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BACTERIA, SPIROCHETES, AND RICKETTSIA AS INSECTICIDES

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Introductory Background

Most bacteria pathogenic to insects are classified in the families Pseudomonadaceae, Enterobacteriaceae, Lactobacillaceae, Micrococcaceae, and Bacilliaceae; spirochetes and rickettsia are in the families Spirochaetaceae and Rickettsiaceae, respectively. Except for Bacilliaceae, these families contain nonsporulating microorganisms. Most spore-forming bacteria pathogenic to insects belong to the family Bacilliaceae. The identification of microorganisms associated with insects is inadequate because of inaccurate descriptions. A comprehensive evaluation of bacterial classification and identification can be found on the sixth and seventh editions of Bergey's Manual of Determinative Bacteriology.[4,5] However illogical the existing scheme may be, we feel strongly that it should be adhered to by the insect pathologist, and the use of generalized names without reference to the specific causative disease agent should not continue. The incorrect hypothesis among insect pathologists that insects harbor special microorganisms is probably the main cause of misnamed bacterial species. Often, microorganisms from diseased insects are named for the insect from which they are isolated and are incriminated as pathogens. This practice should be discouraged. The procedures outlined in Koch's canons for demonstrating that a disease is caused by a microorganism are as follows: finding the specific microorganism in all cases of the disease; isolating it in "pure culture" (description of the pure culture isolate should be by established taxonomic procedures); inoculating (or feeding) the isolate into the host insect and experimentally producing the original disease; and finally, reisolating the microorganism from the experimentally diseased insect and demonstrating it to be the same as the pure culture isolate previously inoculated. In this communication, we shall, when possible, ignore unauthenticated bacterial species claimed to be insecticidal.

Bucher[7,9] classified bacterial insect pathogens as either obligate, facultative, or potential. We shall discuss, according to Bucher's categorization, nonsporulating bacterial pathogens, including true bacteria, spirochetes, and rickettsiae and sporulating bacterial pathogens, including "crystalliferous" types.

Pathogen Invasion Routes

The integument of most insects is contaminated by microorganisms from their environment.[53,56,57] Usually, the types of microorganisms involved reflect the nature of the specific environment. For example, soil-inhabiting insects normally harbor soil microorganisms. Such insects as cockroaches and houseflies, which frequent diverse environments, carry a flora characteristic of that situation. In relatively clean areas these same insects usually possess a flora different from that found on the same species frequenting unclean conditions.

* Agricultural Research Service, U.S. Department of Agriculture. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

The insect integument consists of the cuticle (outer body wall) and the epidermis (inner body wall). Detailed descriptions of insect integument have been published in addition to the procedures and methodology for making visual examination and general inspection of diseased insects.[34,38,57] Considering the sizable number of bacteria reported to attack chitin, the chief substance of insect cuticle, it is surprising to find so little experimentation with bacteria that attack this material. The majority of bacteria that decompose chitin have been isolated from marine and soil sources and are of the genus *Beneckea.* The most often quoted reports of bacteria that attack insect chitin are those of an unidentified bacterium from exuviae of mayflies[3] and micrococci found attached to the cuticle of locusts from Greece.[33] *Staphylococcus acridicida* was the most predominant strain isolated from these locusts, but the bacterium does not invade internal tissues. Certain other bacteria are capable of breaking down the cast-off skin and cuticle of dead insects, but apparently they do not attack the cuticle of living insects. David[16] suggests that epidermal cells of living insects supply substances to the cuticle that protect it from attack.

Two instances of bacteria attacking the epidermis are noteworthy. *Bacillus entomotoxicon* (a nonspore-former improperly named)[4,5] attacks the epidermis of the squash bug (*Anasa tristis*)[18] in addition to the perivisceral cavity, blood, adipose tissue, and cardiac tissue. The infected squash bug becomes sluggish and at death is darker and softer than in life. Microscopical examination shows the body fluids to contain nearly a pure culture of the bacterium. In the other instance, *Micrococcus nigrofaciens* (a soil bacterium) penetrates the integument of the June beetle larva (*Phyllophaga*) and specifically attacks the epidermis.[39] The principal sites of infection are the leg joints, spiracles, and segments of the soft body parts. These body parts become black and shiny upon infection. As the disease progresses, the blackened leg segments drop off. Microscopical examination reveals the micrococcus in the integumental laminae and in the epidermal cells. *M. nigrofaciens* appears to be the only authenticated example of a bacterium entering its host by penetrating the unbroken external body wall although Paillot[40] described a spirochete that occasionally penetrates the integument of *Pieris brassicae,* the European cabbageworm.

Bacteria are secondary invaders of insects through wounds caused by fungi, nematodes, other parasitic or predacious insects, and mechanical injury. *M. nigrofaciens* is pathogenic to the cockroach, *Periplaneta americana,* only if the insect is previously wounded although the bacterium easily penetrates the unbroken integument of June beetle larvae. "Spring disease" is caused in part by entry of *Pseudomonas septica* through damaged integument of cutworm caterpillars (*Euxoa segetum*). The grasshopper, *Schistocerca gregaria,* when infected with a fungus, *Aspergillus flavus,* is easily invaded by *Pseudomonas aeruginosa.* This bacterium also is highly pathogenic to the wax moth larva (*Galleria mellonella*) when entry is possible through a wound in the integument. *Micrococcus pyogenes* var. *albus* is another secondary invader that is pathogenic to the roach, *Blatta orientalis.*

Some pathogenic microorganisms are inoculated into healthy insects by contaminated stingers or ovipositors of predacious and parasitic insects. Usually, the parasitic insect is not itself diseased; the ovipositor is merely contaminated. Although this mode of transmission is uncommon,[6] predators or parasites can facilitate multiplication, distribution, and transmission of pathogens. Larvae of *Ephestia puhniella* can be infected with spores of *Bacillus thuringiensis* by a contaminated ovipositor of the parasite *Nemeritis canescens.*[57]

The oral cavity is the predominant route of entry by microorganisms in nature. Experimental infections can be produced by injecting microorganisms into the hemocoel or by forced feeding. Diseases produced by these means are internal. Generally, the symptoms of internal bacterial diseases of insects are: decreased mobility, diminished appetite, and rectal and oral discharges. After ingestion and subsequent bacterial invasion of the insect body cavity, a septicemia develops that results in the host's death. Upon death, the internal tissues become viscid and develop a unique odor. Later, the dead insect dries and becomes shriveled, with the integument remaining intact. These symptoms occur with many types of insect diseases and, therefore, cannot be the only criteria used for pathogen identification. Most important is microscopical examination of histological sections that reveal the morphology of the causative agent.

Bacteria

Pseudomonadaceae. Internal infection of grasshoppers by *Pseudomonas aeruginosa* is well known. The disease can be produced in adult grasshoppers, *Melanoplus bivittatus* and *Camnula pellucida,* by injection into the hemocoel of as few as 10–20 bacteria. In contrast, adult grasshoppers are more resistant to ingested doses of *P. aeruginosa.* Between 8000 and 60,000 ingested bacteria are required for an LD_{50}.[12,14] *P. aeruginosa* also produces lethal septicemia when injected into the greater wax moth, silkworm, locust, tent caterpillar, cutworm, and hornworm. *Pseudomonas septica* is pathogenic to *Melolontha melolontha* and to many other scarabaeidae; it is also infectious to *Aporia crataegi, Trypodendron lineatum,* and *Phyllopertha* sp.

Interestingly, in this family and under the tribe Spirilliae are bacteria, such as *Vibrio pieris,* that are often found in insects but do not cause disease. *V. pieris* is frequently observed in caterpillars of the European cabbage butterfly (*Pieris brassicae*). Another member of this tribe, *Vibrio leonardii,* on the other hand, is pathogenic to the wax moth and European corn borer.

Enterobacteriaceae. The red varieties of *Serratia marcescens* are historically the best-known pathogens of various insects because their distinctive color enables ready recognition. Steinhaus[54,55] confirmed reports that the chromogenic species are highly pathogenic when inoculated into insect hemocoel but are only mildly pathogenic when ingested. Septicemia precedes death in the infected insects; microscopical examination at the time of death shows considerable tissue destruction caused by *S. marcescens.* The dead insects quickly decompose and putrefy. Nonchromogenic strains of *S. marcescens* also are pathogenic and exhibit no significant biochemical differences from the classical red strains. Outbreaks of disease caused by *S. marcescens* are common in laboratory-reared insects. The bacterium is frequently found in insectary-reared silkworm caterpillars that become infected with a fungus. No collaborative relationship between *S. marcescens* and fungi has been established although Masera[35] believes that silkworm caterpillars previously infected with *S. marcescens* are more susceptible to fungi.

Some organisms of the genera *Escherichia, Aerobacter,* and *Klebsiella,* collectively referred to as "coliform bacteria," are pathogenic to insects. Specifically, *Aerobacter aerogenes* and *Escherichia coli* kill silkworms and other lepidoptera larvae. Considerable attention has been given to the coliform diseases of grasshoppers. However, the role of coliforms in the pathogenesis of disease is not yet clear. Experiments on infection of grasshoppers by *A. aerogenes* suggest that the disease is caused by an ultramicroscopic virus and that the bacterium is a

secondary invader.[55] It is also believed that the bacterium is transmitted through insect eggs and, on occasion, causes a morbid process in its host. Other evidence suggests that the bacterium present in the grasshopper's alimentary tract could become pathogenic to the insect under certain environmental conditions.[10,13]

In the *Proteus* genus, three species are of interest: *P. vulgaris, P. mirabilis,* and *P. rectgeri.* All three bacteria infect grasshoppers, and pathogenicity is correlated with proteolytic activity.[9] Of the *Salmonella–Shigella* group of bacteria, *Salmonella schottmuelleri* var. *alvei* causes acute enteritis in bees. This organism, as well as *S. enteritidis, S. typhosa,* and *Shigella dysenteriae,* can experimentally infect larvae of the wax moth (*Galleria mellonella*). It is doubtful that these organisms are significant insect pathogens.

Lactobacillaceae. Several organisms of this family have been incriminated as insect pathogens. Organisms in the genera *Diplococcus* and *Streptococcus* are dubious pathogens and probably are only secondary invaders or chance opportunists. The species nomenclature of these bacteria, like many other bacteria associated with insects, is based on the insects from which they were isolated; modern taxonomic literature does not recognize a large number of these microorganisms as authentic species. Several diplococci and streptococci have been isolated from the cockchafer (*Melolontha melolontha*), the silkworm (*Bombyx mori*), the gypsy moth caterpillar (*Porthetria dispar*), and the processionary moth caterpillar (*Thaumetoxoea pityocampa*). Of the recognized species, *Streptococcus pyogenes* is often isolated from flies. The bacterium has not been found to cause infection in insects. *Streptococcus faecalis,* a common inhabitant of the intestine of many animals, including insects, is pathogenic for wax moth larvae when injected in moderate doses.

Micrococcaceae. Only the genus *Micrococcus* appears to contain bacteria of any significance to insect disease.The first micrococci reported were isolated from larvae of the nun moth (*Lymantria monocha*).[21] These bacteria also were experimentally pathogenic to other insects, including several lepidoptera species. Such insects as the European corn borer (*Ostrinia nubilalis*), sawfly (*Neurotoma nemoralis*), cutworms of the genera *Agrotis,* and the housefly (*Musca muscae*) are readily killed when experimentally infected with micrococci. One of the best examples of micrococcus infection is that of the June beetle (*Phyllophaga portoricensis*) larva.[39]

Bacilliaceae. This family consists of two genera: *Bacillus* and *Clostridium;* all species form spores. Members of the *Bacillus* genus can use oxygen for growth, whereas those of the genus *Clostridium* cannot. The literature of insect pathology of the genus *Bacillus* is filled with incorrect epithets. Steinhaus[56] attempted to segregate from the genus *Bacillus* those species that are nonsporogenic.

Experimentally, *Clostridium novyi* and *Clostridium perfringens* are pathogenic for the wax moth (*Galleria mellonella*). Rarely have species of clostridia been isolated from diseased insects in nature. Two sporulating obligate anaerobes have been isolated from diseased larvae of *Malacosoma pluviale,* the western tent caterpillar.[11] Similar kinds of microorganisms also were found in the Essex skipper (*Thymelicus lineda*)[8] and in diseased pine processionary moths (*Thaumetopoea pityocampa*).[1] The small number of clostridia isolated from insects in nature is probably due to improper techniques. Particular attention must be given to the lethal effects of oxygen on most species of clostridia. The common isolation methods used in insect pathology allow preferential growth of aerobic bacteria.

Bacillus cereus is widely distributed in nature and is frequently isolated from diseased Coleoptera, Hymenoptera, and Lepidoptera. Early investigators re-

ported the bacterium in the southern armyworm (*Prodenia eridania*), the American cockroach (*Periplaneta americana*),[2] the Indian mealworm (*Plodia interpunctella*),[55] codling moth larvae (*Carpocapsa pomonella*),[58,59] the spruce budworm (*Choristoneura fumi ferana*),[51] and the larch sawfly (*Pristiphora erichsonii*).[25,26] Pathogenicity of *B. cereus* has been attributed to phospholipase C activity.[60]

"Crystalliferous" spore-forming pathogens are closely related to B. cereus. The term crystalliferous is applied to *Bacillus* species that produce a discrete, characteristic inclusion within the sporangium in addition to the endospore. The term parasporal body, suggested by Hannay,[22] is often used to denote an inclusion that lies alongside the spore and is formed during sporulation. Some of the crystalliferous bacteria associated with insects are obligate pathogens; some are facultative pathogens; and others seem harmless if ingested by insects. Obligate pathogens include *Bacillus popilliae* (to be discussed in detail later) and *Bacillus lentimorbus*.

During the past 10 years, a massive amount of data has accumulated about the best-known crystalliferous bacterium, *Bacillus thuringiensis*. This organism is considered to be a pathogenic variety of *B. cereus*.[4] Because most insect pathologists prefer the name *B. thuringiensis*, we shall maintain this practice to prevent confusion in the literature concerning these bacteria. Taxonomic studies of *B. thuringiensis* have been done by Heimpel,[24] deBarjac and Bonnefoi,[17] and Krieg.[31] More than 100 species of lepidoptera insects have been found to be susceptible to varieties of *B. thuringiensis*. Ingestion and dissolution in the gut of the proteinaceous crystalline inclusion kills the insect.[23] Susceptible species have also been found in the orders Hymenoptera, Coleoptera, Diptera, and Orthoptera. Some Diptera described as susceptible are of economic import and include the housefly (*Musca domestica*) and mosquitoes (*Aedes aegypti* and *Anopheles* sp.).[32] Rogoff and associates[42,43] found several varieties of *B. thuringiensis* toxic to the greater wax moth (*Galleria mellonella*), cabbage looper (*Trichoplusia ni*), bollworm (*Heliothis zea*), southern house mosquito (*Culex pipiens fatigens*), and housefly (*M. domestica*). The list of insects susceptible to *B. thuringiensis* strains and substrains is continually increasing.

If spores or vegetative cells of *B. thuringiensis* are injected into the hemocoel of an insect, the bacterium soon proliferates and can cause a fatal septicemia. When ingested by an insect, the fate of the spores depends upon gut conditions, such as pH, oxidation-reduction potential, degree of anaerobiosis, antibiotics of plant origin, and the host digestive enzymes. Several toxic agents have been demonstrated in *B. thuringiensis varieties*.[15] The most significant are: the δ-endotoxin (proteinaceous parasporal inclusion), α-exotoxin (the enzyme phospholipase C), β-exotoxin (thermostable adenine nucleotide), and γ-exotoxin (unidentified thermolabile phospholipase). Probably, the parasporal inclusion acts as a protoxin or toxin and damages the midgut cells sufficiently to inhibit feeding. Such conditions favor growth of the bacterium.[27] Phospholipase C production depends upon vegetative multiplication in the insect gut. TABLE 1 summarizes the toxic agents produced by *B. thuringiensis* and their action upon susceptible insects. *B. thuringiensis* toxins are discussed in detail by Somerville.[52]

Extensive field testing has been performed throughout the world since 1950 on varieties of *B. thuringiensis* as insecticides. The wide-scale field application of *B. thuringiensis* insecticide has been made possible because of commercial production of the crystalliferous bacteria in several foreign countries, as well as in the United States. Trade names of some American commercial products are

TABLE 1

EFFECT OF TOXIC AGENTS PRODUCED BY CRYSTALLIFEROUS BACILLI ON SUSCEPTIBLE INSECTS*

Toxic Agent	Result to Insect Host
Crystalline protein	midgut paralysis in susceptible lepidoptera
Spore	septicemia after penetration to hemocel
Heat-stable soluble exotoxin	interference with pupal development; death
McConnell–Richards factor (may be identical with heat-stable exotoxin)	mortality in various insect larvae
Heat-labile soluble exotoxin	mortality in larch sawfly larvae
Exoenzymes (phospholipases, hyaluronidase)	may be partially responsible for destruction of gut wall epithelium
Vegetative bacterial cells	may produce toxins and enzymes cited above plus other metabolic products after growth in host gut and hemocoel

* Taken from Rogoff.[42]

Biotrol®, Dipel®, Microtrol®, and Thuricide®. These microbial insecticides are applied either as dusts or sprays; both have been used with equal success. *B. thuringiensis* preparations must be ingested. Therefore, spray or dust is applied at a time when the insects are feeding and when weather conditions are favorable. TABLE 2 lists a few insect pests that are controlled with *B. thuringiensis* preparations.

A number of scarabaeid larvae are infected by either of two related bacteria, *B. popilliae* or *B. lentimorbus*. These organisms cause "milky disease" of larvae of the Japanese beetle (*Popillia japonica*). The term milky disease refers to the milky appearance of the hemolymph after it is heavily infested with bacterial spores. Characteristics of these pathogens are those of an effective biological control agent. They grow in the hemolymph of the insect larvae and ultimately accumulate billions of spores before the host dies. The spores permit the pathogen to survive inert for long periods in the soil and are means of disease transmission. These facts, in addition to a review of some 30 years' research, can be found in summary by Dutky,[19] the first to describe the milky disease bacteria and to characterize the disease. In his review, Dutky describes work on type-A milky disease caused by the bacterium, *B. popilliae,* and type-B milky disease caused by *B. lentimorbus*. Since the classical work of Dutky, research has involved development of techniques for isolation and maintenance of *B. popilliae* in pure culture, investigation of growth characteristics and of methods for growing large numbers of cells, and studies of how to evaluate infectivity of artificially propagated cells; the chemistry of healthy and infected larval hemolymph has been investigated to define the natural growth and sporulation environment of the pathogen. A summary of these latter studies appears in a recent review of St. Julian and Bulla.[44] Spores have been produced outside the insect host by growing cells on solid medium.[41,49,50] As much as 30% of the total population sporulates. Sporulation in liquid culture also has been achieved, but fewer cells form spores. Most recently, emphasis has been placed on biochemistry of the milky disease itself.

The spore of *B. popilliae* is accompanied by a characteristic parasporal body; both are encased in a thick-walled sporangium. The spore is unique among those of related bacilli in that it is highly resistant to chemical germinants.[48] Lysozyme, trypsin, snail (*Helix pamatia*) enzyme, L-alanine, and adenosine alone or in combination with various sugars do not substantially increase spore germination

TABLE 2

SOME INSECT PESTS CURRENTLY CONTROLLED WITH *Bacillus thuringiensis* PREPARATIONS

Insect Pest	Species Designation	Plants Affected
Cabbage looper	*Trichoplusia ni*	broccoli, cabbage, cauliflower, celery, lettuce, potato, melon
Imported cabbageworm	*Pieris rapae*	broccoli, cabbage, cauliflower
Tobacco hornworm	*Manduca sexta*	tobacco
Tomato hornworm	*Manduca quinquemaculata*	tomato
Alfalfa caterpillar	*Colias eurytheme*	alfalfa
Gypsy moth	*Porthetria dispar*	forest trees
European corn borer	*Ostrinia nubilalis*	corn
Grape leaffolder	*Desmia funeralia*	grape
Codling moth	*Carpocapsa pomonella*	apples, pears
Green cloverworm	*Plathypena scabra*	soybeans
Orangedog	*Papilio cresphontes*	citrus
Range caterpillar	*Hemileuca oliviae*	range grass
Sugarcane borer	*Diatraea saccharalis*	sugarcane

and outgrowth. Also, heat treatment is only slightly effective for *B. popilliae in vitro*. Outgrowth of unheated spores is approximately 1% of a total population; only 12% of heat-treated (50°C for 20 min) spores outgrow to proliferating vegetative cells. Obviously, the relationship of spore outgrowth to infection of larvae is critical; spores that fail to develop into proliferating vegetative cells cannot cause the disease.[46] The effect of heat treatment of *B. popilliae* spores on infection of Japanese beetle larvae is shown in TABLE 3. The greatest number of *P. japonica* larvae (64%) is infected by spores previously heated at 50°C. By contrast, maximum infection from unheated spores is only 27%.

Milky disease development occurs in four phases (FIGURE 1): Phase I is the initial incubation stage of approximately 2 days, during which few bacterial cells appear in the hemolymph; phase II is the vegetative stage of proliferation that continues until Day 5; phase III (Days 5–10) marks an intermediate change from predominantly vegetative growth to prespore and spore development; thereafter, a sporulation phase (IV) involves massive sporulation and ensuing larval death by Days 14–21. Milky larvae contain an average of 5×10^{10} spores per milliliter

TABLE 3

EFFECT OF HEAT TREATMENT OF *Bacillus popilliae* SPORES ON INFECTION OF JAPANESE BEETLE LARVAE

Spores Injected per Larva*	Outgrowth of Spores Injected per Larva†	Larvae Infected in 21 Days (%)	
		Range	Average
4×10^6	heat-treated 80°C,‡ 2×10^5	33.3–42.6	37
4×10^6	heat-treated 70°C, 2×10^5	42.6–64.8	49
4×10^6	heat-treated 60°C, 2×10^5	46.3–72.2	62
4×10^6	heat-treated 50°C, 2×10^5	55.6–68.5	64
4×10^6	unheated, 1×10^5	22.2–33.3	27

* Average number calculated from direct microscopic counts made with a Petroff-Hausser bacteria counter.

† Average number calculated from vegetative colony counts after spores were plated onto yeast extract high-phosphate growth medium.

‡ All heat treatments were for 15 min.

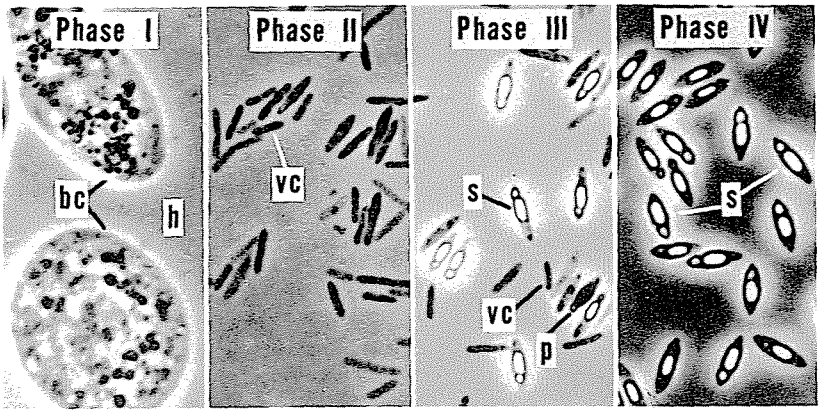

FIGURE 1. Phases I–IV of the infectious process of milky disease in hemolymph of field-infected Japanese beetle larvae. Viewed under phase contrast optics at ×1250. Phase I contains blood cells (bc), transparent hemolymph (h); phase II, vegetative cells (vc) in hemolymph; phase III, vegetative cells (vc), prespores (p), and spores (s) in hemolymph; phase IV, spores of *Bacillus popilliae* in hemolymph.

of hemolymph. Throughout the disease process, microscopic evidence indicates that many vegetative cells die without forming spores; dead cells disappear from the hemolymph by an unknown process.[45] Thus, the massive spore populations that characterize milky disease result from accumulation of spores during a prolonged period of simultaneous vegetative growth and sporulation.

Visual observation of the infectious process of milky disease in Japanese beetle larvae is seen in TABLE 4. Until recently, it was presumed that most field-infected larvae die at phase IV. St. Julian and associates[47] showed that less than 30% of infected larvae survive to phase IV; most larvae die at phases II and III of the infection. Presumably, larval death is due to rapid proliferation of *B. popilliae* vegetative cells and related events. Natural build-up of *B. popilliae* spores in field plots from diseased larvae may not occur so rapidly as previously assumed. To control Japanese beetles effectively, heavy concentrations of *B. popilliae* spores must be applied for immediate availability to larvae. Perhaps, annual or semi-annual application of spores is necessary. A commercial product (called "Doom"), now being used to control Japanese beetles, is prepared from spores developed *in vivo*.

SPIROCHETES

Spirochetes are not true bacteria. One outstanding difference between spirochetes and true bacteria is that spirochetes have a flexible cell wall, in contrast to the rigid cell wall characteristic of true bacteria. Compared to true bacteria, few spirochetes are known associates of insects. Some entomogenous spirochetes are nonpathogenic to man and animals; some are found in chance association with certain other anthropods.

Spirochaeta culicis has been found in salivary glands of *Anopheles* mosquitoes and in the intestinal tract and malpighian tubes of *Culex* mosquitoes.[55] *Spirochaeta pieridis* (isolated by Paillot[40]) causes a septicemia in caterpillars of the cabbage butterfly (*Pieris brassicae*). Interestingly, larvae infected with this spirochete exhibit no external symptoms to distinguish them from healthy larvae.

TABLE 4

VISUAL OBSERVATION OF THE INFECTIOUS PROCESS OF MILKY DISEASE IN JAPANESE BEETLE LARVAE

Time of Incubation (Days)*	Percentage of Larvae in Designated Phase of Infection†							
	Live Larvae‡				Dead Larvae§			
	I	II	III	IV	I	II	III	IV
1	84	16	0.7	0	0	0	0	0
7	18	76	3	2	39	50	11	0
14	4	60	27	9	10	37	47	5
21	3	9	68	16	3	11	80	7
30	6	1	67	26	2	2	85	11
30¶	18	36	39	7	0	28	71	1

* Larvae incubated at 25–28°C in field soil from which they were collected; Day 1 represents the first day larvae were placed in laboratory condition.

† Phase I = no visual evidence of infection; hemolymph is transparent. Phase II = hemolymph slight gray turbidity; vegetative cell proliferation. Phase III = off-white turbidity; concomitant vegetative growth, prespore formation, and sporulation. Phase IV = milky-white hemolymph containing massive spore population.

‡ On each designated day, 450 live larvae were examined.

§ All dead larvae found each designated day were examined.

¶ Observed only after 30-day incubation at which time all larvae found dead or alive were examined.

Paillot found these spirochetes to be principally in insect blood, hypodermal cells, and occasionally between intercellular spaces of fat tissue. Experimental infection of the cabbage butterfly with *S. pieridis* can be initiated only through the larval skin. Often, secondary bacterial septicemia accompanies spirochete infections.

RICKETTSIA

Generally, rickettsia are found in arthropods; however, only a few rickettsia-like organisms cause actual disease in insects. Several species of rickettsiae in the genus Rickettsiella (the "type" species is *Rickettsiella popilliae*) have been named after their insect host. Species of *Rickettsiella* isolated from insects include *R. popilliae*[20] and *R. melolonthae*[63] from Coleoptera, *R. tipulae*[36] and *R. chironomi*[62] from Diptera, and *R. blattae*[28] and *R. schistocercae*[61] from Orthoptera. The *Rickettsiella* sp. grow within the fat body cells of insects and are associated intracellularly with crystalline inclusions. Infected insects usually die within several months.[30]

Regretfully, little work has been done with rickettsia as insecticides. The lack of research with these organisms presumably is due to their apparent pathogenicity to some warm-blooded animals. Realizing this handicap, investigators should direct more attention to learning what animals are harmed by rickettsia used as insecticides. Probably, rickettsia are part of the normal flora of many insects. In our haste to research other insect pathogens, particularly bacteria and viruses, we may be overlooking other organisms that can serve as insecticides. For example, many investigators during the past decade have been preoccupied with developing a bacterial control agent for Japanese beetles. In so doing, the possibility of utilizing *R. popilliae* has been neglected. *R. popilliae* appears to be an effective pathogen of Japanese beetle larvae in the laboratory. Initial infection occurs in the fat bodies of the larvae and then spreads to other tissues once it reaches the hemolymph. Experimentally, larvae become infected upon ingestion of *R. popilliae* in soil. One of the best-known insect rickettsia is *R. melolonthae*,

which causes "Lorsch disease" in larvae of several lamellicorn beetles.[29,37] There is every indication from controlled laboratory studies that this organism, when applied to areas highly infested by lamellicorn larvae, can establish an enzootic disease.

Concluding Remarks

Bacterial control of insects is a reality. Spore-forming bacteria, such as *Bacillus thuringiensis* and *B. popilliae,* are being used to control lepidoptera and scarabeid insects, respectively. These microbial agents are as effective as the best chemical insecticides now available. These bacteria exhibit little change in virulence toward their insect hosts, and the insects develop no apparent resistance to the microorganisms as they do to chemicals. Of paramount importance, these bacterial insecticides do not affect the biocenosis; they pose no hazard to man and animals; and they do not pollute the environment.

In the past 50 years, numerous nonspore-forming bacteria and rickettsia have been observed in association with insects. These microorganisms have been isolated, classified, and sometimes shown to be experimentally pathogenic to their hosts. However, with few exceptions, little effort has been made to develop these isolates into control agents. It is curious that modern knowledge of microbial genetics, biochemistry, and physiology has not been used adequately as a framework for development of microbial control agents. Specifically, development of bacterial strains with host specificity, increased virulence, and stability to environmental changes is not being undertaken on a large scale. Controlled field studies have been limited to only a few known nonspore-forming insect pathogens. Perhaps because of the outstanding success of the two spore-forming *Bacillus* species, microbial control of insect pests has, at long last, the combined support of academe, government, industry, and the general public. Hopefully, greater efforts will be made to combine knowledge of related fields of science toward development of additional microbial insecticides.

Obviously, there is no single answer to our insect pest problems, and we are not suggesting that bacterial insecticides alone will provide the panacea for solving them. What we are suggesting is that microorganisms have a meaningful place in insect control and that greater research efforts in this area will significantly reward us and improve our environment.

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