October 26, 2005

Lecture 18: Hazards to Beneficial Insects; Evolution of Resistance

I. Overview of Effect of Pesticides (especially insecticides) on beneficial insects

A. The use of the term 'beneficial' insect historically denotes all insects, as well as mite species, that are either pollinators (for ex., most bee species, Family Hymenoptera) or feed on other insects (called predators and parasitoids).
   1. Of course, non-pest arthropods, i.e., neutral ones (neither pollinators nor predators/parasitoids) are "beneficial" in the sense they are integral components of a community and ecosystem;
      a. For example, they are prey for other organisms.
      b. They harbor microorganisms and serve as hosts for microbial communities.
      c. When they die and decompose nutrients are released to soil.

B. Predators are distinguished from parasitoids by promiscuity of food choice and relationship with the host.
   1. Predators usually are polyphagous (feed on a wide variety of other arthropod species) in both the adult and larval or sexually immature stage, are mobile by crawling (rather than flying), and will eat both larval and adult forms of other insects and mites.
      a. Most of the predators are spiders, mites (note some mites are phytophagous and are pests), beetles, and some flies.

C. Parasitoids are mostly in the family Hymenoptera (generically called bees, ants, and wasps) and a few are in the Coleoptera (generically called beetles)
   1. Parasitoids are highly mobile by flying searching out hosts in which the eggs are laid.
   2. The eggs hatch within (and sometimes on the outside of the body depending on the species), and the larvae will literally eat the host insect from the inside out.
   3. The larvae will often pupate inside the host's dead body, and then the adult when developed will breakout of the old pupal exoskeleton and fly off in search of a new host.
   4. Parasitoids are typically host specific, feeding on only one or very few species.

D. Since the invention of synthetic organic insecticides with broad spectrum toxicity (i.e., no selectively among pests and nontarget organisms), entomologists have noted widespread effects on beneficial insects, including decimation of predators and parasitoids within agroecosystems, as well as bee kills when certain insecticides are used.

II. Pesticides and Bees

A. If we can make any generalized statement about the state of wildlife populations with respect to environmental conditions, a primary topic would be the state of bee populations. Steep declines in wild bee populations have been noted in recent times, giving concern for ecosystem level effects because of the importance of bees as plant pollinators.

1. However, the causes of these population declines are unknown and probably not due to one factor.
2. Honeybee populations, which are essentially one species, Apis mellifera, derived from a European strain, are susceptible to two different mites. In terms of honeybee population declines, these mites are likely major factors, and also may be important in parasitizing wild bees.
3. Nevertheless, the use of insecticides in agricultural production is routinely cited as one causal factor out of many.

B. Honeybees of course are extremely important pollinator for many of our fruit and vegetable crops.
   1. EPA requires data on toxicity of pesticides to bees prior to registration. If toxicity is very high, then EPA will ensure that language on the pesticide product label warns users of this hazard.
   2. Furthermore, EPA will mandate application practices that will minimize exposure to bees.

C. Another way of comparing hazards of pesticides to honeybees is to integrate the LD50 (as μg of pesticide per bee) with the application (Davis and Williams 1990).
   1. Note in Table 1 that compounds with very low LD50's are not necessarily very hazardous under field conditions. The rate of application must be considered.
      a. Note that imidacloprid ranks as the most hazardous compound, largely driven by its extremely low LD50. On the other hand, another chloronicotinyl insecticide, acetamiprid, has very low toxicity and consequently hazard.
      b. The pyrethroids permethrin and deltamethrin are quite toxic (like imidacloprid and chlorpyrifos), but their very low use rates make them relatively less hazardous.
         1. Pyrethroids are known to have some repellent activity against bees, a property that would further lower their hazard (Moffett et al. 1982).

Table 1. Relationship between LD50 and Hazard Index for selected pesticides against honeybee. 1/

Pesticide	**LD50 (µg/bee)**	**Application Rate (g/ha)**	**Hazard Index =(g/ha)/(LD50*100)**
Imidacloprid	0.0179	224	125.1
Chlorpyrifos	0.059	480	81.4
Malathion	0.27	1260	46.7
Carbaryl	1.3	850	6.5
Permethrin	0.075	40	5.3
Deltamethrin	0.035	7.5	2.1
Acetamiprid	7.07	168.2	0.24
2,4-D	100	908	0.091
Diuron	145	908	0.063

1/ All pesticide data except imidacloprid and acetamiprid from Davis and Williams 1990; imidacloprid and acetamiprid toxicity data from Iwasa et al. 2004)

III. Case Study: Imidacloprid and Bees

A. Imidacloprid is considered to have a very low hazard potential for ecological effects (based on aquatic invertebrate, fish, and bird bioassays; Table 2), but over the last ~seven years, farmers in Canada and France have protested its use.

Table 2. Toxicity of imidacloprid to nontarget organisms (data referenced in Felsot 2001)

Test Organism	Acute LC50 (μg/L)	No Observable Effect Concentration (NOEC) (μg/L)
Aquatic Invertebrates		
Water flea (*Daphnia*)	10,440 – 85,000	
Water flea-reproduction (21 day)		1800 – 3600
Brine shrimp (*Artemia*)	361,230	
Mysid shrimp (*Mysidopsis*)	37	
Hyalella azteca (crustacean)	55	
Mosquito (*Aedes*)	13	
Fish		
Golden orfe	237,000	
Rainbow trout	211,000	
Carp	280,000	
Trout, 21 day		29,000 – 62,000
Birds		mg/kg (Diet)
Canary (force-fed)	25 - 50	
House sparrow (force-fed)	41	
Pigeon (force-fed)	25 - 50	
Japanese quail (force-fed)	31	
Bobwhite quail (force-fed)	152	
Bobwhite quail (5-day dietary)	1420	
Bobwhite quail (reproduction, dietary)		>243
Mallard duck (5-day dietary)	>5000	
Mallard duck (reproduction, dietary)		125

1. These farmers claim that the use of imidacloprid as a seed treatment (with the formulation Gaucho), especially for sunflower production, has seriously damaged honeybee colonies.
   a. The concerns are summed up in a 2000 document issued by a national apicultural association in France. The English translation of the document, "Composite Document of Present Position Relating to Gaucho/Sunflower and Bees" can be found at the URL: http://www.honeybeeworld.com/imidacloprid/compdoc.html.
      1. Gaucho was suspended for use on sunflowers in France in 1999, and the ban was extended in 2003 for three more years (URL: http://www.pan-uk.org/pestnews/Actives/imidaclo.htm).

a. Ironically, bee populations did not return after the ban, but a substitute insecticide, fipronil, was hypothesized to also be toxic to bees and by implication prolonging the problem.

2. In 2004, France temporarily suspended use of Gaucho to treat corn see (URL: http://www.newmediaexplorer.org/sepp/2003/11/26/millions_of_bees_dead_bayers_gaucho_blamed.htm)

b. The Canadian Honey Council reviewed information from beekeepers who complained of hive losses near potato fields treated with imidacloprid (URL: http://www.honeycouncil.ca/users/folder.asp?FolderID=1119).

2. Note that imidacloprid is a systemic insecticide. It easily translocates through the xylem of the growing plant when applied to the seeds before planting. Thus, its residues can easily distribute throughout the plant and potentially concentrate in the flower nectar and or pollen.
3. Imidacloprid is also applied by spraying plants directly, and more infrequently it is applied to the soil through drip irrigation systems. These methods of application seem to be less hazardous to bees.

B. Several studies have been published in recent years to address the issue of whether imidacloprid represents a hazard to honeybees in the field.
1. Imidacloprid is considered extremely toxic to honeybees. On a mass per bee basis, the LD50 for oral exposure (imidacloprid is put in sugar water) is 4.8 ng/bee (48 hour reading), but for dermal exposure, the LD50 was 24 ng/bee (Suchail et al. 2000)
a. Contact or oral intoxication induced rapid neurotoxic symptoms:
1. Problems with movement coordination;
2. Trembling;
3. Tumbling

b. The higher oral toxicity of imidacloprid to honeybee stands in contrast to the OP insecticide chlorpyrifos and pyrethroid insecticide bifenthrin; with these two insecticides, contact toxicity was greater than oral toxicity. Comparatively, imidacloprid is approximately two times more toxic than other extremely toxic insecticides, including the pyrethroids cyhalothrin and deltamethrin, and the OP insecticide triazophos.
1. Interestingly, the group of pest insects that are most susceptible to the effects of imidacloprid, the aphids, have oral LD50 (mass/mass basis) of ~3 ng/g.
a. Imidacloprid has a honeybee oral LD50 (mass/mass basis) of 50 ng/g. Thus the selectivity ratio of pest to honeybee is favorable from the viewpoint of safety.

c. In another toxicity study with honey bees, a metabolite of imidacloprid, called the olefin was actually more toxic than the parent compound (Suchail 2001 et al.).
d. More importantly, a chronic bioassay in which honeybees were allowed to feed on treated sugar solution for 10 days, showed that on a mass/bee or mass/mass basis, imidacloprid was significantly more toxic than in an acute exposure.

1. LD50 after an acute oral exposure was about 400 μg/kg at 72 hours following feeding;
2. For a chronic exposure, the oral LD50 (50% mortality occurred at 8 days of feeding) was at least 10 μg/kg.

C. The studies by Suchail et al. were laboratory based and did not address what happens in the field.
   1. Studies by Schmuck et al. (2001) suggested that the risk to honeybees from field exposure is low, which is in contrast to farmers' perceptions in Canada and France.
   2. Schmuck et al. (2001) treated sunflower seeds with imidacloprid and then analyzed the concentration in pollen and nectar in the grown plant.
      a. In lab studies, Schmuck et al. showed the LD50 to individual bees ranged from 3.7 – 40.9 ng/bee.
         1. The estimated LD50 corresponded to a lethal food concentration between 0.14 and 1.57 mg/kg.
            a. The residue levels in pollen and nectar from field-grown plants were negligible (<0.0015 mg/kg).
               1. Furthermore, imidacloprid was not detected in nectar and pollen of sunflowers planted as a succeeding crop in soils that previously had been cropped with imidacloprid seed-treated plants.
      b. In a feeding field study, honeybees were fed for 39 days with sunflower honey fortified from 0.002 mg/kg to 0.020 mg/kg.
         1. Bees were fed while in colonies in the field. Benchmark parameters that were measured included mortality, feeding activity, wax/comb production, breeding performance and colony vitality.
            a. Even at the highest test concentration, imidacloprid showed no adverse effects on the development of the exposed bee colonies.
               1. Thus, the NOAEC of 0.020 mg/kg compares favorably with a field residue level of <0.0015 mg/kg.
               2. The observations were similar in more than 10 field studies.
   3. Schmuck (2004) also examined different lab studies wherein bees were given a chronic 10-day dose of imidacloprid and a metabolite that was hypothesized to be toxic.
      a. He made this conclusion: "No increased treatment-related mortality or behavioral abnormalities were recorded in four independent research facilities during a 10-day dietary exposure of honeybees of different ages to sucrose solutions spiked with the respective metabolites at 0.0001, 0.001, and 0.010 mg/L 50% sucrose solution."
      b. With regard to parent imidacloprid itself, he concluded, "The majority of data indicated a dietary no observed lethal-effect concentration 0.04 and 0.02 mg/L 50% sucrose solution, respectively, for an acute and a chronic dietary exposure of honeybees to either imidacloprid or its plant metabolites."

4. Interestingly, Suchail had concluded based on his experience with honeybees and imidacloprid, that seed applications of imidacloprid would be less hazardous than spraying the flowers directly.

D. Other studies of behavioral toxicity maintain the uncertainty of effects of imidacloprid residues in nectar on honeybees.
   1. For example, using a behavioral test, "olfactory conditioning of proboscis extension response", Decourtye et al. 2003 found the LOEC for winter bees was 24 μg/kg (i.e., 0.024 mg/kg) and for summer bees 12 μg/kg (0.012 mg/kg).
      a. In an earlier article this research group reviewed behavioral toxicity studies with bees and noted that different NOECs (or LOECs) were obtained depending on the type of behavioral test used (Pham-Delegue 2002).
   2. In another study, the contamination of syrup with imidacloprid (24 µg/kg) induced a decrease in both the foraging activity on the food source and activity at the hive entrance (Decourtye et al. 2004).

E. In a recently published controlled field study, honeybees were fed over multiple days sugar syrup with 0, 0.5, and 5 µg/L imidacloprid (Faucon et al. 2005). Foraging behavior, brood cell area, hive weight, and infestation by Varroa mites were measured. The authors concluded that imidacloprid, at least at 5 µg/L in nectar was not likely to be responsible for the large hive losses experienced by French bee keepers.

F. So what about those wild bees? Not a lot of research has been done on toxicity of insecticides to wild bees, but one group of researchers has examined the effects of imidacloprid on *Bombus terrestris* (common name is the bumblebee) (Tasei et al. 2001)
   1. Tasei et al. (2001) concluded "that applying imidacloprid at the registered dose, as a seed coating of sunflowers cultivated in greenhouse or in field, did not significantly affect the foraging and homing behavior of B. terrestris and its colony development."

G. The moral of this story is that even though a compound has definite neurotoxic properties, and all researchers agree that is extremely toxic to a beneficial organism like the honeybee, whether the hazard is actually manifested under field conditions is dependent on the dose.
   1. Only through a thorough study of toxicity parameters for honeybees, confirming the mode of action on the nervous system, and analysis of pollen and nectar, was it concluded that the perception of imidacloprid's grave danger to honey bees from its use as a seed treatment was not likely to be the cause of hive mortality in the field.
   2. Admittedly uncertainty will still persist because of the variable in residue analysis results and the different behavioral toxicity endpoints employed by different researchers. However, it is unlikely that imidacloprid, if it has any adverse effect on bees in the field, is uniquely different than other insecticides known to be toxic at very low doses to honey bees.

IV. Beneficial Insects Important in Controlling Insect Populations (Natural Regulatory Control; sometimes called Biocontrol; Organisms called natural enemies)

A. DDT became widely commercialized for agricultural use in the late 1940's/early 1950's. It was hailed as a miracle cure for pests, and its discoverer of insecticidal activity, Paul Mueller, was given the Nobel Prize in 1949.
   1. The elation was short-lived, however. Entomologists observed in the 1950's that DDT treatments would knock back pest populations but then they would resurge, often to even higher levels.
      a. It was known that DDT had a broad spectrum of activity (i.e., it was not selective) and had a long residual period (i.e., residues were very persistent).
   2. Furthermore, secondary pests would emerge to become dominant in the treated crops.
   3. Observations of the biological mortality controls on insect populations, i.e., the predators and parasitoids, showed that their populations were also decimated by DDT.
      a. Thus, it was hypothesized and borne out by observation, that loss of these natural regulatory control organisms allowed secondary pests to survive unchecked and primary pest populations to resurge because their natural enemies could not recover as quickly (owing to a differential reproductive potential and also greater susceptibility).
         1. Note that so-called secondary pests are relegated to this status presumably because natural biological controls prevent their populations from becoming large enough to be economically damaging to a crop.

B. Thus, one area of study commonly carried out by entomologists, but not published about within the "environmental toxicology" community is the effects on beneficial insects known as natural enemies (predators and parasitoids) that keep pest populations lower than they might ordinarily be in the absence of these important mortality factors.

C. Another way to look at selectivity from the perspective of controlling pests is whether the pesticide is preferentially toxic to the pest compared to the natural enemies.

V. Evolution of Resistance in Repeatedly Exposed Populations

A. Development of pesticide resistance is one consequence of the use of pesticides that is not traditionally considered as an ecological effect, yet nevertheless is costly to growers and may stimulate ever greater use of per acre application rates.
   1. While the phenomenon of pesticide resistance development has been of research interest among entomologists, weed scientists, and plant pathologists for decades, only recently has it been noted in populations of nontarget organisms chronically exposed to recalcitrant contaminants like heavy metals and TCDD.
      a. For example, Fundulus sp. (killifish, mummichogs) collected from heavily contaminated Newark Bay, NJ seem to be more resistant to the effects of

TCCD than fish collected from a subpopulation at Tuckerton, NJ. (Prince and Cooper 1995a)

b. The hypothetical mechanism for the difference in susceptibility between the populations of NJ killifish is an Ah receptor in the Newark population that is comparatively insensitive to induction by TCDD. (Prince and Cooper 1995b)

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