WILD BEE ABUNDANCE AND SEED PRODUCTION IN CONVENTIONAL, ORGANIC, AND GENETICALLY MODIFIED CANOLA

Lora A. Morandin[1] and Mark L. Winston

Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, British Columbia, V5A 1S6, Canada

Abstract. The ecological impacts of agriculture are of concern, especially with genetically modified and other intensive, modern cropping systems, yet little is known about effects on wild bee populations and subsequent implications for pollination. Pollination deficit (the difference between potential and actual pollination) and bee abundance were measured in organic, conventional, and herbicide-resistant, genetically modified (GM) canola fields (*Brassica napus* and *B. rapa*) in northern Alberta, Canada, in the summer of 2002. Bee abundance data were collected using pan traps and standardized sweep netting, and pollination deficit was assessed by comparing the number of seeds per fruit from open-pollinated and supplementally pollinated flowers. There was no pollination deficit in organic fields, a moderate pollination deficit in conventional fields, and the greatest pollination deficit in GM fields. Bee abundance was greatest in organic fields, followed by conventional fields, and lowest in GM fields. Overall, there was a strong, positive relationship between bee abundance at sampling locations and reduced pollination deficits. Seed set in *B. napus* increased with greater bee abundance. Because *B. rapa* is an obligate outcrossing species, the lack of pollination deficit in the organic (*B. rapa*) fields likely was due to the high bee abundance rather than a lower dependence of *B. rapa* on pollinators than *B. napus* canola. Our study illustrates the importance of wild bees to agricultural production and suggests that some agroecosystems may better sustain wild bee abundance, resulting in greater seed production. Further research on why some cropping systems, such as genetically modified, herbicide-resistant canola, have low wild bee abundance would be useful for management of agroecosystems to promote sustainability of food production.

Key words: agriculture; bees; Brassica rapa; Brassica napus; *canola; conventional; genetically modified; organic; pollination; sustainable development.*

Introduction

One of the greatest challenges for ecologists and conservation biologists in recent years has been to understand the impact of established and novel agricultural systems on biodiversity. The onset of genetically modified crops has stimulated considerable research in this area, and in the process, revealed large gaps in our knowledge concerning how conventional and alternative agroecosystems interact with the environment around them.

One vital area that has been particularly understudied is the relationship between agriculture and pollinator populations, in terms of both agricultural impacts on biodiversity and effects of diminished bee abundance on crop production (Allen-Wardell et al. 1998). This is important ecologically, but also agriculturally, since lower pollinator abundance may lead to reduced yields. While there has been at least some research in conventional systems (e.g., Kremen et al. 2002), no studies have examined how agroecosystems having genetically modified (GM) crops compare to other cropping systems in their effects on wild bee populations, and how interactions between bees and these new technologies relate to yield and crop production.

Pollination requirements of many crop plants are not well known, and the contribution of native bee communities is unclear (Kearns and Inouye 1997, Kevan and Phillips 2001). Approximately 66% of the world's crop species either benefit from or require animal pollination, primarily provided by bees, and fruit production resulting from animal pollination is essential for about one-third of human food in developed countries (O'Toole 1993).

There has been a suspected decline of native pollinators (e.g., Torchio 1987, Matheson et al. 1996, Allen-Wardell et al. 1998). Wild pollinator declines have been associated with low crop yields and even total crop failures (see Kevan 1977 and Allen-Wardell et al. 1998 for examples). Further, non-*Apis* bees are of incalculable value for pollination of natural vegetation. Paradoxically, expansion of agriculture both in size and intensity is reducing available foraging and nesting habitats for bees, which may result in increased pollination deficits and lower crop yields (O'Toole 1993, Kremen et al. 2002). Yet, information on the role of

wild pollinators in agriculture and the effects of agricultural methods on pollinators is largely speculative, making research critical for understanding this interaction.

Although few studies have examined the relationship between pollinator communities and their environment, Potts et al. (2003) have recently shown a positive relationship between bee diversity and plant diversity (primarily annuals) in a non-agricultural setting. Comparisons of bee populations in natural or uncultivated areas and agricultural areas have found higher bee abundance and/or diversity in natural areas than in agricultural ecosystems (MacKenzie and Winston 1984, Scott-Dupree and Winston 1987, Banaszak 1996, Calabuig 2000). Williams (1986) found that the number of bumble bees on crops was positively correlated with the crops' proximity to uncultivated land. Calabuig (2000) surveyed solitary bees and bumble bees in semi-natural areas within an agricultural landscape and found that plant species richness and cover in field edges and hedgerows was positively correlated with bee diversity. She suggested that continuity in pollen and nectar availability was beneficial for bumble bee colonies, while a high diversity of plant species could support a large number of oligolectic solitary bee species. In addition, many bees other than bumble bees and oligolectic bees would benefit from floral resources other than local crops in at least three situations: (1) if individual bees live longer than the blooming period of the crop, (2) if the bee's life span does not completely overlap with the crop bloom, or (3) if the crop's nectar or pollen does not supply the bee with adequate nutrition. Farming practices that reduce weed diversity in or surrounding crops may result in lower bee abundances and/or diversity (Osborne et al. 1991, Mand et al. 2002, Haughton et al. 2003), possibly lowering seed set.

Studies of pollination deficits of entomophilous (insect pollinated) plant species have been used to infer pollinator declines (see Thomson 2001). In a literature review of pollination supplementation experiments, Burd (1994) found that 62% of 258 wild plant species were pollen limited. Few data are available on pollen limitation of crop species, but a similar literature review found that 59% of 16 cultivars representing 11 species were pollen limited (Mayfield 1998 in Thomson 2001). Comparison of pollination deficits across and within various agricultural cropping systems can provide insight into the abundance and efficacy of associated pollinator populations, and the dependence of a crop on insects for pollen transfer and seed set. Significant differences in pollination deficit in different cropping systems may indicate that some types of agroecosystems better promote agriculturally beneficial pollinator populations.

Canola (*Brassica* spp.) is Canada's fourth most important crop by acreage seeded (Statistics Canada 2003*a*) and the most important oilseed crop in Canada (Statistics Canada 2003*b*). Currently, Canada's annual exports of canola seed, oil, and meal alone are valued at over two billion Canadian dollars (CCC 2001). Approximately 50% of canola crops worldwide are transgenic herbicide resistant (GM; James 2000). Organic canola constitutes ~0.07% of the canola grown in Canada (Brooks and Barfoot 2004).

Canola flowers secrete large amounts of nectar and are very attractive to many wild bees, including species of *Andrena*, *Halictus*, and *Bombus*. Although the data are conflicting, and differ among canola varieties, there is evidence that insect visits increase canola yield (reviewed in Free 1993, Delaplane and Mayer 2000). There are two species of *Brassica* that have been developed into canola varieties, *B. napus*, or Argentinian canola, and *B. rapa*, Polish canola (formerly described as *B. campestris* by Linnaeus, but later grouped with *B. rapa*; Toxeopus et al. 1984). *Brassica napus* is self-compatible, yet studies largely show that insect pollination increases seed production, whereas *B. rapa* is self-incompatible (an obligate outcrosser), and pollinator visits are required for seed production (Zuberi and Sarker 1992, Mishra et al. 1988, reviewed in Free 1993, Delaplane and Mayer 2000). Zuberi and Sarker (1992) found that without adequate cross pollination, rape seed (*B. campestris* (*rapa*) var. Toria) could not produce high yields and cite multiple examples of similarly inadequate pollen transfer in *B. rapa* under open-pollination conditions (Singh 1954, Zuberi and Sarker 1982, Zuberi et al. 1987).

Different cropping methods associated with GM, conventional, and organic canola may affect wild bee abundance in fields. Transgenic herbicide-resistant canola fields can be treated with broad-spectrum herbicides after canola emergence, resulting in more effective weed control than in conventional systems. Organic canola growers primarily rely on pre-sowing tillage and fast growing canola varieties for weed management and, consequently, organic fields tend to have larger amounts and greater diversity of weeds than conventional and GM fields (L. A. Morandin and M. L. Winston, *unpublished data*). Other differences in cropping methods such as pesticide treatments and field size may also affect wild bee abundance and pollination in different types of canola fields. Chemical pesticide use in conventional and GM crops may cause lower bee numbers in these types of fields than in organic fields, which tend not to have pesticide applications, or employ pesticides that are less toxic to bees. In addition, smaller fields, as is characteristic for organic crops (L. A. Morandin, *personal observation*), may have more bees simply because there is less crop area in relation to uncultivated adjacent area.

We assessed pollination deficits in organic, conventional, and genetically modified canola (*Brassica* spp.) in relation to wild bee abundance as part of a study on the effects of agroecosystems on native bee diversity and abundance. We also examined the relationship be-

tween increasing distances into fields and wild bee abundance and seed production. We hypothesized that (1) native bee pollination was required for canola to reach full seed set, (2) different field types would differ in their bee abundance, (3) sampling locations with greater bee abundance, regardless of field type, would have a lower pollination deficit, and (4) bee abundance would diminish with distance into fields.

MATERIALS AND METHODS

Data were collected during July–August 2002 near La Crete, Alberta, Canada (~58° N, 116° E). The region is open parkland with boreal forest and is a mosaic of cultured fields, aspen woodland, grassland, shrubland, wetland, and cattle pasture, adjacent to the Peace River. Four replicate fields were selected in each of three field types: organic, conventional, and GM, for a total of 12 fields. Within field types, fields were matched for size and crop variety. Organic fields were *B. rapa* Reward (SeCan Association, Ottawa, Ontario, Canada) canola and certified organic by the Peace River Organic Producers Association (Dawson Creek, British Columbia, Canada). Conventional canola fields were non-genetically modified *B. napus* HyLite Clearfield system 45A71 (Advanta Seeds, Winnipeg, Manitoba, Canada), treated with the herbicide Odyssey. GM fields were *B. napus* Roundup Ready DK3235 (Monsanto, St. Louis, Missouri, USA), treated with the herbicide Roundup. In addition to herbicide treatment, conventional and GM fields were all treated once during bloom for pests with the insecticide Matador (Syngenta, Guelph, Ontario, Canada), with the exception of one conventional field and one GM field that were not sprayed with insecticides during bloom. Pesticide application dates were different among fields and we make no attempt in this paper to quantify direct effects of pesticide application on bee populations. No insecticides were used on any of the organic fields in this study. Because organic canola in our study area was *B. rapa*, it would have been more susceptible to pollination deficits under low pollinator conditions than *B. napus* canola, a self-fertile species.

Conventional and GM fields were ~64 ha (quarter section, 800 × 800 m), and organic fields were smaller, ranging from 20 ha to 50 ha. All fields began blooming within one week of each other (late June) and continued blooming until mid to late July. Canola fields bloom for two to four weeks depending on location and environmental conditions. Fields were chosen so that treatment replicates were spread throughout the ~200 000-ha study location, in order to minimize spatial autocorrelation of treatments and possible confounding effects of environmental similarity. The La Crete, Alberta, area of this study is only recently undergoing intense conversion of forested land to agricultural land, and subsequently each study field was in close proximity (no greater than 800 m) to a forested area of at least 16 ha.
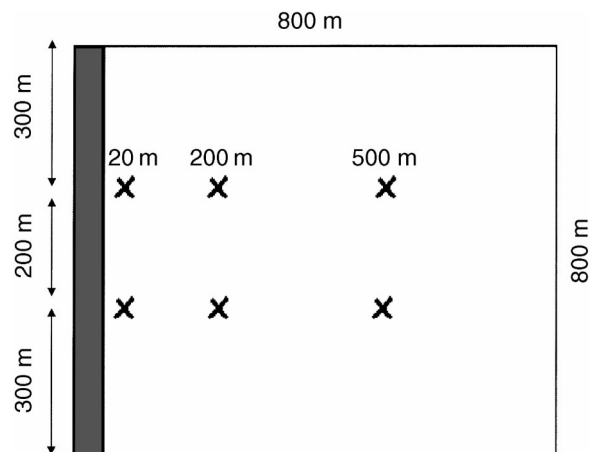

FIG. 1. Bee and pollination sample locations in canola fields. The dark region on the left represents a seminatural hedgerow located at the side of each field from which sample distances were measured.

Sampling locations in each field were oriented in relation to an uncultivated hedgerow along one side of the field. Hedgerows all had trees and understorey vegetation and were at least 5 m wide. Sampling of bees and pollination limitation were conducted at the same distances from the hedgerow, at 20 m, 200 m, and 500 m into fields, with two sample locations at each distance, 200 m apart, for a total of six sample locations per field (Fig. 1). Because organic fields were mostly smaller than conventional and GM fields, only one of the organic sites had sampling locations 500 m into the field. At most sites, only one side of a field had a hedgerow and the remaining sides were typically canola, other crops, or roads. Therefore, 500-m collection locations were 300 m from field edges, but usually 500 m from seminatural areas. However, we included distance from any field side (edge) as a factor in our analyses. To maintain equal sampling effort of bees among locations, fields without 500-m collection sites had one additional collection location at 20 m and 200 m.

Pollen limitation

At each sample location, supplemental pollination experiments were conducted in order to compare seed number in fruit from open- (naturally) and supplementally pollinated flowers (also referred to as control and experimental, respectively). We used methods modified from Zimmerman and Pyke (1988). Six pairs of plants were marked with flagging tape at each sampling location while the field was in full bloom. Within each pair of plants there was one control and one experimental plant and three pollination treatments: three control flowers on control plants (CC), three control flowers on experimental plants (EC), and three experimental flowers on experimental plants (EE), resulting in 18 CC, EC, and EE flowers at each sampling location and a total of 108 flowers per pollination treatment in

each field. Three of the four organic fields did not have 500-m collection locations, and in these fields the pollination experiment was conducted at four locations resulting in 72 flowers per pollination treatment in each field. Overall, 3564 flowers were used in the experiment. If resources necessary for seed production are limited, fruits produced from open-pollinated flowers on the same plants as supplementally pollinated flowers may have lower seed set (Zimmerman and Pyke 1988). Therefore, we incorporated controls on both experimental plants and on adjacent plants to ensure that differences in seed number between open- and supplementally pollinated fruits were due to differences in pollen transfer and not resource availability. A greater number of seeds from fruit in the CC control treatment than in the EC control treatment would indicate that EC controls were suffering lower seed set as a result of shared resources with supplementally pollinated fruit and not from inadequate pollen transfer per se.

Stems of flowers were marked with different colors of non-toxic acrylic paint (DecoArt, Stanford, Kentucky, USA). EE flowers were supplementally pollinated with a mixture of pollen, collected with a paintbrush into a Petri dish, from 10 to 15 adjacent flowers, all from different plants. This pollen mixture was then wiped onto the stigma of EE flowers. Seedpods (siliques) were collected no less than 12 d following supplemental pollination, and the numbers of seeds per silique were counted. Because some siliques could not be found at collection time, the actual number of siliques collected was lower than the number of flowers marked.

Bee collections

In the northern Canadian area of our study, there were few honey bee colonies, and honey bees made up <2% of all bee captures, so consequently we were able to assess the importance of native bee populations to canola yields in different types of agroecosystems.

Bees were collected during canola bloom at each previously described location, from 2 July to 31 July, using pan traps and standardized sweep netting. Each field was sampled with pan traps once during bloom for 48 h. Pans were left out longer if necessary to compensate for rain, which results in virtually no bee activity, so that all effective collection durations were as similar as possible. When possible, pan trapping was done concurrently at each of one organic, conventional, and GM site. One set of three (blue, white, and yellow), straight-sided, $30 \times 50 \times 20$ cm pan traps was placed on the ground, with the tops of the traps approximately even with the lowest flowers on the racemes at each sampling location. Each pan trap had 1.5 L water, ~5 mL glycerol to lower surface tension, and 10 mL of honey. Bees were collected from traps and stored in 70% ethanol for later identification.

Two days of standardized sweep net samples were taken in each field, generally by three different people concurrently in one organic, conventional, and GM field, although it was not always possible to follow this design because of slight differences in the onset of bloom and travel times between fields. Sweep net samples were only conducted on days that were mostly sunny, when the temperature was above 18°C from the beginning to the end of the collection period (~10:00–17:00 hours). The collector followed a standard route between the previously described sampling locations in such a way that two collections were taken each day at each sample location, one between 10:00 and 13:00 hours, and the second from ~14:00–17:00 hours. At each sampling location the collector walked a 30-m transect while making 100 180°-sweeps of the flowering vegetation with a 30 cm diameter sweep net.

Data analyses

All analyses were done using SAS (SAS Institute 1999). Across all field types, least squares means of number of seeds per silique did not differ between CC and EC control flowers ($t_9 = -1.64$, $P = 0.137$) and hence open-pollinated data were pooled. We categorized all siliques as either having seeds or having no seeds. The number of siliques in each category was compared between flower treatments within each field type in order to assess if there was a significant difference in the proportion of siliques that set seeds compared to the proportion that did not set seeds between open- and supplementally pollinated flower treatments in each field type. Flower treatments were contrasted within field type using the Logistic Procedure (Wald chi-square), with a binary logit function for binomial distributions. We also categorized each set of siliques (set = three siliques on same plant, either CC, EC, or EE) from 1 to 4, with 1 = all siliques with between 3 and 10 seeds, 2 = two out of three siliques with between 3 and 10 seeds, 3 = one out of three siliques between 3 and 10 seeds, and 4 = no siliques between 3 and 10 seeds. Silique sets were categorized based on 3 to 10 seeds per silique because there was a left-hand tail in the histograms of seeds per silique of the open-pollinated flowers that differed in the supplementally pollinated histograms in this range. This categorization may also be biologically relevant as it may correspond to siliques that were self-pollinated in self-fertile varieties (see *Discussion*). Categories were compared across field treatments (organic, conventional, and GM) with respect to flower treatment (control vs. experimental) using the Logistic Procedure (Wald chi-square), with a cumulative logit link function for multinomial distributions. Contrasts were conducted within each field treatment comparing flower treatment.

Silique seed number was compared between flower treatments (open and supplementally pollinated) and among distances (20, 200, and 500 m) using a type three sum of squares mixed analysis of variance model (Proc MIXED; covariance structure = variance components) within field types. In this analysis we included

individual plants, collection locations, and fields as random factors, controlling for lack of independence in the data. Plants were of different varieties in conventional and GM treatments and a different species in organic fields, as well as being subject to different cropping practices. Therefore, pollination deficit (see next paragraph) rather than absolute seed numbers in siliques was used for most analyses to compare differences among field treatment types and in relation to bee abundance.

Pollination deficit was calculated as the difference in mean seed number per fruit between supplementally ($N = 3$) and open-pollinated flowers ($N = 6$) for each plant pair. Variation in pollination deficit among field treatments and distances were analyzed using a type three sum of squares mixed analysis of variance model (Proc MIXED; covariance structure = variance components), again including all main effects, interactions, and random factors. Where appropriate, orthogonal pairwise comparisons were conducted ('‘estimate statements'' in Proc MIXED). We used separate residual analyses to determine if there was either an effect of distance from the designated hedgerow (always perpendicular to the 20-, 200-, and 500-m sampling locations) and/or distance from the closest edge on seed deficit. We first derived residuals from the relationship between deficit and distance from the closest edge while controlling for field treatment. With the derived residuals we tested for a relationship between distance from the designated hedgerow and seed deficit (Proc GLM), expecting a negative trend if there was a relationship. A similar analysis was also performed on residuals from the relationship between seed deficit and distance from the hedgerow, while controlling for treatment, and testing for an effect of edge distance (Proc GLM).

Absolute seed deficit values (number of seeds in supplementally pollinated siliques—number of seeds in open-pollinated siliques) can be readily comprehended and are direct indicators of the contribution of pollinators to seed output and crop yield. However, because mean supplementally pollinated seed number differed among canola varieties and species, we include analyses to control for this factor. Proportional seed deficit was calculated as the number of seeds in siliques from open-pollinated flowers divided by number of seeds in siliques from supplementally pollinated flowers. Some of the values of proportional seed deficit were greater than 1 and therefore, values were divided by 2, the largest proportional seed deficit value, enabling us to normalize the data with an arcsine square-root transformation. Proportional seed deficit data were analyzed using a general linear model (Proc GLM). All reported data in graphs are absolute seed deficit values.

Bee abundances were calculated for each sampling location as the total number of bees collected in both pan traps and sweep nets. The total number of bees collected at each sampling location in each field was compared among treatments using a categorical model with chi-square distribution statistics (Proc CATMOD) followed by pairwise contrasts of maximum likelihood estimates between organic, conventional, and GM fields.

To elucidate whether pollen limitation was related to differences among sampling locations in bee abundance, we regressed pollination deficit at each sampling location on the corresponding bee abundance across all field treatments.

RESULTS

In all field types and flower treatments, there was a maximum of between 35 and 40 seeds per silique. The number of seeds per silique (mean ± SE) from open- and supplementally pollinated flowers was 17.5 ± 0.36 and 18.8 ± 0.49, 16.2 ± 0.38 and 19.6 ± 0.42, and 17.7 ± 0.32 and 23.6 ± 0.31 in organic, conventional, and GM fields, respectively. In conventional fields, the percent siliques with no seeds was much higher in control siliques (20.0%) than in experimental siliques (6.3%) (Contrast tests, Wald $\chi_1^2 = 37.77$, $P < 0.0001$. Similarly, the percentage of siliques with no seeds was much greater in control siliques from GM fields (10.3%) than from experimental siliques (0.5%) (Wald $\chi_1^2 = 20.26$, $P < 0.0001$). The percentage of siliques with no seeds in control and experimental siliques was similar in organic fields, 11.5% and 9.6%, respectively (Wald $\chi_1^2 = 0.96$, $P = 0.327$; Fig. 2). Across all field treatment types, there was a lower proportion of siliques with between 3 and 10 seeds from the open- vs. supplementally pollinated flowers (Wald $\chi_1^2 = 29.81$, $P < 0.0001$). However, there was a field treatment by flower treatment interaction (Wald $\chi_2^2 = 9.94$, $P = 0.007$). The difference in the proportion of siliques with 3 to 10 seeds between control and supplementally pollinated siliques was greatest in GM fields (Contrast tests, Wald $\chi_1^2 = 25.82$, $P < 0.0001$), followed by conventional fields (Wald $\chi_1^2 = 4.88$, $P = 0.027$), and there was no difference in organic fields (Wald $\chi_1^2 = 3.10$, $P = 0.078$).

Pollination deficit

Across all field types, there was a strong effect of flower treatment (open-pollinated control and supplementally pollinated) on the number of seeds per silique ($F_{1,9} = 28.73$, $P = 0.0005$). There was also an interaction between flower treatment and field treatment (organic, conventional, and GM; $F_{2,9} = 4.49$, $P < 0.044$). There was no difference between supplementally pollinated and open-pollinated flowers in organic fields ($t_9 = 1.12$, $P = 0.292$), but there was a difference in seed number between the two flower treatments in conventional ($t_9 = 3.31$, $P = 0.0091$) and GM ($t_9 = 5.47$, $P = 0.0004$) canola fields.

There was no relationship between distance from the hedgerow and pollination deficit (Fig. 3). However, because the 500-m locations were only 300 m from the
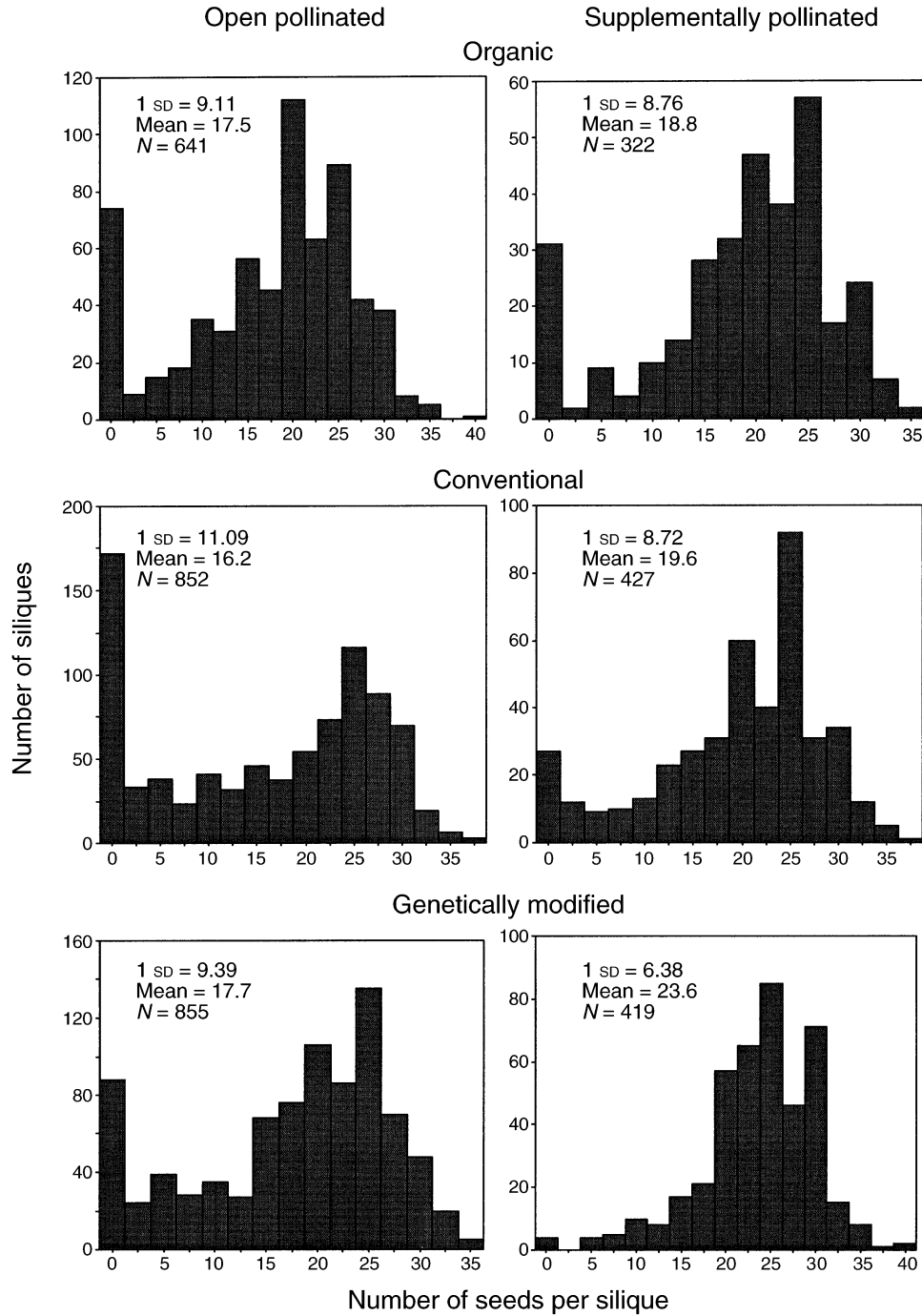
Fig. 2. Histograms of the number of siliques vs. seed number per silique from flowers that were open-pollinated (left-hand graphs) and from flowers that were supplementally pollinated (right-hand graphs) in three types of canola fields: organic (*Brassica rapa*), conventional (*B. napus* 45A71), and genetically modified (*B. napus* DK3235).

nearest edge of the field, we conducted analyses of residuals, controlling for either distance from closest edge or distance from the hedgerow, while controlling for treatment. We found no relationship between seed deficit and distance from hedgerow or edge ($F_{1,394} = 0.24$, $P = 0.623$ and $F_{1,394} = 0.13$, $P = 0.723$, respectively).

There was significant variation in pollination deficit among organic, conventional, and GM fields ($F_{2,9} = 16.02$, $P < 0.0001$; Fig. 4). Pairwise comparisons showed a difference in pollination deficit between organic and GM fields ($t_9 = -5.02$, $P < 0.0001$), with a mean deficit of -1.09 ± 0.63 seeds in organic and
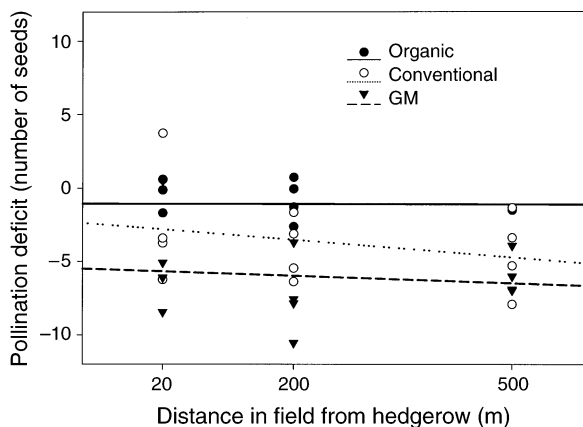
FIG. 3.   Pollination deficit measured as the difference in the number of seeds from siliques between open and supplementally pollinated canola flowers at different distances into four replicate organic, conventional, and genetically modified (GM) fields. $P > 0.05$ for all regressions.

-6.07 ± 0.52 seeds in GM fields. The mean pollination deficit in conventional fields was intermediate between organic and GM at -3.70 ± 0.61 and different from the other field treatments (conventional vs. organic $t_9 = -2.58$, $P = 0.010$; conventional vs. GM $t_9 = -2.99$, $P = 0.003$). The mean percentage of seed set in open-pollinated plants in each field treatment (number of seeds in siliques from open-pollinated flowers divided by number of seeds in siliques from supplementally pollinated flowers for each plant pair) was 99% $\pm$ 4%, 84% $\pm$ 4%, and 78% $\pm$ 2% in organic, conventional, and GM fields, respectively. There was no interaction between field treatment and distance from the hedgerow ($F_{4,15} = 0.44$, $P = 0.776$), or effect of distance on proportional deficit ($F_{2,15} = 0.95$, $P = 0.388$). Overall, there was a difference in the percentage of seed set of open-pollinated flowers among field treatments ($F_{2,9} = 9.94$, $P < 0.001$), with the greatest proportional seed set in organic fields (organic vs. conventional $t_9 = 3.28$, $P = 0.001$; organic vs. GM $t_9 = 4.44$, $P < 0.001$). There was no difference between conventional and GM in percentage of seed set in open-pollinated flowers ($t_9 = 1.41$, $P = 0.159$).

Bee abundance

There were 342, 230, and 101 total bees collected in each treatment (organic, conventional, and GM fields, respectively) during bloom, with a proportion of bumble bees to other bees of 1.54, 4.17, and 0.38, respectively. There was no effect of distance from the hedgerow by treatment interaction on bee abundance ($F_{4,14} = 0.57$, $P = 0.690$) or effect of distance on bee abundance ($F_{2,14} = 1.29$, $P = 0.3073$). Therefore, although only one organic field had 500-m collection locations, this distance was not excluded from the analyses. Mean numbers of bees collected within fields were 85.5 $\pm$ 7.1, 57.5 $\pm$ 7.3, and 25.3 $\pm$ 6.5 bees in

organic, conventional, and GM canola respectively, and were different among field types ($\chi^2 = 118.13$, df = 2, $P < 0.0001$). Pairwise comparisons showed that each field treatment was different from the others (organic vs. conventional $\chi^2 = 21.64$, df = 1, $P < 0.0001$; organic vs. GM $\chi^2 = 116.00$, df = 1, $P < 0.0001$; conventional vs. GM $\chi^2 = 47.53$, df = 1, $P < 0.0001$; Fig. 4). Species composition and population diversity will be described in a future publication.

The number of bees collected at each sampling location in each field was used as an index of bee abundance and regressed with pollination deficit at each location. Within each field, bee abundance and pollination deficit data were averaged between replicates, and there was a highly significant decrease in pollination deficit with increasing bee abundance among all field treatment types (inverse exponential decay regression; $y = -8.71e^{(-0.05x)}$, $R^2 = 0.56$, $F_{1,31} = 40.08$, $P < 0.001$; Fig. 5). When $B. napus$ varieties (conventional and GM) were analyzed, excluding $B. rapa$ (organic), again there was a highly significant relationship between bee abundance and pollination deficit ($y = -8.72e^{(-0.05x)}$, $R^2 = 0.48$, $F_{1,22} = 20.57$, $P < 0.001$).

DISCUSSION

Supplementally pollinated flowers in conventional and GM sites produced siliques with more seeds than adjacent open-pollinated flowers, suggesting that (1) yield in $B. napus$ canola in the Peace River region could benefit from increased bee-mediated pollen transfer,
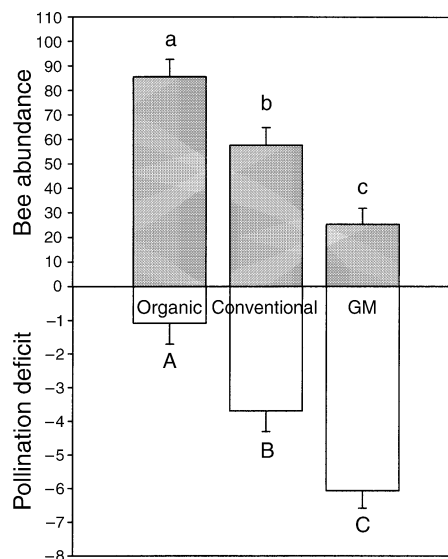

FIG. 4.   Bee abundance and pollination deficits (mean $\pm$ SE) for each field type (number of fields per treatment = 4). Different lowercase letters above bars indicate a difference in bee counts at $P < 0.05$ among field types (CATMOD followed by pairwise contrasts of maximum likelihood estimates; SAS Institute 1999). Different capital letters below bars indicate different levels of pollination deficit at $P < 0.05$ between field treatments (Proc MIXED followed by comparison of least-squares means; SAS Institute 1999).
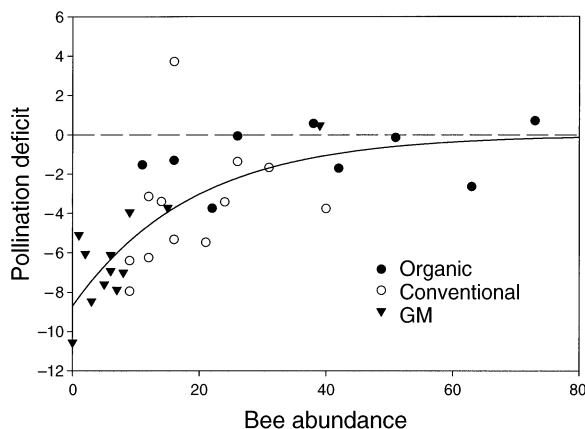
Fig. 5. Mean pollination deficit in canola flowers calculated as the difference in the number of seeds per silique in open and supplementally pollinated flowers. Means of pollination deficit and bee abundance are from two replicate distances in each field at 20-, 200-, and some 500-m distances from a chosen hedgerow. The best-fit regression was with an inverse exponential decay function for all field types combined (organic *Brassica rapa*; and conventional and genetically modified *B. napus*): ($y = -8.71e^{(-0.05x)}$, $R^2 = 0.56$, $F_{1,31} = 40.08$, $P < 0.001$), and when the *B. napus* fields were analyzed alone ($y = -8.72e^{(-0.05x)}$, $R^2 = 0.48$, $F_{1,22} = 20.57$, $P < 0.001$).

and (2) there were not enough bees and/or other pollinators in the conventional and GM sites to produce full seed set.

Brassica napus is self-fertile, yet insect pollination can increase seed set (summarized in Free 1993) and/or density of siliques (Manning and Boland 2000). However, the degree to which insects increase seed production is variable, possibly due to different cultivars tested, different environmental conditions, and different experimental methods. In this study, GM fields with *B. napus* DK3235, supplemental pollination caused a 33% increase over open-pollinated flowers in seeds per silique, and there was a 21% increase in conventional *B. napus* 45A71 canola. Thus, pollen transfer by wild pollinators was not sufficient for the canola in these fields to reach their full yield potential.

The *B. napus* in our conventional and GM fields were different varieties, and therefore the greater seed deficit in GM fields could have been due to a higher dependence on pollinators for pollen transfer and seed set than the conventional variety examined. However, from our data, it seems unlikely that the conventional canola variety had a lower requirement for pollinators because at collection sites with low bee abundance, pollination deficit values were comparable to pollination deficit values in GM fields with similar pollinator abundances (see Fig. 5). Our data suggests that the low number of pollinators in the GM fields resulted in the high pollination deficits. Pollinator exclusion experiments would be required to directly test the pollinator requirements of these canola varieties.

In contrast, we found no pollination deficit in organic fields. Since organic canola was *B. rapa*, we were not able to make direct comparisons of absolute seed numbers with GM and conventional canola. However, because *B. rapa* is self incompatible (Ohsawa and Namai 1987, Mishra et al. 1988, Zuberi and Sarker 1992), we predicted that *B. rapa* would be more vulnerable to pollination deficits under inadequate pollinator conditions. Lack of difference in seed number between open-pollinated and supplementally pollinated flowers in organic canola was likely a result of sufficient bee numbers to produce full seed set.

The pattern we found in the proportion of siliques with no seeds between open- and supplementally pollinated flower treatments showed a much greater effect of supplemental pollination in conventional and GM fields than in organic fields. In the organic fields, the proportion of siliques with no seeds was similar between open- and supplementally pollinated siliques (11.5% vs. 9.6%), suggesting that the proportion of siliques with no seeds had little to do with lack of pollen transfer. The high proportion of siliques with no seeds from flowers that were supplementally pollinated in organic fields was not anticipated and requires some explanation. In some fields, we observed high levels of lygus bug (*Lygus* spp.), a sucking insect that feeds on the sap of reproductive tissue, causing damage to siliques and seeds in canola. No pesticides were used in the organic fields in our experiments, and lygus bug damage appeared to be substantial, likely causing the relatively high proportion of siliques with no seeds in both open- and supplementally pollinated organic flowers. We made no systematic observations of lygus bug infestation, and although lygus damage is a plausible explanation for the similarly high proportion of flowers that did not set seeds from open- and supplementally pollinated flowers in the organic fields, there are a number of other explanations, including a possible lack of nutrients in organic fields resulting in seedless siliques or it could be a characteristic of the *B. rapa* variety that we examined.

The larger difference in the percentage of siliques with no seeds between open- and supplementally pollinated flowers in conventional (20.0% vs. 6.3%) and GM (10.3% vs. 0.5%) fields suggests that ~69% of the siliques with no seeds in conventional and 95% of siliques with no seeds in GM fields were a direct result of lack of pollen transfer. This is a marked contrast to the organic fields where the proportion of siliques with no seeds had little to do with pollen transfer. The lower response of siliques with no seeds to pollen transfer in conventional fields than in GM fields may have been a result of greater lygus bug damage in conventional fields; however, other explanations include resource limitation or greater competition with weeds in conventional fields. Conversely, in GM fields, our data indicate that lack of pollen transfer was the primary cause of siliques with low and no seeds.

We found a relatively equal number of seeds per silique from open-pollinated flowers in organic, conventional, and GM fields. One interpretation is that there was a similar ''pollinator force'' in all field types. However, for a number of reasons, this does not appear likely. We measured greater pollinator abundance in organic, followed by convention, and lowest in GM fields. As organic canola is self-incompatible, it would therefore require a greater pollinator force to achieve the same number of fertilized ovules as a self-pollinated species. In addition, greater lygus bug damage and/or other factors not related to pollinator abundance caused reduced seed set in organic and conventional fields. Similarly, the lower mean number of seeds per silique from supplementally pollinated flowers in organic and conventional fields than in GM fields resulted from a larger proportion of siliques with under 11 seeds and was likely not a result of lower potential seed set given ideal conditions (see Fig. 2).

Conventional and GM canola are partially self-fertile and the high proportion of siliques with 3–10 seeds in the open-pollinated treatment vs. the supplementally pollinated treatment may have resulted from flowers that were self pollinated in the absence of insect-mediated pollen transfer. The significantly lower proportion of siliques in the 3–10 seed range in supplementally pollinated flowers than from open-pollinated flowers in conventional and GM fields supports this hypothesis.

The ''diminishing'' relationship we found between bee abundance and pollination deficit across all fields suggests that seed set increased with bee pollination within canola varieties, up to a threshold. Across field types, pollination deficits approached zero with a bee abundance index above ~20 bees per sampling distance, suggesting a threshold level for bee abundance sufficient for full pollination in both *B. napus* and *B. rapa* fields. Similar diminishing returns relationships have been found between contact duration of the drone fly, *Eristalis tenax* L. on sweet pepper flowers and fruit quality (Jarlan et al. 1997), and in greenhouse tomatoes pollinated by bumble bees (*Bombus impatiens* Cresson) between anther cone bruising levels (a measure of extent of bumble bee contact) and fruit quality (Morandin et al. 2001). In *B. napus* canola fields, we found a striking positive relationship between bee abundance at sampling sites and pollination deficit, with 48% of the variation in pollination deficit being explained by differences in bee abundance, suggesting that fine-scale differences in bee abundance in the fields we tested were associated with measurable differences in pollination levels.

Canola is Canada's most important oil seed crop, and honey bees are sometimes used to supplement pollination and increase plant yields. However, current declines in managed honey bee colonies and increasing demands due to agricultural expansion are focusing attention on the contribution of wild bee populations to crop yields. Our findings support recent concerns over the economic consequences of native bee declines (e.g., Westerkamp and Gottsberger 2000, Kevan and Phillips 2001). The northern area where our research was conducted is a patchwork of agricultural acreage, logged areas, and uncultivated areas. Our research suggests that native pollinator abundance in organic canola fields is adequate for seed set, while in conventional and GM fields it is not. MacKenzie and Winston (1984) and Scott-Dupree and Winston (1987) examined pollinator diversity and abundance in berry and orchard crops, and in adjacent uncultivated areas. In both studies, they found that wild bee abundance and diversity was greater in the uncultivated areas than on the crops, and, similar to our findings in conventional and GM canola, they concluded that pollinators were not abundant enough in the crop areas to provide full pollination.

Our results also suggest that flight distances for wild bees were sufficient for pollination throughout the canola fields we studied, since we found no relationships between bee abundance or seed deficit, and distance into the fields. However, our sites had abundant adjacent uncultivated areas in which wild bees could nest. Research in regions with larger crop acreages and fewer nesting opportunities near fields might reveal different patterns. In addition, bumble and other bees have very different foraging ranges from each other, and species composition could change with distance into the field (Calabuig 2000).

Seminatural habitat is thought to benefit bumble and other bees by providing nesting and continuous, diverse foraging resources in agricultural landscapes (Corbet 1995, O'Toole 1993, Dramstad and Fry 1995). Kremen et al. (2002) found that areas of intense agriculture remote from seminatural areas have lower pollinator diversity and abundance, insufficient for adequate pollination of watermelon (*Citrullus lanatus*). In their study, organic watermelon farms in close proximity to natural habitat had their pollination requirements met by wild pollinators without supplementation from honey bees, while organic and conventional sites far from natural habitat did not receive adequate pollination.

In the present study, all of our fields had native vegetation nearby. Canola fields were in bloom from two to a maximum of four weeks, making it likely that a single canola field might not have provided enough pollen and nectar resources for bees with life spans longer than bloom, or bees without complete overlap of life and crop bloom timing. Thus, hedgerows, other uncultivated areas, and in-field weeds may provide vital foraging resources pre- and post-bloom. However, one study has found that seminatural areas are not as important resources for bumble bees as they may be for other bees, possibly because bumble bees' large foraging ranges allow them to access multiple mass flowering crop species at successive times (Westphal et al. 2003). Further work is needed to assess the importance

of landscape-level factors and cropping-system differences on bee abundance, diversity, and community structure in agroecosystems.

Although insecticide treatments were similar between GM and conventional fields, GM fields were treated with Roundup, a highly effective herbicide, which resulted in lower weed diversity and abundance within GM fields than in conventional fields (*unpublished data*), possibly affecting bee abundance. Williams (2002) suggested that herbicide-tolerant crops such as oilseed rape (*B. napus* and *rapa*), because they employ more effective weed control strategies than non-GM rape, will possibly reduce weedy and non-weedy farmland plants, causing a reduction in food resources for insects, including bees. The recent Farm Scale Evaluations in Europe (Firbank et al. 2003), are the first large-scale studies comparing GM herbicide-tolerant crops to their conventional counterparts. They found that weed diversity and biomass, and bee abundance were lower in GM herbicide tolerant spring oilseed rape (*B. napus*) than in conventional varieties (Haughton et al. 2003, Heard et al. 2003). They proposed that the lower bee numbers in GM herbicide-tolerant varieties was an indirect result of herbicide treatments that effectively reduced weeds, and consequently, forage for bees. Because organic canola growers in our study relied solely on pre-seeding tillage for weed control, organic fields had the greatest weed diversity and abundance (*unpublished data*). In addition, the smaller organic fields may have resulted in greater bee densities simply as a consequence of similar bee source areas supplying smaller field areas. However, this would not account for differences found between GM and conventional fields that were of similar size. We currently are exploring these and other factors.

Uncultivated regions in our study area are rapidly being cleared and converted to agriculture. The demonstrated limitation in seed set in *B. napus* caused by pollinator scarcity may, over time, become more pronounced as northern agricultural areas become farther removed from natural ecosystems and weed control technologies become more advanced. Long-term studies of this and similarly changing regions will be important in determining the importance of wild pollinators to agriculture and food production.

Our study has demonstrated an interesting pattern in which wild bee abundance is related to improved crop yields, but a genetically modified crop variety designed to improve yields through weed management might have the undesired consequence of reducing bee abundance in the field. However, it is important to note that other factors may be correlated with field type and be as important to bee abundance. For example, organic farmers tend to locate farther from established farm areas in order to satisfy minimum distance regulations regarding proximity to GM fields, possibly resulting in greater amounts of seminatural habitat around fields. It is vital to explore these interactions further over a number of years at multiple locations before making broad generalizations concerning particular agroecosystem interactions with pollinator communities. Nevertheless, our research highlights an interaction in which cropping systems may influence bee distribution and abundance within fields, and in turn pollinator deficits may result in decreased yields. Further studies would clearly be of interest for both ecologists and agronomists.

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