Chapter 7
INSECT PATHOLOGY
IN BIOLOGICAL CONTROL

I. Insect Pathology

A. There are 3 major subdivisions of biological control:
1. BC of athropod pests;
2. BC of weeds; and
3. Insect Pathology.

B. Disease classifications:

1. Infectious: those ailments resulting from actions of living organisms. Pathogenic agents include viruses, bacteria, protozoa, fungi,
rickettsia, and nematodes.
2. Non-infectious: those ailments not resulting from actions of living organisms or micro-organisms.

These include:
a. Those caused by mechanical injury;
b. Those caused by chemical agents;
c. Nutritional disturbances or deficiencies;
d. Those caused by physiological or metabolic arrangements;
e. Genetic diseases;
f. Congenital (non-genetic) abnormalities; and
g. Tumors (abnormal growth of tissue).

C. Insect pathogens can be used as BC agents in IPM in 3 different ways:
1. By the maximum utilization of naturally occurring diseases;
2. By the introduction of insect pathogens into the insect population as permanent mortality factors;
3. By the repeated application of insect pathogens as microbial insecticides for temporary control of an insect pest.

D. **Definition of Microbial Control**: that control which includes all aspects of the utilization of micro-organisms or their by-products in the control of insect pest species.

II. Infectious Pathogens: Inclusion Viruses - submicroscopic, obligate, intracellular, pathogenic organisms.

A. Nuclear polyhedrosis viruses (NPV): virus particles are rod-shaped and are encased in an outer envelope which may enclose one or several virus rods (dependent on the particular NPV). The viruses enclosed in the envelope are occluded (encased) in protein crystals called polyhedra.
1. NPV is normally transmitted by oral ingestion of polyhedra. Ingested polyhedra dissolve, releasing virus rods into the lumen of the insect host's midgut.
2. Symptoms of NPV infected host include:
a. Larval skin darkens and may have yellow patches or appear oily;
b. Skin becomes fragile;
c. Hemolymph becomes turbid;
d. Prior to death, infected larva usually climbs to highest point available and dies; and
e. After death, integument frequently ruptures, releasing millions of polyhedra.

B. Granulosis viruses (GV): GV particles are surrounded by an envelope similar to NPV's envelope. Particles surrounded by these membranes are occluded in a proteinaceous capsule similar to the polyhedral protein that occludes NPV's.
1. GV usually contains only one GV particle rather than many virus particles contained in NPV's and Cytoplasmic Polyhedral Viruses
(CPV's).
2. The fat body of lepidopterous larvae is the primary site of infection.
3. GV's are transmitted orally and via the egg.
4. Symptoms of a GV infected host include:
a. Larvae frequently develop lighter color;
b. Blood of infected larvae is usually turbid and contains large numbers of capsules; and
c. GV infections involving the epidermis cause liquefaction of larvae similar to NPV infections (but only when epidermis is infected).
C. Cytoplasmic polyhedrosis viruses (CPV): particles are not enclosed in membranes as are NPV's, but are occluded in protein crystals similar to those of NPV's.
1. CPV infects the cytoplasm of the midgut epithelium of lepidopterous larvae.
2. Symptoms of CPV infected hosts include:
a. Developmental times of host larvae are longer than normal;
b. Infected larvae appear to have small bodies and large heads; and
c. Body color may change.

III. Bacteria

A. Nonspore-forming bacteria
1. Potential pathogens
2. Live in digestive tracts of most insects
3. Gain entrance to hemocoel due to stress factors
a. Temperature extremes
b. Other pathogens
c. Parasites
d. Poor food
B. Spore-forming bacteria - most important bacterial pathogens
1. *Bacillus popilliae* - causes milky disease in white grubs of scarab beetles such as the Japanese beetle.
a. Transmitted orally by ingestion of spore
b. After ingestion, spores germinate and penetrate the alimentary canal (probably through the Malpighian tubules)
c. Found in blood ca. 30 hrs after initial invasion (30°C)
d. 7 to 10 days later, ca. 2-5 billion spores/larva
e. Larva's blood appears milky due to spores
f. Larva dies shortly thereafter
g. Commercial production of spores must be produced in living hosts - no artificial media available
2. *Bacillus thuringiensis* - very pathogenic to many lepidopterous larvae and immatures in 4 other orders.
a. Transmitted orally
b. When the bacteria sporulates, it forms a toxic crystal (parasporal body)
i. Different lepidoptera exhibit different responses to the various combinations of crystal and *B. thuringiensis* spores. Some are susceptible to action of either the crystal or the spore alone
(some to both):

- Type I: develop a general paralysis and die 1 to 7 hrs after ingestion;
- Type II: do not develop a general paralysis and die 2 to 4 days after ingestion;
- Type III: susceptible to a combination of crystals and spores; and
- Type IV: some lepidoptera not susceptible.

c. After ingestion of spores, first symptom in both Type I and II is cessation of feeding

d. Activity of crystal is dependent on the pH of the larval foregut and midgut (pH 9-10.5) and the action of proteolytic enzymes within the gut

e. The crystal is a protoxin activated by enzymic hydrolysis

f. *Bacillus thuringiensis* can be grown on artificial media. Hundreds of tons are manufactured in the U.S. The variety in the commercial preparation Dipel is *B. thuringiensis var. kurstaki*

IV. Fungi

A. More than 36 different genera of fungi contain species which cause insect disease.

B. Identification of fungus species is difficult.

C. Most fungi are transmitted from one host to another by a spore, usually a conidium

1. Conidia germinate and form a special structure which penetrates the insect cuticle
2. Fungus then grows in insect's body until the insect is filled with mycelia (insect is usually dead at this point)
3. Under favorable conditions, fungus continues to grow and produces structures which protrude through the cuticle and forms spores or conidia

D. Development of fungus infections is dependent on environmental conditions such as high humidity and temperature and high population densities.

E. Examples of Fungi are:

1. *Metarrhizium anisopliae*
2. *Beauveria bassiana*
3. *Entomophthora* spp.

V. Protozoa

A. Flagellates, ciliates, amoebas, coccidians, and haplosporidians have pathogenic relationships with insects, but are considered the least important groups.

B. Neogregarines and microsporidians are the most important entomopathogenic protozoa.

1. They are transmitted orally from one insect to another (have a resistant spore)
2. They can be transmitted transovarially from infected females to her progeny
3. They produce diseases in insects which range from very pathogenic to chronic debilitating infections
4. They can be important naturally occurring mortality factors
5. They are obligate parasites which cannot complete their life cycles in artificial media

VI. Nematodes

A. Several entomopathogenic nematode families (Mermithidae, Steinernematidae, Heterorhabditidae) contain species that are parasites of insects during at least part of the nematodes development.

B. Normally, they have 4 molts between the egg and adult stages and between stages are referred to as juveniles.

C. Most nematodes infect insect hosts as infective stage juveniles.

1. They may enter through the host cuticle or through the midgut
2. After entrance into the hemocoel, juvenile undergoes a period of rapid growth, then leaves the host, enters the soil and molts to form the adult nematode

D. Mating and oviposition occur external to the host in the Mermithidae.
E. Some species kill their hosts upon leaving.
F. Some species transport bacteria when they enter the cavity of the host and the insect dies from bacterial septicemia and the nematode feeds on the bacteria in the dead host tissue.
G. Most nematodes are difficult to culture on artificial media.
H. Only obligate endoparasitic nematodes are found in the genus *Steinernema* (= *Neoaplectana*).

VII. Introduction and application of insect diseases for long-term suppression.

A. Control by insect pathogen is dependent on the relationship between the economic level of the host density and the threshold level of the disease.
1. If the threshold level of the disease is significantly lower than the economic threshold for the pest, then long term control can be achieved by introduction of the pathogen into the system.
2. If the threshold of the disease is significantly higher than the economic threshold for the pest, then only short term control will be achieved analogous to the use of chemical insecticides.
B. Several factors should be considered when introducing a pathogen into an insect population. These include:
1. The concentration of the insect pathogen must be high enough to produce infection in at least some of the insect hosts.
2. The host population in which the pathogen is to be applied should have a relatively high density in order to assure propagation of the pathogen and its survival from one generation to the next.
3. The pathogen should be applied when a susceptible stage of the host is present.

Chapter 8
BIOLOGICAL CONTROL OF WEEDS

I. General Considerations Concerning Weeds

A. Definition of weed: A weed is a plant growing where it is not wanted. In other places or times a weed species may be considered either neutral or of some benefit. Plants are able to establish themselves in almost every conceivable habitat.

B. There are ca. 2,200 weeds of importance in the entire U.S. More than half of the weed species were either accidentally introduced as seed, feed, or ship's ballast contaminants or purposely introduced as ornamentals which escaped cultivation.

C. Direct crop losses from weeds are estimated at 10 Billion dollars annually. Weed control costs 6.2 billion dollars/year of which $3.6 billion is spent on herbicides. Herbicides account for 57% of all pesticides sold. Weeds are the second most important agricultural and forest problem. The amount of losses is second only to soil erosion. Losses far exceed insect problems.

D. Types of losses due to weeds:

1. Quantity and quality of crop yields reduced due to weed competition for common resources;
2. Higher cultivation costs (machinery, fuel, manpower) used for weed control;
3. Reduced seed quality and increased costs for special seed and grain cleaning;
4. Loss in food quality due to off-flavor and color of agricultural products (dairy products especially);
5. Direct injury caused to man and livestock from internal and external poisoning, mechanical injury (thorns), and pollinosis (hay fever);
6. Blockage of waterways due to aquatic weeds which stop water flow, provide environments for mosquito breeding, ruin recreational activities, increase evaporation from reservoirs (through transpiration), and affect color and flavor of drinking water; and
7. Serve as alternate hosts for insect pests and plant pathogens.

II. Considerations in the Biological Control of Weeds:

A. Definition of BC of Weeds: The use of plant-feeding organisms or diseases to reduce the population of a plant species that has risen to the status of a weed. (Note that objective is reduction not eradication of weed species)

B. Some consider BC of weeds as inverse of BC of pests.

C. Theoretical considerations: Many of the worst weeds in the U.S. are of foreign origin. Their aggressiveness in the U.S. may not always be due to the absence of their phytophagous enemies. Aggressiveness of an alien weed in a new area may also be due to more favorable climatic and/or edaphic conditions or relative freedom from competition with other plants. However, the fact that many alien weeds have no or only a few insects specifically feeding on them in the areas of introduction as compared to their country of origin is the basis upon which the study of BC of weeds is based. Theoretically, the introduction of host specific phytophagous insects (minus the insects' own complements of natural enemies) should reduce the abundance of an alien weed.

D. Distinctions in BC of weeds:

1. Phytophagous species rather than entomophagous organisms are utilized
2. There can be "conflict of interest" problems
3. Necessity to apply extremely host specific organisms as BC agents

E. To date introduction of host specific weed feeding insects into regions invaded by alien weeds remains the major approach to BC of weeds. Insects constitute the largest group of natural enemies of weedy plants. Effective BC agents found in the orders: Hemiptera, Homoptera, Thysanoptera, Coleoptera, Lepidoptera, Diptera, and Hymenoptera. Organisms in other groups include plant pathogens, spider mites, fish, snails, ducks, manatee, and parasitic plants.

F. Insects have been used most in BC of weeds because:

1. There is great variety and number of species;
2. They frequently exhibit a high degree of host specificity;
3. They are intimately adapted to their host plants; and
4. There is a great range of natural enemies suited to particular ecological situations.

G. Recent increase in use of plant pathogens. These were once thought not to be host specific but turns out in some cases they are too host specific (will not attack different variety of same plant).

III. Preliminary Considerations to BC Projects on Weeds

A. Eradication of the weed may really be desired (i.e., weed toxic to livestock-may become dead stock), thus BC may not be the answer.

B. Biological control is selective and usually aimed at one species only. It is best used when weed is devastatingly abundant and aggressively spreading in dense stands.

C. Biological control is a relatively slow method. Usually it takes about 5 years between initiation of a project and importation of the first natural enemy. It may require several more years after the introduction of the first natural enemies to get control.

D. There may be risks involved in importing plant-feeding organisms and therefore BC of weeds is often resorted to only after other methods of control have failed or the weed covers large areas of land with (1) such low value (i.e., rangeland) or (2) such rough terrain that cultural or chemical treatments are precluded. However, potential agents are now screened for host specificity.

E. Biological control agents cannot be limited to an area, like chemical and cultural treatments, because living organisms will disperse from the introduction areas into adjacent areas. It must be agreed that everyone regards the targeted plant species as a weed. In some cases not everyone will feel that way. Then a "conflict of interests" arises. This especially happens when one wants wide spread control of a weed. Some might see weed as an ornamental or as in many cases as a valuable food for animals (prickly pear cactus, haole koa). Objections should be resolved prior to initiating the project. This may be done by arbitration (losses vs. gains).

IV. Methods used in BC of weeds (Classical BC, augmentation, conservation)

A. Steps in classical BC of weeds after objectives are defined.

1. Two surveys should be conducted prior to the foreign exploration.

These include:

a. A survey to obtain from the literature and specialists as much information as possible pertaining to the target weed and its natural enemies (refer to notes on foreign exploration - "Accumulation and evaluation of available information")

b. A survey to determine what organisms are feeding on the target weed in the area for which control is desired. This survey is concerned with determining what native insects are already present on the target weed and the extent of the damage they cause. This is very important to do and can result in savings of funds and time. Effective local insects may be present or some insects may already have been introduced.

2. Most of the other steps in foreign exploration are similar to that conducted for natural enemies of arthropod pests (refer to earlier lecture notes mentioned above) until it is time to make the final selections for candidate species to import.
3. Selection of candidate phytophagous insects for importation are based on two criteria:
a. The width of the range of host plants (none can be crop plants); and
b. The plant structures attacked by the plant-feeding stages (larvae, nymphs, adults) that are vital to the plant (growing tips, stems, seeds, etc.).
4. After collection of phytophagous insects, studies can be divided into those conducted (1) abroad where insects were collected and (2) those conducted at the site of introduction (domestic studies)
a. Studies Abroad. These should be conducted near the habitat of the target weed and its natural enemies. This allows the avoidance of the problem of crossing political borders with plants and insects. It also insures a ready supply of material for study and allows time for observations on natural enemy/plant relationships under natural conditions.

<u>Two types of laboratory studies should be conducted:</u>

i. Starvation Tests: provide insect with only one type of plant and see if it will attack it or lay eggs.
ii. Multiple Choice Tests: Follows starvation tests and allows determination of insects' preference for test plants.

<u>Types of plants used in testing include:</u>

i. Representative economic and desirable plants to which the insects would be exposed in the target weed area.
ii. A systematically arranged spectrum of plants related to the target plant.
iii. Plants that are known to contain chemical constituents those are similar to those of the target plant.
iv. Plants from which the candidate insect has been reported collected or associated with in the literature. After above information is obtained then permission is requested to import the candidate species into quarantine in the country of introduction.
b. Domestic Studies. After shipping to quarantine, complete host plant specificity testing is a major phase of work at the domestic laboratory. Additional studies conducted include feeding tests on:
i. Ornamentals, crop, and forage plants that would have been difficult to obtain or grow abroad
ii. Native plants in the vicinity of introduction that provide browse for domestic and wild animals, are necessary as food and shelter for wildlife, or have other redeeming qualities. If any of the above plants were taken abroad they could potentially become a weed problem.
5. Domestic Release. When ready to release a phytophagous BC agent, accumulated data must be submitted to the "Working Group on Biological Control of Weeds" (Joint Committees of the USDA and the US Dept. of Interior (in charge of National Parks). If importation is approved by the "working group" then permits are sought from APHIS (USDA) and the associated state DOA's. When permits are obtained then BC agent can be released.
B. Manipulative methods (Augmentation and Conservation). These methods involve the manipulation of the BC agents or their environment to enhance their effectiveness. Some work has been done in this area with inundative releases of native insects and pathogens.

V. Evaluation of BC of weeds projects through 1984.

A. There have been 174 projects worldwide against 101 weed species. In all, 39% of the projects resulted in successful control and 48% of the 101 weed species were controlled in at least one project.

B. A total of 499 species of natural enemies have been released against the 101 weed species in 70 countries. Establishment of natural enemies occurred in 64% of the releases. Agents were effective in controlling the weed in 29% of all releases or 47% of the releases which resulted in establishment.

C. Releases of native natural enemies resulted in effective control in 62% of the cases compared to 29% when exotic natural enemies were used. This may result from native agents being used in inundative programs compared to exotic agents which are often released without any follow up programs or additional aid.

VI. Examples.

A. *Lantana camara* L.: First major program for BC of weeds. In 1902, sugarcane planters in Hawaii were concerned with spread of weed. HSPA sponsored exploration for phytophagous BC agents in 1902. Albert Koebele searched for BC agents in Mexico. Attempted to introduce 18 insects into Hawaii, but only 8 species became established (problems encountered due to difficulties in transportation - slow rail and boat transportation). At that time no specificity tests were conducted and 2 of the released organisms fed on other plants (eggplant, pepper, and some ornamentals). Despite that, moderate control was achieved in the drier areas of the islands. More control was desired and from 1954-1970 (50 years later) 7 additional species were established in Hawaii. A noctuid moth, *Hypena strigata* F., was extremely effective in the drier areas of Hawaii during the cooler months. During the hotter months, the lace bug, *Teleonemia scrupulosa* Stal., was effective in the dry areas. It was one of the original 8 species released in 1902. In the wetter areas of the islands, 3 beetles appear to have reduced the problem somewhat. Two of the beetles (*Octotoma scabripennis* (Guerin-Meneville) and *Uroplata girardi* Pic.) are chrysomelids which mine the leaves. The other a cerambycid, *Plagiohammus spinipennis* (Thomson), bores into the stems of the plants. Currently work in being conducted in Australia for control of Lantana. The lace bug has done quite well against some Lantana varieties in some areas, but complete control is still lacking. Insects <u>Introduced into Hawaii for Biological Control of Lantana (Established species only)</u>

1. Original Introductions (1902--Koebele)

Lantana butterflies (Lycaenidae)
smaller - *Strymon bazochii gundlachianus* (Bates)
larger - *Thmolus echion* (L.)
Lantana lace bug (Tingidae): *Teleonemia scrupulosa* Stal
Lantana galls fly (Tephritidae): *Eutreta xanthochaeta* Aldrich
Lantana seed fly (Agromyzidae): *Ophiomyia lantanae* (Froggatt)
Lantana plume moth (Pterophoridae): *Lantanophagapusillidactyla* (Walker)
Lantana tortricid moth (Tortricidae): *Epinotia lantana* Busck
Lantana leafminer (Gracillariidae): *Cremastobomycia lantanella* Busck

2. Recent Introductions (1954-1970)

Lantana cerambycid, *Plagiohammus spinipennis* Thomson - 1960
Lantana defoliator caterpillar (Noctuidae), *Hypena strigata* (Fabricius) - 1957
Lantana stick caterpillar (Noctuidae), *Neogalea esula* (Druce) - 1955
Lantana leaf beetles (Hispidae), *Uroplata girardi* Pic - 1961;

Octotoma scabripennis (Guerin-Meneville) - 1954
Lantana leaf-tier (Pyralidae), *Salbia haemorrhoidalis* Guenee - 1956
Second lantana lace bug (Tingidae), *Leptobyrsa decora* Drake - 1970
B. *Opuntia* spp., Prickly pear cacti: Greatest BC success with weeds. In early part of this century (1910 - 1920), prickly pear became extremely bad in Australia where it covered up to 60 million acres (the cacti excluded all grasses and shrubs). Searches for natural enemies began in 1920 under the auspices of the Commonwealth Prickly Pear Board. A total of 150 species were discovered whose life cycles were restricted to *Opuntia* spp. The best insect found was the tunneling caterpillar, *Cactoblastis cactorum* (Berg) from Argentina. Releases led to almost complete control. It was ineffective in cooler regions where cochineal scale insects (*Dactylopius* spp.) are required for control. After the successes in Australia, *Cactoblastis* and the dactylopiid scales were introduced into South Africa, India, and Hawaii where partial to complete control was achieved in various areas.
C. *Hypericum perforatum* L., St. Johnswort or Klamath weed: First weedy plant for which insects were imported into the continental U.S. In 1952, ca. 2.3 million acres were infested in northern California plus 2.7 million acres in the surrounding northern states. USDA and the University of California established a joint research team in 1944. The first insect liberated in the field was the northern European leaf beetle, *Chrysolina hyperici*, in 1945. The second species released was the southern European leaf beetle, *Chrysolina quadrigemina*, in 1946. The insects were obtained from Australia due to the political problems in Europe at the time (WWII). Immediate success was achieved with *C. quadrigemina* in sunny areas. Further control in shady areas was achieved with the introduction of a flathead borer, *Agrilis hyperici* whose larvae bore into the root crown and tap root of the plant. species.