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ABSTRACT Pest management practices may be contributing to a decline in wild bee populations in or near canola (*Brassica napus* L.) agroecosystems. The objective of this study was to investigate the direct contact toxicity of five technical grade insecticides—imidacloprid, clothianidin, deltamethrin, spinosad, and novaluron—currently used, or with potential for use in canola integrated pest management on bees that may forage in canola: common eastern bumble bees [*Bombus impatiens* (Cresson); hereafter bumble bees], alfalfa leafcutting bees [*Megachile rotundata* (F.)], and *Osmia lignaria* Cresson. Clothianidin and to a lesser extent imidacloprid were highly toxic to all three species, deltamethrin and spinosad were intermediate in toxicity, and novaluron was nontoxic. Bumble bees were generally more tolerant to the direct contact applications > *O. lignaria* > leafcutting bees. However, differences in relative toxicities between the three species were not consistent, e.g., whereas clothianidin was only 4.9 and 1.3× more toxic, deltamethrin was 53 and 68× more toxic to leafcutting bees than to bumble bees and *O. lignaria*, respectively. Laboratory assessment of direct contact toxicity, although useful, is only one measure of potential impact, and mortality under field conditions may differ greatly depending on management practices. Research conducted using only honey bees as the indicator species may not adequately reflect the risk posed by insecticides to wild bees because of their unique biology and differential susceptibility. Research programs focused on determining nontarget impact on pollinators should be expanded to include not only the honey bee but also wild bee species representative of the agricultural system under investigation.

KEY WORDS bumble bee, alfalfa leafcutting bee, *O. lignaria*, insecticides, direct contact toxicity

Bees are ecologically and economically important pollinators of many wild plants and cultivated crops, with some being entirely dependent on bees for pollination (Free 1993, Allen-Wardell et al. 1998, Kearns et al. 1998, Delaplane and Mayer 2000). Although the honey bee (*Apis mellifera* L.) is generally regarded as the most important bee pollinator (Allen-Wardell et al. 1998, Kearns et al. 1998, Delaplane and Mayer 2000, Westerkamp and Gottsberger 2000), wild bees also are important (Free 1993, Williams 1996, Kevan 1999, Westerkamp and Gottsberger 2000, Kremen et al. 2002). There has been growing concern about suspected declines in wild bee populations and the implications for agricultural and natural ecosystems (Matheson et al. 1996, Allen-Wardell et al. 1998, Kevan and Phillips 2001, Klein et al. 2007, NRC 2007).

Wild bee declines have, in part, been attributed to insecticide use (Kearns et al. 1998, Westerkamp and Gottsberger 2000, Tasei 2002). Bees maybe unintentionally exposed to insecticides during or after spray application while foraging in crops and nesting in hedgerows adjacent to treated fields, or by consuming insecticide residues in nectar and pollen from treated crops. Wild bees are particularly vulnerable to foliar insecticides because, unlike honey bees, nesting sites cannot be moved or protected during spray application (Tasei 2002), and different foraging behaviors may bring wild bees into contact with insecticides applied at times designed to reduce foraging honey bee exposure (Corbet et al. 1993). Although there is limited information on the toxicity of pesticides to nontarget beneficial insects such as common eastern bumble bees [*Bombus impatiens* (Cresson)] (hereafter bumble bees), alfalfa leafcutting bees [*Megachile rotundata* (F.)], and *Osmia lignaria* Cresson compared with honey bees (Tasei 2002), in recent years, several studies have been conducted that focus on exposure and susceptibility to insecticides in groups of non-*Apis* bees (Morandin and Winston 2003, Franklin et al. 2004, Morandin et al. 2005, Malone et al. 2007, Abbott et al. 2008). Morandin and Winston (2003) concluded that ≤7 ppb of imidacloprid in pollen

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would not harm *B. impatiens* and *B. occidentalis* colony health or foraging ability; however, 30 ppb in pollen (i.e., ≈4× the highest imidacloprid residues found in field situations) resulted in sublethal effects on foraging. Franklin et al. (2004) determined that colony health and foraging ability of *B. impatiens* was not adversely affected by six ppb of clothianidin in pollen (representing the highest residues in pollen found in field studies) or 36 ppb. Studies on the impact of the biopesticide spinosad on *B. impatiens* at concentrations bees would be exposed to in the field (0.2 and 0.8 ppb) had minimal impact on colony health, whereas 8 ppb caused significant mortality of brood and adults (Morandin et al. 2005). Sublethal effects to workers exposed to 0.2 and 0.8 ppb spinosad during larval development also were demonstrated (Morandin et al. 2005). Novaluron, an insect growth regulator (IGR) (i.e., chitin synthesis inhibitor) was fed to microcolonies of *B. terrestris* in pollen at concentrations of 0.015 (a realistic field exposure concentration), 0.09, and 0.135 ppb for 35 d followed by monitoring to day 70 (Malone et al. 2007). Significantly shorter life spans of drones produced by workers in microcolonies were determined only at 0.135 ppb. In laboratory and field studies, Abbott et al. (2008) determined there were no lethal effects of either imidacloprid on *O. lignaria* or clothiandin on *M. rotundata* at three (low), 30 (intermediate), or 300 (high) ppb. Minor sublethal effects on *O. lignaria* were detected at 30 and 300 ppb imidacloprid resulting in increased larval development, whereas no sublethal effects were detected with clothianidin at any concentration on *M. rotundata.*

Canola (*Brassica napus* L.) is the second most important field crop in Canada in terms of area, with ≈5.9 million ha planted in 2007 (Statistics Canada 2007). It is an excellent source of nectar and pollen and is highly attractive to bees (Delaplane and Mayer 2000). Management of economically important insect pests including flea beetles (*Phyllotreta* spp.), bertha armyworm (*Mamestra configurata* Walker), diamondback moth [*Plutella xylostella* (L.)], lygus bugs (*Lygus* spp.), root maggots (*Delia* spp.), and the weevil *Ceutorhynchus obstrictus* Marsham requires use of seed and/or foliar insecticide treatments (Canola Council of Canada 2007), which can be highly toxic to bees (Mayer et al. 1998, OMAFRA 2006). The large area of land devoted to canola production coupled with the frequency and toxicity of insecticides applied for pest management may be important factors contributing to suspected declines of wild bee populations in canola agro-ecosystems (NRC 2007).

Our objectives were to 1) investigate the direct contact impact of five insecticides—the systemic neonicotinoids imidacloprid and clothianidin; the microbial bioinsecticide spinosad; the pyrethroid deltamethrin; and the IGR novaluron—currently used, or with potential for use in canola pest management on three bee pollinators that may forage on this crop: bumble bees, alfalfa leafcutting bees, and *O. lignaria;* and 2) determine whether toxicity of these insecticides was similar between the pollinators.

Materials and Methods

Test Bees. The test bees were selected because they are important indigenous North American pollinators of native plants and cultivated crops and reliable rearing techniques have been established for each of them.

Bumble bee colonies containing a queen and 40–60 workers were purchased from Biobest Canada Ltd. (Leamington, ON, Canada). Each colony consisted of a well-ventilated plastic container (≈20 by 28 by 18 cm) housed in a cardboard box, with the nectar substitute Biogluc (Biobest Canada Ltd.) available ad libitum. Pollen was collected from honey bee colonies at the Townsend House Bee Research Facility at the University of Guelph in 2003 and was frozen until use. Each colony was given ≈5 ml of pollen twice weekly. Female worker bees were aspirated under red light from each colony and were placed into 1 liter mason jars until testing. Each jar of bees was randomly assigned to a treatment.

Alfalfa leafcutting bee pupae were purchased from Northstar Seeds Inc. (Neepawa, MB, Canada) and were stored at 5°C until needed. They were incubated until fully developed according to a standard emergence protocol (Agriculture Canada 1989) in plastic containers (20 by 10 by 8 cm) with metal mesh lids. Emerging adults were transferred to new containers free of pupae and debris, containing two cotton dental wicks (2 by 0.9 cm), one soaked in 50% sucrose, and one soaked in tap water. Seven-day-old bees (sex ratio of 2 females:1 male) were removed from the containers and were placed into a 1-liter mason jar that was randomly assigned to a treatment.

O. lignaria were purchased from Beediverse Products of CPC Ltd. (Coquitlam, BC, Canada) in September and were stored at 2–4°C and 40–60% RH. They were incubated until fully developed according to a standard emergence protocol (Dogterom 2005) and were maintained in the same manner as alfalfa leafcutting bees until required for testing. The sex ratio of *O. lignaria* is 1 female:1.7 males in nests with a typical tunnel diameter of 7.5 mm and length of 14 cm (Dogterom 1999).

Bumble bees, *O. lignaria,* and alfalfa leafcutting bees were all commercially available, but for our purposes we considered them to be wild bees because commercial stocks are invigorated with wild caught specimens annually. In recent years, *B. impatiens* has increasingly been used for pollination in commercial greenhouses. *M. rotundata* is used in western Canada and the United States for pollination of alfalfa and canola. *O. lignaria* is used for pollinating fruit crops, e.g., apple, cherry, almonds and berry bushes (blueberries) and other flowering crops wherever they are found (canola).

Insecticides. Insecticides tested were: clothianidin, imidacloprid, deltamethrin (Bayer CropScience Inc., Calgary, AB, Canada), spinosad (Dow AgroSciences Canada Inc., Calgary, AB, Canada), and novaluron (Makhteshim-Agan North America via Crompton Co./Cie, Middlebury, CT). Clothianidin and imidacloprid are systemic neonicotinoid insecticides cur-

Table 1. Direct contact toxicity of technical grade insecticides after 48 h to bumble bees, alfalfa leafcutting bees, and *O. lignaria*

Insecticide	No. adult bees tested	Slope ± SE	χ^2	LC_{50}[a]	95% FL[b]
Bumble bees (♀ only)					
Clothianidin	253	1.9 ± 0.34	0.1	0.39a[c]	0.30–0.59
Imidacloprid	251	1.9 ± 0.19	3.6	3.22b	2.54–4.10
Deltamethrin	199	4.4 ± 0.53	2.0	6.90c	6.06–7.76
Spinosad	199	4.2 ± 0.70	0.8	8.95d	7.92–10.76
Novaluron	163			>100[d]	
Alfalfa leafcutting bees (♀ and ♂)					
Clothianidin	297	2.4 ± 0.26	6.1	0.08a	0.07–0.09
Deltamethrin	253	1.2 ± 0.23	0.1	0.13b	0.10–0.19
Imidacloprid	299	2.0 ± 0.23	1.5	0.17b	0.14–0.21
Spinosad	250	4.5 ± 0.49	4.3	1.25c	1.13–1.40
Novaluron	270			>100[d]	
O. lignaria (♀ and ♂)					
Clothianidin	380	2.7 ± 0.35	1.1	0.10b	0.09–0.10
Deltamethrin	580	2.3 ± 0.43	15.4	8.90d	6.00–11.70
Imidacloprid	400	2.2 ± 0.34	0.2	0.07a	0.06–0.09
Spinosad	500	3.09 ± 0.37	6.4	4.70c	4.00–5.40

[a] Concentrations are expressed as percentage of solution (wt:vol) ($\times 10^{-3}$).
[b] Fiducial limits ($\times 10^{-3}$).
[c] For each bee species tested, values followed by the same letters are not significantly different as determined by overlap of 95% fiducial limits.
[d] No mortality at the highest concentration tested (0.1% solution).

rently registered as canola seed treatments. The seeds are treated with formulated insecticide before planting and it is translocated through the growing plant. Deltamethrin is a pyrethroid insecticide, registered for use in canola. Spinosad, a microbial bioinsecticide and novaluron, a benzoylphenyl urea IGR, also may have potential for use on canola in Canada. All insecticides tested were technical grade (>95% purity), with exception of spinosad (90% purity). Stock solutions (1.0%, wt:vol) were prepared by dissolving each insecticide in a 19:1 acetone:olive oil solvent mixture (Harris and Svec 1969).

Insecticide Application. Direct contact toxicity of the insecticides was determined using a Potter spray tower (PST) (Potter 1952) located at the Southern Crop Protection and Food Research Centre-Agriculture and Agri-Food Canada-London, ON, Canada. Four to six concentrations of each insecticide were prepared by serial dilutions of the appropriate stock solutions. For each concentration, four to six replicates, each containing nine to 11 bees, were tested. Control insects, treated with acetone:olive oil only, were included with each insecticide. Control mortality did not exceed 10%. Before treatment, bees were anesthetized with CO_2 for 3 s, placed ventral side up in a 10-cm glass petri dish containing a single sheet of 9 cm diameter filter paper and placed in the PST. Five ml aliquots of the desired concentration of each insecticide were then applied.

After treatment bees were transferred to waxed paper Dixie cups that were covered with 10-cm plastic petri dishes. Cotton dental wicks (2 by 0.9 cm) were soaked in Biogluc for bumble bees, and 50% sucrose-water solution for alfalfa leafcutting bees and *O. lignaria* and stapled to the inside of the cups. Posttreatment containers were placed at 25 ± 1°C in the dark immediately after treatment. Mortality was assessed 48 h posttreatment. Bees that could not move when touched with a blunt probe were considered dead.

Data Analysis. Statistical analyses were performed using SAS version 8.02 (SAS Institute 2001). The probit procedure was used to determine the median lethal concentration (LC_{50}), 95% fiducial limits, and chi-square goodness-of-fit for each insecticide tested. Each LC_{50} determination was based on between four and six concentrations causing ≈15–90% mortality. Abbott's formula was used to correct for control mortality (Abbott 1925). Direct contact toxicity data for the three wild bees were compared with data from Bailey et al. (2005) on honey bees to determine the relative susceptibility of both wild and domesticated bees frequenting the agroecosystems.

Results

The descending order of contact toxicity to bumble bees was clothianidin > imidacloprid > deltamethrin > spinosad > novaluron; to alfalfa leafcutting bees was clothianidin > deltamethrin ≥ imidacloprid > spinosad > novaluron; and to *O. lignaria* bees was imidacloprid > clothianidin > spinosad > deltamethrin (Table 1). Novaluron, not tested on *O. lignaria,* caused no significant adult mortality at the highest rate tested (0.1% solution) to either alfalfa leafcutting or bumble bees.

The neonicotinoid insecticides clothianidin and to a lesser extent imidacloprid were highly toxic to the three bee species. For example, clothianidin was ≈23, 16, and 47× more toxic to bumble bees, alfalfa leafcutting bees, and *O. lignaria* bees, respectively, than was the microbial bioinsecticide spinosad. The pyrethroid insecticide deltamethrin varied considerably in toxicity, being highly toxic to alfalfa leafcutting bees

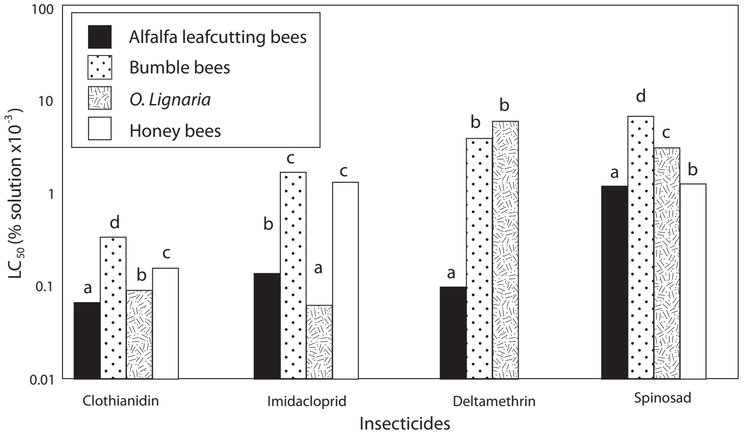

Fig. 1. Comparison of direct contact toxicity of clothianidin, imidacloprid, deltamethrin, and spinosad to alfalfa leafcutting bees, bumble bees, *O. lignaria* bees, and honey bees. LC_{50} values for honey bees are from Bailey et al. (2005). Bars within each category followed by the same letter are not significantly different as determined by overlapping 95% fiducial limits.

but much less toxic to bumble bees and *O. lignaria* bees (Table 1; Fig. 1).

Bee species have been reported to vary in susceptibility to insecticides (Mayer et al. 1998, Devillers et al. 2003), and our data followed a similar pattern with alfalfa leafcutting bees being more susceptible than *O. lignaria* bees or bumble bees (Table 1; Fig. 1). Bees with larger surface area to volume ratios are thought to generally be more susceptible to direct contact insecticide applications than those with smaller ratios because they are exposed to relatively larger doses (Tasei 2002, Johansen et al. 1983). However, differences in relative toxicity of the insecticides between the three species were not consistent, e.g., whereas clothianidin was only 4.9 and 1.3× more toxic, deltamethrin was 53 and 68× more toxic to alfalfa leafcutting bees than to bumble bees and *O. lignaria* bees, respectively.

Bailey et al. (2005), using the same bioassay technique, determined the direct contact toxicities of clothianidin, imidacloprid, and spinosad to honey bees. Comparison of their data with those obtained in this study showed some significant differences in insecticide toxicity. Although clothianidin was highly toxic to honey bees, imidacloprid and spinosad were only moderately toxic. *O. lignaria* and alfalfa leafcutting bees were more susceptible to the neonicotinoid insecticides than honey bees (Fig. 1).

Discussion and Conclusions

Canola growers tend to rely on self-fertilization and ambient wild bee populations to ensure seed set in commercial oil-producing canola. Although the honey bee is an important pollinator of canola, recent dramatic losses in honey bee populations worldwide due to colony collapse disorder (Oldroyd 2007), reduced efficacy to the miticide fluvalinate used to control *Varroa destructor* Anderson & Trueman (Macedo et al. 2002), and the impact of *Nosema ceranae* (Williams et al. 2008) likely will result in an increasingly important pollination role for wild bees because seed set has been shown to increase as a result of increased wild bee abundance (Morandin and Winston 2005). Thus, it is essential to assess the impact on wild bee pollinators of insecticides considered suitable for canola pest management. The study showed that the direct contact toxicity of the insecticides tested varied, sometimes very substantially, with insecticide and species. Clothianidin and imidacloprid were generally highly toxic, deltamethrin and spinosad being less so, with bumble and honey bees being more tolerant than mason and leafcutter bees (Table 1; Fig. 1). It was not unexpected that novaluron was nontoxic to adult bumble and alfalfa leafcutting bees (Table 1) because as a chitin synthesis inhibitor its activity is restricted to immature life stages (Cutler and Scott-Dupree 2007a).

Laboratory assessment of direct contact toxicity is only one measure of potential impact, and mortality may differ greatly under other laboratory or field conditions (Stark et al. 1995, Bailey et al. 2005) depending on management practices such as methods, rates, and timing of pesticide applications. For example, although highly toxic by direct contact, the neonicotinoid insecticides are applied as seed treatments on canola, thus minimizing impact other than through possible minimal exposure to very low residues in nectar and pollen (Tasei et al. 2001, Maus et al. 2003, Cutler and Scott-Dupree 2007b). Conversely, although generally less toxic by contact, foliar applications of deltamethrin or spinosad could have immediate impact on foraging bees. Situations where potential hazard has been identified in laboratory studies must be validated under field conditions using formulated product at recommended application rates before reaching conclusions about insecticide impact on either domesticated or wild bees.

Research conducted using only honey bees as the indicator species may not adequately reflect the risk posed by insecticides to wild bees because of their differential susceptibility and unique biology

(Thompson and Hunt 1999). Pesticide impacts on social (e.g., honey and bumble bees) and solitary (e.g., alfalfa leafcutting bees and *O. lignaria*) bee pollinators may vary substantially depending on foraging patterns, nesting behavior, seasonal activity, and diurnal flight activity; all need to be considered in future research.

The significance of these data goes much beyond just canola agroecosystems because these, and other insecticides, are used on numerous horticultural and field crops that benefit from wild bee pollination. Protecting wild bees from exposure to insecticides is essential, particularly given their increasing importance in pollination as honey bee populations are compromised. To this end, it is important to establish globally accepted laboratory and field study protocols for comparative assessment, before registration, of lethal and sublethal pollinator responses to novel insecticides and other control products.

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