REVIEW ARTICLE

Microorganisms and their byproducts, nematodes, oils

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The insect and mite control potential of natural and biological toxins has been recognized for several centuries. Bacteria, viruses, protozoa and fungi are the primary groups of microorganisms known to reduce insect populations; they often occur naturally in fields and function as components of biological control. Beneficial nematodes are also being used for pest control, especially against soil insects. The isolation of toxic metabolic compounds from microorganisms continues to be a fruitful research area, although there are barriers to their successful marketing and distribution. Another, more controversial way to deliver these insect-specific toxins to the target pest is through genetically modified plants, such as those modified to express* Bacillus thuringiensis *(Bt) toxins. Oils and particle films also have important niche uses for pest control.

Natural and botanical products have been used to control insect pests for centuries. Kaolin is a mineral-based particle film that is sprayed onto crops as a barrier to repel insects and prevent feeding.

As pest management moves forward in the 21st century, alternative control measures are needed to suppress insects and mites. Federal regulators are closely scrutinizing the organophosphate (OP) and carbamate insecticides under the Food Quality Protection Act (NRC 2000); in California, surface-water contamination is of particular concern in the Sacramento and San Joaquin river water basins (USGS 2000). The recognized adverse effects of synthetic chemical pesticides (such as the OPs, chlorinated hydrocarbons and carbamates) on the environment and human health emphasize the need to advance and refine current pest-management strategies (see page 7).

The insect-control potential of natural and biological toxins has been recognized for several centuries. As early as 2700 B.C., unintended epizootics (outbreaks of disease affecting many animals of one species at the same time) by natural enemies (microorganisms) were reported in beneficial insects such as silkworms and honeybees. The first record of microorganisms being intentionally used to control crop pests was in the 18th century (a fungus against a weevil pest). Bacteria, viruses, protozoa and fungi are the primary groups of microorganisms known to reduce insect populations. These organisms often occur naturally in fields and function as a component of biological control. Research on these microorganisms as "biopesticides" has resulted in the ability to isolate, culture and formulate some for use in integrated pest management (IPM) programs. These formulations have improved the shelf life of the resulting products, their miscibility with water or oil, and the ability to spray them with commercial application equipment, as well as provided some protection against environmental extremes that occur after application.

Entomopathogenic (insect-parasitic) nematodes are another example of biological agents that are being used for pest control, especially against soil insects. Several species of beneficial nematodes are commercially available, and the animal itself acts as a distribution tool for symbiotic bacteria that actually kill the target pest. As with the microorganisms, the ability to produce and formulate these nematodes into insecticidal products has enabled their use in pest management programs.

A common feature of some microorganisms, principally bacteria and fungi, is their natural ability to produce metabolic byproducts that are toxic to many organisms. For example, the antibiotic penicillin was isolated from a fungus and is used to combat bacterial infections in humans. Other metabolic byproducts have toxic activity against arthropod pests. Instead of relying on the microorganism to produce these arthropod-active toxins in the field, the microorganism can be cultured in fermentation facilities and the resulting metabolites can be harvested, purified, formulated and used effectively against major arthropod pests. Two widely used commercial insecticides, spinosad and abamectin, were developed using this approach. Over the centuries, as microorganisms have evolved in their envi-

In most cases, the use of living organisms — with their inherent growth and survival criteria — introduces considerable complexity into arthropod management schemes.

ronment, the metabolites they produce have provided a competitive advantage; consequently, the isolation of these compounds continues to be a fruitful agricultural research area for the suppression of both arthropod pests and plant pathogens.

One additional way of protecting a microbial-derived toxin and efficiently delivering it to the target pest is through genetically modified plants that express an insecticidal protein, such as *Bacillus thuringiensis* (Bt). Although there is considerable controversy worldwide regarding the applicability and sustainability of this technology, it does undeniably represent an effective way to deliver a toxic dose to the pest (Shelton et. al 2002). Other types of natural and biological toxins that are useful in pest management include plant-derived compounds (such as rotenone, pyrethrum, sabadilla and azadiractin), inorganic products (sulfur), mineral/refined petroleum oils and mineral-based particle films.

Many of the alternative control agents that we discuss are not new to science. However, through innovative approaches and with a better understanding of how they kill insects and mites, many have come to the forefront in pest management. Others are still plagued with high production costs, inconsistent efficacy or special handling requirements, thereby limiting their usefulness in agricultural systems and as alternatives to broad-spectrum insecticides such as the OPs.

The natural epizootics of insect pathogens occur commonly in native and managed systems, significantly assisting pest management. However, except for Bt, the application of microorganisms for pest control in agricultural systems in California is extremely limited (Flint 1992). The reasons for this include: (1) the high cost of *in vitro* or *in vivo* production; (2) limited persistence and efficacy due to UV light degradation, high humidity requirements or temperature sensitivity in the field; (3) slow speed of kill; (4) poor shelf-life or special handling needs; and (5) high levels of specificity. The latter can preserve populations of natural enemies following application, but also makes it difficult to balance market size with registration costs. In addition, microorganisms generally only have one mode of entry into the host. Bacteria, viruses and protozoa must be ingested to cause an infection, whereas fungi cause an infection when the conidium (spore) attaches to and penetrates the insect cuticle.

Bt in widespread use

The notable exception, Bt, was used on nearly 800,000 acres in California in 2001 (DPR 2003). This bioinsecticide is registered on all California field, vegetable, orchard and floriculture crops. Bt strains (also known as subspecies) are active against particular groups of insects (Tanada and Kaya 1993). These include Bt *kurstaki* and Bt *aizawai*, which are both active against lepidopterous larvae (but differ in which caterpillar species are most susceptible); Bt *tenebrionis*, active against certain beetles; and Bt *israelensis,* active against mosquitoes and black flies.

Different Bt products are also characterized by different insecticidal proteins, (δ-endotoxins) known as Cry endotoxins A, B, C, and so on, and are further subdivided into Cry1Aa, Cry 1Ab, Cry1Ac, and so on. These differ in their toxicity to specific pests. Because these products must be ingested, they require warm weather for active feeding and are most effective on early instars. Crop damage may occur for a short time, since Bt products are slow-acting, but the problems of slow kill and short residual activity are offset by their lack of toxicity to other natural enemies (such as predators and insect parasites). Bt products are especially important for pest control in organic cropping systems. However, some Bt products are prohibited by some certifying agencies, either because they contain inert ingredients that are prohibited, or because they have been genetically modified using molecular techniques not acceptable for organic production and marketing.

Photos: Jack Kelly Clark

In the U.S. South, the cotton bollworm, *top*, is controlled with cotton genetically engineered to express *Bacillus thuringiensis* (Bt), but this pest is of minor importance in San Joaquin Valley cotton. *Bottom*, a cabbage looper killed by a foliar application of Bt, the most widely used "bioinsecticide" in the state.

Activity of other microorganisms

Active bacteria. Other bacterial species have activity against insects, such as *Paenibacillus* (formerly *Bacillus*) *popilliae* and *B. sphaericus,* with efficacy against Coleoptera (white grubs) and Diptera (mosquitoes), respectively. Despite their commercial potential, these bacteria currently have limited market share.

Nucleopolyhedroviruses. Nucleopolyhedroviruses (NPV) can potentially be used against lepidopterous larvae. A number of NPVs are registered in the United States, with two of particular significance to California agriculture. The NPV from the beet armyworm *(Spodoptera exigua)* is registered in the United States with a provisional registration for use in California in 2005 (the California Department of Pesticide Registration is requiring further data with the actual product before full registration is granted), for use in field, vegetable and floriculture crops. A similar product contains an NPV from corn earworm *(Helicoverpa zea)* and controls this species and tobacco budworm *(Heliothis virescens)*; it has a provisional registration in California. Both viruses are expected to get full registration for use in California in 2005 or 2006.

Photos: Jack Kelly Clark

Naturally occurring microorganisms, as shown above, can be isolated, cultured and formulated into commercial products for pest control. ***Left to right,*** **a cabbage looper killed by a nucleopolyhedroviris, and a rose grass aphid and spirea aphid, both killed by fungal diseases.**

Other microbial products that have been used commercially in the United States or are registered with the U.S. Environmental Protection Agency (EPA) include a granulovirus (GV) of the codling moth *(Cydia pomonella)*, an NPV of the gypsy moth *(Lymantria dispar)*, an NPV of the Douglas-fir tussock moth *(Orgyia pseudotsugata)* and a fungus *(Lagenidium giganteum)* with activity on mosquitoes. Other microorganisms, such as the GV of the Indian meal moth *(Plodia interpunctella)* and a GV of the grapeleaf skeletonizer *(Harrisina brillians)*, have generated some interest but their market share may be too small for a commercial venture.

Barriers to acceptance. Despite their effectiveness, several important factors have hindered the acceptance of microorganism-based pest control products, as well as their limited commercialization and marketing. For instance, the slow speed of kill of the codling moth GV allows larvae to inflict shallow wounds in apples, reducing their marketability compared with apples in synthetic-chemical management programs (Kienzle et al. 2002). There is a narrow window for the virus to infect codling moth larvae, because once the larvae enter the fruit they escape viral infection. Moreover, the GV has short persistence on foliage and fruit (necessitating frequent applications) and a narrow host range, which is an advantage for preserving natural enemies but a detriment in terms of providing insecticidal control of other arthropod pests (Cross et al. 1999). With fungi, considerable research on pest control has been conducted on *Metarhizium anisopliae* and *Beauvaria bassiana* (the latter registered as a commercial product) to control foliage pests, but field uses are limited in California, especially by the naturally arid conditions present in many of the state's agricultural production areas.

Potential as replacements. At present, microorganisms appear to have limited potential for offsetting the loss of OP and carbamate insecticides in traditional agricultural production in California. Usage is limited, except for the significant use of Bt. In most cases, the use of living organisms — with their inherent growth and survival criteria — introduces considerable complexity into arthropod management schemes. The selection of microorganisms adapted for specific conditions where control is needed — Central Valley cotton fields, for example — may enhance their applicability. In addition, the use of molecular and classical genetics to improve the pathogenicity of microorganisms may improve their performance as insecticides. Classic research in this area has been done at UC Davis, where the *Autographa californica* NPV was engineered to encode an insect-selective neurotoxin isolated from the venom of a scorpion *(Androctonus australis)*(McCutchen et al. 1991). This greatly enhanced the activity and speed of kill of the NPV. However, the public's acceptance of genetic modifications to insect-pathogenic microorganisms is uncertain at this time.

Genetically modified Bt plants

Bt crops, genetically modified to express the Bt toxin, have been widely used for pest control in many parts of the United States as well as internationally. Cotton and field corn are the most commonly grown crops utilizing this technology in the United States (registrations are also in place on sweet corn and potatoes), and thus far their efficacy against target pests, primarily lepidopterous larvae, has been exceptional. However, genetically modified crops with efficacy on other groups of arthropod pests, such as sucking insects or mites, have not been marketed. The controversial aspects of this technology include resistance management, possible effects on nontarget insects, the escape of modified genes into wild plants or other cultivars, and allergenicity and other health concerns in humans. These issues, among others, are beyond the scope of this article, but the reader should be aware that the controversy exists.

In terms of replacements for synthetic insecticides (OPs) in California, Bt crops have had little impact through 2004. For crops available with the Bt technology in California's Central Valley, lepidopterous pests are of minor importance or are effectively managed by nonchemical means (such as cultural and pheromone management strategies for pink bollworm [*Pectinophora gossypiella*] in cotton (see pages 16, 23). The unique environmental and cultural conditions in California, compared with the rest of

Photos: Jack Kelly Clark

Other commercially available pest-control products utilize natural chemistries, functioning as components of pest control in agro-ecosystems. *Left,* the rice water weevil is infected by the fungus *Beauvaria bassiana* (bottom). *Center,* healthy northern masked chafer larva (left) and larva infected by the nemotode *Heterorhabditis bacteriophora* (right). Healthy spider mite adults, *right,* are killed by a microbial-based acaricidal product, abamectin, the metabolite of a microorganism.

the United States, often result in a unique suite of arthropod pests. The crop diversity of minor-acreage crops in California has thus far limited the development of Bt crops, as high volume sales are needed to offset development costs.

Entomopathogenic nematodes

Two genera of nematodes, *Steinernema* and *Heterorhabditis*, have been commercialized for pest control (Koppenhöfer and Kaya 2002). The nematodes are associated with bacteria in the genera *Xenorhabdus* for *Steinernema* and *Photorhabdus* for *Heterorhabditis*. The nematode penetrates into the insect host and releases bacteria that kill the insect within 2 days. Unlike other insect pathogens, nematodes are regulated by the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) and, therefore, are exempt from registration with the U.S. EPA.

These biological control agents can be produced *in vivo* or *in vitro*, formulated and applied like other soil pesticides. Because their natural habitat is the soil, they require moisture. They function best against insect pests in cryptic habitats (such as soil-borne pests and stem borers). Some use occurs in greenhouses (for fungus gnats), landscape crops and mushroom production in California. Entomopathogenic nematodes have been proven effective against root weevils in citrus and scarab larvae in turf; however, in most cases synthetic insecticides are more widely used against these pests in California.

Byproducts from microorganisms

Abamectin. Abamectin (Agri-mek, Zephyr, Avid) is a microbial-based insecticide and acaricide (kills mites and ticks) that is widely utilized and performs like a conventional, synthetic insecticide. This toxicant, produced by the soil bacterium *Streptomyces avermitilis*, was isolated from a soil sample collected in Shizuoka Prefecture, Japan. Abamectin acts on insects by interfering with neural and neuromuscular transmission and paralyzes arthropods, resulting in the cessation of feeding and death 3 to 4 days after exposure. Abamectin is most effective when ingested by target arthropods, but also works on contact.

Abamectin penetrates leaf tissue and provides long-term (3 to 5 weeks) control of various mite species in field-grown roses and other ornamentals, strawberries, citrus, cotton and pears. A unique attribute of abamectin is that its acaricidal properties are coupled with activity on a few other insect species, especially dipterous and lepidopterous leafminers (*Liriomyza* and *Gracillariidae*, respectively), the lepidopterous tomato pinworm *(Keiferia lycopersicella)*, citrus thrips *(Scirtothrips citri)*, fire ants in turf and the homopterous pear psylla *(Cacopsylla pyricola)*. As with synthetic insecticides, the development of resistance is a concern; however, this has been largely avoided to date, except in greenhouse systems. With the exception of predatory mites, abamectin is fairly nontoxic to natural enemies.

Spinosad. Spinosad (Success) was fermented from the actinomycete bacterium *(Saccharopolyspora spinosa)*. The process yields several metabolites called spinosyns, of which two biologically active compounds form the basis for the insecticide. The active ingredients are derived from a soil-dwelling bacterium reportedly collected from an abandoned rum distillery on a Caribbean island in 1982. Spinosad kills susceptible species by causing the rapid excitation of the insect nervous system, leading to involuntary muscle contractions, tremors and paralysis. Insects must ingest spinosad; therefore, it has little effect on sucking insects and most nontarget predatory insects (it is highly toxic to syrphid fly larvae). Spinosad is relatively fast-acting — the insect usually dies 1 to 2 days after ingesting the active ingredient and there appears to be no recovery.

Spinosad has excellent activity on lepidopterous larvae including the cotton bollworm, peach twig borer *(Anarsia lineatella)*, armyworms (except western yellow-striped armyworm), loopers and the saltmarsh caterpillar *(Estigmene acrea)*. It also controls thrips. Registrations are in place on almonds, stone and pome fruits, citrus, cole crops and cotton. Spinosad is very short-lived, sometimes necessitating additional applications. A new formulation called Naturalyte was developed for the organic crop industry.

The organic mining of fermentation products (byproducts from microorganisms) could be a fruitful area for the discovery of other compounds with insecticidal properties in the future. The soil environment where actinomycete bacteria flourish is extremely complicated and diverse. For instance, milbemycins are structurally related to the avermectins, and pesticidal products based on these byproducts are under development. More than 30 different spinosyns have been isolated from *S. spinosa* alone, and these are being evaluated for pesticidal properties. Numerous other microbial species may also produce useful compounds.

Natural toxins from plants

Plant-derived compounds are another fruitful area for the discovery of natural toxins for IPM. Botanical insecticides are mainstays for pest control in organic systems, but receive limited use in conventional systems. They are used on vegetables, fruits and ornamentals.

Pyrethrum. Pyrethrum (as distinct from synthetic pyrethroids) is the most widely used plant-derived material, with about 125,000 acres treated in California in 2001. Pyrethrins are produced by certain species of chrysanthemums. The natural pyrethrins are contact poisons, which quickly penetrate the cuticle to the insect's nervous system. A few minutes after exposure, insects are paralyzed. This quick knockdown is one of the strong attributes of pyrethrins. However, enzymes in the insect swiftly detoxify the natural pyrethrins and some pests will recover. To delay the enzyme action so a lethal dose is assured, OPs, carbamates or synergists may be added. The short residual activity of pyrethrum on plant surfaces allows crops to be harvested shortly after application.

Azadiractin. Azadiractin is extracted from seeds of the neem tree *(Azadirachta indica)*. Chemically, azadiractin falls within a class of compounds known as tetranortriterpenoids. The properties of these insecticidal compounds include feeding and ovipositional deterrence, repellency, growth disruption, reduced fitness and sterility in a number of insect species. The active ingredient is structurally similar to insect hormones called ecdysones, which control metamorphosis. Therefore, the disruption of the insect's development and molting process (interfering with their life cycle) is likely to follow exposure.

Azadiractin is primarily used to control whiteflies, aphids, thrips, fungus gnats, mealybugs, leafminers and other arthropods on food, greenhouse crops, ornamentals and turf. Its acceptance and usage in conventional agriculture have been low, however, due to its erratic performance on major-crop insect pests. The more extensive use of this product in conventional agriculture is limited by its narrow application window (during the susceptible life stage), the need for frequent applications and a narrow pest spectrum.

Rotenone. Rotenone is a nonspecific insecticide with some acaricidal properties, which is used for pest control in a variety of crops. It is extracted from several plants in the pea family. Because of its toxicity to aquatic animals, especially fish, it should not be used near waterways.

Sabadilla. Sabadilla is extracted from seeds of the Mexican lily *(Schoenocaulon officinale)*, which contain the alkaloid veritrine as the active ingredient. It is mixed with sugar or molasses and applied as a baited toxicant for citrus thrips and avocado thrips. Activity is affected by the percentage of alkaloids in the extracted product and by the pH of the water used for the application. Because of sabadilla's variability in activity for thrips control and its rapid breakdown in light, spinosad and abamectin have largely replaced it in citrus and avocado. However, when thrips develop resistance to spinosad and abamectin, as citrus thrips has to OPs, carbamates and pyrethroids, interest in sabadilla is likely to resume.

Above, **in 1915 a worker sprayed dry sulphur on young prune trees infected with brown mite near Yuba City. Today, sulfur is still widely used as a pesticide to control mites on grapes, citrus and other crops. *Right*, an air-blast sprayer with tower treats citrus crops with petroleum-based oils to control citrus red mite and California red scale.**

Other plants. Several other plants produce compounds that are reportedly toxic to arthropods. Cinnamaldehyde, derived from cinnamon oil, is the toxic element in the product Cinnamite. This product controls mites and aphids, as well as powdery mildew, on a range of crops. Oils of rosemary, wintergreen, clove, garlic and lemon are also sold and reported to have insecticidal properties. Certainly as insects and plants coevolved, plants developed defense compounds to ward off attack by these herbivores. The isolation of these compounds could provide useful crop-protection tools in the future, but they are not cost effective in commercial agriculture today.

Inorganic products. For arthropod control, sulfur is the most commonly used inorganic product. Sulfur has strong fungicidal properties and secondarily controls spider mites. Significant usage for spider mite control occurs annually on grapes, citrus, ornamental plants and cotton.

Petroleum oils and particle films

Petroleum-based oils have been used for a number of years to control insect pests, especially mites and scale insects. Early formulations caused several problems because they had components that were phytotoxic to plants. More recently, petroleum oils have become highly refined and rarely show phytotoxic effects. Their common distillation points are 415 and 440. The heavier 440 oils show greater efficacy, but also more potential for phytotoxicity. The 440 oils tend to be used in fruit and nut trees as dormant applications for mites and lepidopterous and armored scale pests, often in combination with other pesticides. The lighter 415 oils are used for in-season treatments alone or in combination with other pesticides such as spinosad and abamectin. In citrus, 0.5% to 2% oil is used in spring for citrus red mite *(Panonychus citri)* and in the summer as a 1.4% spray for California red scale *(Aonidiella aurantii)*. Phytotoxicity can be avoided by treating during the coolest periods of the day and ensuring that the orchard is well irrigated prior to treatment.

Presently, one product is available in the mineral-based particle film category. Kaolin (Surround) is a naturally occurring product, generally inert to mammals, which does not react with other materials (Glenn et al. 1999). When used as a pesticide, kaolin is sprayed as a powdered suspension on crops, where it forms a barrier film that repels and prevents target pests from penetrating

Jack Kelly Clark

Certified organic growers often rely on plant-derived materials such as pyrethrum (extracted from chrysanthemums), azadiractin (from neem seeds), rotenone (from several pea species), sabadilla (from Mexican lilly seeds), cinnamaldehyde (from cinnamon oil) and other natural insecticides.

the leaves or other parts of the plant. To be effective, the suspension must coat all parts of the plants. Kaolin was registered in California in 2000 for home use and commercial agriculture. Its target pests are widespread and include earwigs, thrips, true bugs, aphids, hoppers, whiteflies, scales, beetles, caterpillars and mites. Crop registrations include fruit, vegetable, and field and ornamental crops. Kaolin has been used extensively in grape vineyards to repel glassy-winged sharpshooters *(Homalodisca coagulata)* from landing and transmitting the bacterium *Xylella fastidiosa,* which causes Pierce's disease. However, kaolin caused outbreaks of California red scale in citrus because it prevents natural enemies from gaining access to the scales.

Contributions to IPM programs

Insect pathogens, their insecticidal byproducts and natural insecticides and acaricides will make important contributions to IPM programs in a post-OP era. These pathogens and natural toxins have been known for their pest control properties for decades and will continue to be important niche products in IPM systems. However, the utility of insect pathogens is limited by their specific requirements for growth, survival and infectivity. Certain toxic byproducts of microorganisms are important and robust tools for arthropod management today and may take on an added role in the future. Research advances could overcome the limitations of these control agents and will likely discover and refine other types of natural control strategies.

One such strategy is the area of "induced resistance," whereby plants, once injured by an arthropod, are more resistant to subsequent feeding and injury (Karban and Baldwin 1997). This induction has been shown for mite, thrips, aphid and leafminer injury in crops such as grapes, cotton and celery (Karban et al. 1997; Omer et al. 2001; Black et al. 2003). The isolation of the compounds involved in this type of plant defense and the defining of the scope of the activity is ongoing. One commercial product (Messenger) utilizes this technology through induction with harpin proteins.

Additional new natural pest-control tactics, microorganism byproducts, and natural insecticides and acaricides will likely be discovered to facilitate IPM programs. In conventional agriculture, insecticides and acaricides from microorganism byproducts are the "standards" for the control of certain important arthropods, and some natural products have important niche uses. These products are already important tools against many pests in organic production systems.

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