Pollinating Efficacy of *Osmia cornifrons* and *Osmia lignaria* subsp. *lignaria* (Hymenoptera: Megachilidae) on Three Brassicaceae Species Grown Under Field Cages[1]

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Abstract Until 1997, honey bees, *Apis mellifera* L., were used to pollinate Brassicaceae grown in field cages at the North Central Regional Plant Introduction Station (NCRPIS), at Ames, IA. At this time, a solitary bee, *Osmia cornifrons* (Radoszkowski), was first employed to pollinate the crop in field cages; however, a native bee species, *Osmia lignaria* subsp. *lignaria* Say, out-competes *O. cornifrons* in central Iowa for artificial nesting sites erected for rearing. The purpose of this study was to investigate the efficacy of these two solitary bee species for producing *Brassica napus, B. rapa,* and *Sinapis alba* seed in field cages. There was no difference in seed production for the *S. alba* accession, PI 209022, or the *B. rapa* accession, PI 278766, between the two species of *Osmia*. But, the *B. rapa* accession, PI 392025, and the *B. napus* accession, PI 469944, produced significantly more seed when pollinated by *O. lignaria* subsp. *lignaria* than by *O. cornifrons*. Because the native bee is easier to rear and maintain, it will be the pollinator of choice for control pollinating collections of Brassicaceae species maintained at NCRPIS. The commercial use of *O. lignaria* subsp. *lignaria* or the related subspecies, *O. lignaria* subsp. *propinqua*, should be investigated for improving the production of canola and rapeseed.

Key Words Plant germplasm, pollination, *Brassica napus, Brassica rapa, Sinapis alba,* canola, *Osmia cornifrons, Osmia lignaria lignaria,* honey bee

The North Central Regional Plant Introduction Station (NCRPIS) at Ames, IA, conserves extensive germplasm collections as one of about 25 active sites in the U. S. National Plant Germplasm System (NPGS) (Widrlechner 1998). The NCRPIS specializes in managing crop and wild species germplasm that is primarily allogamous (outbreeding) in nature. An important aspect of managing these extensive germplasm collections is to regenerate seeds in the field and in greenhouses by controlling pollination to preserve the genetic integrity of the collections. Many of the allogamous crops maintained at the NCRPIS are pollinated by using honey bees,

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Apis mellifera L., confined to field cages that cover the germplasm accessions (Ellis et al. 1981, Widrlechner et al. 1997). In the 1990s, we initiated research to investigate the pollination efficacy of additional insect species to produce seed in field cages for the various plant species maintained by the NCRPIS.

The active germplasm collection of oilseed Brassicaceae is maintained by NCRPIS. The majority of the collection is composed of two species, *Brassica rapa* L. and *B. napus* L. These are used primarily for the edible (canola) and industrial (rapeseed) oil markets. White mustard, *Sinapis alba* L., is another important oilseed maintained by the NCRPIS. Until 1998, the NCRPIS used only *A. mellifera* to pollinate Brassicaceae in field cages. Wilson et al. (1999) found that a solitary bee, *Osmia cornifrons* (Radoszkowski), was equal to or better than alfalfa leafcutting bees, *Megachile rotundata* (F.), or *A. mellifera* for pollination of Brassicaceae species in field cages as measured by seed production.

Osmia cornifrons is a univoltine megachilid bee that was introduced into the U. S. from Japan in 1977 (Batra 1979). Recently, the NCRPIS has been using *O. cornifrons* in comparative pollinator studies and control pollination of oilseed Brassicaceae germplasm. However, a native bee species, *O. lignaria* subsp. *lignaria* Say, has been out-competing *O. cornifrons* for artificial nesting sites erected for rearing in central Iowa. Thus, the native bee may potentially be easier to rear and maintain and may be the pollinator of choice if it is as effective as *O. cornifrons* for control pollinating Brassicaceae. The primary purpose of this study was to compare the efficacy of these two solitary bee species for producing *B. napus, B. rapa,* and *S. alba* seed in field cages.

Materials and Methods

Seeds of two accessions of *B. rapa* L. (PI 278766 and PI 392025) one accession of *S. alba* L. (PI 209022) and one accession of *B. napus* L. (PI 469944) were each planted in 5.1-cm^2 peat pots (135 seeds per accession) in the greenhouse from 16 February to 7 March 1995. The seedlings were transplanted into 64 field plots (four accessions × four pollinator treatments × four blocks) from 17 to 24 April 1995. For each plot, approximately 130 plants were grown within a 6.0 m row with a space of 4.8 m between rows (plots) for placement of the cages and alleyways. The experiment was conducted as a randomized complete block design with four replications. The plots were covered with insect-proof field cages (6.4 × 1.6 × 1.6 m) before the onset of flowering (Widrlechner et al. 1997).

The *O. cornifrons* and *O. lignaria,* subsp. *lignaria* used for this study were either purchased from Orchard Bees (Auburn, IN) or reared by the NCRPIS in central Iowa. The bees were stored and held at 1 to 2°C (Wilson and Abel 1996). Voucher specimens (deposited at NCRPIS, Ames, IA) were collected to identify bees emerging from the nesting straws from both groups.

At the onset of plant flowering in the field cages, the stored nesting straws were removed from cold storage. Individual nesting straws were placed in clear plastic bags and the bags were closed with a rubber band. The bags containing the nesting straws were placed in an insect rearing room at 23°C ± 2°C. Each day at approximately 0700, 1100, and 1530 h, the bags were checked and emerged bees identified according to species and gender before being released into the field cages. Approximately 30 males and 18 females of a single species were released into each of the 64 cages. A domicile was placed in the northwest corner of each cage at the onset of flowering. The domicile consisted of a 5.1-cm diam PVC pipe containing enough empty nesting straws (10 to 12) to tightly fill the pipe. The empty nesting straws (Custom Paper Tubes, Inc., Cleveland, OH) were 40-cm long × 7-mm diam cardboard tubes with each tube bent in half forming two 20-cm long nests. The cardboard tube wall was 1.5-mm thick. The soil in the field cages was heavily mulched with shredded newspaper to control weeds. The mulch also held sufficient soil moisture to provide the nesting bees with a supply of moist soil for constructing cell partitions and nest plugs.

Osmia adults were introduced into the field cages from 18 to 24 May for the *S. alba* accession and the two *B. rapa* accessions and from 26 to 31 May for the *B. napus* accession. *Apis mellifera* were used to compare the relative merits of *Apis* vs. *Osmia* pollination and were introduced into cages on 18 May for the *S. alba* accession and the two *B. rapa* accessions and on 26 May for the *B. napus* accession. The *Apis* treatment consisted of one nucleus colony containing 4,000 to 6,000 worker bees and a fertile queen for each cage. The control treatment was 16 field cages without bees. We discontinued insect pollination for all four accessions on 3 July 1995 when pollinators were removed from the field cages. The insect-proof screens which covered the cages were not removed until the plots were harvested. The plots were harvested once the plants reached maturity.

All means for the weight of seed produced for each field cage plot were log-transformed and analyzed using REML-ANOVA (PROC MIXED, Littel et al. 1996). All main effects and their interactions were considered fixed. Orthogonal contrast statements were included to test specific hypothesis about the pollinator treatments and their interactions with plant accessions. Pollinator treatment means for each plant accession were separated using the LSMEANS option of PROC MIXED (Littel et al. 1996).

Results

Field cages containing pollinators produced significantly more seed than cages without pollinators (Control vs. Pollinators contrast, $F = 44.70$; df = 1,48; $P < 0.0001$). The two-way ANOVA indicated significant differences among pollinator treatments ($F = 16.03$; df = 3.48; $P < 0.0001$). Differences among pollinator treatments were not dependent on plant accession (Pollinator × Plant accession, $F = 1.55$, df = 9,48, $P = 0.1566$). The remaining contrasts for pollinator treatment effects were not significant.

Because there was a large difference between the control and bee treatments, a second two-way ANOVA was conducted that excluded the control treatment. The control treatment was excluded to better evaluate differences among the bee species in their affect on seed production. Pollinator species significantly differed in their influence on seed weight (Table 1). There was no over-all difference in seed production in *Apis*-pollinated and *Osmia*-pollinated cages (*Apis* vs. *Osmia* species, Table 1); however, cages pollinated by *O. lignaria* subsp. *lignaria* produced significantly greater seed weights than cages pollinated by *O. cornifrons* (Tables 1 and 3).

Differences among pollinator species in their affect on seed production varied with plant accession (Pollinator species × Plant accession interaction and corresponding contrast statements, Table 1). Because there were significant interactions between pollinator species and plant accessions, separate one-way ANOVA's were conducted to examine the effect of pollinator species on seed production for each of the four Brassicaceae accessions. The results indicate that pollinator species had no affect on

Table 1. Two-way ANOVA with contrast statements evaluating differences among bee species for their affect on seed production of *S. alba, B. rapa,* and *B. napus*

Source	ndf	ddf	*F*	*P*
Pollinator species	2	36	3.95	0.028
Apis vs. *Osmia* spp.	1	36	0.48	0.493
O. cornifrons vs. *O. lignaria* subsp. *lignaria*	1	36	7.42	0.010
Plant accession	3	36	65.17	<0.001
Pollinator species × Plant accession	6	36	4.15	0.003
(*Apis* vs. *Osmia* spp.) × Plant accession	3	36	5.58	0.003
(*O. cornifrons* vs. *O. lignaria*) × Plant accession	3	36	2.72	0.059

seed production for the *S. alba* accession, PI 209022 (Table 2). *Apis mellifera* and *O. lignaria* subsp. *lignaria* significantly improved seed production when compared to *O. cornifrons* for the *B. rapa* accession, PI 278766. For the *B. rapa* accession, PI 392025, *O. lignaria* subsp. *lignaria* significantly improved seed production when compared to all other pollinators, and *O. cornifrons* was a significantly better pollinator when compared to *A. mellifera. Osmia lignaria* subsp. *lignaria* significantly improved seed production when compared to the other pollinators, and *A. mellifera* was a significantly better pollinator when compared to *O. cornifrons* for the *B. napus* accession, PI 469944 (Table 3).

Discussion

In general, *B. rapa* is considered to be self-sterile and requires insect pollination for seed production (Downey et al. 1970, Fries and Stark 1983, Williams 1978). There have been conflicting views on the need for insect pollination of *B. napus.* Downey et al. (1970) reported that *B. napus* is 70% self-pollinated. Free and Nutall (1968) re-

Table 2. Log-transformed mean ± SEM weight of seed produced by three insect pollinator species on four accessions of Brassicaceae

Pollinator	*S. alba* PI 209022	*B. rapa* PI 278766	*B. rapa* PI 392025	*B. napus* PI 469944
A. mellifera	5.72a ± 0.11	6.36a ± 0.10	6.58c ± 0.09	5.87b ± 0.07
O. cornifrons	5.94a ± 0.11	5.90b ± 0.10	6.80b ± 0.09	5.68c ± 0.07
O. lignaria subsp. lignaria	5.81a ± 0.11	6.17a ± 0.10	7.01a ± 0.09	6.07a ± 0.07

Weight of seed was log transformed prior to analysis.
Means in column followed by the same letter are not significantly different (α = 0.05, PROC MIXED, LSMEANS [Littel et al. 1996]).

Table 3. Mean seed production in g per field cage for four Brassicaceae accessions pollinated by three insect species

Pollinator	*n*	*S. alba* PI 209022	*B. rapa* PI 278766	*B. rapa* PI 392025	*B. napus* PI 469944
Osmia lignaria subsp. *lignaria*	4	348.0	487.9	435.9	1125.0
Osmia cornifrons	4	383.0	372.9	297.0	905.3
Apis mellifera	4	309.0	583.4	357.1	730.8
Control	4	195.1	285.4	200.4	656.1

ported only a 13% increase in seed yield for *B. napus* plots pollinated with bees compared to plots without bees, bringing into question the economic benefits of using bees for commercial seed production of *B. napus.* Other research have found supporting evidence for the use of insect pollinators for improving seed production of *B. napus* (Eisikowitch 1981, Langridge and Goodman 1975, Mesquida et al. 1988). Our results showed a significant seed production increase from cages that used pollinators with 56.1, 58.5, 46.0, 58.3% seed increase when using *O. lignaria* subsp. *lignaria* compared to the control (no pollinator) for the *S. alba* accession, PI 209022, the *B. rapa* accessions, PI 278766 and PI 392025, and the *B. napus* accession, PI 469944, respectively (Table 3). Continued research should be conducted to determine the most efficient pollination systems for *S. alba, B. rapa,* and *B. napus* seed production.

Our results indicate that *O. lignaria* subsp. *lignaria* and *O. cornifrons* are equally effective as pollinators of *S. alba* (Tables 2 and 3). This finding is important because the native bee, *O. lignaria* subsp. *lignaria,* has been out-competing *O. cornifrons* at artificial nesting sites erected by NCRPIS to rear *Osmia* each year. This study demonstrates that both bee species are suitable pollinators for *S. alba.* Because the native bee is easier to rear and maintain, it will be the pollinator of choice at NCRPIS for control pollinating this plant species when grown in field cages.

Apis mellifera was as effective as the *Osmia* species for pollinating *S. alba* (Tables 2 and 3). *Apis mellifera* was also as effective as *O. lignaria* subsp. *lignaria* for pollinating the *B. rapa* accession, PI 278766 (Tables 2 and 3). An important consideration when pollinating plant germplasm collections is the cost of the pollination system. Wilson et al. (1999) determined a seven-fold cost increase when using *A. mellifera* field-cage pollinations of Brassicaceae when compared with *O. cornifrons,* making *Osmia* a more economical choice.

Notably, our results indicated that *O. lignaria* subsp. *lignaria* is a superior pollinator of *B. rapa* accession, PI 278766, when compared to *O. cornifrons.* For *B. rapa* accession, PI 392025, and the *B. napus* accession, *O. lignaria* subsp. *lignaria* was significantly better than all other pollinators. The significantly greater seed production, in conjunction with reduced costs associated with choice of pollinating insect, could result in considerable cost savings for Brassicaceae regeneration efforts at the NCRPIS. The finding that *B. napus* pollination was significantly improved by using *O. lignaria* subsp. *lignaria* may have important commercial implications because the majority of canola grown in Canada is *B. napus* (Westcott and Nelson 2001).

In a field-cage study, Abel and Wilson (1999) showed that *O. cornifrons* produced sufficient progeny on *B. napus, B. raga,* and *S. alba* to provide enough bees for pollinating the following season. The number of progeny-filled nesting straws produced by *O. cornifrons* on *B. napus* was so great that all of the available empty straws were filled in three of the four caged plots. Abel and Wilson (1999) concluded that a high nectar/pollen ratio in *B. napus* (Ermakova 1959, Haragsimová-Neprăsová 1960, Kubišová et al. 1980, Petkov 1963, Torchio 1985) improved the nesting ability of *O. cornifrons.* If *O. lignaria* subsp. *lignaria* is as successful at being reared on Brassicaceae grown in field cages as is *O. cornifrons,* sufficient progeny could be produced during the field-cage pollination season to supply bees needed for the following year, resulting in a self-sustaining system.

The flowering of most accessions of Brassicacea grown under field cages at NCRPIS is asynchronous with the spring emergence of the univoltine *O. lignaria* subsp. *lignaria* in its native environment. The bee typically emerges early April in central Iowa, coinciding with the bloom of juneberries, *Amelanchier spp.,* and plums, *Prunus spp.,* with bee activity ceasing after 6 wks. Accessions from the diverse collection of Brassicaceae species maintained at the NCRPIS flower from mid-April until late-June. Therefore, it is critical that the storage method described in Wilson and Abel (1996) be used to maintain the bees in optimal condition until needed for pollination. This method extends the normal winter diapause period by maintaining the bees at a constant 1 to 2°C.

When control pollinating germplasm collections to make adequate seed available for distribution, techniques should be employed that minimize changes in the relative abundance (frequencies) of genes within an accession (Roos 1984). Inadvertant selection by a plant pollinator for certain plant or floral characteristics is highly undesirable and may result in the eventual loss of useful genes within an accession. Some bees, such as *A. mellifera,* are known for floral preference, i.e., the propensity of an individual foraging bee for visiting flowers with similar characteristics (Clement 1965, Jaycox 1970, Loper and Waller 1970). A pollinator species that is more random in its foraging behavior would reduce the degree of genetic change occurring in the plant accessions being regenerated. Research should be conducted to determine the degree of random foraging behavior exhibited by the pollinator species used in this study. The degree of random intercrossing performed by a pollinator could be estimated by using flower-colored or hypocotyl-colored marker lines of a Brassicaceae species, similar to a study conducted by Steiner et al. (1992) which examined pollinator effects on a three-flower-color alfalfa population.

Currently, *A. mellifera* is the primary pollinator used for the commercial production of canola and rapeseed (Westcott and Nelson 2001). Our data showed seed production increased by 11.2, 18.1, and 35.0% for the *S. alba* accession, the *B. rapa* accession P1 278766, and the *B. napus* accession, respectively, when *O. lignaria* subsp. *lignaria* were used compared to *A. mellifera* (Table 3). Research should be conducted to determine the potential benefits of using *O. lignaria* subsp. *lignaria,* which is adapted to the eastern U.S., or *O. lignaria* subsp. *propinqua,* which is adapted to the western U.S., for improving canola and rapeseed pollination and hybrid-canola seed production.

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