine Sunflower Association (ANPCyT-ASAGIR, PICTOS-13151), the Secretaría Gral. de Ciencia Tecnología (SeGCyT-UNS) and the Comisión de Investigaciones Científicas (CIC, La Plata) Argentina. The valuable comments on the manuscript made by Dr. D. Sammataro (Dept. Entomology, Univ. Arizona, USA) are greatly appreciated. The invaluable cooperation of N. Vischer (Swammerdam Institute for Life Sciences, University of Amsterdam) in developing a special plug-in for his software Object Image and Mrs. G.M. Abrego for collaborating in the field experiments are also specially appreciated. Sunflower seed was kindly provided by Dow Agrosiences of Argentina.

#### REFERENCES

- Alkio, M., and E. Grimm. 2003. Vascular connections between the receptacle and empty achenes in sunflower (*Helianthus annuus* L.). *J. Exp. Bot.* 54:345-348.
- Alkio, M., M. Diepenbrock, and E. Grimm. 2002. Evidence for sectorial assimilate supply in the capitulum of sunflower (*Helianthus annuus* L.). *New Phytol.* 156:445-456.
- Berry, P.E., and R.N. Calvo. 1991. Pollinator limitation and position dependent fruit set in the high Andean orchid *Myrosmodes cochleares* (Orchidaceae). *Plant Syst. Evol.* 174:93-101.
- Birch, E.B., and J.C. van der Sandt. 1985. Bee pollination of sunflower. p. 255-260. In: Proc. 11th Int. Sunflower Conf. Mar del Plata, Argentina.
- Cantagallo, J.E., D. Medan, and A.J. Hall. 2004. Grain number in sunflower as affected by shading during floret growth, anthesis and grain setting. *Field Crops Res.* 85:191-202.
- Connor, D.J., and A.J. Hall. 1997. Sunflower Physiology, p. 113-182. In: A.A. Schneiter, [ed.], *Sunflower Technology and Production*, Agron. Ser. 35, ASA, CSSA, SSSA, Madison, WI, USA.
- DeGrandi-Hoffman, G., and M. Chambers. 2006. Effects of honey bee (Hymenoptera: Apidae) Foraging on seed set in self-fertile sunflowers (*Helianthus annuus* L.). *Environ. Entomol.* 35:1103-1108.

- Diggle, P.K. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Ann. Rev. Ecol. Syst.* 26:531-542.
- Diggle, P.K. 1997. Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. *Int. J. Plant Sci.* 158 (supplement): S99-S107.
- Durrieu, G., C. Percie du Sert, and A. Merrien. 1985. Anatomie du capitule de tournesol consequences sur la nutrition des akenes. p. 7-12. In: Proc. 11th Int. Sunflower Conf. Mar del Plata, Argentina.
- Fell, R.D. 1986. Foraging behaviors of *Apis mellifera* L. and *Bombus* spp. on oil sunflower (*Helianthus annuus* L.). *J. Kansas Entomol. Soc.* 59:72-81.
- Gawleta, N., Y. Zimmermann, and T. Eltz. 2005. Repellent foraging scent recognition across bee families. *Apidologie* 36:325-330.
- Giurfa, M. 2004. Conditioning procedure and color discrimination in the honeybee *Apis mellifera*. *Naturwissenschaften* 91:228-231.
- Giurfa, M., and J.A. Núñez. 1992. Honeybees mark with scent and reject recently visited flowers. *Oecologia* 89:113-117.
- Hernández, L.F., and G.A. Orioli. 1991. Role of different leaves of the sunflower (*Helianthus annuus* L.) plant during the grain filling period (in spanish). *Turrialba* 41:330-334.
- Hernández, L.F., and J.H. Palmer. 1992. Incorporation of <sup>14</sup>C labeled metabolites into the developing sunflower capitulum. p. 564-570. In: Proc. 13th Int. Sunflower Conf. Pisa, Italy.
- Hernández, L.F., C.N. Pellegrini, and L.I. Lindström. 2002. Prediction of the generation of seedless fruits in the sunflower capitulum (*Helianthus annuus* L.) (in spanish). XI Latin American Meeting of Plant Physiologists. Punta del Este, Uruguay.
- Lindström, L.I., M.E. García, and L.F. Hernández. 2004. Morphology and distribution of incompletely developed fruits in sunflower (*Helianthus annuus* L.) capitula. p. 333-337. In: Proc. 16th Int. Sunflower Conf., Fargo, ND, USA.
- Lindström, L.I., C.N. Pellegrini, L.A.N. Aguirrezábal, and L.F. Hernández. 2006. Growth and development of sunflower fruits under shade during pre and early post-anthesis period. *Field Crops Res.* 96:151-159.
- Lindström, L.I., C.N. Pellegrini, and L.F. Hernández, L.F. 2007. Histological development of the sunflower pericarp as affected by pre and early post-anthesis canopy shading. *Field Crops Res.* 103:229-238.
- Medan, D., M. Chamer, M. Devoto, N.H. Montaldo, A.I. Mantese, N.J. Bartoloni, A. Roig-Alsina, and S. Leguizamón. 2003. Do the argentine sunflowers need pollinators? (in spanish). II Argentine Sunflower Association Meeting, Buenos Aires, Argentina, ASAGIR.
- Medrano, M., P. Guitián, and J. Guitián. 2000. Patterns of fruit and seed set within inflorescences of *Pancreatium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? *Am. J. Bot.* 87:493-501.
- Parker, F.D. 1981a. Sunflower pollination: abundance, diversity and seasonality of bees and their effect on seed yields. *J. Apic. Res.* 20:49-61.
- Parker, F.D. 1981b. How efficient are bees in pollinating sunflowers? *J. Kansas Entomol. Soc.*, 54:61-67.
- Schneider, A.A., and J.F. Miller. 1981. Description of sunflower growth stages. *Crop Sci.* 21:901-903.
- Sammataro, D., P.K. Flottum, and E.H. Erickson. 1984. Factors contributing to honeybee preferences in sunflower varieties. p. 20-21. In: Proc. Sunflower Res. Workshop, Bismark, ND.
- Sammataro, D., E.H. Erickson, and M.B. Garment. 1985. Ultrastructure of the sunflower nectary. *J. Apic. Res.* 24: 150-160.
- Skinner, J.A. 1987. Abundance and spatial distribution of bees visiting male-sterile and male-fertile sunflower cultivars in California. *Environ. Entomol.* 16:922-927.
- Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Ann. Rev. Ecol. Syst.* 12: 253-279.
- Thomson, J.D. 1989. Deployment of ovules and pollen among flowers within inflorescences. *Evolutionary Trends in Plants* 3:65-68.
- Vischer, N.O.E., P.G. Huls, and C.L. Woldringh. 1994. Object-Image: An interactive image analysis program using structured point collection, BINARY, (Bioline), Vol. 6.
- Zimmerman, M., and G.H. Pyke. 1988. Reproduction in *Polemonium*: assessing the factors limiting seed set. *Amer. Nat.* 131:723-738.