TOXICITY OF RED CLOVER PESTICIDES TO A NATIVE BUMBLE BEE POLLINATOR

K.M. Skyrm, S. Rao and G.C. Fisher

Introduction

Red clover is a biennial forage legume crop grown for seed in the Willamette Valley of western Oregon. A key factor in maximizing red clover seed production is obtaining adequate seed set through cross pollination. Bees serve as the primary pollinating agents for red clover seed production. While honey bee hives are rented for pollination, wild bees such as bumble bees are considered to be efficient pollinators of red clover (Rao and Stephen, 2009). Currently, there are worldwide declines in the availability and abundance of both honey bee and native bee species. One of the principal causes of this decline has been attributed to the extensive use of pesticides in agricultural ecosystems (Goulson et al., 2008). Bees are affected by pesticides via direct contact with sprays, contaminated nectar, pollen sources from flowers, and residues on plants (Johansen, 1966).

Historically, honey bees have attracted the majority of interest with regard to evaluating the toxicological effects of pesticides on bees (Devillers et al., 2003). Few studies have examined the effects of pesticides on feral bee populations. As such, pesticide label precautions regarding rates and timing for applications to crops requiring cross pollination have been established based on the responses of honey bees to pesticide exposure (Riedl et al., 2006). Given the differences in physiology, life history, ecology, and foraging behavior of bees, these data are not appropriate for assessing and comparing the toxicity of pesticides on populations of wild bee pollinators such as bumble bees (Thompson, 2001). Hence, there is a need to evaluate the toxicity of pesticides to feral bumble bees. The objective of this study was to determine the toxicity of insecticides used in the production of red clover seed, to a dominant feral bumble bee, *Bombus vosnesenskii*.

Methods

Plant material and bees. Red clover plant material was collected from an unsprayed field located in Polk County, OR. Three insecticides currently registered in Oregon and used on red clover to control clover aphid and pea aphid were tested: Metasystox-R (MSR® Spray Concentrate), Lorsban® Advanced and Brigade® 2EC (Fisher and Dreves, 2009). Labels for Lorsban® Advanced and Brigade® 2EC specifically indicate that these products are not to be applied to blooming crops or crops with actively foraging pollinators. The MSR® Spray Concentrate label allows for an early morning application prior to pollinator activity. A fourth unregistered product, Admire® 2, was also evaluated because of recent publicity concerning the toxicity of its active ingredient, imidacloprid to honey bees. Its label specifically prohibits the use of imidacloprid as Admire® to crops grown for seed. The minimum, maximum and 2X maximum recommended field rates for aphid control were used as treatments in the experiment (Fisher and Dreves, 2009). Each chemical was prepared and applied as a dilute spray in the equivalent of 100 gallons per acre. Treatments were replicated six times. Worker bumble bees, *Bombus vosnesenskii,* were used given their late season abundance in red clover during bloom. A total of 384 wild caught worker bees were field collected and subsequently exposed to the test materials in these bioassays on the day of collection.

Bioassay. Standard cylinder cages described by Johansen et al. (1983) consisting of a 15 cm plastic petri dish and a 45.7 cm x 5.1 cm strip of metal screen (6.7 meshes per cm) were used for all bioassays. Prior to application, plant samples consisting of flowers, leaves and stems were condensed to 2.5-5 cm lengths and 15.5 g were placed inside cages (Johansen et al., 1983). Pesticides were applied to plant material within cages using a Potter precision laboratory spray tower at a rate of 2ml of solution per cage (Potter and Way, 1958) (Figure 1). After application, residues were allowed to dry for a period of 1-2 hours prior to the introduction of bees. Bees were anesthetized to facilitate handling and randomly assigned to treatments (Figure 2). Each cage received four worker bees. Bees were provided a 50% nectar solution by a cotton wick feeder attached to the bottom of each cage (Johansen et al., 1983). Prior to experimentation, cages were situated on a tray consisting of all four treatments spaced equidistantly from one another. Cages were kept under controlled environmental conditions of humidity (50-60%), temperature ($28^{o}C \pm 2^{o}C$) and photoperiod (D7:L17). Mortality was assessed after 24, 48 and 72 hours by recording the number of dead bees within each cage.

Results and Discussion

After just 24 hours of exposure, Lorsban® Advanced and Brigade® 2EC resulted in 83% to 100% bumble bee mortality at all three rates evaluated (Table 1). For both Lorsban® Advanced and Brigade® 2EC mortality increased significantly over that of the untreated check as the rate increased for the three periods of exposure. The minimum, maximum and 2X maximum field rates did not differ from each other. In contrast, bumble bee mortalities in the Admire® 2 and MSR® treatments were not significantly different from the natural mortalities observed in the untreated controls over the three days the trial was conducted. There were no significant differences across all treatments and periods of exposure for Admire® 2 and MSR®. Based on these results, growers are encouraged to time pesticide applications to reduce or avoid non-target impacts on the mortality of feral pollinators. Aphid control in red clover with insecticides should be made prior to bloom to avoid killing native bumble bees that are essential for increased seed yields.

References

Devillers, J., Decourtye, A., Budzinski, H., Pham-Delegue, M.H., Cluzeau, S. and Maurin, G. 2003. Comparative toxicity and hazards of pesticides to *Apis* and non-*Apis* bees. A chemometrical study. *Environ. Res.*, 14: 389-403.

Fisher, G. and Dreves, A. 2009. Clover seed pests. *In* Hollingsworth, C.S., Ed. Pacific Northwest Insect Management Handbook, 57-62.

Goulson, D, Lye, G.C., and Darvill, B. 2008. Decline and conservation of bumble bees. *Ann. Rev. Entomol*., 53: 191-208.

Johansen, C.A. 1966. Digest on bee poisoning, its effects and prevention. *Bee World*, 47(b): 9-25.

Johansen, C.A. Mayer, D.F., Eves, J.D. and Kious, C.W. 1983. Pesticides and bees. *Environ. Entomol*.,12(5): 1513-1518.

Potter, C. and Way, M.J. 1958. Precision spraying *In* Shepard, H.H., Ed., Methods of testing chemicals on insects, Vol. 1, Burgess Publishing Company, Minneapolis.

Rao, S. and W. P. Stephen. 2009. Bumble bee pollinators in red clover seed production. *Crop Science*, 49: 2207-2214.

Riedl, H., Johansen, E., Brewer, L. and Barbour, J. 2006. *How to reduce bee poisoning from pesticides*. Pacific Northwest Extension publication, 591 pp.

Thompson, H.M. 2001. Assessing the exposure and toxicity of pesticides to bumblebees (*Bombus* sp.). *Apidologie*, 32: 305-321.

Figure 1. The Potter precision laboratory spray tower used in pesticide applications.

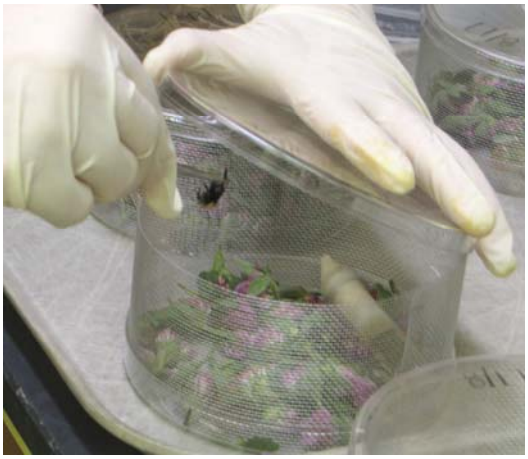

Figure 2. A marked bumble bee (*Bombus vosnesenskii*), worker being assigned to a standard cylinder cage used in bioassays.

Table 1. Mean mortality (% ± SE) values of worker bumble bees after exposure to field rates of pesticides applied to red clover plant material.

Pesticide (chemical - a.i. %)	Mortality (Mean % ± SE)[a b] Rate/acre				Analysis[c]	
	Untreated check	Minimum	Maximum	2X maximum	*F*	*P*
Admire 2®	--	0.03 lb	0.04 lb	0.08 lb		
(imidacloprid - 21.4 %)						
24 hrs	8.33 ± 5.27	0.00 ± 0.00	0.00 ± 0.00	12.50 ± 8.54	1.63	n.s.
48 hrs	20.83 ± 11.93	33.33 ± 5.27	33.33 ± 12.36	33.33 ± 10.54	0.61	n.s.
72 hrs	20.83 ± 11.93	41.67 ± 5.27	54.17 ± 15.02	62.50 ± 10.70	2.69	n.s.
Metasystox-R® (MSR)	--	0.38 lb	0.50 lb	1.00 lb		
(oxydemeton-methyl - 25.5 %)						
24 hrs	16.67 ± 8.33	0.00 ± 0.00	0.00 ± 0.00	16.67 ± 8.33	3.08	n.s.
48 hrs	25.00 ± 9.13	37.50 ± 10.70	25.00 ± 6.45	33.33 ± 12.36	0.26	n.s.
72 hrs	37.50 ± 14.07	41.67 ± 8.33	45.83 ± 11.93	58.33 ± 10.54	0.89	n.s.
Lorsban Advanced®	--	0.50 lb	1.00 lb	2.00 lb		
(chloropyrifos - 40.18 %)						
24 hrs	20.83 ± 4.17a	83.33 ± 8.33b	100.00± 0.00b	100.00 ± 0.00b	41.35	<0.001
48 hrs	20.83 ± 4.17a	95.83 ± 4.17b	100.00± 0.00b	100.00 ± 0.00b	80.67	<0.001
72 hrs	29.17 ± 7.68a	100.00 ± 0.00b	100.00 ± 0.00b	100.00 ± 0.00b	80.00	<0.001
Brigade 2EC®	--	0.06 lb	0.10 lb	0.20 lb		
(bifenthrin - 25.1 %)						
24 hrs	0.00 ± 0.00a	87.50 ± 8.54b	91.67 ± 8.33b	95.83 ± 4.17b	45.48	<0.001
48 hrs	8.33 ± 5.27a	95.83 ± 4.17b	91.67 ± 8.33b	100.00 ± 0.00b	47.75	<0.001
72 hrs	33.33 ± 10.54a	95.83 ± 4.17b	91.67 ± 8.33b	100.00 ± 0.00b	19.55	<0.001

[a] n=6.
[b] Data analyzed using ANOVA with $\alpha = 0.05$. Data were arcsine(sqrt) transformed prior to analysis to meet the assumptions of normality.
[c] Mean values with the same letter are not significantly different at $P = 0.05$ (*n.s*), according to Tukey multiple means comparison.