Re: **Bee learning behaviour affected by GMO toxin**

To the editor:
The question of exposure, (i.e. estimating how much transgene product pollinators will eat) is likely to be crucial in accurately predicting the effects of Bt proteins on bees, but is only briefly discussed by Ramirez-Romero et al. (2008). To answer this question, regulators may be tempted to use feeding measurements from other ecosystems to estimate exposures. In general, however, when exposure is critical for determining safety, those measurements should come from the specific jurisdiction making the decision.

To illustrate, consider the following story. The amount of pollen that is ingested by bees has been estimated at 6.5 mg per day (reported in Ramirez-Romero et al., 2008), but studies report high variation between sites (e.g. Babendreier et al., 2004). Pollen is the main natural source of protein in the bee diet (Crailsheim, 1990). Pollen collection is influenced by the plants producing it. Maize produces up to 50 million grains per plant over a week, and 80% of honeybee intake can come from maize when this crop is in monoculture (Li et al., 2008, Ramirez-Romero et al., 2008). Additionally, ingestion will vary by age (Crailsheim, 1990), and behavior, as indicated by the slower feeding on Cry toxin-containing food (Ramirez-Romero et al., 2008). Lifetime exposure will also vary with category of bee. All young bees and the hive throughout winter (~ 90 days) are fed a mixture of honey and beebread which is a composite of pollen. Worker larvae then begin to eat harvested pollen over about 5 days beginning at about the age of 3.5 days, increasing until they become nurses (Crailsheim, 1990). During development, pollen is ingested by drone larvae for 6.5 days, nurses for 10 days, nectar and pollen foragers daily (Halm et al., 2006). In addition, the amount of toxin produced in pollen will vary by crop, place and time (Nguyen and Jehle, 2007, Schmidt et al., 2008). Decision-makers thus should be wary of working with averages because they can be very unrepresentative of normal extremes (UCS, 2002) (see Table).

Table: Estimated Cry toxin ingestion through pollen by honey bees based on reported Cry toxin concentrations in commercial GM corn

Event / Study:	Ramirez-Romero et al.[1]	Babendreier et al.
Bt 176 (1-11 μg Cry1Ab/g fw) (Babendreier et al., 2004, Schmidt et al., 2008)	(nurse) 62-686 ng/12 days	(larva) 2-22 ng total
MON863 (10-101 μg Cry3Bb/g fw) (Schmidt et al., 2008)	624-6302 ng/12 days	20-202 ng total
[1]Note that harm was detected in nurse bees at 600 ng Cry1Ab per bee in 12 days, but may occur at lower doses.		

The study by Ramirez-Romero et al. concluded that adverse effects on bee learning behavior caused by Cry1Ab were detectable at doses around 600ng/12 days, which were estimated to be about a factor of two higher than the average exposure expected for a nurse honey bee. This conclusion would fail an appropriate safety test on several grounds (Andow and Hilbeck, 2004). Firstly, the Cry1Ab toxin used by Ramirez-Romero et al. was isolated from a surrogate source (bacteria) rather than a commercial GM plant and structural equivalence between that produced in GM plants and that produced in bacteria was not demonstrated. Secondly, the applied uncertainty factor was far too small (less than 2-fold between 312 and 600ng). Generally, uncertainty factors of 10-10,000 are used to account for variance in a number of estimates based on averages (Andow and Hilbeck, 2004). Worryingly, the exposure is based on 5 µg Cry1Ab/g pollen, which is a factor of two below the maximum reported amounts of Cry 1Ab in Bt 176 pollen. When the more appropriate maximum field dosage (11 µg/g) is substituted, then it is clear that Bt 176 is capable of delivering a biologically meaningful dose. Finally, only two dosages were reported: 3 ppb and 5000 ppb (=600ng/12 days). The minimum "no observed effect level" was not determined and may be far less than 5000 ppb.

Taken together, the Ramirez-Romero et al. study should be taken as an important indicator of probable adverse effect to honey bees from at least some Cry toxin-producing GM plants. The authors do say that their findings "highlight the importance of developing studies assessing how exposures to transgenic crops can affect honey bee foraging capacities", because the "preservation and accumulation of toxins coming from GM crops in the hive is not investigated when assessing the risk of GM crop for pollinators." But they incautiously also conclude "that negative effects of Cry1Ab protein on foraging behaviour of honey bees are unlikely in natural conditions." Contrary to what the authors conclude, the amount of Cry 1Ab toxin produced in some commercial GM corn varieties is sufficient to cause a measurable effect on learning behavior. The conclusions of the authors were oddly based on

- systematically substituting selected low averages of both bee pollen ingestion and Cry toxin concentrations into their final calculations; and
- two measured data points separated by a factor of nearly 1,000 concentration units. This implicit dose-response analysis is fundamentally flawed.

The effect on bee learning that these researchers noted was different to the effect other insecticides have. The Ramirez-Romero et al. study and others report that the insecticide imidacloprid reduces conditioning in bees. That is, the insecticide interferes with the ability of bees to transfer knowledge of new food sources to long-term memory. Cry 1Ab seemed to affect the other end of the process, interfering with the ability of bees to remember that a food source is depleted and therefore they should abandon that location and search elsewhere. The combination of the two insecticides coupled with the slow-feeding effect of Cry 1Ab, could form a reasonable hypothesis as to why bees may be disappearing, and certainly justify careful follow-up using improved experimental designs.

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