● INSECTICIDE FACT SHEET

BACILLUS THURINGIENSIS (B.T.)

Bacillus thuringiensis (B.t.) is a live microorganism that kills certain insects and is used to kill unwanted insects in forests, agriculture, and urban areas.

In a purified form, some of the proteins produced by B.t. are acutely toxic to mammals. However, in their natural form, acute toxicity of commonly-used B.t. varieties is limited to caterpillars, mosquito larvae, and beetle larvae. B.t. is closely related to *B. cereus*, a bacteria that causes food poisoning and to *B. anthracis*, the agent of the disease anthrax. Few studies have been conducted on the chronic health effects, carcinogenicity, or mutagenicity of B.t. People exposed to B.t. have complained of respiratory, eye, and skin irritation, and one corneal ulcer has occurred after direct contact with a B.t. formulation. People also suffer from allergies to the "inert" (secret) ingredients. People with compromised immune systems may be particularly susceptible to B.t.

Viable B.t. spores are known to exist for up to one year following application. Insect resistance to B.t. has been well documented. Genetic engineering may greatly expand use of B.t., speeding up the development of more resistance.

Large-scale applications of B.t. can have far-reaching ecological impacts. B.t. can reduce dramatically the number and variety of moth and butterfly species, which in turn impacts birds and mammals that feed on caterpillars. In addition, a number of beneficial insects are adversely impacted by B.t.

B.t. is less toxic to mammals and shows fewer environmental effects than many synthetic insecticides. However, this is no reason to use it indiscriminately. Its environmental and health effects as well as those of all other alternatives must be thoroughly considered before use. B.t. should be used only when necessary, and in the smallest quantities possible. It should always be used as part of a sustainable management program.

BY CARRIE SWADENER

As hazards of conventional, broad acting pesticides are documented, researchers look for pesticides that are are toxic only to the target pest, have less impact on other species, and have fewer environmental hazards. *Bacillus thuringiensis* (B.t.) insecticides result from this research. However, there is evidence suggesting that B.t. is not as benign as the manufacturers would like us to believe, and that care is warranted in its use.

B.t. is a species of bacteria that has insecticidal properties affecting a selective range of insect orders. There are at least 34 subspecies of B.t.[1] (also called serotypes or varieties) and probably over 800 strain isolates.[2] B.t. was first isolated in 1901 in Japan from diseased silkworm larvae. It was later isolated from Mediterranean flour moths and named *Bacillus thuringiensis* in 1911.[3] It was not until 1958 that B.t. was used commercially in the United States.[4] By 1989, B.t. products had captured 90-95 per cent of the biopesticide market.[5]

Bacillus thuringiensis products available in the United States are comprised of one of five varieties of B.t.: B.t. var. *kurstaki* and var. *morrisoni*, which cause disease in moth and butterfly caterpillars; B.t. var. *israelensis* which causes disease in mosquito and blackfly larvae; B.t. var. *aizawai* which causes disease in wax moth caterpillars); and B.t. var. *tenebrionis*, also called var. *san diego*, which causes disease in beetle larvae.[6,7] Other strains of B.t. have been discovered that exhibit pesticidal activity against nematodes, mites, flatworms, and protozoa.[5]

B.t. products are used to control moth pests in fruits, vegetables, and beehives; blackfly and mosquito pests in ponds and lakes; and several beetle pests in vegetables and shade trees.[6] (See Fig. 1,2, and 3 for more details.) Common brand names include Dipel, Foray, Thuricide (all B.t. *kurstaki*), Vectobac, Mosquito Attack (all B.t. *israelensis*), and M-Trak (B.t. *tenebrionis*).[6]

Mode of Action

When conditions for bacterial growth are not optimal B.t., like many bacteria, forms spores. Spores are the dormant stage of the bacterial life cycle, when the organism waits for better growing conditions. Unlike many other bacteria, when B.t. creates spores it also creates a protein crystal. This crystal is the toxic component of B.t..

After the insect ingests B.t., the crystal is dissolved in the insect's alkaline gut. Then the insect's digestive enzymes break down the crystal structure and activate B.t.'s insecticidal component, called the delta-endotoxin. The delta-endotoxin binds to the cells lining the midgut

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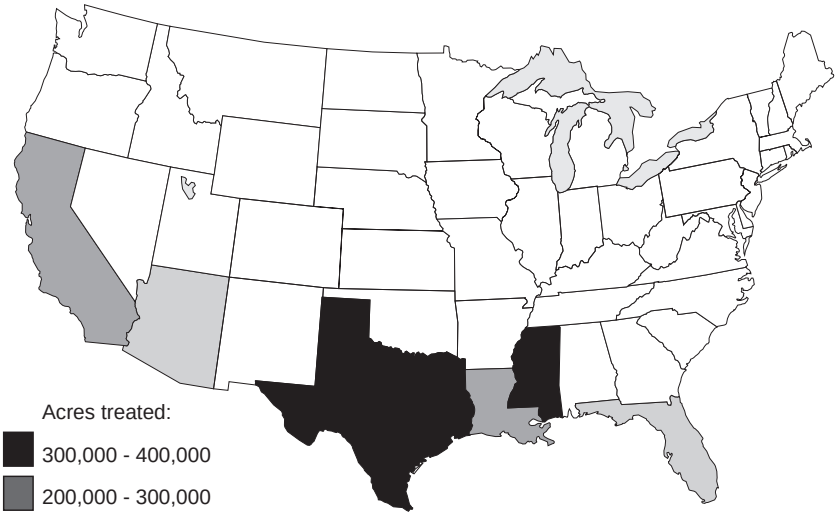
Figure 1
B.t. Used in U.S. Agriculture



Sources:
U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economic Research Service. 1994. Agricultural chemical usage: 1993 field crops summary. Washington, D.C. (March.)
U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economic Research Service. 1993. Agricultural chemical usage: Vegetables 1992 summary. Washington, D.C. (June.)
U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economic Research Service. 1992. Agricultural chemical usage: 1991 fruits and nuts summary. Washington, D.C. (June.)

B.t. is widely used in cotton production in Texas, Mississippi, and Louisiana as well as in the production of fruits and vegetables in California, Arizona, and Florida.

membrane and creates pores in the membrane, upsetting the gut's ion balance. The insect soon stops feeding and starves to death.

If the insect is not susceptible to the direct action of the delta-endotoxin, death occurs after B.t. starts vegetative growth inside the insect's gut. The spore germinates after the gut membrane is broken; it then reproduces and makes more spores. This body-wide infection eventually kills the insect.[8]

Factors Affecting Selectivity

One of B.t.'s most desirable characteristic is its selectivity; only certain insects are susceptible to the delta-endotoxin. Scientists have identified at least 29 different crystals and delta-endotoxins.[5] Each is effective against specific insects. Each variety of B.t. can produce one or more of these toxins.[7] Alkaline (basic; pH greater than 7) solutions activate the delta-endotoxin, and different varieties may require different pHs.[9] Certain enzymes must also be present in the insect's gut to break the crystal into its toxic elements.[8] In addition, certain cell characteristics in the insect gut encourage binding of the endotoxin and subsequent pore formation.[7] The age of the insect is also a factor, the younger larvae being more susceptible than older larvae.[8]

Health Effects Testing

Since B.t. is a live microbial organism, testing for the possible hazards of B.t. is conducted differently that for conventional pesticides. Microbial toxicity is described using pathogenicity (the ability of the microbe to cause disease) and infectivity (the ability of the organism to reproduce within the body.) The United States Environmental Protection Agency (EPA) requires no testing of B.t. for carcinogenicity, mutagenicity, or chronic toxicity.[10]

Laboratory Tests of Acute Toxicity

Each of the more than 800 strains of *Bacillus thuringiensis* may exhibit different toxicity to insects, rodents and humans. This fact complicates any discussion about the toxicity of B.t. The following are summaries of the acute toxicity data available for two commonly used commercial varieties of B.t..

Bacillus thuringiensis var. *kurstaki (B.t.k.):* B.t.k. and commercial products containing B.t.k. generally have low oral acute toxicity to rats. In tests with laboratory animals, researchers did not observe any adverse effects after feeding large doses.[11-13]

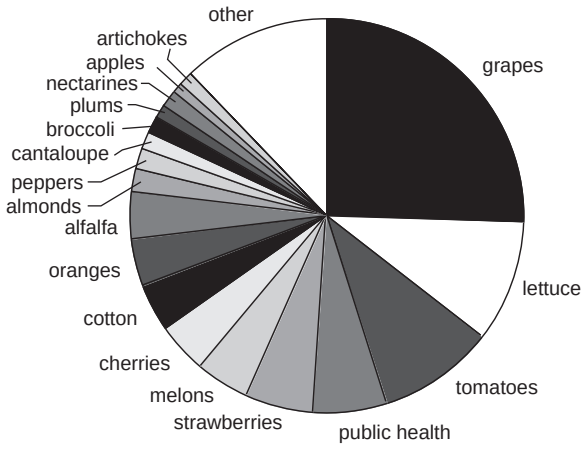
Other types of exposures have some acute effects. Rats who breathed air containing B.t.k. spores experienced respiratory depression,[14] and B.t.k. spores injected into rats' veins aggravated preexisting disease.[15] Both B.t.k. and Foray 48B are irritating to rabbit skin,[16] and Foray 48B is moderately irritating to rabbits' eyes.[12]

Bacillus thuringiensis var. *israelensis (B.t.i.):* In studies assessing B.t.i.'s acute toxicity to mammals, mortality only occurred when B.t.i. was injected into the abdomen or the brain. In one study conducted on rats, 79 percent mortality occurred after a single injection into the brain.[17] Effects other than mortality can also occur. For example, in mice injected with a B.t.i. suspension, spleens became enlarged.[18]

B.t.i. is irritating to both eyes and skin. Injection of both viable and inactivated B.t.i. spores under the skin resulted in abscesses in mice.[17] Rabbits' eyes are irritated by B.t.i.[18] The irritancy of B.t.i. to eyes depends on the physical characteristics of the formulation; a dry, dusty formulation with smaller particles is less irritating and cleared from the eye more quickly than a clumped formulation with larger particles.[17]

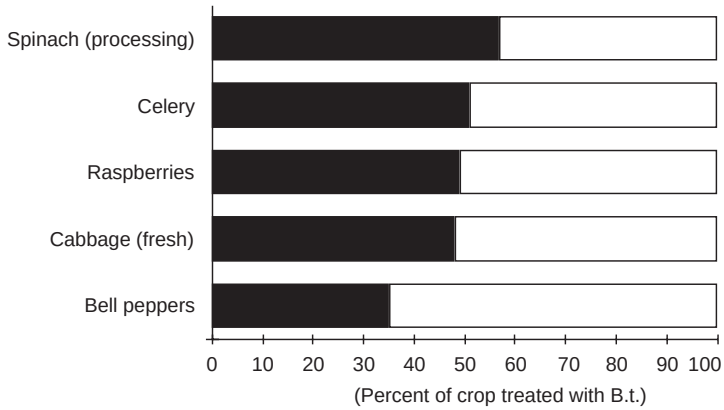
In a purified form, B.t.i.'s endotoxin is clearly toxic to mammals. When the delta-endotoxin from B.t.i. was injected intravenously into mice, they exhibited rapid paralysis, followed by death within 12 hours. When the same dosage was injected under the skin of suckling mice, death occurred in 2-3 hours. The delta-endotoxin also caused destruction of rat, mouse, sheep, horse, and human red blood cells.[19] When a small protein isolated from the endotoxin was administered to mice at sublethal levels, mice suffered from severe hypothermia and their heart beat slowed.[20]

Figure 2
Uses of B.t. in California



Source: California Environmental Protection Agency. Dept. of Pesticide Regulation. 1993. Summary of pesticide use report data: Annual 1991. Indexed by chemical. Sacramento, CA. (January.)

Figure 3
Crops Frequently Treated with B.t.



Sources:
U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economic Research Service. 1993. Agricultural chemical usage: Vegetables 1992 summary. Washington, D.C. (June.)
U.S. Dept. of Agriculture. National Agricultural Statistics Service. Economic Research Service. 1992. Agricultural chemical usage: 1991 fruits and nuts summary. Washington, D.C. (June.)

In California, where pesticide use reporting is more comprehensive than in other states, almost 52,000 pounds of B.t. were used on diverse crops in 1991. Grapes, lettuce, and tomatoes account for almost half the B.t. used in California (left). B.t. is extensively used nationawide in the production of certain fruit and vegetable crops (right).

Acute Toxicity to Humans

Bacillus thuringiensis var. *kurstaki*: There have been few experimental studies assessing the toxicity of B.t.k. to humans. Most information comes from occupational exposures, or from exposures occurring during large-scale B.t.k. programs.

One case of B.t.k. infection resulted from a farmer splashing a B.t.k. formulation, Dipel, in his eye. The man developed an ulcer on his cornea from which positive B.t.k. cultures were taken.[21] Another man working on a spray program splashed B.t.k. on his face and eyes. He then developed skin irritation, burning, swelling, and redness. B.t.k. was cultured from a sample taken from his eye.[22] Ground-spray applicators using Foray 48B reported symptoms of eye, nose, throat, and respiratory irritation. The frequency of their complaints was found to be related to the degree of exposure. Workers with similar preexisting health problems were more likely to report adverse effects from the ground spray.[23]

A woman exposed to an B.t.k. formulation as a result of drift went to the hospital due to burning, itching and swelling of her face and upper chest. She later exhibited a fever, altered consciousness, and suffered seizures.[24] No B.t. was cultured from tissue samples, but her doctor believed that B.t. was the cause of the clinical symptoms.[25]

Monitoring studies following large-scale B.t. spray programs have shown that exposed people carry B.t. in their tissues. For example, more than 11 percent of nasal swab samples taken from patients surveyed by doctors in Vancouver (Canada) following a gypsy moth spray program were found to contain B.t.k.[23] B.t. was also found in cultures taken from patients in Lane County, Oregon following a gypsy moth spray program there. Monitoring studies also show that exposed people report a variety of health problems that they believe to be associated with B.t. exposure.[22] For example, during the Vancouver spray program, almost 250 people reported health problems, mostly allergy-like or flu-like symptoms. During a Washington gypsy moth spray program, over 250 people reported health problems and 6 were treated in emergency rooms for allergy or asthma problems.[26] Physicians have so far been unable to definitively link B.t. exposure to these health problems.[22,23,26]

Bacillus thuringiensis var. *israelensis:* There has only been one case of documented adverse effects of B.t.i. on humans. This case involved a researcher who accidentally injected himself with a mixture of B.t.i. and another kind of bacteria commonly found on human skin.[20] He suffered from a toxic reaction and irritated lymph vessels. When these two bacteria were later injected into rodents the combination was consistently lethal, but each bacteria injected separately caused only slight inflammation.[8]

Special Concerns about B.t. Toxicity

Exotoxins: The earliest tests done regarding B.t.'s toxicity were conducted using B.t. var. *thuringiensis*, a B.t. strain known to contain a second toxin called beta-exotoxin. The beta-exotoxin is toxic to vertebrates, with an LD_{50} (median lethal dose; the dose that kills 50 percent of a population of test animals) of 13-18 milligrams per kilogram of body weight (mg/kg) in mice when injected into the abdomen. An oral dose of 200 mg/kg per day killed mice after eight days.[20] Beta-exotoxin also causes genetic damage to human blood cells.[27] B.t. formulations containing beta-exotoxin have

not been used in most countries[20] although attempts are currently being made to register beta-exotoxin as an insecticide in the United States.[8] Another toxin produced by B.t. is the alpha-exotoxin that is highly acutely toxic to mice.[20] Current B.t. production methods are such that alpha-exotoxin is not a "significant component" of B.t. formulations.[8]

Related Bacteria: B.t. belongs to a small group of closely related *Bacillus* species, including *B. cereus*, a bacteria that is an agent of food poisoning, and *B. anthracis*, the pathogen of the virulent animal disease, anthrax. These three bacteria are so similar it has been theorized that they are all varieties of the same species.[28,29] If *B. cereus* is cultured with B.t. cells, genetic material is transferred to the *B. cereus* cells that allows *B. cereus* to produce B.t.'s crystal proteins.[28] Transfers of genetic material between *B. anthracis* and B.t. have also occurred.[30]

A toxin produced by *B. cereus* that causes diarrhea in monkeys is also produced by certain strains of B.t.,[30] although this toxin is not likely to be present in B.t. spore formulations.[28] Human volunteers suffered from nausea, vomiting, diarrhea, colic-like pains, and fever after eating food contaminated with one B.t. strain, B.t. var. *galleriae*.[31] These examples indicate the close relationship between B.t. and disease-causing pathogens.

Increased Susceptibility: People with compromised immune systems or preexisting allergies may be particularly susceptible to the effects of B.t. In mice with reduced immune function, the dose required to kill more than 50 percent of the mice when injected was several orders of magnitude smaller than the highest dose tested in normal mice.[32] Mice with impaired immune function also showed higher mortality than regular mice when one dose of B.t.i. was injected into the abdominal cavity.[33] Although no definite cases have been reported of B.t. infecting humans with compromised immune systems, the Oregon Health Division suggested before a B.t.k. spray program that "individuals with...physician-diagnosed causes of severe immune disorders may consider leaving the area during the actual spraying."[34]

A memo from Novo Nordisk, the manufacturer of Foray 48B, states that the amount of the spray a person would be exposed to would be too small to develop new allergies. However, "It is possible that someone that already has developed an allergy to one of the components of Foray 48B or has asthma … could be affected by exposure to small quantities of Foray 48B."[35] The 1991 Material Safety Data Sheet for Foray 48B states "Repeated exposure via inhalation can result in sensitization and allergic response in hypersensitive individuals."[36]

Contaminants: In the mid 1980s, several B.t. products were contaminated with other bacteria, including *Streptococcus faecium* and *S. faecalis*.[37] While B.t. products are routinely monitored for bacterial contaminants,[2] the risk of contamination with a disease-causing bacteria is always present.[25]

Inert Ingredients

All B.t. products contain ingredients other than B.t.. These are identified only as "inert" ingredients and are called trade secrets by the manufacturers of the products. The "inert" ingredients are potentially the most toxic components of the formulations.[8] For example, during the 1992 Asian gypsy moth spray program in Oregon, a woman who was exposed to Foray 48B had a preexisting allergy to a carbohydrate that was present as an inert ingredient. Within 45 minutes of exposure, the woman suffered from joint pain and neurological symptoms.[38]

Because "inerts" are called trade secrets, there is little public information about their identity, but the information that is available indicates they could cause health problems. Foray 48B has contained sodium hydroxide, sulfuric acid, phosphoric acid,[39] methyl paraben,[40] and potassium phosphate,[41] as "inerts." While these ingredients make up less than 10 percent of Foray 48B,[39] they pose hazards. Sodium hydroxide, more commonly known as lye, causes "severe corrosive damage to the eyes, skin, mucous membranes and digestive system .... Breathing sodium hydroxide dust or mist leads in mild cases to irritation of the mucous membranes of the nose ... and in severe cases to damage of the upper respiratory tract."[42] Sulfuric acid and phosphoric acid are both corrosive. Sulfuric acid can cause severe deep skin burns and permanent loss of vision. When inhaled as a mist, sulfuric acid may cause severe bronchial constriction, and bronchitis.[43] Phosphoric acid is an irritant to skin and mucous membranes, and its vapors may cause coughing and throat irritation.[43] Both methyl paraben and potassium phosphate were once registered by EPA as pesticide active ingredients.[44]

Sodium sulfite has been identified as an inert ingredient of the B.t.k. formulation Dipel 8AF.[45] Up to ten per cent of asthmatics (about one million people in the United States) may react to sulfites, particularly those people who are treated with steroids.[42] Symptoms of exposure in those sensitive to sulfites usually involve the respiratory system, and can also include nausea, diarrhea, lowered blood pressure, hives, shock, and loss of consciousness.[42]

Environmental Fate

Very little is known about the natural ecology of B.t. It occurs naturally in many soils. In one study, B.t. was isolated from 70 per cent of soil samples taken from around the world, and was most abundant in samples taken in Asia. More than half of these isolates were undescribed varieties of B.t.[46] B.t. has also been isolated from insect bodies, tree leaves and aquatic environments.[7] It has even been recovered from paper.[47]

Soil: B.t. generally persists only a short time in soil. The half life of the insecticidal activity (the time in which half of the insecticidal activity is lost) of the crystal is about 9 days.[48] However, small amounts can be quite persistent. In one experiment, B.t. spore numbers declined by one order of magnitude after 2 weeks, but then remained constant for 8 months following application.[49]

B.t. does not appear to move readily in soil. In one study, two varieties of B.t. were applied in adjacent plots, but did not become cross-contaminated, indicating that B.t. does not move laterally in soil.[2,8] Other

studies found that B.t. was not recovered past a depth of 6 centimeters after irrigation, and that movement beyond the application plot was less than 10 yards.[7,50]

Foliage: B.t. deposited on the upper side of leaves (exposed to the sun) may remain effective for only 1-2 days, but B.t. on the underside of leaves (i.e. protected from the sun) may remain active for 7-10 days.[2,8] It is possible for it to be significantly more persistent, however. Viable spores of B.t.k. were recovered from white spruce foliage one year after application.[51] In one experiment conducted in Japan, B.t. persisted for two years in a citrus orchard and remained toxic to caterpillars.[52]

Water: B.t.k. has been recovered from rivers and public water distribution systems after an aerial application of Thuricide 16B. Standard water treatment processes are not adequate to destroy B.t.k. spores.[53]

B.t.i. spores and crystals bind readily to sediments in the water column,[54,55] which reduces their efficacy by making them inaccessible to mosquito and blackfly larvae.

In one test, B.t.i. was applied to water, then allowed to contact mud particles. Over 99 percent of the B.t.i. spores were found in the mud, rather than in the water, after 45 minutes. The B.t.i. retained viability and toxicity for at least 22 days, killing 90 percent of the mosquito larvae when the mud was stirred and reintroduced to the water column.[54]

In another experiment, viable cells were recovered from the water for up to 200 days and in the sediment for up to 270 days after application.[55]

Air: B.t.k. has been found to drift over 3,000 meters downwind during an aerial application. The distance B.t.k. is capable of drifting depends upon the amount and method of application,[56] as well as the climatic conditions. B.t. *thuringiensis* was measured in air for up to 17 days following an application.[4]

Biotechnology

Examples of genetic manipulation and genetic engineering with B.t. include the following:[7]

• In the agricultural product Foil, the gene for a toxin with activity against beetles was transferred through conjugation (sexual reproduction in bacteria) to a B.t.k. cell that only affected butterflies and moths. The resulting cell showed insecticidal properties against beetles, butterflies, and moths. Since EPA considers the organisms resulting from conjugation to be genetically manipulated rather than genetically engineered, Foil was registered for use in the U.S. in 1990.

• *Pseudomonas fluorescens* cells can be engineered to produce the B.t. delta-endotoxin without production of a spore. The crystal protein remains inside the *P. fluorescens* cell wall. In the products MVP and M-Trak, the *P. fluorescens* cell is killed after it produces the crystal protein. When the product is applied, the delta-endotoxin remains protected within the now dead cell wall. In this way, the B.t. delta-endotoxin retains its effectiveness for two to three times longer than other B.t. formulations. MVP and M-Trak were the first genetically engineered products to be registered by EPA, since the transgenic organism was not alive when released into the environment.

> "During the 1992 Asian gypsy moth spray program in Oregon, a woman who was exposed to Foray 48B had a preexisting allergy to a carbohydrate that was present as an inert ingredient. Within 45 minutes of exposure, the woman suffered from joint pain and neurological symptoms."

• B.t.i. used to control mosquito and blackfly larvae that live on the water surface begins to sink, away from the target larvae, within 24 hours. Bacteria that naturally live on the water surface (in the same environment as mosquito or blackfly larvae), have been engineered to produce the B.t.i. crystal proteins.

• Over thirty different crops have been engineered to produce the B.t. crystal protein throughout their plant structure. Any pest that feeds on any part of these plants will be exposed to the B.t. delta-endotoxin, and those susceptible to the toxin will be killed.

Clearly, the possibilities for the genetic engineering of B.t. delta-endotoxins seem endless. However, researchers know so little about the ecology and genetic stability of B.t., that the potential ecological effects of these transgenic organisms are impossible to predict with certainty.

Resistance

Scientists once thought that the mode of action of B.t. was complex enough to prevent the development of pest insect resistance. However, time and further research proved this to be untrue. Eight insect species have been studied because of their ability to develop resistance to B.t.[57] The Indian meal moth, a pest of grain storage areas, was the first insect to develop resistance to B.t.k.[58] in laboratory experiments. Resistance progresses more quickly in laboratory experiments than under field conditions due to higher selection pressure in the laboratory.[59] No indications of insect resistance to B.t. were observed in the field, until the development of resistance was observed in the diamondback moth in crops where B.t. had been used repeatedly. Since then, resistance has been observed in the laboratory in the tobacco budworm, the Colorado potato beetle and other insect species.[57] The gypsy moth also shows potential for developing B.t. resistance.[60] Some insects, such as the diamondback moth and the tobacco budworm, exhibit resistance to multiple B.t. strains.[61,62] Development of resistance occurs faster when larger amounts of a pesticide are used, so that use of crop plants genetically-engineered to produce the B.t. toxin could dramatically increase the number of B.t.-resistant insects.

B.t.'s Ecological Impacts

Some of the most serious concerns about widespread use of B.t. as a pest control technique come from the effects it can have on animals other than the pest targeted for control. All B.t. products can kill organisms other than their intended targets. In turn, the animals that depend on these organisms for food are also impacted.

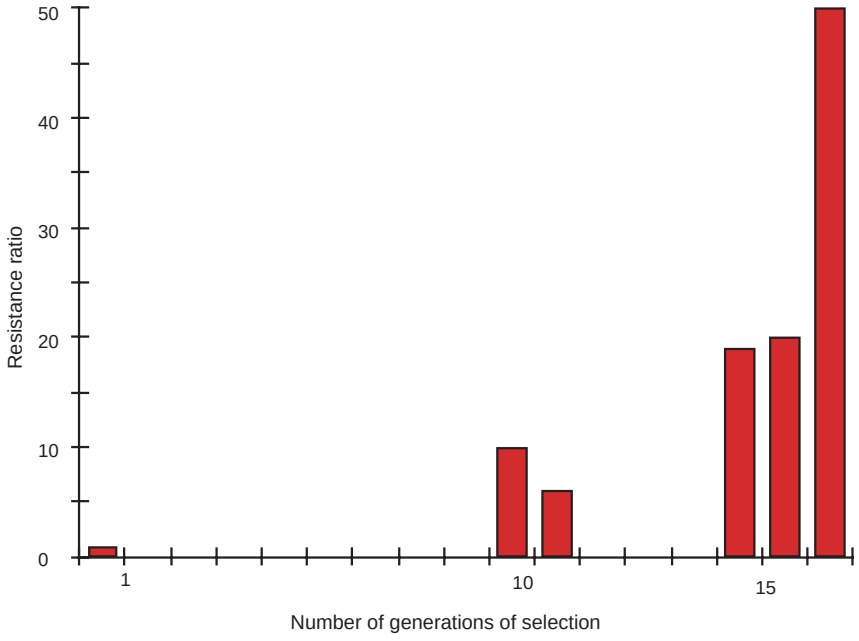
Beneficial insects: Many insects are not pests, and any pest management technique needs to be especially concerned about those that are called beneficials, the insects that feed or prey on pest species. B.t. has impacts on a number of beneficial species. For example, studies of a wasp that is a parasite of the meal moth (*Plodia interpunctella*) found that treatment with B.t. reduced the number of eggs produced by the parasitic wasp, and the percentage of those eggs that hatched.[63] Production and hatchability of eggs of a predatory bug were also decreased.[63] On collards, aphid-eating flies in the family Syrphidae were reduced by Dipel treatment.[64] Both B.t.*tenebrionis* and Dipel have caused mortality of predatory spider mites.[65] Dipel also has caused mortality of the cinnabar moth, used for the biological control of the weed tansy ragwort.[66] Finally, B.t.i. has caused mortality of a moth (*Synclita obliteralis*) that helps control aquatic weeds in Florida.[67]

Other insects: Many insects that do not have as directly beneficial importance to agriculture are important in the function and structure of ecosystems. A variety of studies have shown that B.t. applications can disturb insect communities. Research following large-scale B.t. applications to kill gypsy moth larvae in Lane County, Oregon, found that the number of oak-feeding caterpillar species was reduced for three years following spraying, and the number of caterpillars was reduced for two years.[68] Similar results were found in a study of caterpillars feeding on tobacco brush following a B.t.k. application to control spruce budworm in Oregon.[69] In untreated areas, the number of species was about 30 percent higher, and the number of caterpillars 5 times greater, than in B.t.k.-treated areas two weeks after treatment. The number of caterpillars was still reduced in treated areas the following summer. In Washington, B.t. applications in King and Pierce counties to kill gypsy moths reduced spring moth populations by almost 90 percent.[70] In addition, one rare species appeared to have been eradicated from the treatment zone, and moth populations were "heavily impacted in an area more than double that which was actually sprayed" as moths moved into the treatment zone from surrounding areas.[70] In West Virginia, applications of Foray 48B reduced the number of caterpillar species and the number of caterpillars. The year following application, the number of moth species and the number of moths were both reduced.[71] A recent (1994) study in four different Oregon plant communities found that total weight of caterpillars was reduced between 90 and 95 percent by B.t. treatment; the number of caterpillars was reduced by 80 percent; and the number of caterpillar species was reduced by over 60 percent.[72]

Aquatic insects are also affected by B.t. treatments. Canadian studies found that certain stream insects (*Simulium vittatum* and *Taeniopteryx nivalis*) were killed by applications of Thuricide and Dipel respectively.[73,74] Midges (chironomids) have repeatedly been shown to be killed by B.t.i.[75-77]

Birds: Because many birds feed on the caterpillars and other insects affected by B.t. applications, it is not surprising that impacts of B.t. spraying on birds have been documented. In Lane County, Oregon studies of chickadees following a gypsy moth spray program found that birds nesting in B.t.-treated areas brought fewer caterpillars to their nests than did birds nesting in untreated areas. The birds were able to find other food, so that nesting success was not significantly impacted.[78] In New Hampshire, when B.t.-treatment reduced caterpillar abundance, black-throated blue warblers made fewer nesting attempts and also brought fewer caterpillars to their nest-

Figure 4
Development of Resistance to B.t. in the Tobacco Budworm



Note: The resistance ratio is determined by dividing the dose required to kill a B.t.-resistant insect by the dose required to kill a susceptible insect.

Source: Gould, F. et al. 1992. Broad-spectrum resistance to *Bacillus thuringiensis* toxins in *Heliothis virescens. Proc. Nat. Acad. Sci.* 89:7986-7990.

Under appropriate conditions, resistance to B.t. can develop quickly. After only seventeen generations of selection, resistance to B.t. increased 50-fold in the tobacco budworm.

lings.[79] A Canadian study found that numbers of caterpillars, followed by numbers of two species of warblers and a thrush, were reduced by B.t. treatment. In addition, there were fewer spruce grouse chicks in B.t. treated areas, and the chicks in those areas grew more slowly than chicks in untreated areas.[80]

There is also some evidence that B.t. can be directly toxic to birds. A study of the effects of application of Dipel to ring-neck pheasant eggs found that hatching was only half as successful as hatching of untreated eggs. Because the Dipel was applied with a spreader-sticker compound (Plyac) the decrease in hatching may be a result of the Plyac and not the B.t. product.[81]

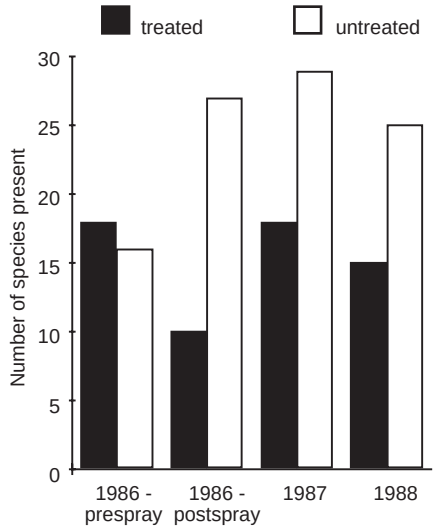
Other animals: Because shrews often feed on caterpillars, impacts from B.t. treatments are likely. A study in northern Ontario (Canada) found that treatment with Dipel changed the structure of the shrew population. Adult males emigrated, so that the proportion of juveniles increased. The juveniles and adult females who did not emigrate shifted from a diet of caterpillars to alternative prey.[82]

Foray 48B at high concentrations (about 3 percent) is acutely toxic to rainbow trout, probably because the product is highly acidic.[83]

B.t.i. treatments can also affect other animals. Low concentrations of B.t.i. endotoxins decrease the weight of tadpoles and delay their metamorphosis.[84] The B.t.i. formulation Vectobac is acutely toxic to fathead minnows, probably because "inerts" in the product deplete the dissolved oxygen in water.[85] The B.t.i. formulation Teknar was acutely toxic to brook trout fry, probably because of xylene used as an "inert" in the product.[86]

Comparison with synthetic insecticides: Where comparative studies have been done, the ecological impacts of a B.t. treatment are almost always less than those of synthetic insecticides. For example, B.t. treatment of collards caused less of an increase in aphid numbers than did treatment with carbaryl, which killed many aphid predators.[64] Vectobac was much less acutely toxic to an estuary fish than other mosquito insecticides including temephos, fenoxycarb, diflubenzuron, and methoprene.[87]

Figure 5
Effect of B.t. Treatment on the Number of Oak-feeding Moths near Elmira, Oregon



Source: Miller, J.C. 1990. Field assessment of a microbial pest control agent on nontarget Lepidoptera. *Amer. Entomol.* (Summer):135-139.

B.t. applications to kill gypsy moths in Oregon caused a three-year decrease in the number of oak-feeding moth species.

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