SYMPOSIUM: SELECTED TOPICS IN MICROBIAL ECOLOGY[1]

II. The Importance of Environmental Factors in the Insect-Microbe Ecosystem

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Introduction

It is a genuine pleasure for me to participate in this symposium for the reason that in a sense it reflects a growing concern shown by microbiologists in a field of endeavor that should be of great interest to them, but one that they have largely ignored. There was little general enthusiasm for the fields of insect microbiology and insect pathology until, in recent years, they found a haven of interest among the entomologists. As is so often the case with borderline fields, insect microbiology, although a surprisingly large field, somehow fell between the microbiological and entomological chairs, so to speak. Perhaps largely because of unfamiliarity with one another's fields, the microbiologist and the entomologist each avoided this area in which their disciplines overlapped. Some rapprochement has existed in areas of medical entomology and in the study of the transmission of plant pathogens by insects, but important as these areas are they represent only a part of what might be included in or be associated with insect microbiology.

In recent years, the rapid development of the study of microorganisms pathogenic for insects has created another point of intense activity. Insect pathology is now recognized as a distinct branch of entomology or, depending on one's viewpoint, of invertebrate pathology. It constitutes an extremely fertile blending of the microbiological sciences with entomology, and goes on to include noninfectious maladies as well. Consulting the purely microbiological journals, one would probably be unaware of the fact that a significantly great amount of research is now being conducted throughout the world on insect pathogens—bacteria, viruses, rickettsiae, fungi, and protozoa—but in only a few instances are microbiologists as such availing themselves of the opportunities presented by the study of entomogenous microorganisms as research tools, and the fascinating relationships they have with their insect hosts. (Consider, for example, what virologists would have missed had they not used a mosaic disease of the tobacco plant as a research tool!) These relationships are, of course, part of the ecology of both the microorganism and the insect and hence are appropriately included in a symposium of this kind.

The general neglect of this field on the part of microbiologists is peculiarly mysterious when one considers that the first instance in which a microorganism was shown experimentally to be the cause of a disease in an animal was when Agostino Bassi in 1834 presented proof that the fungus *Beauveria bassiana* (Balsamo) Vuillemin was responsible for a disease, muscardine, in the silkworm. Moreover, Louis Pasteur's work on two other diseases (pébrine and flacherie) of the silkworm induced this French scientist to become interested in the microbial diseases of other animals and of man. We may trace the existence of even our modern medical technician to the fact that Pasteur employed his daughter, Marie-Louise in his laboratory to diagnose diseased silkworms—Pasteur's being the first laboratory in which a microscope was used to diagnose infectious disease. Similarly, Elie Metchnikoff, who initiated his studies on cellular immunity by observing the activity of phagocytes in a crustacean (*Daphnia*) suffering from a yeast infection, is acknowledged to have begun his researches on infectious diseases with his investigation of a fungus disease (green muscardine) of the wheat cockchafer. And whether it was d'Herelle or Twort who first discovered bacteriophage, it is worth noting that d'Herelle first observed the phenomenon in cultures of bacteria that he isolated from diseased locusts. So, from an historical standpoint at least, the insect pathologist or insect microbiologist should feel as at home here among his fellow microbiologists as do his colleagues concerned with the microbial maladies of other forms of life.

[1] This symposium was held at the 60th Annual Meeting of the Society of American Bacteriologists in Philadelphia, Pennsylvania, on May 4, 1960, with Dr. Willard O. Nelson as convener.

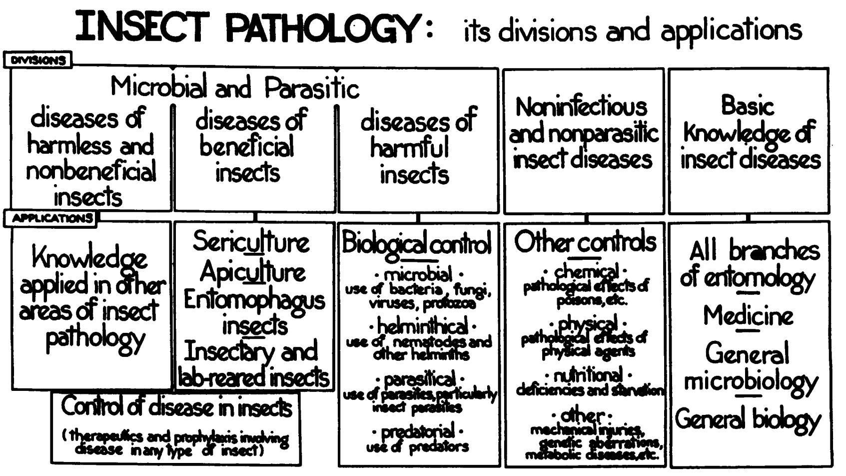

Figure 1. Diagram showing the different divisions of insect pathology and their respective areas of application.

An Orientation

Because of this background—this neglect by microbiologists of a field that overlaps with that of entomology—I believe it is useful to digress somewhat to provide a brief orientation with respect to the field of insect pathology. Insect pathology embraces the general principles of pathology (disease in its broadest sense) as they may be applied to insects; and from a standpoint of convenience it is usually considered to include the general field of insect microbiology and certain of the biological relationships existing between insects and microorganisms not pathogenic to them. Insect pathology finds its applications in agriculture, medicine, and biology generally. Both entomology and microbiology have received an abundance of benefits and contributions from the study of insect diseases and their causative agents, and from the study of microorganisms normally associated with healthy insects. As far as agricultural practices and crop protection are concerned, one of the most significant applications of insect pathology has been found in the use of microorganisms to control insect pests—an application commonly designated as "microbial control" (a form of "biological control"), but the suppression of disease in beneficial insects, such as the silkworm and the honey bee, is also of great practical significance.

Figure 1 diagrams what might be considered the different divisions or branches of insect pathology and their respective areas of application. In considering these relationships it is important to remember that we are using the broad concept of disease, and hence of insect pathology: "disease" signifies a departure from the state of health or normality; a diseased insect is one that is not healthy, regardless of the cause. Thus there are microbial (infectious) diseases, parasitic diseases, and noninfectious diseases of different types. Abiding by this concept, parasitic insects are agents of disease, and the study of the activities and effects of these insects on their hosts may properly be considered a part of insect pathology. Similarly, the effects of chemical poisoning, nutritional deficiencies, and genetic and metabolic disturbances in insects are the concern of insect pathology. (From a purely pragmatic standpoint, and frequently from an administrative and organizational one, it has been found convenient, in most of the entomological world, to consider that microbial and helminthic diseases are the concern of insect pathology, that insect parasites and predators are the concern of entomophagology, or "biological control," that the pathological effects of chemical poisons are, in part, the concern of insect toxicology, and that nutritional and metabolic diseases are the concern of insect physiology as well as insect pathology. But these associations reflect arrangements of convenience and do not invalidate the premise that anything that causes disease or anything that "goes wrong" with an insect is, at least theoretically and in principle, the concern of insect pathology.)

Insect pathology may be placed in another frame of reference, namely invertebrate pathol-

Zoology		Pathology (and Microbiology)
↕		↕
Invertebrate Zoology	↔	Invertebrate Pathology
↕		↕
Entomology	↔	Insect Pathology

Figure 2. Diagram showing the fundamental relationships between pertinent branches of zoology and pathology. According to this concept, just as entomology is a branch of zoology concerned with invertebrates, insect pathology may be considered as a branch of either entomology or invertebrate pathology.

ogy. As indicated in figure 2, insect pathology, as a field of scientific activity, may be considered as a branch of either entomology or of invertebrate pathology. Entomology, of course, is a part of that branch of zoology concerned with invertebrates, and therefore insect pathology is a logical part of invertebrate pathology. As I see it, agricultural and marine microbiologists, as well as those interested in microorganisms pathogenic for animals, have also been neglecting this broader field of endeavor. Although 97 per cent of all animals on earth are invertebrates, nowhere in the United States are organized studies being made of the diseases and abnormalities of these animals as a group. As a result, there is an astonishingly large field waiting to be explored and developed. Fortunately, investigations of the diseases of insects, the largest group of invertebrates, are well under way in a number of laboratories both in this country and abroad; but large and important groups remain completely ignored in this respect. Leading authorities (*e.g.*, Walford, 1958) testify to the fact that "one of the most serious gaps" in our knowledge of invertebrates is the study of their diseases. From the standpoint of the agricultural sciences it is the one remaining large area of pathology in need of development, study, and exploitation, the other two areas being plant pathology and vertebrate pathology.

Whereas many groups of invertebrates are of direct agricultural importance, others are important as food, as enemies of man, animals, and plants, in various industries, and in the role they play in the balance of nature. For example, the destructive properties of snails, slugs, barnacles, nematodes, and others are well known, but little is known of their diseases, or of the possible use of disease agents in the control of these invertebrates. On the other hand, useful and beneficial invertebrates such as earthworms, oysters, shrimps, and others, have destructive diseases, but we are virtually ignorant of how these diseases may be suppressed and controlled. The economic importance of invertebrates, other than insects, is indicated by the fact that in 1957 the world catch in marine invertebrates alone was 30 million tons. The urgency of some of the problems relating to the diseases of these animals is highlighted by the fact that a disease of oysters has become so serious that there are bills before Congress authorizing loans to oystermen until the disease can be suppressed. Furthermore, most authorities, looking to the future, see the time rapidly approaching when the seas of the world will have to be "farmed," just as the land is today; much of this "farming" will concern invertebrates.

Types of Ecological Relationships

To be sure, microorganisms are associated with insects (and other invertebrates) in ways other than as pathogens. Upon examination, we find that the biological relationships between insects and microorganisms are of numerous distinct types and that they vary all the way from obligate mutualism on the one hand to obligate parasitism on the other. Some of these relationships are very intimate and others are casual; all of them, however, are pertinent to ecological studies involving these two forms of life. Although the role of microorganisms in the ecology of insects has been largely neglected by entomologists and microbiologists alike, most authorities will agree that to understand properly the biology and activities of an insect it must be studied as a component of an ecological system, and microorganisms are a very important part of this system.

The types of relationships existing between microorganisms and insects may be arranged (Steinhaus, 1954) into a number of arbitrary categories, as follows: (a) Insects feeding on substrates previously broken down or changed by the activity of one or more microbial species; such as yeasts bringing about the fermentation of grapes in the field, thus providing optimal conditions for the developing larvae of drosophila flies. (b) Free-living microorganisms, especially bacteria and yeasts, serving directly as food for insects;

e.g., mosquito and fly larvae feeding directly on bacteria in their environment. (c) Insects and microorganisms existing separately but in a more-or-less common or regular association. Insects acting as carriers or intimate hosts only occasionally, or to ensure continuation of the relationship, or when specially cultivated microorganisms are ingested as food. Examples of this type of relationship are the fungus-growing ants, termites, and beetles, and the fungi they cultivate. (d) Insects as hosts to adventitious microorganisms fortuitously present in or on the insects; thus it is common to find on insects bacteria that occur in their environment, such as soil bacteria being found on insects inhabiting the soil. (e) Insects as hosts to commensal microorganisms found associated with them; just as *Escherichia coli* (Migula) Cast. and Chal. is a commensal regularly present in the intestinal tract of healthy man, so are certain species of microorganisms constantly present as commensals in the alimentary tracts of insects. (f) Insects as vectors of microorganisms pathogenic to animals or to plants; such as the classic examples of the mosquito transmission of the agents of malaria and yellow fever, or the leafhopper transmission of the virus of curly top. (g) Insects as hosts to extracellular symbiotes (*i.e.*, mutualists), as exemplified by the protozoa that live in the gut of termites enabling the latter to obtain nutriment from ingested wood, or the bacteria that regularly inhabit the gastric caeca of many insects. (h) Insects as hosts to intracellular symbiotes (*i.e.*, mutualists); such as the bacteria and yeastlike microrganisms that regularly inhabit the mycetomes, or other tissues of insects, and which are apparently necessary for the normal life of the insect. (i) Insects as hosts to microorganisms that are semiparasitic for them; such as fungi of the ascomycete order *Laboulbeniales* which obligately live on the integument of insects, or those basidiomycetes (genus *Septobasidium*) which parasitize some individuals of *Aspidiotus* while benefiting other individuals of the same species. (j) Insects as definitive hosts of microbial agents to which they are susceptible; in other words, a relationship in which microorganisms cause true disease in the insects. It is this category with which we are usually concerned in insect pathology, although actually all categories are of interest to the insect pathologist because he must also be thoroughly familiar with the general field of insect microbiology.

Environmental Stress Factors

It is hoped that the partial orientation of insect microbiology and insect pathology (and their relation to their invertebrate counterparts) just presented helps to emphasize the breadth and depth of what is involved in considering the microbial ecology of insects. The scope of the subject is so great that it would be foolish even to attempt to do justice to it in the time available. When I was invited to participate in this symposium, I was asked to place the emphasis of my remarks on the use of microorganisms to control insects. But even this limitation leaves too broad a subject to be covered adequately here. Moreover, the use of microorganisms in the control of insect pests and the effects of disease on insect populations have been heavily and extensively reviewed of late (*e.g.*, see Steinhaus, 1957, 1959; Tanada, 1959; Hall, 1960; Heimpel and Angus, 1960). Even such a basic matter as the classification, nomenclature, and identification of microorganisms has been related to the "ecology of microorganisms in biological control of insects" (Lysenko, 1959). There would be little purpose in repeating the essence of these reviews here. Instead, I should like to use this opportunity primarily to emphasize one very important aspect of what might be called the ecology of insect disease, namely environmental stress factors.

Aided by the problems associated with the use of chemical insecticides in leaving toxic residues, and in bringing about resistance in insects to many of these insecticides, there has been, in recent years, an increased interest in the use of biological agents to control insect pests. One segment of this interest has concerned itself with the use of microorganisms pathogenic for the pests, *i.e.*, so-called "microbial control." Several microbial insecticides have appeared on the market and more are in the offing. Among the more promising microbial insecticides are bacteria, such as *Bacillus popilliae* Dutky and *Bacillus thuringiensis* Berliner, and viruses, such as those causing polyhedroses of certain sawflies, the alfalfa caterpillar, the cabbage looper, and others. In addition to these agents, definite potentialities are becoming apparent with certain entomogenous fungi, protozoa, and nematodes. There is every indication that the commercial production of microorganisms for insecticide purposes is fast becoming an important segment of industrial and agricultural microbiology. But regardless of how successful man's use of microorganisms may be in this connection, the natural occurrence of disease in insect populations is of much broader significance in insect ecology, and we must extend our inquiries into every aspect of the insect-microbe ecosystem. As Sir Macfarlane Burnet says in his *Natural History of Infectious Disease*, "If the pests are to be controlled or the valuable species saved from extermination, every detail of their life histories, their physical environments, and of the numbers and habits of their enemies may be necessary. It is the task of the trained ecologist to provide this knowledge and to show how it can be applied to the desired end."

It is interesting to speculate on the ecological aspects of *Bacillus thuringiensis* Berliner, and its varieties, now being used as a microbial insecticide against a number of lepidopterous pests. The pathogenic action of this bacillus is caused apparently by a toxic crystal, formed in the sporangium at the time of spore formation, which, when ingested by a susceptible insect, causes paralysis and death. It appears to be harmless for all forms of life other than certain insects. Except for its pathogenicity for insects (and the presence of the toxic crystal), *B. thuringiensis* is virtually indistinguishable from *Bacillus cereus* Frankland and Frankland, commonly found in soil. To my knowledge, *B. thuringiensis* has not been found in nature unassociated with insects. Inasmuch as ecology deals with the interrelationships of organisms and their environment, it is natural to inquire as to what factors in the entomic environment of this sporeforming bacillus caused it to acquire a crystallized toxin lethal for certain insects.

There have been claims (Toumanoff, 1956; Le Corroller, 1958) to the effect that by repeated controlled passage through larvae of the wax moth (*Galleria*), certain strains of *B. cereus* acquire the ability to form the toxic crystal. Therefore, are the crystalliferous sporeformers merely selected, or specially adapted, strains of *B. cereus*? Does the insect habitat induce certain strains of *B. cereus* to become crystal-bearing? How stable or permanent a character is this crystal formation? It is hoped that research now in progress or contemplated will answer these questions which, so far, lack definitive answers. It is to be further hoped that the investigations will probe deep enough to elucidate the environmental factors involved in the intentional and rather rapid formation of crystalliferous strains, if such can occur. If such does not occur, we are still left with speculating as to how or by what steps (genetically or environmentally) the crystalliferous strains evolved in nature.

One may raise similar questions concerning what there is about the cellular environment of most insect viruses that causes them to become embedded in a characteristic protein matrix (the polyhedral and capsular inclusion bodies), or why they attack primarily lepidopterous insects, or why only immature stages (larvae and pupae) but not adults are susceptible to frank infection.

Unfortunately, in much of insect pathology there is a tendency, in field observations as well as in laboratory research, to concentrate our attentions on the pathogen and on its insect host without fully appreciating the role of environmental factors. Any epizootic affecting an insect population is concerned with three primary natural entities: the infectious agent, the insect host, and the environment. Each of these factors has certain attributes which, when properly related to the attributes of the others, play their appropriate roles in determining the initiation, rise, and decline of an epizootic. Generally appreciated is the fact that optimal conditions of temperature and moisture, for example, aid in enhancing the activity of pathogens and thus in promoting the outbreak of an epizootic. Not so well known or appreciated, however, is the fact that the environmental factors (both physical and biological in character) may also serve as stressors making the insect more susceptible to attack by disease agents.

Putting this another way, we might say that our knowledge as to just how disease affects insect populations or as to how to use microorganisms to control insect pests has as yet not advanced to the point where we know all the many factors involved and how they operate. It is true that we are able to spray or dust crops with preparations containing bacterial spores and toxins, or with virus inclusion bodies, and regularly and successfully to reduce the population to a point below its destructive level; and that we are able to introduce pathogens into insect populations in such a manner that they take a steady and significant toll of the pest year after year. However, the beneficial effects derived from spraying and dusting frequently are really obtained by overwhelming the insects with the pathogen. If environmental conditions are satisfactory, well and good; if not, the pathogen may not work or may give only partially satisfactory results. But only rarely do we know enough or are we able to manipulate the different factors in the environment involved. Sometimes the operation of these factors is very subtle and difficult to discern. At other times it may be quite obvious. For example, the role of moisture in certain virus diseases is so indefinite that although some workers believe it has little or no effect, others feel that it might be quite important. On the other hand, there is no question that with most of the entomogenous fungi, infection of new hosts is impossible unless the moisture available to the spores is sufficiently high to cause these structures to germinate. And on the matter of moisture (the lack of it or an excess amount of it) serving as a stressor, we know very little. I refer here to the effect of moisture on the insect, making it more susceptible to invasion by microbial pathogens.

An interesting example of the role of environmental factors within the insect itself in determining the capacity of a microorganism to invade the body cavity of its host has recently been suggested by Bucher (1960). This investigator uses the term "potential pathogen" to indicate bacteria that are capable of multiplying in the hemocoele of insects (causing septicemia and death) from small inocula but which do not multiply significantly in the gut of these arthropods. Such bacteria have two important characteristics that aid in explaining their pathogenic behavior: they produce strong proteolytic enzymes that may attack the tissues of the host once they gain entrance into the hemocoele, and they are aerobes capable of multiplying in the aerobic blood of insects but are inhibited from multiplying in the relatively anaerobic alimentary tract. Inability to multiply in the insect gut because of the anaerobic conditions there precludes the formation of large populations of the bacteria and the production of enzymes in this location. Thus, the ability of the bacteria to invade the body cavity of the insect is limited, and epizootics in nature caused by these bacteria are not likely. Their effective use in microbial control, if possible at all, would seem to depend on circumventing these limitations, such as by possibly concomitantly using certain stressors.

For many years now, those who have studied the infectious diseases of silkworms and honey bees have observed that certain "predisposing causes" (*e.g.*, age, stage, and sex of the insect; climate) were involved. Although these predisposing causes were sometimes vague and ill-defined, it was apparent that they could play an important role in the microbial diseases of these insects. In the case of certain diseases of the honey bee, it has become increasingly clear, as through the work of Bailey and Lee (1959) and Bailey (1960), that unfavorable circumstances that lead to food deficiencies enable disease to flourish where it otherwise would not.

Permit me to give another example or reason which illustrates why ecologists and applied insect pathologists should be concerned with stress factors in the environment. In our work at the University of California on the use of a nuclear polyhedrosis virus to control the alfalfa caterpillar, we often observed the natural outbreak of the disease in the field. One could frequently visit a field of alfalfa and observe a dense population of caterpillars, all apparently healthy and vigorous. A day or two later one could return to the same plot and this time observe the entire population suffering from polyhedrosis. The epizootic appeared to develop throughout all of the population at once. It did not, as one might expect, begin slowly in one corner of the field and gradually spread through the remainder of the caterpillar population. The question thus presented itself as to what factors were involved in bringing about this apparent outbreak of disease simultaneously and more-or-less uniformly throughout the population. What triggered the epizootic? Was the virus already well distributed in the environment so that exposure and infection occurred uniformly? Was the virus already present uniformly in the insects themselves, perhaps through transovarial transmission? Was the virus present in an occult form (*i.e.*, as a latent infection) until activated by some incitant? Were factors operating in the environment that, through stress or otherwise, uniformly and simultaneously lowered the resistance of the host to a point where it became more susceptible to the virus, or to the occult form of the virus? We still do not have definitive answers to these questions, but confronting them did cause us to focus more attention on stress factors in the environment.

Perhaps I should make clear just what I mean when I refer to stressors and to stress factors in the environment.

The work of Selye (1950, 1952, 1955, 1956) and his associates has, in recent years, called attention to the role of stress factors in diseases of vertebrate animals. Evidence is accumulating that stress also plays a role in the manifestation of disease in invertebrates, especially insects, although apparently a different set of mechanisms may operate as it pertains to the matters we are discussing. When we speak of stress we are really speaking of the effect of certain ecological or environmental factors (physical and biological) on the insect-microbe relationship. For the purposes of this paper I shall use the word "stress" simply to refer to a state manifested by a syndrome, or bodily changes, caused by some force, condition, or circumstance (*i.e.*, by a "stressor") in or on an insect or on one of its physiological or anatomical systems.[2] A stressor may also be thought of as any stimulus, or succession of stimuli, that tends to disrupt the homeostasis of an animal. Our concern here is the role of stress (*i.e.*, the effects of stressors) in diseases of insects caused by entomogenous microorganisms both as these diseases occur in nature and as they may be intentionally initiated by man. Concomitantly, we are of necessity concerned with the effect of these same stressors on the pathogens themselves. In other words, disease may result not only when a pathogen directly attacks and invades a susceptible host, but it may be induced or promoted when stress is manifested in or weakens the host, or when the pathogen is incited, stimulated, or activated in a manner that enhances its capacity to produce disease. Whereas we say that "stressors" are factors that produce stress, we may say that "incitants" are factors which incite or activate pathogens or potential pathogens. (The terms "inducing agent" and "induction" are similarly used.) Frequently, the same factor can act either as a stressor or as an incitant.

Among the stressors studied in connection with the diseases of insects are certain chemicals, excessive heat, excessive cold, crowding, excessive moisture, excessive drought, starvation, abnormal nutrition, physical injuries, and the like. The exact role of these stressors in insect disease, either in nature or in experimental infections, is not clear. Indeed, we appear, momentarily at least, to be faced with the paradoxical situation that the more experimentation is reported from different laboratories the more confusing the picture becomes. Each of the above-mentioned stressors has been found, by one investigator or another, to induce or to promote infectious disease in certain insects. Such manifestations of disease have been observed most often in connection with viral and bacterial infections, but also with certain protozoan infections. Claims have been made that the administration of certain chemicals, both with and without the stressor of cold, has produced polyhedrosis virus *de novo* in silkworms. In other instances, it has been concluded that the application of certain stressors (chemicals, excessive temperatures, crowding) has activated latent infections. Frequently, the fundamental effects of stress, or their manifestations, are not at all clear (*e.g.*, see Steinhaus and Dineen 1960).

Laboratory experiments (Steinhaus, 1958*a*)[3] have indicated that crowding may act as a stressor at least in the case of certain nonaggregating insects. (Of course, crowding may also serve as a "condition" or "circumstance" that causes other, more direct, stressors to act.) Such information might be transferred to field situations where disease is frequently observed to break out at times of high population densities. Although most authorities consider that, in general, disease is density dependent, it should be made clear that such is not always the case, that although there may be a correlation between disease and density, there is not necessarily a causal relationship between the two, that disease may manifest itself at low density especially if the pathogen is adequately distributed, and that when microbial agents are applied as insecticides they can act in a density-independent manner as do chemical insecticides. Of pertinence to our discussion here is the fact that while high densities or crowding of insects may serve as stressors, other stressors may be acting independently of the high density factor, or may be superimposed upon it. Furthermore, the outbreak of disease in

[2] In a previous paper (Steinhaus, 1958*b*) the author used the word stress in a somewhat different, and dual, sense. Better usage requires that the state of stress be clearly differentiated from the stressor.

[3] The research being done in our laboratory on the role of stress in insect diseases is supported by a U. S. Public Health Service research grant (No. E-1000).

populations of low density may be the result of stressors other than the crowding stressor which may exert an effect during periods of high density. I might add that whenever we consider the effect of disease on insect populations, in addition to the mechanisms involved in population dynamics, it seems to me that we should also keep in mind what Pavlovskii and other Russian workers (see Audy, 1958) call the "doctrine of nidality," that is, disease itself has a natural habitat (in the same way that a species does), frequently in well-defined ecosystems.

Without going into detail, it is safe to say that the great importance of environmental factors, including stressors, in insect disease is becoming more and more clear. Moreover, it is becoming increasingly apparent that those who wish to exploit the use of entomogenous microorganisms in the control of insect pests must have a greater understanding of these factors and their manipulation. To those of you who are concerned with the process of disease in higher animals or in plants, it may appear that I am emphasizing the obvious, that the importance of environmental factors in disease is well-known. Unfortunately, until recently it has not been well-enough appreciated in the study of the disease of insects to provoke the amount of study and investigation that it deserves. The amount of research being accomplished on this aspect of entomic disease is sadly out of balance with that being done on the pathogen and the host.

It has been my simple, but primary, purpose in this paper to emphasize the triangle involved in the diseases of insects: pathogen-host-environment; and especially to spotlight the environmental member of the triad. In general, disease in insects must be thought of as more than simply the result of a pathogen encountering a susceptible host regardless of the conditions. All three, pathogen, host, *and environment*, are necessary elements in the disease complex. This fact must be understood and appreciated not only in the study of insect diseases as they occur naturally, but in the proper use of microorganisms in the control of insect pests. Also, I have tried to make the additional point that among the important but frequently forgotten factors in the environment are those ("stressors") that produce stress in insects and thus promote the manifestation of disease among these animals. With proper manipulation of these stress factors it is not unreasonable to expect that we may be able to utilize, for pest control purposes, microorganisms that ordinarily do not show overtly invasive or highly pathogenic properties toward insects. Moreover, through the appropriate use of stressors it may be possible to induce epizootics of disease among insects carrying occult as well as virulent entomopathogens.

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