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IMPACT OF DISEASES ON INSECTS AND PROCEDURES FOR DETECTING AND ELIMINATING THEM FROM CULTURES PRIOR TO RELEASE FOR BIOLOGICAL CONTROL

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ABSTRACT

Insect diseases of the baculovirus and fungi groups cause acute infections terminating in death, whereas members of the microsporidia and cytoplasmic viruses produce debilitating symptoms which cause reductions in fecundity and longevity, and a general loss of vigour. Pathogens may be transmitted externally or in some cases transovarially.

The elimination of diseases from an insect colony requires time, cleanliness, and patience. Procedures to accomplish this task and steps to be considered prior to the release of candidate insects will be discussed.

INTRODUCTION

The release of beneficial insects for the purpose of suppressing pests has been well documented in general by DeBach (1965) and Anonymous (1971), and for weeds by Bennett (1974). The causes of the success or failure of a biological control agent have often been neglected. Although the failure of a biological control agent to adapt to a new ecosystem may be due to ecological and physical factors, the prospect of the candidate organism being constrained by its own natural enemies or those of closely related species (particularly diseases) has not been adequately considered. This aspect has, however, been accented in recent years, particularly by researchers working with insects for aquatic weed control.

Insects are subject to a wide range of pathogens including bacteria, viruses, protozoa, fungi, rickettsia, nematodes, and other microorganisms. Members of these groups exert varying degrees of influence on their hosts, ranging from debilitating effects to wide-spread epizootics or epidemics. The purpose of this paper is to address the major types of diseases associated with insects, particularly those orders being considered as biological control agents for weed pests.

ETIOLOGY OF INSECT DEATH

A high incidence of mortality, particularly in laboratory or insectary reared insects, is related to non-pathological causes including such predisposing factors as nutritional, physical, and genetic stresses.

Physical factors

Various ecological factors may act as 'stressors' on insect colonies, predisposing the individuals to death or opportunistic microorganisms capable of causing death. Generally the primary stressors include temperature, humidity, and handling. Significant changes in the optimal temperature ranges of the insect usually cause death independent of microbial involvement; however marginally high temperatures may cause death due to opportunistic bacteria or viruses.

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The major stress factor usually encountered in the mass rearing of insects is 'overcrowding' which causes temperature changes, behavioural changes, or damage due to cannibalism. Several authors have reported the interaction of insect crowding and the opportunistic bacterium *Serratia marcescens* (Steinhaus 1959, McLaughlin and Keller 1964, and Greany *et al.* 1977). I have routinely isolated this bacterium from specimens submitted for diagnosis by persons who culture massive numbers of crickets for sale as fishing bait. Thorough disinfection and reduction in insects per container usually eliminates cricket mortality due to *S. marcescens.*

Jaques (1962) reported that larvae of *Trichoplusia ni* reared in crowded conditions showed an eightfold increase in mortality due to *nuclear polyhedrosis virus* (NPV). Crowding also reduced rates of growth and development, and influenced feeding behaviour.

The occurrence of *cytoplasmic polyhedrosis viruses* (CPV) and some microsporidia may also be pronounced in lepidopterous larvae cultures predisposed by crowding.

Nutritional factors

In general insects require for growth the ten common essential amino acids, six or more B vitamins, a sterol such as cholesterol, and a number of inorganic salts. There is, as might be expected, a wide range in nutritional requirements among insect species and between larval and adult stages.

Slow or arrested growth and development, high mortality of the immature stages, and reduced or no reproduction in the adult, are familiar symptoms of nutritional defects.

Genetic factors

The study of genetics has long used the genus *Drosophila* as an experimental tool to demonstrate genetic variations. Although the occurrence of genetic mutants in insect cultures is probably insignificant, extensive inbreeding utilizing a limited genetic base can cause important changes. Genetic effects are usually characterized by aberrations in the developmental process; i.e., morphological malformations.

It is essential that an adequate genetic base be maintained in insect cultures, either by introduction from other culture stocks or from 'wild' stock that is free of disease.

THE ROLE OF PATHOGENS IN INSECT MORTALITY

Entomogenous pathogens of the baculovirus and fungi groups cause acute infections terminating in death, whereas members of the microsporidia and CPV groups produce debilitating diseases which cause reductions in fecundity and longevity, general stress, and eventual death. Bacterial pathogens are usually opportunistic and cause mortality following some form of stress.

Baculoviruses

The baculovirus group consists of two types: NPVs and *granulosis viruses* (GV). This group is characterized by rod-like virions, composed of DNA protected by a nucleoprotein crystal. Both NPV and GV replicate in the nucleus of cells of the epidermis, fat body, blood cells, and tracheae. In some sawfly larvae, NPV replication occurs in the nuclei of midgut cells.

Although there are several groups of viruses attacking insects, our discussion will be restricted to the most common ones.

Nuclear polyhedrosis viruses

As the name implies, the nucleus is infected and polyhedral protein crystals are formed.

Symptomatology and pathology – As is the case with all viruses the NPVs must be consumed by a susceptible host. Then the protective nucleoprotein is dissolved by digestive enzymes, thus releasing the free virions which are the infective units. After replication, virions are released into the bloodstream where they are transported to susceptible tissues (usually fat body).

Hypertrophy of the cell nucleus is the first sign of NPV infection. The nucleus eventually fills with mature polyhedra and ruptures, destroying the cell and releasing polyhedra and free virions. The polyhedra are not capable of re-infecting other cells; however the free virions can continue the process. Other tissues are infected until the complete larval system is destroyed.

Generally six to seven days are required for the complete infection-death process to occur. The first external symptom observed in the larva is a change in epidermal coloration caused by the disintegration of fat body cells (hence the old term 'jaundice'). In the final stages of infection the epidermis becomes fragile and the larva fails to respond to probing. After death the cadaver readily ruptures, releasing its liquid, polyhedra-filled contents.

Types of NPVs – Based on the number and arrangement of the virions within the protein crystal the NPVs are divided into two groups. In cases where the virions are arranged in single units the term '*single embedded virus*' (SEV) is applied. In contrast, some NPVs have the virions arranged in pockets of two to ten or more and are referred to as '*multiple embedded viruses*' (MEVs).

Host specificity of NPVs – In general the SEVs are host specific with some infecting up to the generic level in insects. In contrast, the MEV group is often infective at the family level (Vail *et al.* 1973).

In late stages of NPV infection, lepidopterous larvae exhibit the behavioural characteristic of seeking the highest available point on host plants or rearing containers. At death the flaccid cadavers are generally found hanging by their prolegs in the typical 'wilt' fashion.

The only report to date of an NPV associated with aquatic Lepidoptera is by Webb and Levine (1975) of the yellow water lily borer, *Bellura gortynoides*. I have not observed any type of virus infection in *Arzama densa*, *Sameodes albiguttalis*, or *Acigona infusella*.

Granulosis viruses

The GVs differ from the NPVs in nucleoprotein morphology and in the fact that the crystals generally contain only one to two infected virions.

Symptomatology and pathology – The GV group is thus far found only in lepidopterous larvae and the fat body is the primary tissue attacked. Typical external changes in coloration occur as the fat tissue disintegrates. GV-killed larvae are similar to NPV-killed larvae in that both are flaccid, liquefied, and found hanging from the prolegs.

The infective process for GVs is generally much longer than that for NPVs, often ranging from 15 to 30 days.

Transmission of GVs – Attempts have failed to establish the transovarial transmission of GVs in lepidopterous insects (Etzel and Falcon 1976, David and

Taylor 1976, and Melamed-Madjar and Raccah 1979). The authors did, however, show that transstadial transmission (larvae to pupae to adult) did occur to some degree.

Cytoplasmic polyhedrosis viruses

As the name implies the cell cytoplasm is the site of replication and polyhedral crystal formation. The group is characterized by spherical virions composed of RNA protected by a nucleoprotein crystal. The CPV's attack the midgut cells of lepidopterous larvae, causing altered growth patterns.

CPV-infected larvae exhibit stunted bodies with disproportionately large head capsules, and a loss of appetite resulting in a reduced growth rate. Infected larvae do not respond to probing and may linger for extended periods before dying.

The effects of CPVs on their hosts are debilitating rather than acute. Characteristically CPVs cause reduction in vigour, extended development time, production of small or malformed pupae (Simmons and Sikorowski 1973, Bullock *et al.* 1970), reductions in fecundity and longevity, and aberrations in adults (Neilson 1965, Sikorowski and Thompson 1979, Vail and Gough 1970, and Vail *et al.* 1969).

Subtle CPV infections in laboratory colonies render the culture virtually useless for experimental purposes. This virus group has a wide range, and rearing facilities should be arranged to prevent cross-infection among different insect species.

As previously mentioned, in laboratory colonies carrying sub-lethal CPV levels the infection is accelerated following the introduction of stress factors such as temperature changes, crowding, and poor nutrition. Crowding encourages the transmission of polyhedra passed in the faeces or regurgitated by infected larvae.

Members of the CPV group are readily transmitted transovarially on the surface of the egg (Mery and Dulmage 1975, Sikorowski *et al.* 1973).

Protozoa

A wide range of symbiotic or parasitic protozoa is associated with insects. For the purpose of this discussion I have restricted my comments to the microsporidia, the group most often causing acute infections in insect colonies and showing the greatest promise as microbial control agents.

Microsporidia

Microsporidia and other members of the same subphylum are characterized by the presence of a spore stage with one or more polar filaments and sporoplasms. The microsporidia are characterized by spores of unicellular origin, a single sporoplasm, and usually one long tubular polar filament through which the sporoplasm emerges. The spore is an important taxonomic feature, particularly with respect to the arrangement of this tubular polar filament. Most species are parasites of arthropods, although many species are found in fishes.

Symptomatology and pathology – When ingested by a susceptible insect the tubular polar filament is extruded and the sporoplasm freed into the lumen of the host, where vegetative development ensues within midgut cells. Later the infection reaches the fat body or other tissues, where the pathogen develops within the cytoplasm. Death may occur from the direct destruction of host cells by the microsporidia or from septicemia by secondary bacterial infection from the microsporidia-damaged gut cells.

Studies have shown that members of the genus *Nosema* can be transovarially transmitted within the insect egg (Brooks 1968, Kellen and Lindegren 1973). In cases where this phenomenon exists special selection procedures have to be used in order to obtain microsporidia-free insect cultures.

Infected insects such as certain lepidopterous and coleopterous species may exhibit such symptoms as reduced feeding, slow growth, colour changes in localized areas of the body, incomplete metamorphosis, pupal aberrations, and reduced adult fecundity and longevity (Gaugler and Brooks 1975).

Microsporidia associated with aquatic weed insects – Host specificity of the microsporidia varies widely. Some, such as *Varimorpha* (=*Nosema*) *necatrix* are cosmopolitan with a wide host range. This species is one of the most virulent of the microsporidia and is under consideration as a microbial control agent for agricultural pests.

I have routinely isolated *V. necatrix* from field and laboratory specimens of *A. densa* in Florida and *A. infusella* and *S. albiguttalis* from Trinidad. These three lepidopterous species have either been released or have been evaluated for potential biological control of water hyacinth in the U.S., Trinidad, and Australia. I might note here that the *V. necatrix* isolate from *A. densa* in Florida in 1974 was one of the most virulent strains known at that time against a wide range of agricultural pests. Since *A. densa*, *S. albiguttalis*, and *A. infusella* inhabit the same niche in the aquatic ecosystem and are all susceptible to *V. necatrix*, caution should be exercised when considering the release of any of these insects into an area where at least one of them already exists with a natural infection. Populations of *S. albiguttalis* and *A. infusella* are under heavy pressure by *V. necatrix* in Trinidad, as is *A. densa* in Florida.

A *Nosema*-like microsporidia was reported in shipments of *Neochetina eichhorniae* from Argentina by the CIBC station in Bangalore, India in 1974. Specimens could not, however, be confirmed by Dr J. Weiser, a renowned expert in the field. I made extensive surveys of *N. eichhorniae* and *N. bruchi* field speciments in Argentina, Trinidad, and lab cultures in the U.S. for *Nosema* infection. I observed microsporidia spores in only one pupal specimen of *N. bruchi* in Argentina in 1975, and I have concluded that any involvement of microsporidia in the *Neochetina* complex is secondary in nature and not the result of a primary pathogen.

It is my understanding that a *Nosema*-like infection has been observed in *Samea multiplicalis* by Dr Ken Harley and his group at the CSIRO Long Pocket Laboratories, Brisbane.

Fungi

Insects are attacked by several families of fungi, more specifically the Phycomycetes (*Entomophthora, Coelomomyces*), Ascomycetes (*Ascosphaera, Cordyceps*), and Deuteromycetes, better known as the Fungi Imperfecti (*Beauveria, Metarrhizium*). For the purposes of this discussion I will discuss only the Fungi Imperfecti group, which contains the majority of primary pathogens affecting the insects which have either been released as weed control agents or are under investigation.

Symptomatology and pathology

The infective unit of entomogenous fungi is the spore or conidium. Conidia germinate on the cuticle and penetrate with the assistance of enzymes or mechanical forces. Yeast-like fragments of mycelium called hyphal bodies are produced, float freely, and multiply within the blood system of the host insect.

During this stage some species produce toxins capable of causing death without tissue invasion. In most cases tissues, particularly of the fat body, are invaded. After three to four days the hyphae fill the body cavity, rendering it firm. Death follows shortly thereafter. Conidiophores then erupt through the cuticle and produce external spores or conidia. The cycle from germination to sporulation usually takes six to seven days. Large numbers of conidia are produced per cadaver. For example, Kish and Allen (1976) reported the production of conidia for the entomogenous fungus *Nomuraea rileyi* to reach 13 x 10^6 per mm^2 on larvae of *Pseudoplusia includens*, a lepidopterous pest of soybean. Entomogenous fungi require high humidity and temperature ranges of 28 to 32°C.

Fungi associated with biological control agents

A number of fungi isolated in my laboratory from insect candidates for biological control of aquatic weeds since 1973 are presented in Table 1.

Table 1. Fungi isolated from insects considered as biological control candidates for water hyacinth 1973–1977.

Insect	Fungal pathogen	Collector
Sameodes albiguttalis	*Penicillium, Aspergillus, Geotrichum, Paecilomyces, Beauveria*	G. Buckingham
Neochetina eichhorniae	*Beauveria bassiana* *Metarrhizium anisopliae*	D. Perkins
Neochetina bruchi	*Beauveria brongniartii* *Beauveria bassiana* *Aspergillus flavus*	D. Perkins
Arzama densa	*Paecilomyces fumoso-rosus*	T. Center

Host range and transmission

Entomogeous members of the Fungi Imperfecti have wide host ranges across a number of insect familes. *Beauveria bassiana* and *Metarrhizium anisopliae* are cosmopolitan insect pathogens which are generally transmitted by direct exposure to conidia.

Bacteria

Bacteria pathogens associated with insects include both sporeformers and non-sporeformers. The principle sporeformers are members of the *Bacillus cereus* group. The major member of this group is *B. thuringiensis* which is now a world-wide commercial product used to control a wide range of agricultural lepidopterous pests.

The primary bacteria associated with laboratory insect cultures fall into the non-sporeforming group. They attack a wide range of insect families and are generally considered secondary or 'opportunistic'.

Syptomatology and pathology

Non-sporeforming bacteria multiply extracellularly in the blood system of insects and produce a lethal septicemia. Infected larvae show signs of sluggishness and diarrhea. In this discussion I will only consider the two most

encountered species, *Pseudomonas aeruginosa* and *S. marcescens*.

P. aeruginosa is the major bacterium associated with grasshoppers. The original infecting bacteria occur in the foam of the egg pod. The nymphs die rapidly in the laboratory or field and the disease may become epizootic. The bacteria are transmitted when healthy individuals feed on contaminated food or water, or on their sick or dead siblings.

As previously mentioned, *S. marcescens* has been shown to be closely associated with stressed Lepidoptera and Coleoptera. Typically the bacterium produces a bright red chromogenic pigment readily seen in infected eggs (Bell 1969) and moribund larvae and adults. It is interesting to note that *S. marcescens* has been shown to be transmitted to hymenopterous parasites (Bracken and Bucher 1967, Bucher 1963, and Greany *et al.* 1977).

PROCEDURES FOR OBTAINING CLEAN INSECT CULTURES

Prior to the release of an insect for biological control purposes steps should be taken to ensure clean or disease-free stock. In order to avoid post-release infection, care must be taken to determine not only the candidate insect's pathogens, but also those of closely related species. In order to ascertain the effect of pathogens on the candidate insect population, the following steps should be taken with the assistance of an insect pathologist or microbiologist:

(a) determine the major pathogens associated with the candidate insect in its native habitat;
(b) determine the diseases associated with closely related insect species already occupying the target habitat;
(c) determine the susceptibility of the candidate insect to pathogens associated with the closely related species; and
(d) evaluate the impact of the pathogens on the potential success of the candidate species.

Guidelines for insect rearing

In order to establish and maintain a disease-free insect culture in the laboratory or insectary, proper care must be taken. The primary factors to be considered include the following:

(a) Rearing facilities should be restricted to personnel directly involved in the rearing.
(b) Prevent contamination from outside air by adequate filtration.
(c) Artificial diets capable of supporting adequate growth of the candidate insect should contain certain inhibitors such as formalin to help preserve the medium. A special 'off-limits' room should be maintained for the purpose of media preparation. Special care should be taken to clean working surfaces with a strong disinfectant. If plant tissues are used as the rearing substrate, a confined, screened facility should be used. If possible, this facility should be away from the rearing area but close enough for ready access.
(d) Separate rooms should be maintained for holding larvae and adults. Adult holding areas generally require adequate space for emergence and mating cages.
(e) Maintaining adequate temperature and humidity regimes is extremely important.

(f) The use of disposable rearing containers for larvae will maintain a degree of cleanliness. Cages for adults as well as all instruments used in the rearing process should be able to withstand autoclaving or disinfecting.
(g) Supplies should be stored in separate quarters.

Methods for eliminating disease from insect cultures

Maintaining a disease-free insect culture is a challenge to say the least. Some of the earliest research conducted on insect diseases was by pioneers such as Pasteur who worked on the microsporidia 'pebrine' disease of the silkworm, *Bombyx mori*. We are still trying to master such maladies as sacbrood, chalkbrook, and *Nosema* disease of the honeybee.

In cases where disease is evident in an insect culture, the steps required will be determined by the pathogen involved. Therefore positive identification of the causative agent is necessary before laborious and expensive procedures are attempted. Historically, attempts have been made to use disinfectants, antibiotics, and other chemicals to eliminate disease. In some cases it may be necessary to enter into extensive breeding and selection processes.

Use of chemicals to eliminate diseases

Antibiotics, oxidizing agents, and fumigants have been used to suppress diseases in insect cultures.

Antibiotics and related compounds – Various commercial preparations of antibiotics have been investigated for the elimination of bacteria from insect cultures. McLaughlin and Keller (1964) reported that the use of Novabiocin and Tetracycline eliminated *S. marcescens* from colonies of *Anthonomus grandis*. Afrikian (1960) successfully used Streptomycin, Biomycin, Terramycin, and Tetracycline to suppress a series of bacteria associated with the silkworm in Russia. King *et al.* (1975) suppressed a *S. marcescens* outbreak in the sugarcane borer, *Diatraea saccharalis*, and its dipterous parasite, *Lixophaga diatraea*, using methenamine mendelate and nalidixic acid. However, Greany *et al.* (1977) failed to control *S. marcescens* with these materials in similar systems involving the Caribbean fruit fly and its parasitoid.

Several attempts have been made to use chemicals to suppress microsporidia in insect cultures. Lynch and Lewis (1971) reported that the antibiotic fumagillin (Fumidil B) suppressed the microsporidia *Perezia pyraustae* in the European corn borer as long as the compound was present in the diet; however the infection recurred following the elimination of the antibiotic.

Hsiao and Hsiao (1973) reported that benomyl, a systemic fungicide, eliminated *Nosema* from colonies of the alfalfa weevil. Brooks *et al.* (1978), however, failed to eliminate *N. heliothidis* from the corn earworm using benomyl even in high concentrations.

It should be noted that antibiotics suppress not only target bacteria but also essential commensal bacteria in the insect digestive tract which assist in digestion. Generally, bacterial infections, such as *S. marcescens*, can be prevented by keeping working areas clean. The presence of these organisms are also a sign of stress and indicate that a re-evaluation of the procedures used in the rearing system may be necessary.

Disinfectants and fumigants – Disinfectants have proven effective in disease prevention in insect cultures. Sodium hypochlorite solutions of one to two per

cent are routinely used in most rearing facilities to disinfect eggs of external virus contaminants (Thomas 1974). Vail *et al.* (1968) showed that the incorporation of 10 per cent formalin in artificial diets restricted NPV activity in the cabbage looper. Tompkins and Cantwell (1975) successfully eliminated NPVs and GVs from an insectary using the fumigant ethylene oxide. The treatment did not affect the CPVs in the test.

Martignoni and Milstead (1960) reported the successful sterilization of insect larvae and pupae using the quaternary ammonium compounds Zephiran chloride and Hyamine 10-X.

Ultraviolet Light (UV) – Considerable data have been generated concerning the susceptibility of insect pathogens to UV. Kelly and Anthony (1979) successfully eliminated spores of the microsporidia *N. algerae* using UV. Witt and Stairs (1975) inactivated an NPV of the greater wax moth using 2537Å. Natural sunlight is highly effective against all pathogen groups.

Methods for eliminating transovarially transmitted pathogens

In cases where pathogens such as CPVs and microsporidia are transmitted transovarially within the egg there is little choice but to undertake a selective breeding program. Since such an effort is laborious and costly, the value of the culture should first be evaluated.

Generally, selected adult pairs are placed in individual mating chambers where they breed and the female oviposits. A significant number of these pairs is necessary to maintain an adequate genetic base.

The eggs are disinfected with a suitable oxidizing agent and placed individually in small containers of food for the larvae to feed on as they emerge. Larvae are maintained individually until they pupate.

The resulting pupae are surface-sterilized with an oxidizing agent, sexed, and placed in a chamber along with an individual of the opposite sex. After the emerging pair have mated the process is repeated.

Stresses such as temperature changes and starvation contribute to the appearance of debilitating diseases in the larvae of lepidopterous insects. A sudden temperature change causes enough stress for the expression of CPVs and microsporidia. Cold stress can be achieved by overnight exposure to normal refrigerator temperatures; heat stress can be achieved by increases of two to four degrees Celsius above the normal rearing temperature. Stress results when larvae are starved for 24 to 48 hours. Generally, larvae are stressed first when they are young (second instar), and again in a later instar.

Any individual or stage that appears diseased should be discarded along with the container and substrate. Random samples of apparently healthy insects should be examined for disease using staining procedures if available and light microscopy or electron microscopy. A similar procedure was used successfully by David and Gardiner (1966) for the European cabbage worm, *Pieris brassicae*. A similar approach has been employed at C.S.I.R.O. in Brisbane to clean up cultures of *Sameodes albiguttalis* and *Samea multiplicalis*.

Determination of 'clean' stock prior to release

Prior to the official release of a candidate insect as a biological control agent a final attempt should be made to confirm that the stock is disease-free. The following is the procedure we used in assisting U.S. scientists to determine if *N. eichhorniae* was disease-free prior to release:

a) A random sample of 20 larvae was prepared for microscopic examination. First, smears were made with Giemsa stain for the detection of vegetative stages of microsporidia.
b) Second, fat body tissue was examined under phase microscopy to screen for microsporidia spores or polyhedral bodies of NPV or CPV.
c) Next, the same tissues were prepared for electron microscopy to detect the presence of vegetative stages of microsporidia or virions of NPV, CPV, or GV.

This procedure was repeated once a week for three weeks.

SUMMARY

Insect pathogens can determine whether or not an insect becomes established in a new ecosystem. Although we have recorded a long list of successful biological control agents, we have had some unexplained failures. The question is: Could undetected diseases have been released with the insect, causing it to fail? This question must be resolved before a definitive biological control program can be established.

REFERENCES

Afrikian, E.G. (1960). Causal agents of bacterial diseases of the silkworm and the use of antibiotics in their control. *J. Insect Pathol.* 2:299–304.

Anonymous (1971). Biological Control Programmes Against Insects and Weeds in Canada 1959–1968. Commonwealth Inst. Biol. Control Tech. Commun. No. 4, (England:Commonwealth Agr. Bur.), 266 p.

Bell, J.V. (1969). *Serratia marcescens* found in eggs of *Heliothis zea*: Tests against *Trichoplusia ni*. *J. Invertebr. Pathol.* 13:151–2.

Bennett, F.D. (1974). Biological control. In 'Aquatic Vegetation and its Use and Control'. pp.99–106. (Ed. D.S. Mitchell.) (Paris:UNESCO), 135 p.

Bracken, G.K., and Bucher, G.E. (1967). Mortality of hymenopterous parasite caused by *Serratia marcescens*. *J. Invertebr. Pathol.* 9:130–2.

Brooks, W.M. (1968). Transovarian transmission of *Nosema heliothidis* in the corn earworm, *Heliothis zea*. *J. Invertebr. Pathol.* 11:510–2.

Brooks, W.M., Cranford, J.D., and Pearce, L.W. (1978). Benomyl: Effectiveness against the microsporidian *Nosema heliothidis* in the corn earworm, *Heliothis zea*. *J. Invertebr. Pathol.* 31:239–45.

Bucher, G.E. (1963). Transmission of bacterial pathogens by the ovipositor of a hymenopterous parasite. *J. Invertebr. Pathol.* 5:277–83.

Bullock, H.R., Martinez, E., and Stuermer, C.W., Jr. (1970). Cytoplasmic-polyhedrosis virus and the development and fecundity of the pink bollworm. *J. Invertebr. Pathol.* 15:109–12.

David, W.A.L., and Gardiner, B.O.C. (1966). Breeding *Pieris brassicae* apparently free from granulosis virus. *J. Invertebr. Pathol.* 8:325–33.

David, W.A.L., and Taylor, C.E. (1976). Transmission of a granulosis virus in the eggs of a virus-free stock of *Pieris brassicae*. *J. Invertebr. Pathol.* 27:71–5.

DeBach, P. (Ed.) (1965). 'Biological Control of Insect Pests and Weeds.' (Reinhold Publ. Corp.:New York), 844 pp.

Etzel, L.K., and Falcon, L.A. (1976). Studies of transovum and transstadial transmission of a granulosis virus of the codling moth. *J. Invertebr. Pathol.* 27:13–26.

Gaugler, R.R., and Brooks, W.M. (1975). Sublethal effects of infection by *Nosema heliothidis* in the corn earworm, *Heliothis zea*. *J. Invertebr. Pathol.* 26:57–63.

Greany, P.D., Allen, G.E., Webb, J.C., Sharp, J.L., and Chambers, D.L. (1977). Stress-induced septicemia as an impediment to laboratory rearing of the fruit fly parasitoid *Biosteres (Opius) longicaudatus* (Hymenoptera:Braconidae) and the Caribbean fruit fly *Anastrepha suspensa* (Diptera:Tephritidae). *J. Invertebr. Pathol.* 29:153–61.

Hsiao, T.H., and Hsiao, C. (1973). Benomyl: A novel drug for controlling a microsporidian disease of the alfalfa weevil. *J. Invertebr. Pathol.* 22:303–4.

Jaques, R.P. (1962). Stress and nuclear polyhedrosis in crowded populations of *Trichoplusia ni* (Hubner). *J. Insect Pathol.* 4:1–22.

Kellen, W.R., and Lindegren, J.E. (1973). Transovarian transmission of *Nosema plodiae* in the Indian-meal moth, *Plodia interpunctella*. *J. Invertebr. Pathol.* 21:248–54.

Kelly, J.F., and Anthony, D.W. (1979). Susceptibility of spores of the microsporidian *Nosema algerae* to sunlight and germicidal ultraviolet radiation. *J. Invertebr. Pathol.* 34:164–9.

King, E.G., Bell, J.V., and Martin, D.F. (1975). Control of the bacterium *Serratia marcescens* in an insect host-parasite rearing program. *J. Invertebr. Pathol.* 26:35–40.

Kish, L.P., and Allen, G.E. (1976). Conidial production of *Nomuraea rileyi* on *Pseudoplusia includens*. *Mycologia* 68:436–9.

Lynch, R.E., and Lewis, L.C. (1971). Reoccurrence of the microsporidian *Perezia pyraustae* in the European corn borer, *Ostrinia nubilalis*, reared on diet containing Fumidil B. *J. Invertebr. Pathol.* 17:243–6.

Martignoni, M.E., and Milstead, J.E. (1960). Quaternary ammonium compounds for the surface sterilization of insects. *J. Insect Pathol.* 2:124–33.

McLaughlin, R.E., and Keller, J.C. (1964). Antibiotic control of an epizootic caused by *Serratia marcescens* Bizio in the boll weevil, *Anthonomus grandis* Boheman. *J. Insect Pathol.* 6:481–5.

Melamed-Madjar, V., and Raccah, B. (1979). The transstadial and vertical transmission of a granulosis virus from the corn borer *Sesamia nonagrioides*. *J. Invertebr. Pathol.* 33:259–64.

Mery, C., and Dulmage, H.T. (1975). Transmission, diagnosis, and control of cytoplasmic polyhedrosis virus in colonies of *Heliothis virescens*. *J. Invertebr. Pathol.* 26:75–9.

Neilson, M.M. (1965). Effects of a cytoplasmic polyhedrosis on adult Lepidoptera. *J. Invertebr. Pathol.* 7:306–14.

Sikorowski, P.P., Andrews, G.L., and Broome, J.R. (1973). Trans-ovum transmission of a cytoplasmic polyhedrosis virus of *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.* 21:41–5.

Sikorowski, P.P., and Thompson, A.C. (1979). Effects of cytoplasmic polyhedrosis virus on diapausing *Heliothis virescens*. *J. Invertebr. Pathol.* 33:66–70.

Simmons, C.L., and Sikorowski, P.P. (1973). A laboratory study of the effects of cytoplasmic polyhedrosis virus on *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Invertebr. Pathol.* 22:369–71.

Steinhaus, E.A. (1959). *Serratia marcescens* Bizio as an insect pathogen. *Hilgardia* 28:351–80.

Thomas, G.M. (1974). Diagnostic techniques. In 'Insect Diseases'. pp.1–48. (Ed. G.E. Cantwell.) (Marcel Dekker, Inc.:New York), 300 p.

Tompkins, G.J., and Cantwell, G.E. (1975). The use of ethylene oxide to inactivate insect viruses in insectaries. *J. Invertebr. Pathol.* 25:139–40.

Vail, P.V., and Gough, D. (1970). Effects of cytoplasmic–polyhedrosis virus on adult cabbage loopers and their progeny. *J. Invertebr. Pathol.* 15:397–400.

Vail, P.V., Hall, I.M., and Gough, D. (1969). Influence of a cytoplasmic polyhedrosis on various developmental stages of the cabbage looper. *J. Invertebr. Pathol.*. 14:237–44.

Vail, P.V., Henneberry, T.J., Kishaba, A.N., and Arakawa, K.Y. (1968). Sodium hypochlorite and formalin as antiviral agents against nuclear-polyhedrosis virus in larvae of the cabbage looper. *J. Invertebr. Pathol.* 10:84–93.

Vail, P.V., Jay, D.L., and Hunter, D.K. (1973). Infectivity of a nucleous polyhedrosis virus from the alfalfa looper *Autographa californica*, after passage through alternate host. *J. Invertebr. Pathol.* 21:16–20.

Webb, S.R., and Levine, E. (1975). A nuclear polyhedrosis virus of *Bellura gortynoides* (Lepidoptera:Noctuidae). *J. Invertebr. Pathol.* 25:141–3.

Witt, J.W., and Stairs, G.R. (1975). The effects of ultraviolet irradiation on a baculovirus infecting *Galleria mellonella*. *J. Invertebr. Pathol.* 26:321–7.