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Review

Biorational insecticides in pest management

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We present herein a review article of the latest developments of the biorational approaches in pest management appeared in the literature from 1997 to date. The proposed advantages of the biopesticides including their specificity, safety to non-target organisms, particularly mammals, and utilization in low, sometimes minute, amounts have led to an intensive research program by public and private institutions resulting in an avalanche of reports in attempts to discover and develop newer and safer pesticides, particularly in the past three decades. This review is divided into three main chapters, including microbial insecticides in pest control, utilization of semiochemicals, and botanical insecticides, paying particular attention to those practical approaches that are respectful to the environment.

Keywords: Biopesticides, microbial insecticides, semiochemicals, insect pheromones, botanical insecticides, integrated pest management (IPM).

Introduction

Until few years ago, the biopesticide share represented little more than 1% of the total world pesticide market, which was estimated in 1998 to be around 32 billion $.[1)] Currently, there is a catalogue of over 800 pesticides formulated in 21,000 different products and registered at the US Environmental Protection Agency (EPA) for use in the US. Insecticides account for more than 94% of the total market for biological control products and more than 90% of the total insecticide sales are based on the bacterium *Bacillus thuringiensis* Berliner (*Bt*).[2)] While chemical pesticides occupy approximately 99% of the market, concerns about the use of these compounds in nature are permanently growing. The ideal pesticide should be toxic only to the target organisms, biodegradable, and should not leach into the groundwater. Unfortunately, this is rarely the case and the widespread use of pesticides in modern agriculture is of increasing worry. On the other hand, the low level of acceptance of biopesticides is the result of several factors: inconsistent practical results, uncompetitive price in comparison to classical insecticides, inappropriate formulations and application to a limited range of pests. However, the expected advantages of biopesticides, that is their specificity, safety to non-target organisms, use in very limited amounts, which often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides, have led to numerous scientific works on new and safer pesticides, particularly in the last three decades.[3)] In this context, several products of biological origin have been approved. The EPA has taken the lead in developing guidelines for the regulation of these pesticides in order to accelerate commercialization of safer natural or synthetic biorational products replacing the more toxic conventional active ingredients. There are over 190 active ingredients and more than 800 commercial formulations registered in the US. Biopesticides are classified by EPA into three main groups: *microbial pesticides*, in which a microorganism (bacterium, fungus, virus, or protozoan) is the active ingredient; *plant pesticides*, pesticidal substances that plants produce from genetic material which has been added to the plant; and biochemical pesticides, naturally occurring substances that control pests by non-toxic mechanisms. A list of new active ingredients approved by EPA for 2007 is shown in Table 1.[4)]

In addition to be inherently less harmful than conventional pesticides, biopesticides have been of immense value in specific integrated pest management (IPM) strategies and are very effective when they are produced and delivered correctly. In this case, the use of biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high. Another important attribute is the specificity of action on tar-

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Table 1. Biopesticides new active ingredients approved by EPA for fiscal year 2007

Chemical	Pesticide Type	Uses	Registrant
Mir Cry 3A	PIP Insecticide	Corn Root Worm	Syngenta
Beauveria bassiana H123	Insecticide	Chicken manure	JABB of the Carolinas
Z-9-Tetradecen-1-yl Acetate	Insecticide (pheromone)	Codling moth and leaf roller moths on orchard crops	Pacific Biocontrol Co.
Z-11-Tetradecen-1-ol	Insecticide (pheromone)	Codling moth and leaf roller moths on orchard crops	Pacific Biocontrol Co.
Z-11-Tetradecenal	Insecticide (pheromone)	Codling moth and leaf roller moths on orchard crops	Pacific Biocontrol Co.
Fir Needle Oil	Repellent	Rodents	Earth-Kind
Pythium ologandrum DV74	Fungicide	Food crops & ornamentals	Biopreparatory Co. Ltd.
Calcium lactate	Attractant	Mosquitoes	Ticks or Mosquitoes
Activated sewage sludge	Repellent	Deer; around gardens	Milwaukee Metropolitan Sewage District
Chenopodium ambrosiodes extract	Insecticide	Ornamental Greenhouse Turf	Codena, Inc.
Potassium dihydrogen phosphate	Fungicide	Apples, grapes, cucumbers, melons, summer & winter squash, watermelons, mangoes, peaches, nectarines, plums, cherries, peppers, tomatoes, roses	Cal Agri
(*R*)-1-Octen-3-ol	Attractant	Mosquitoes	Bedoukian
Quillaja extract	Nematicide/Fungicide	Grapes, citrus, pome fruits, stone fruits, nut crops, avocados, vegetable crops, and ornamentals	Desert King
Chitosan hydrolysate	Bactericide/Fungicide	Food crops, ornamentals, turf	Morse Enterprises
Salicylic Acid	Bactericide/Fungicide	Food crops, ornamentals, turf	Morse Enterprises
Indole 3-Acetic Acid	Plant regulator	Food crops and ornamentals	Stoller Enterprise
Avirulent zucchini yellow mosaic virus	Fungicide	Cucurbits	Bio-Oz Biotechn. Ltd.

get pests and closely related organisms in contrast to broad-spectrum conventional pesticides that may affect organisms as different as birds, insects, and mammals. In addition and due to the fact that, in many instances, for example in *Bt*, multiple toxins are involved in killing the insect, the possibility of creating resistance is greatly diminished, in spite of a large scale utilization over time.

1. Microbial pesticides

Microbial pesticides are those pesticides in which the active ingredient is a microorganism, either bacterium, fungus, virus, or protozoan. These types of pesticides can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest(s); for example, there are fungi that control certain weeds and other fungi that kill specific insects. The most widely used microbial pesticides are subspecies or strains of *Bt*. Each strain of this bacterium produces a different mix of proteins, and specifically kills one or a few related species of insect larvae. While some *Bt* strains control moth larvae found on plants, other strains are specific for larvae of flies and mosquitoes. The target insect species are determined by whether the particular *Bt* produces a protein that can bind to a larval gut receptor, thereby causing the insect larvae to starve.

1.1. Bacteria

The most widely used insecticidal bacterium is *Bt*, a Gram-positive soil bacterium that forms crystalline protein inclusion during sporulation.[5] During this process, this bacterium produces δ-endotoxin, an insecticidal crystal protein that is encoded on bacterial plasmids. The formation of crystal proteins (usually referred to as Cry proteins) is an essential feature that distinguishes *Bt* from a very close related species *B. cereus*, which can produce emetic and diarrheal toxins that cause food poisoning.[6] Cry proteins are produced as protoxins that are proteolytically converted into a combination of up to four smaller toxins upon ingestion. These proteins bind to specific receptors in the larval midgut epithelium causing formation of large cation-selective pores that increase the water permeability of the cell membrane. A large uptake of water then causes cell swelling and eventual rupture of the midgut.[7] The detailed mode of action of these toxins, however, is still un-

Table 2. List of strains and constructions of *Bacillus thuringiensis* approved by EPA in the period 2000–2007[a)]

Name	Active ingredient and possible use.	Date approved
Bt. modified Cry3 (67979-5)	This Syngenta Seeds Inc. product is used for control of the corn rootworm in field corn, sweet corn, and popcorn.	10/3/06
Cry3Bb1	Protein and the genetic material necessary for its production (Vector ZMIR39) in Event MON 88017 corn. The new construct of this protein is deployed in corn for rootworm protection, and stacked with Cry1Ab in a second corn product for corn borer protection also.	12/13/05
Var. aizawai strain PS811 Cry1F	The moCry1F protein, like the poCry1F protein, protects corn from certain lepidopteran insect larvae including European corn borer (*Ostrinia nubilalis*), southwestern corn borer (*Diatraea grandiosella*), fall armyworm (*Spodoptera frugiperda*) and black cutworm (*Agrotis ipsilon*).	5/27/05
Var. aizawai strain NB200	Targeted against larvae of lepidopteran (moth) agricultural pests.	6/10/05
Cry34Ab1 and Cry35Ab1	Proteins and the genetic material necessary for their production (plasmid insert PHP *17662*) in Event DAS-59122-7 corn. The new corn plant-incorporated protectant, Event DAS-59122-7 corn, is derived from *Bacillus thuringiensis* (*Bt*).	8/31/05
Var. aizawai Cry1F	The genetic material (from the insert of plasmid pGMA281) necessary for its production in cotton and *Bacillus thuringiensis* var. *kurstaki* Cry1Ac and the genetic material (from the insert of plasmid pMYC3006) necessary for its production in cotton.	9/30/04
Cry3Bb 1	Protein and the genetic material necessary for its production (Vector ZMIR13L) in Event MON863 corn.	2/24/03
Cry2Ab2	Protein and the genetic material necessary for its production in cotton.	6/14/02
Cry1F	Protein and the genetic material necessary for its production (plasmid insert PHI8999) in corn plants.	5/18/01

[a)] http://www.epa.gov/oppbppd1/biopesticides/product_lists/new_ai_2007.htm

known although a number of recent studies have added new data to disclose their mechanism of action.[8–11)] Nine different toxins have been described in *Bt* strains.[12)] These include an α-exotoxin of phospholipase C type, β-exotoxin (nucleotide thermostable, non selective toxic), γ-exotoxin (toxic to sawflies), δ-endotoxin (exploited commercially for pest control), louse factor exotoxin (only toxic against lice), mouse exotoxin (active to mice and lepidoptera), water soluble toxin Vip3A (vegetative insecticidal protein) and enterotoxin.[13,14)] These toxins bind to different receptors (phospholipids, phosphatidylcholine and sphingomyelin) in different insect species and with varying intensities, thus explaining species specificities.[15,16)] The potential presence of the β-exotoxin in certain *Bt* subspecies prompted the EPA to require acute toxicity testing of many *Bt* microbial pesticide formulations in rodents to confirm its absence. The presence of β-exotoxin in commercial *Bt* pesticide formulations has been prohibited by the EPA.[17)]

Bt strains containing mixtures of up to 6–8 different Cry proteins have been widely used as microbial pesticides since *Bt kurstaki* strain HD1 was originally commercialized in 1961. Cry protein-encoding genes were then visualized as an obvious and interesting choice to protect crops against pests. The first Cry gene was cloned and expressed in *E. coli*,[18)] and later soon the first genetically modified *Bt*-protected tomato, tobacco and cotton plants were produced.[19–21)] Nowadays, *Bt* protected cotton, potato, and corn have been commercialized in the US and one or more of these products are marketed in Argentina, Mexico, China, Canada, South Africa, France, Australia, Spain, Ukraine and Portugal,[22)] so that they represent about 1–2% of the global insecticide market.[16)] A list of the new *Bt* strains approved by the EPA in the period 2000–2007 is shown in Table 2.

These plants express one or more Cry proteins for the control of the Colorado potato beetle, tobacco budworm, cotton bollworm, pink bollworm, European corn borer, southwestern corn borer and corn earworm, among others. The total surface planted in the US for *Bt*-protected cotton, corn and potato exceeded 16 million acres in 1998, that is 17%, 18% and 4% of the total cotton, corn and potato acreage, respectively.[23)] This has represented a significant reduction (metric tons annually) in the use of insecticides to control these pests. More recently, a new insect-protected *Bt* corn variety has been developed that provides protection against corn rootworm, a pest that causes significant damage to the roots of corn grown in the Midwest and Eastern US.[24)] In China, where there has been widespread adoption of *Bt* cotton in the last few years, the number of insecticide applications have been reduced from 20 to 7/ha/season, which means a decrease of organophosphate and organochlorine insecticides by up to 80%.[25)] In terms of

cost, this represents a reduction of the insecticide cost by $762/ha/season.

In Europe, a number of *Bt*-derived products are used to control Lepidoptera pests in vegetables, tomatoes, top fruit, vines, olives and forestry. They include *Bt* serotype 3, *Bt* subsp. *azawai*, *Bt* subsp. *kurstaki* (*Btk*), δ-endotoxin of *Bt* subsp. *kurstaki*, *Bt* subsp. *tenebrionis*, *Bt* subsp. *israelensis* and *B. sphaericus*.[26] An illustrating example of the use of *Bt* in forestry was the control of an unexpected outbreak of the nun moth *Lymantria monacha*, which attacked more than 600,000 ha of forest in Poland in 1994. An emergency program, in which several public and private institutions were involved, was put into practice by applying a special forestry formulation of *Btk* from Novo Nordisk called Foray 48B. This material is suitable for spraying from the air at low volumes (4 l/ha). The results were excellent with *Btk* providing *ca*. 95% control of the pest while having a minimum impact on non-target and beneficial organisms. Since then, similar programs are being used in adjacent countries to control the pest. In North America, *Btk*-based products have become the first choice for controlling forest pest outbreaks, particularly for the gypsy moth *Lymantria dispar*.[26]

With regard to toxicity, *Bt*-protected plants provide a safety degree which is unmatched by any other pest control product. First, Cry proteins are non toxic to vertebrate species (mice, rats, monkeys, humans) even at doses higher than 1×10^6 μg/kg body weight, while dosages acutely toxic to susceptible insects are about μg/kg body weight.[17,27] Second, the acidic environment of the mammalian stomach does not favor solubilization and activation of the Cry proteins. Third, these proteins are readily degraded very fast (often in some seconds), from 60–130 kDa to polypeptides less than 2 kDa that corresponds to peptides with 10 amino acids in length. The rapid degradation of these proteins by proteases in the mammalian gastrointestinal tract precludes their toxicity in mammals. Fourth, several studies in vertebrates have failed to find high affinity Cry protein binding sites on gut epithelial cell membranes.[28,29]

In addition, the Cry proteins produced in *Bt*-protected crops (Cry3Aa in *Bt* potato and Cry1Ab and Cry1Ac proteins in *Bt* cotton) have been shown to rapidly degrade in soil at rates comparable to the rate of degradation of Cry proteins in microbial *Bt* products.[30,31] In summary, the benefits of using *Bt*-protected plants include: reduction of chemical insecticide treatments for the target pests, highly effectiveness in pest control, increase of crop yields, reduction of levels of fungal toxin, and additional pest control by preserving or enhancing populations of beneficial organisms.

While *Bt* is currently among the most widely used microbial insecticides, many other strains of *Bacillus* sp., including *Bacillus sphaericus* and *Bacillus subtilis*, as well as *Pseudomonas* sp. are also currently used as biopesticides.[6] A list of the bacterial biopesticides recently registered in the US is available at the EPA Office of Pesticide Program website.[32]

1.2. Viruses

Insects are attacked by many different types of virus, baculovirus being the most promising in insect control, particularly of Lepidoptera and Diptera,[33] because of their specificity. In addition, insect baculovirus are safe to vertebrates, plants and non target organisms, and they are pathogenic, the death of the host is generally the outcome of a baculovirus infection. The major successes of microbial control with viruses have taken place in forestry, particularly for sawflies control in Europe and North America through the NPV of the European pine sawfly, *Neodiprion sertifer*.[34]

Baculovirus control of pest insect populations was demonstrated in the 1940's but the first viral insecticide registered was that of *Helicoverpa* (*Heliothis*) *zea* in 1971 under the tradenames Viron/H and later Elcar.[35] This virus had been developed by the USDA for control of the tobacco budworm (*Heliothis virescens*), corn earworm (*Heliothis zea*) and *Heliothis armigera* on cotton, row crops, fruits and vegetables. Since then, a number of baculovirus insecticides have been registered and produced commercially.[36] They are all wild-type baculovirus, and like most microbial insecticides, have had limited success for various reasons. These include narrow spectrum of biological activity, slow mode of action (5–7 days after ingestion of NPVs and 7–14 days in the case of GV infections, see below) and photolability (particularly to solar radiation). There have been different approaches directed to enhance the role of baculovirus as effective biopesticides. For instance, the effect of baculovirus may be enhanced by the synergistic action of specific chemical insecticides, such as the synthetic pyrethroids deltamethrin and permethrin.[37] To improve potency and speed of action, several recombinant baculovirus have been developed.[38,39]

Baculoviruses are classified into two genera: nuclear polyhedrovirus (NPV) and granulovirus (GV). The NPVs are further segregated into two groups based on the phylogenetic relationships of 20 different genes.[40] The baculovirus are characterized by double-stranded circular DNA genomes ranging from 88 to 153 kb. Baculoviruses produce two types of progeny: the budded virus (BVs) and the occluded virus (OVs). The OVs of the NPV and GV are termed polyhedron (plural polyhedra) and granule, respectively. BVs are responsible for the systemic or cell-to-cell spread of the virus within an infected insect. OVs, in turn, are responsible for the larva-to-larva transmission of the virus.[41] During infection, the host larva is debilitated, resulting in reduction of development, feeding, and mobility and increasing exposure to predation.[42] Post larval effects may include lower pupal and adult weights, as well as reduced reproductive capacity and longevity.[43] Infected larvae usually climb to the upper parts of the plants, dying in 5–8 days although cessation of feeding may occur in 2–4 days depending on biotic and abiotic factors. Diseased and dead larvae serve as inoculums for virus transmission, which may occur by rain and movement of arthropods on plants, or *via* predators and parasitoids. Fortunately, not many

mammalian viruses are as successful and prevailing as baculoviruses in infecting insects.[41)]

The BVs offer several unique advantages as a vector for the expression of a foreign gene within insect cells and insect larvae. Currently, *Autographa californica* NPV (AcNPV) is the choice for gene insertion or gene deletion research both in industry and academic institutions. Methods for the construction and use of recombinant BVs for the expression of heterologous genes have been described and are identical to those that were later used to generate recombinant Bv insecticides.[44,45)] In this context, it should be noted the efforts conducted with AcNPV engineered to express an insect specific toxin derived from the Algerian scorpion *Androctonus australis*.[46)] The recombinant AcNPV showed equal selectivity towards beneficial insects and pest species to that of the native AcNPV. In addition, the AcNPV-AaIT killed noctuid moth larvae in half the time required by the native AcNPV and several private companies have conducted promising field trials in cotton, tobacco and other crops.[47)] Other recombinant baculovirus systems reported include: genes encoding the juvenile hormone esterase have been inserted in AcNPV,[48)] genes insertions and genes deletions to express development-disrupting enzymes,[49)] diuretic hormones,[50)] specific toxins such as *Diguetia canities* and *Tegenaria agrestis* spider toxins,[51,52)] scorpion toxins,[53–56)] and specific neurotoxin from the venom of *Leiurus quinquestriatus hebraeus* against *Trichoplusia ni* and other lepidoptera pests.[57)]

1.3. Fungi

Another major class of microbial biopesticides includes fungi. Many fungi are pathogenic to the insect host, and these are referred to as entomopathogenic fungi. Many of the genera of entomopathogenic fungi currently under research either belong to the class Entomophthorales or Hyphoymycetes. Species within these classes can behave very differently, and thus, for example, insect host, infection levels, germination rates and optimum temperature of action can vary between species and isolates.[58,59)] Members of Hyphoymycetes are generally considered opportunistic pathogens infecting many species of several insect orders, and host death is commonly associated with toxin production overwhelming host defense mechanisms. In contrast, other groups of fungi are thought to have been evolved into higher parasitic forms. For example, infection and host death by Entomophthorales usually occurs by tissue colonization with little or no use of toxins.[60)]

In contrast to other microbials acting *via* ingestion by the insect host, entomopathogenic fungi attack insects by direct penetration through spiroacular openings in the cuticle. Fungal infections occur in arthropods but other non-host insects and/or species which are not pest of cultivated crops, such as spiders and ants, may also be infected. The fungal infection and transmission is produced by asexual fungal spores or conidia that are dispersed throughout the environment in which the insect host is present. The spores germinate in the insect's blood and germinating mycelia gradually kill the host.[47)] Interactions between the entomopathogenic fungi and their arthropod hosts and the induced altered behaviors on the invertebrates resulting thereof has been recently reviewed.[61)]

Although over 750 fungal species are known to infect insects, only a few have been commercialized in USA, Europe, Latin America, China and the former Soviet Union. A list of the commercially available fungi, the current producer and the target pests has been reported.[62)] The most notable mycoinsecticides currently in use are: *Beauveria bassiana*, *Beauveria brongniari*, *Metarhizium anisopliae*, and *Metarhizium flavoviride*. Among them, the most prominent is *B. bassiana*, from which several strains have been developed and marketed in the US, Europe, Russia, South America and probably also in China.[26,63)] The application of *B. bassiana* for the control of the pine moth *Dendrolimus* spp. in China probably represents the largest use of a biocontrol agent. The programs started in 1950 and it is calculated that at least one million hectares of pine forest are now involved in the treatments.[34)] *B. bassiana* strain Bb-147 is registered on maize in Europe for control of the European corn borer *Ostrinia nubilalis* and the Asiatic corn borer *Ostrinia furnacalis*. *B. bassiana* strain GHA, in contrast, is registered in the US for control of the whitefly, thrips, aphids and mealybugs. *B. bassiana* strain ATCC 74040 is registered against many soft-bodied insects of the orders Homoptera, Heteroptera and Coleoptera. *B. brongniari* is registered on sugarcane and barley for control of white grubs and cockchafers *Melolontha melolontha*. *Melolontha anisopliae* is one of the first fungi used in biological control (the first account of its utilization dates from Russia in 1879[64)]) because it has not adverse effects in mammalian toxicology tests, and presents no risk of infectious complications because the organism in incapable of growth at mammalian body temperatures. It is registered in the US for control of termites but is also produced for control of coleopteran and lepidopteran greenhouse pests. In addition, recent developments have disclosed that new formulations of *M. anisopliae* are being used in Africa, Australia and Brazil against locusts and grasshoppers.[65)] *Melolontha flavoviride* (*M. anisopliae* var. *acridum*) has been found effective against the brown locust *Locustana pardalina* in Africa,[66)] *Locusta migratoria* in Madagascar,[67)] and the Australian plague locust *Chortoicetes terminifera* and *L. migratoria* in Australia.[68)] With variable success, *M. flavoviride* has also been tested against the tree locust *Anacridium melanorhodon* in Sudan,[69)] the rice grasshopper *Hieroglyphus daganensis* in Benin, Mali and Senegal[70)] and the desert locust *Schistocerca gregaria* in Mauritania.[71)] In the case of this highly mobile insect, an estimated plot size larger than 1000 ha would be necessary and such trials are expected to take place within the FAO Emergency Prevention Program. Very recently, *M. anisopliae* has been modified to express an insect-specific neurotoxin from the scorpion *A. australis*, under the control of a promoter that is active only in the presence of insect hemolymph.[72)] This restricts expression of the

neurotoxin to the time after the fungus has penetrated the cuticle. The toxin dramatically increased pathogenicity and virulence against tobacco hornworm *Manduca sexta* and yellow fever mosquitoes *Aedes aegypti*. In comparison with the wild fungus, the modified fungus achieved the same mortality rates in tobacco hornworm at 22-fold lower doses and reduced survival times of infected mosquitoes by over 40%.[72)]

Compared with most chemical insecticides, fungi are less toxic to mammals and have negligible environmental impacts. One obstacle in exploiting fungi for insect control, however, is that they may kill their host too slowly. Even highly virulent isolates take 2–5 days to kill an insect, and infected host can survive much longer, depending on dose and environmental conditions. Another constraints which may hamper a wider utilization of entomopathogenic fungi include dessication, UV light, host behavior, temperature, pathogenic strength, etc.[73)] Many of these constraints are currently being addressed by recent advances in formulation technology.[62)]

1.4. Nematodes

Although it was known for more than 60 years that certain nematodes infect and kill soil-inhabiting grubs, only until recently practical attention has been paid to entomopathogenic nematodes (EPNs) as biopesticide agents.[47)] Nematodes are simple roundworms, colorless, unsegmented, and lacking appendages. They are not actually microbial organisms but are always included as part of the microbial control of insects. In fact, the non-feeding infective juveniles possess attributes of both insect parasitoids or predators and microbial pathogens. Like parasitoids/predators they have chemoreceptors and are motile; like pathogens they are highly virulent, killing their hosts quickly.[74)] In addition, in contrast to chemicals which should have to decay within few days, EPNs are persistent and recycle inside the host causing long-term and sustainable effects on pest populations.[75)] They can be cultured easily *in vitro* and have a high reproductive potential. EPNs are environmentally friendly agents with no detrimental effects on non target organisms, mammals or plants.[76)] In almost all countries EPNs are exempt from registration, which enables small and medium-sized companies to develop them as plant protection agents. Moreover, they can be stored for months and can be applied with conventional spraying equipment. On the other hand and like other biological control agents, nematodes are constrained by being living organisms that require specific conditions to be effective; they are fragile, subject to desiccation and sensitive to temperature changes and solar radiation.[47)] They are ubiquitous and have been found in a wide range of ecologically diverse soil habitats including cultivated fields, forests, grasslands, deserts and even ocean beaches. Today, nematodes are mainly used where chemical insecticides fail, for instance in soil, galleries of boring insect pests or where resistance to insecticides has appeared.[76)]

Of all nematodes studied, those from the genera *Steinernema* and *Heterorhabditis* have aroused the greatest interest and developed for insect control. These nematodes are characterized by the ability to carry specific pathogenic bacteria, *Photorhabdus* with *Heterorhabditidae* and *Xenorhabdus* with *Steinernematidae*, which are released into the insect hemocoel after penetration of the 3-stage infective juveniles (called "dauer juveniles", DJ). The DJ is a free-living, third juvenile stage that is well adapted to long-term survival in the soil. When a host is located, the nematodes penetrate into the insect body cavity, usually *via* natural body openings (mouth, anus, spiracles), and the DJ release the symbiont cells and the symbiotic bacteria (*Xenorhabdus* or *Photorhabdus*), which multiply rapidly and cause rapid insect death (within about 2 days after nematode invasion). The bacteria proliferate and produce suitable conditions for nematode reproduction. Feeding on the symbiont cells, juveniles develop into adults and produce offspring. When the nutrients are consumed, the offspring develop into DJ, which retain the symbiotic bacteria in the intestine. The life cycle is completed in a few days, and hundreds of thousands of new infective juveniles emerge in search of fresh host.[76,77)] Only a few strains of the symbiotic bacteria have been studied in detail from a genetically point of view, but they have gained considerable attention since their genes represent a possible alternative to *Bt* genes for expression in transgenic plants.[78)]

As noted, the EPNs are lethal to a notably broad range of insect pests in the laboratory, although in the field the host range is considerably more restricted. Important soil insects against which there are no effective nematodes commercially available include wireworms, grape phylloxera, fire ants or corn rootworms.[77)] Of the nearly 30 steinernematid and heterorhabditid nematodes identified to date, eight species are commercially available.[62)] *Seiternema carpocapsae* is the most studied, versatile, and available of all EPNs and is particularly effective against lepidopterous larvae, including webworms, cutworms, armyworms, girdlers and wood borers. Other EPNs marketed are *Seiternema feltiae*, *Seiternema glaseri*, *Seiternema kushidai*, *Seiternema riobravis*, *Seiternema scapterisci*, *Heterorhabditis bacteriophora*, and *Heterorhabditis megidis*,[77)] the target insects being mushroom flies, fleas, fungus gnats, coleopterous larvae particularly scarabs, mole crickets, root weevils, white grubs, sciarid flies, slugs, *etc*.[79)] In some cases, the efficiency of the EPNs particularly against white grubs has been inconsistent and unsatisfactory.[80)] Nematode efficacy and consistency may be improved by combining EPNs with insecticides, particularly with the nicotinoid insecticide imidacloprid.[81)] Combination of both agents induced a synergistic increase in scarab mortality between 4 and 13 fold higher than in treatments with the nematodes alone and the insecticide did not interfere with nematode recycling.[81)]

1.5. Protozoa

Entomopathogenic protozoa are an extremely diverse group of organisms in relationship with insects ranging from com-

mensal to pathogenic. They are generally host specific and slow acting, often producing chronic infections characterized by a general debilitation of the host.[73] The infections cause sluggishness and irregular or slowed growth finally resulting in reduced feeding, vigor, fecundity and longevity of the insect host. However, although they are undoubtedly important in natural biological regulation of insect populations, they do not possess the required attributes for a successful microbial insecticide.[62]

Out of the approx. 15,000 protozoa described, around 1000 species, primarily microsporidia, are known to attack invertebrates, including many insect species such as grasshoppers and Heliothine moths.[47] Most microsporidia must be eaten to infect an insect, but there may also be natural transmissions, for instance by predators and parasitoids, within a pest population. The spore formed by the protozoan is the infectious stage in susceptible insect. They are ingested by the host and germinate in the midgut. Sporoplasm is then released invading target cells and causing massive infection to the host.[82]

The most notable entomopathogenic protozoa belong to *Nosema* spp. and *Vairimorpha necatrix*. Among the *Nosema* spp., *Nosema locustae* is the only commercially available species of microsporidium, and marketed under several labels for control of grasshoppers and crickets. It was first described against the grasshopper *L. migratoria*, but it is now known that its potential host range involves at least more than 100 orthopteran species.[83] *N. locustae* has been the subject of extensive molecular investigations regarding metabolism, evolution, and phylogenetics, and its complete genome is currently being sequenced, but details on the ultrastructure of intracellular stages were unknown until recently.[84] It has been extensively tested in the field in the US, Canada, Argentina, Cape Verde, China and Mali.[65] In Argentina, comprehensive studies have indicated a considerable decline in locust populations in treated areas[85] and in China surfaces of *ca*. 100,000 ha have been treated annually.[86] However, the utility of *N. locustae* as a grasshopper control agent remains questionable mainly because of the great difficulty underlying an adequate efficacy assessment in this highly mobile insect.[73] Other microsporidia with far greater virulence against grasshoppers than that of *N. locustae* have been described, including *Nosema enthomophaga*, *Nosema cuneatum* and *Johenrea locustae*. However, their great virulence hampers development of an effective production system.[65]

Nosema pyrausta is another beneficial microsporidium that reduces fecundity and longevity on adults of the European corn borer[87] and also slow down larval development increasing mortality.[88] In the laboratory, *N. pyrausta*-infected females laid a significantly reduced number of eggs at low temperatures in comparison to that laid by non-infected insects.[89] These data have been claimed to be useful in predicting *O. nubilalis* populations.[89]

2. Semiochemicals

Chemical substances or mixtures that mediate interactions between organisms are named semiochemicals, and includes pheromones, kairomones, allomones, and other classes of behaviorally active compounds. Semiochemicals are considered to be safer and environmentally more acceptable than conventional pesticides because they occur naturally, are able to target the pest species only, elicit low acute toxicities to vertebrates, and are usually volatile chemicals that do not leave behind harmful residues.[90] Semiochemicals may be used to monitor populations or in direct pest control strategies reducing populations by mass trapping, lure-and-kill or mating disruption.

2.1. Monitoring

Development of effective monitoring systems provides valuable information for coordination of the treatment schedule with pest phenology. Semiochemicals are used in traps to monitor changes in population levels allowing a better knowledge about the onset of adult emergence and the flight peak. Highly sensitive pheromone-based monitoring is crucial for detection of incipient infestations of introduced or exotic insects, such as the Mediterranean and Mexican fruit flies,[91] wood boring and bark beetles[92] or for detection of pests with constantly expanding ranges, such as the gypsy moth,[93] the pink bollworm[94] or oriental beetles.[95]

The most widely used attractants in monitoring systems are sex pheromones to monitor,[96,97] aggregation pheromones to monitor coleopteran species,[98,99] and host plant odors for dipteran species.[100] Although in the vast majority of cases a good correlation between catches and level of damage has been noticed,[101–103] sometimes the number of adults caught in traps was not correlated with the number of eggs laid and larvae found in plants.[104,105] In order to get a good prediction of the damage caused by the larvae of the next generation, several features should be considered. Pheromone composition of the bait including dose and purity, both chemical and stereomeric, is the first parameter to bear in mind. The stereomeric purity of the lure is very important for an efficient monitoring in many species, as proved to be for the oak processionary moth *Thaumetopoea processionea*,[106] the Israeli pine bast scale *Matsucoccus josephi*,[107] and the leafminer *Phyllonorycter blancardella*.[108] In addition, it should also be considered the possible geographical variation in pheromone composition, as in the European pine sawfly *N. sertifer*,[109] or in the apple leafminer moth, *Phyllonorycter ringoniella*.[110] Variables like color, form and position of the traps may have also a substantial influence in the number of catches, and optimization needs to be performed for every species. Trap color may be also a significant variable in certain species, like stink bugs,[98] and the processionary moth *Thaumetopoea pityocampa* males,[111] whereas other species do not show any preference for any specific color.[112] The type of trap can also

Table 3. Some recent cases of mating disruption experiments using sex pheromones or pheromone analogues

Common name	Species	Crop	Reference
Corn stalk borer	*Sesamia nonagrioides*	maize	158,178
Codling moth	*Cydia pomonella*	apple	132,179,180
European corn borer	*Ostrinia nubilalis*	corn	158,181
	Tecia solanivora	potato	182
Oriental fruit moth	*Grapholitha molesta*	apple orchard peach/pear orchard	135,183
Jasmine moth	*Palpita unionalis*	olive groves	184
Cherry tree borer	*Synanthedon hector*	cherry orchard	185
Lightbrown apple moth	*Epiphyas postvittana*	citrus	186
Olive pyralid moth	*Euzophera pinguis*	olive groves	187
Clearwing moths	*Paranthrene robiniae*	poplar	188
Yellow stem borer	*Scirpophaga incertulas*	rice	189
Coneworm moths	*Dioryctria* spp.	pine seed orchard	190
Pink bollworm	*Pectinophora gossypiella*	cotton field	191

be essential for an optimum level of catches, as demonstrated for the Japanese beetle *Popillia japonica*,[113] or for the Nantucket pine tip moth *Rhyacionia frustana*.[114] Position of the traps[115,116] and the relative distance among them within the plot should also be considered.[117,118]

2.2. Mating disruption

Mating disruption is the most widely and successfully used control method for a variety of insects.[119] It prevents mating and, hence, reduces the incidence of larvae in the next generation. This is normally done by releasing a large amount of pheromone or pheromone analogue in the treated area, and has been used against lepidopteran species,[120] and other orders like Coleoptera,[121,122] Hemiptera,[123] and Heteroptera.[124] Some recent cases of mating disruption are shown in Table 3.

Mating disruption has a number of advantages as pest management tactic. It is species-specific, has low environmental impact and is more sustainable than other broad spectrum techniques without evidence of resistance. In fact, it has proven to be one of the preferred control methods against insecticide-resistant populations. On the other hand, mating disruption has also important drawbacks. Due to its specificity, it is often more costly than the broad spectrum insecticides, particularly when more complex and/or unstable components are used. In addition, secondary pests can often emerge as important problems.[120] Monitoring success of the experiments is often difficult and sometimes inconsistent, since possible reduction in trap catches in pheromone-treated blocks may not be corresponded with egg mass densities and damage produced by larvae of the next generation.[125–128]

There has been considerable debate about the mechanisms underlying mating disruption,[129,130] although there is general agreement now that more than one mechanism may be operational at the same time and that they may vary between species.[131] Two possible mechanisms are recognized in the literature for mating disruption: competitive (competitive attraction=false-plume-following) and non-competitive (camouflage, desensitization, and sensory imbalance).[129,130] Competitive attraction appears to be the prevalent mechanism in mating disruption when the pheromone is deployed in specific point sources. This is the suggested mechanism operating, for instance, in the mating disruption of the codling moth *Cydia pomonella*.[132] However, studies on *Choristoneura rosaceana* (Lepidoptera: Tortricidae) suggest that disruption of the mate-finding behavior can be explained by a combination of mechanisms including adaptation of antennal receptors, camouflage of the female-produced plume, and false-trail following.[133]

The main parameters to be considered for a successful mating disruption experiment include: type of dispenser, dispenser release rate and blend composition. In addition to the classical dispensers, such as hollow-fibers, microencapsulated sprayables, laminates, polyethylene tubing, and aerosols, emulsified paraffin wax has also been recently introduced as a long-season dispenser that works efficiently against *Grapholitha molesta*, offering several advantages like low cost, self adhesion and biodegradation.[134] Also, new sprayable microencapsulated pheromone formulations have been recently effective against *G. molesta*.[135] Another type of controlled-release dispensers of pheromone recently used are MSTRS (Metered Semiochemical Timed Release System) devices, which are very efficient in food stores,[136] cranberry fields,[137] and apple orchards.[138] With respect to the dispenser release rate, this should be constant and long life to cover entirely the flight duration of the moth. It should be noted that increasing pheromone concentration does not always provide improved control of high populations. An optimization of the pheromone concentration is then necessary to allow reduction

of the applied pheromone and therefore lowering the cost of the treatment.[139] Blend composition is also important since the different components should be stable under environmental conditions, and optimized to have the least expensive formulation possible, for instance using only the major component.[140] Other important factors for success imply to know the infestation level and the pheromone concentration in the treated areas. Low levels of infestation are clearly preferred for mating disruption since the lower number of insects flying the lower the probability to encounter each other for mating. This has been confirmed in field experiments against the gypsy moth and codling moth.[131] In addition, when the population density of the pest is very high, neither false trails nor plume masking significantly affect the ability of males to find the females and, furthermore, individuals from the surrounding zones may be attracted to the treated areas by the applied pheromone.[141] To measure pheromone concentrations in the field three different techniques are commonly used: chemical analysis, field electroantennogram recordings and single sensillum recordings in the field.[142,143] These methods, however, do not indicate the success of the experiment directly, which should be assessed by other methods.

Recently, there have been assayed successfully blends of attractive formulations targeting more that one species at the same time, like four different leaf roller species in apple orchards,[144] the codling moth *C. pomonella* and the Oriental fruit moth *G. molesta*,[145,146] and the pest vineyard *Lobesia botrana*.[147]

Other ways to improve mating disruption techniques have been tried in the context of IPM programs. For instance, integration of mating disruption with deployment of transgenic apples appeared to offer prospects for delaying the evolution of resistance to *Bt* toxins in the lightbrown apple moth *Epiphyas postvittana*.[148] Also, programs using combination of mating disruption with insecticide treatments have been developed,[138,149,150] but in some cases the use of insecticides has not improved the level of damage.[151,152]

Little work in pest control has been done using pheromone antagonists.[153–156] These compounds, that alter the behavior or physiology of the insect communication system,[157] may be pheromone components of closely-related species with a sufficiently similar structure to that of the natural pheromone to bind to the pheromone receptor sites, and therefore competing with the natural attractant.[131] Successful control of the navel orangeworm *Amyelois transitella*[154] and the pea moth *Cydia nigricana*[153] has been reported using pheromone inhibitors. Very recently, a significant reduction of damage by the Mediterranean corn borer (MCB) *Sesamia nonagrioides* and the European corn borer *O. nubilalis* in maize fields has been noticed by utilization of a trifluoromethyl ketone analogue of the MCB pheromone.[158] Effectiveness of the treatment was particularly high for the most damaging second generation of both pests.

2.3. Mass trapping

In mass trapping, a very high proportion of insects must be caught in traps baited with chemical lures before mating or oviposition to reduce the pest population. This reduction must then be translated into a reduction in plant damage. For success it is required that the lure be very attractive, over surpassing if possible the effect of the naturally occurring attractant. For Lepidoptera it is essential that males are trapped before mating and this is most likely to occur with insects that mate only once. For Coleoptera it is highly recommended that both sexes are caught (if trapping is based on aggregation pheromones) before eggs are laid or damage is inflicted by feeding adults.

Mass trapping techniques have been used successfully to control a wide range of insect pests, typically species in Lepidoptera, Coleoptera and Diptera orders. The mechanism of population reduction *via* trapping differs depending on the type of semiochemicals used. In Lepidoptera[97] and some Coleoptera,[159] the female sex pheromone is the agent used to attract males. In other Coleoptera, for instance Scolytidae, aggregation pheromones are generally used, either alone[160,161] or in combination with food attractants.[162,163] In other cases, insects can be attracted only by volatiles from the host as the beetle *Hoplia communis*, which can be caught in great numbers by 2-phenylethanol, a major volatile component of the host flowers *Rosa* spp.,[164] or just by synthetic food-based baits as the Mediterranean fruit fly *Ceratitis capitata*.[165]

Several studies have compared the efficiency of mass trapping with other methods of pest management. In some of them mating disruption was considered to be more effective than mass trapping in trials against the pink bollworm *Pectinophora gossypiella*.[166] Trematerra[167] considered mass trapping better than mating disruption on small, hilly sites, whereas other found mating disruption too expensive.[168] There may be cases in which a combination of mass trapping and mating disruption would be effective, such as mass trapping females by using kairomones and using sex pheromone to disrupt males.[169]

One of the handicaps of this technique is the cost. A cost reduction should be taken into account for mass trapping success, particularly if it implies a reduction in the use of insecticides.[170] In Costa Rica, for instance, control of the American palm weevil *Rhynchophorus palmarum* as vector of the red ring nematode *Bursaphelencus cocophilus*, the infecting agent of the red ring diseased oil palms, was successfully achieved using less than one trap per five hectares.[171] Also, in Ethiopia, the implementation of an adaptive tsetse population management system along with the application of geostatistical methods to discover patches with increased fly densities allowed the authors to reduce the number of traps from 216 to 127, maintaining previously achieved levels of occurrences of the pest.[172] On the other hand, the number of catches should also be as high as possible. For instance, in mass trapping experiments against *Monochamus clamator* and *Monochamus*

scutellatus using pheromone components of scolytid bark beetles, traps baited also with ethanol, α-pinene and ipsenol captured twice as many beetles as traps baited with host volatiles alone.[99] Another way to reduce costs is the control of more than one species at the same time. Thus, experiments in Costa Rica and Honduras allowed control of the Indian sugarcane weevil *Metamasius hemipterus* and the American palm weevil *R. palmarum* by using lures emitting a mixture of their male-produced aggregation pheromones.[173]

2.4. Lure and kill

Lure and kill tactics combine lures with insecticides. Many preliminary studies carried out on dipteran and lepidopteran species have been performed with no conclusive results.[169] Charmillot and Hofer[174] developed one attract and kill formulation against the codling moth, which was registered in Switzerland by Novartis. More recently, a bait of (*Z*)-9-tricosene, the only commercially available pheromone for use in lure and kill approaches to housefly control, in combination with sugar/insecticide has been widely used in indoor livestock farms.[175] The formulation was, however, inefficient to provide adequate control of houseflies outdoors. Williams *et al*.[176] have claimed that pyrethroid bifentrin-treated lethal ovitraps can be used as a lure and kill device against the dengue vector *A. aegypti* and that they should be effective in the field for at least four weeks. Kairomonal attractants can also be used in lure and kill tactics, as shown by the attracticides developed against the navel orangeworm *A. transitella* in almonds.[177]

3. Botanical insecticides

Herbs, animal products and inorganic materials have been for centuries the ingredients of drugs and pesticides. Long before knowing the structure of plant naturally occurring chemicals (=botanicals), plants or derivatives thereof were extensively used in agriculture as insecticides. Pyrethrum from *Chrysanthemum cinerariifolium* Vis. (Compositae), rotenone from *Lonchocarpus nicou* or *Derris elliptica* (Leguminosae), and nicotine from *Nicotiana tabacum* (Solanaceae), are outstanding among other examples (Fig. 1).[192]

Use of botanical insecticides in commercial agriculture was dramatically reduced when synthetic insecticides were developed. DDT was used extensively to keep World War II soldiers free of head and body lice and also proved very effective against mosquito-transmitted diseases, such as malaria or yellow fever.[193] Discovery and increasing development of synthetic insecticides followed (major classes: organochlorinated, organophosphate, carbamate) driven by their lower cost, effectiveness and longer lasting properties.[193] But public concern for long-term risks for health or the environment, brought by toxicological problems (acute and chronic poisoning of applicators, farmworkers or consumers) and environmental contamination (wildlife destruction or disruption of pollination and natural pest control), resulted in severe regulatory restrictions or even banning.[194] Pyrethroid, being one class based on a natural product model is an example of successful synthetic pesticide chemistry.[195]

As a consequence, increased attention and interest have followed on botanical insecticides as natural pesticides for IPM strategies.[196,197] However, "natural" plant-derived compounds may be as toxic (or more toxic) to humans and beneficial insects as many common "synthetic" insecticides are, and a "reduced risk" status is to be proved.[198]

At present, current botanicals in commercial use for insect control fall into four major types (pyrethrum, rotenone, neem and essential oils) and a few more (ryania, nicotine and sabadilla) are of limited use.[194] The expanding body of literature reporting new plant derivatives with prospective bioactivity against insect pests is also considered here.

3.1. Pyrethrins

Pyrethrum is an extract from dried flowers of *Tanacetum* (*Chrysanthemum*) *cinerariifolium* (Asteracae) or related species, and the active principles "pyrethrins" may reach a 3% content. Chemical components of the extract are sesquiterpenes, triterpenes and sterols, flavonoids, *n*-alkanes and fatty acids. The "pyrethrins" are meroterpenes (mixed biosynthesis: a terpene-derived unit is attached to a non-terpene moiety), esters of the chrysanthemic or pyrethric acid with ketocyclopentene alcohols (Fig. 1): pyrethrolone (pyrethrins I and II), cinerolone (cinerins I and II) and jasmolone (jasmolin I and II). Often available at 25–50% concentration, pyrethrin I and II are present in greatest amounts.[195] Pyrethrins show neurotoxic action by blocking voltage-gated sodium channels (a mechanism qualitatively similar to that of DDT), prolonging their opening and thereby causing a rapid knock-down effect and death.[194]

Synthetic analogs (pyrethroids) have been developed to overcome low stability to UV light without compromising biodegradability, and to maintain insecticidal potency while minimizing fish toxicity. The structure of modern pyrethroids differs quite a lot from that of model pyrethrins as well as their molecular mode of action.[193]

3.2. Nicotinoids

Nicotine is an alkaloid isolated from tobacco plants, mainly *Nicotiana tabacum* and *Nicotiana rustica* (Solanaceae), and it is the most toxic botanical insecticide, with an LD_{50} (rat oral) at 50 mg/kg, and extremely harmful to humans. As a fast-acting nerve toxin, it works as a contact poison (symptons similar to those caused by organophosphate or carbamate insecticides). Acting at the nicotinic acetylcholine receptor it leads to uncontrolled continuous firing of the neuroreceptor. Nicotine is most effective on soft-bodied insects and mites, including aphids, thrips, leafhoppers and spider mites.[194,198]

Commercial tobacco contains other alkaloids such as nornicotine (found specially in *Nicotiana glutinosa* and *Nicotiana sylvestris*) and anabasine (*Nicotiana glauca* being its

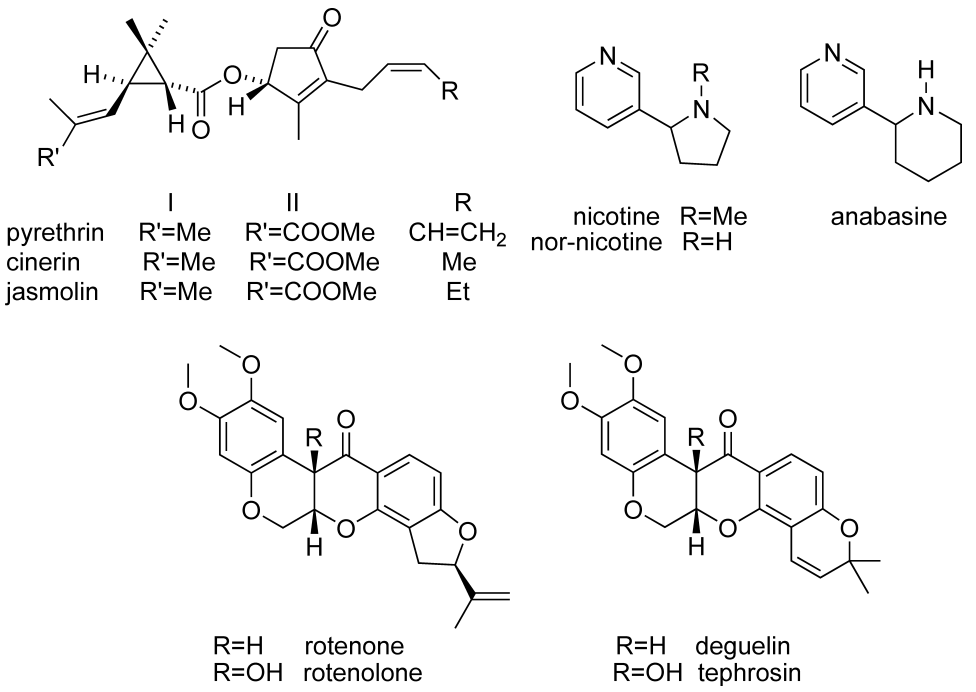

Fig. 1. Naturally occurring insecticides.

most important source) and a range of other "minor" related alkaloids (Fig. 1).

Related to nicotine in action and partly in structure, the highly insecticidal neonicotinoids originated from screening novel synthetic chemicals. This newest major class of neuroactive insecticides is increasingly used because of their outstanding potency and systemic action for crop protection against plant piercing-sucking pests, and being highly effective for flea control on cats and dogs.[199)]

3.3. Rotenoids

Rotenone is an isoflavonoid molecule, and the major constituent of insecticidal, acaricidal, and piscicidal cubé resin. It is commonly available as a dust containing 1–5% of active ingredients from rhizomes or roots of the tropical *Lonchocarpus*, *Derris* or *Tephrosia* (Leguminosae), or extracts (as resins) usually with up to 45% total rotenoids. The four major active ingredients of cubé resin are rotenone, deguelin, rotenolone and tephrosin (Fig. 1), totaling 77 wt %. From the resin 12 new compounds have been isolated and identified, out of 29 rotenoid constituents, and their biological activities assayed for all of them.[200,201)]

Fifty-two rotenoids and seven rotenoid glucosides were reported in the period 1855–1981,[202)] and fifty more new ones, and again seven rotenoid glucosides, were isolated in the period 1982–1999.[203)] A recent review on isoflavonoids covers the period 1997–2004.[204)]

3.4. Neem and azadirachtin-related tetranortriterpenoids

Derived from *Azadirachta indica* (=*Melia azadirachta*) (Meliaceae), neem-based insecticides contain azadirachtin, a complex tetranortriterpene, as the most important active principle (Fig. 2). Enthusiasm for neem was a direct result of the numerous and beneficial reported effects on insects: repellence, feeding and oviposition deterrence, interference with reproduction (sterility in adult female insects) or growth and development (ecdysteroid synthesis and release). An overwhelming research effort has been devoted to study also other trees of the family Meliaceae (*Melia*, *Toona*, *Trichilia*, *Khaya*, *Turrea*, *Aglaia* and many others).[205–207)] A large number of intact triterpenes (protolimonoids, as very likely biosynthetic precursors of the limonoids), and limonoids (degraded triterpenes=tetranortriterpenoids; remainder side-chain as a furan ring) of different ring systems (intact; A-seco; B-seco; C-seco; D-seco) have also been isolated and characterized, along with azadirachtin and closely related compounds (such as the meliacarpins) where the furan ring has been modified.[206)]

Three International Neem Conferences and later on World Neem Conferences (WNC: Bangalore, India, 1993; Gatton College near Brisbane, Australia, 1996; University of British Columbia, Vancouver, Canada, 1999; Mumbai, India, 2002; Coimbatore, India, 2007) have been held and the proceedings from several are available.[208–211)]

3.5. Annonaceous acetogenins

Pesticide activity of Annonaceae species was attributed to their content of isoquinoline alkaloids, according to papers (288) reporting isolated compounds (320) from 150 species/41 genera (out of *ca*. 2,300/130) by 1982. However, the insecticidal activity was traced to a newly isolated type of

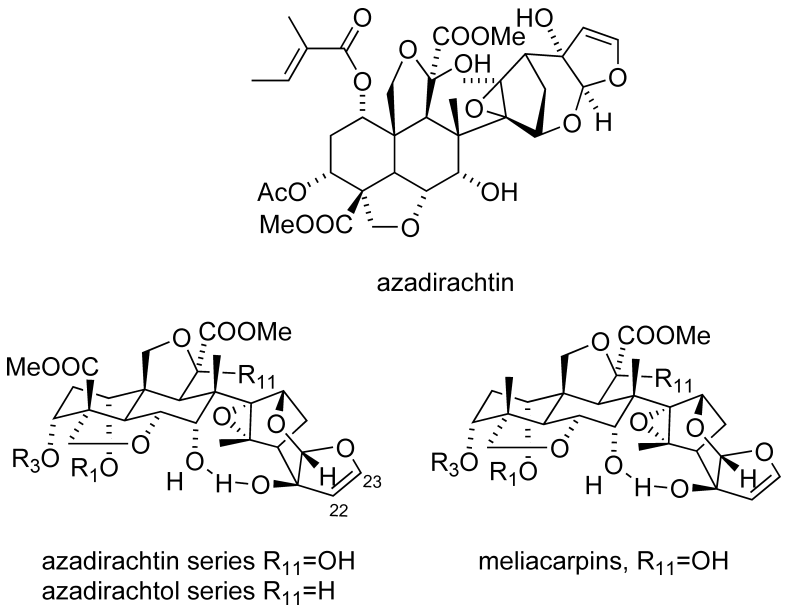

Fig. 2. Azadirachtin and closely related compounds.

compound, displaying a long-chain fatty acid structure (usually C-32 or C-34, such as for asimicin) (Fig. 3). Since then, well over four hundred of them have been reported.[212] Their structures might be described by four partial units as ZHCL. L stands for a γ-lactone head and H for a characteristic heterocyclic fragment, whereas C is a linking chain from L to H, and Z a terminal unit. Both C and Z may contain functional groups (hydroxyl as most usual, occasionally double or triple bond, epoxy ring or carbonyl).

One major group displays a 4-methyl-2-substituted butenolide (L1, as in asimicin) as γ-lactone head. Less common moieties within this group are 4-hydroxy-L1, 4-(hydroxymethylene)butanolide, 3-hydroxybutanolide, and 3-methoxy-4-methylenebutenolide. The second major skeletal type displays a 2-(2-oxopropyl)-4-substituted butanolide (L2) with the corresponding butenolide as less common function (see Fig. 3). The most common heterocyclic fragments are built out of tetrahydrofuran rings [mono-THF, adjacent bis-THF (as in asimicin), non-adjacent bis-THF, adjacent tris-THF] (Fig. 3). Less common heterocycles are tetrahydropyran or epoxy rings. These compounds are potent inhibitors of complex I (NADH:ubiquinone oxidoreductase) in mitochondrial electron transport systems, and slow-acting stomach poisons, a mode of action identical to that of rotenone.

3.6. Piperamides and isobutylamide-related compounds

Insecticidal activity of unsaturated aliphatic isobutylamides was first reported for an active principle of "pyrethri radix" drug (roots of *Anacyclus pyrethrum* DC, Compositae), and named initially as "pyrethrine". Isolated as pellitorine (Fig. 4), this last name was adopted to avoid confusion with the isolate from *Chrysanthemum cinerariifolium* Vis. (Compositae). It was recognized as a complex mixture and finally elucidated the main component as (2*E*,4*E*)-*N*-isobutyl-deca-2,4-dienamide. Other than in Asteraceae species (*Anacyclus*, *Achillea*, *Spilanthes*, *Echinacea*, *Acmella*, *Artemisia*, *Brachycome*)/ Compositae (*Chrysanthemum*), unsaturated aliphatic isobutylamides are common in Piperaceae (*Piper*, *Peperonia*, *Ottonia*), Rutaceae (*Zanthoxylum*, *Heliopsis*), Aristolochiaceae and Poaceae (*Ctenium*). The secondary metabolites iso-

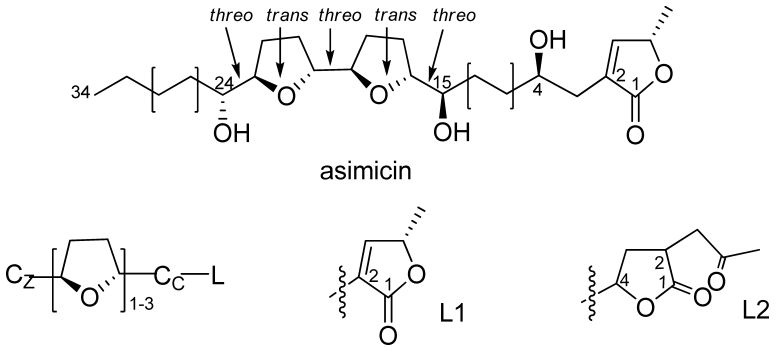

Fig. 3. Acetogenins.

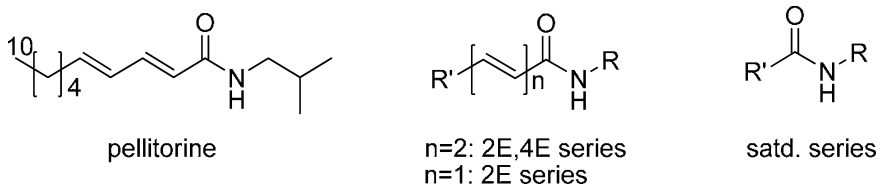

Fig. 4. Isobutylamide-related naturally occurring insecticides.

lated from Piper species were near to six hundred (classified under twelve categories) by 1997[213] and recently the phytochemistry, insecticidal activity and mode of action of *Piper* spp. (Piperaceae) has been reviewed.[214] In addition to isobutylamides, derivatives of pyrrolidine and piperidine, with different degrees of unsaturation and end-groups, are also common amongst the alkaloid/amide group (see Fig. 4). The compounds cause rapid knockdown and kill of flying insects, acting as voltage-dependent blockers of the sodium channel.

3.7. Essential oils

Plant essential oils (or their constituents) have been valued as insecticides, owing to their broad spectrum of activity. Direct toxicity, oviposition and feeding deterrence, repellency or attraction appear to result from interaction with the insect nervous system, either by acetylcholinesterase inhibition or antagonism of the octopamine receptors. The oils consist of complex mixtures (hydrocarbon or oxygenated mono and sesquiterpenes; aliphatics; aromatics; *etc*) with a few major constituents such as 1,8-cineole (rosemary, eucalyptus), menthol (mints), pulegone (pennyroyal), eugenol (clove), *etc*. (Fig. 5). Most come from highly aromatic Lamiaceae (basil, mints, salvia, lavender, sage, rosemary, thyme, *etc*.), Rutaceae (lemon, lime, amyris), Myrtaceae (myrtle, clove), *etc*., plant species also known as flavorings and spices and many considered to have medicinal use.[215,216]

Applications to crop protection (stored product pests), mosquito repellency (citronella oil), control of domestic pests (cockroaches, ants, fleas, *etc*.), *Varroa* mite control, as aphicides and acaricides (cinnamon oil) and urban insect control (eugenol-based products from basil or clove) have been reported.[192,217] A range of insecticide products may be also blends of plant oils, such as clove, peanut oil, thyme, lemon grass, cinnamon or pennyroyal as components.[217]

3.8. Minor plant insecticides

The powdered stemwood of *Ryania speciosa* (Flacourtiaceae) has been used as "ryania insecticide", the activity attributed to ryanodine, a pyrrole-2-carboxylic ester of a complex diterpene (ryanodol) (Fig. 6). Eleven ryanoids have been obtained from different preparations and their insecticidal activity, mammalian toxicity and potency at the Ca^{2+} release chanel complex determined.[218] Related ryanodane diterpenes have been isolated from *Persea indica*.[219]

The veratrum alkaloids derived from *Sabadilla* and *Veratrum* species are effective by blocking the sodium channel in insects, but they also have rather high mammalian toxicity. They were used extensively in Europe and the USA between the late nineteenth and mid-twentieth centuries. Major "sabadilla" bioactive principles, cevadine and veratridine (see Fig. 6), are esters of a steroidal alkaloid named veracevine.[220,221]

Quassia is a weak insecticide derived from the wood of *Quassia amara* L (Simarubeaceae/Ailanthus) and related genera such as *Aeschrion excelsa* ("Jamaica quassia"). Aqueous extracts of Quassia chips were used as an insecticide from the late 18th to the mid 20th centuries. Quassinoids are highly oxygenated degraded triterpenes with a variety of interesting biological properties.[222] Recent new quassinoid isolations have been reported from *Ailanthus, Brucea*, *Picrasma*, *Eurycoma*, and *Simaruba* species.[223,224]

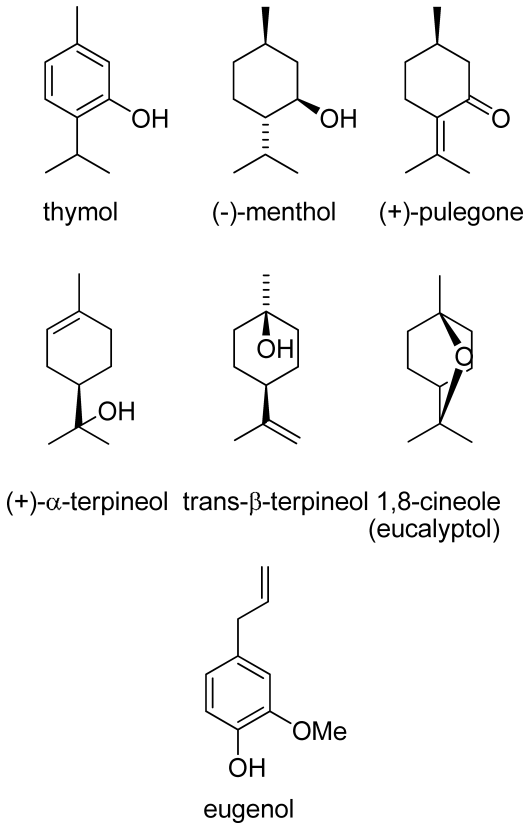

Fig. 5. Essential oils components.

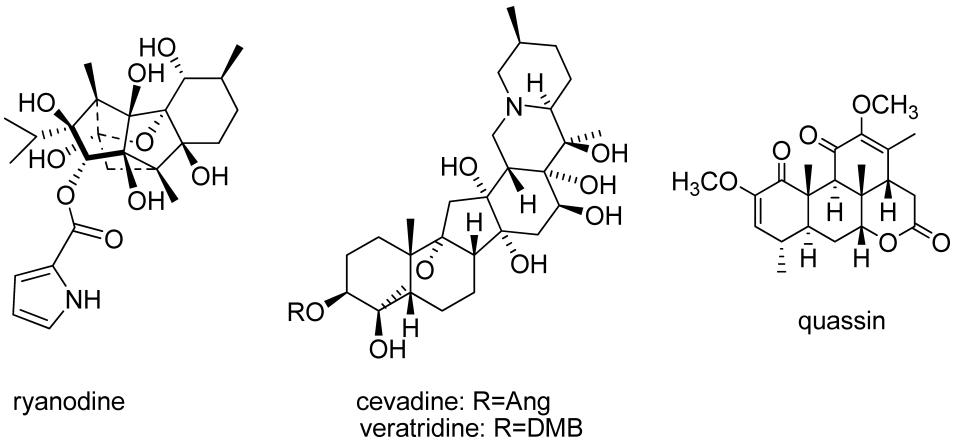

Fig. 6. Minor plant insecticidal compounds.

Conclusion

Natural products have had a great impact in crop protection over the years. It is remarkable the number of compounds that have reached the market place and remain there after a long period of time. A more tolerant regulatory policy from the developed countries will ease the registration of new natural products that together with advances in mass production technologies will benefit the commercialization of new bioactive control agents. On the other hand, utilization of the skeletons of natural products as base for the development of new biopesticides is one of the most successful applications so far of natural products discovery to crop protection. The commercial success of the synthetic pyrethroids is a classic example, with their share in today's market being over 1 billion US $. It is likely that discoveries will continue in the future to identify new areas of chemistry that justify the efforts directed in the synthesis of such natural products analogues.

Pheromones will continue to play a significant role in pest control although its economical impact in the market is limited. There is an ever-increasing interest by consumers in developed countries on the use of much more ecologically acceptable pesticides and more benign production systems. This trend towards a greater market acceptance of new alternatives is coupled with the increasing failures of broad-spectrum pest management tactics due to insecticide resistance or other undesirable side effects resulting thereof. This is the case, for example, of the Colorado potato beetle which is resistant to almost all available synthetic insecticides. Very recently, the male aggregation pheromone (*S*)-3,7-dimethyl-2-oxo-6-octen-1,3-diol, is being investigated as an attractant for control of this pest.[225] Mating disruption has shown remarkable successes, particularly in cotton for the control of the pink bollworm, and in orchards, vineyards and olive groves for control of the olive fly, codling moth and grape berry moth. However, the specificity of the compounds is a major constrain for crops suffering attack from two or more different insect species. Therefore, new blends of attractive formulations targeting more than one species at the same time have been assayed successfully, for example to control the codling moth, the Oriental fruit moth and the grape vine moth.

With regard to living organisms, more work should be done to overcome the drawbacks inherent to the living systems, particularly to enhance the shelf life, the speed of kill, the biological spectrum and the field efficacy. However, great successes have been noticed in the last years, particularly with *Bt* transgenic crops. Molecular biology, not covered in this review, and specially molecular genetics of microorganisms and genetic engineering technology will help to identify the modes of action of many biocontrol agents and set up the basis for the development of new strategies for their subsequent improvement and use.

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