SOME LIMITING FACTORS IN THE USE OF FUNGUS DISEASES FOR COMBATING INSECT PESTS*

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Insects are attacked in nature by many fungus diseases. Sporadic cases of such diseases are common, and may be found by a diligent collector at almost any time. Occasionally, however, some of these diseases appear in epidemic form so as almost to exterminate locally, insect species which previously had been abundant.

That house flies are commonly decimated in autumn by a fungus disease of that insect, *Empusa muscae,* is well known. Even the most superficial observer can

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scarcely have failed to notice dead bodies of house flies in Autumn sticking to window panes and to other surfaces, with the abdomen whitened by the spores and spore producing bodies of this fungus, and often with a broad circle of the discharged spores adhering to the surrounding surface so as to form a sort of halo about the dead insect. Other species of *Empusa* attack grasshoppers and plant lice, as well as many other kinds of insects, and epidemics of another fungus disease, *Sporotrichum globuliferum*, have often contributed materially to the suppression of the chinch bug during outbreaks of that notorious pest. It would be exceedingly difficult to grow citrus fruits profitably if the scale insects which attack these fruits were not largely destroyed and thus held in check by fungus diseases. Indeed, one of the authors has himself observed a mortality of 95 per cent in the scale insect, *Aspidiotus ancylus,* on pecans in Georgia, due entirely to fungus disease.

The fact that fungus diseases at times cause the destruction of great numbers of insects has aroused much popular as well as scientific interest; and since many of these fungi may easily be cultivated in the laboratory on non-living culture media, the question whether artificially produced epidemics of such diseases might not afford a ready means for controlling outbreaks of injurious insects has received much serious attention. The idea of employing their fungus diseases to control the ravages of noxious pests is not a new one. DeBary, the Tulasne brothers and others, as early as the middle of the last century, called attention to the important natural check on destructive insects afforded by the "white muscardine", *Isaria densa* Link., and similar organisms, and since that time many workers have attempted to produce epidemics of such diseases by artificial means for the purpose of combating insect pests.

Among the host of investigators who have worked on this general problem, Krassilschik (1884) employed the so-called "green muscardine", *Metarhizium anisopliae* Metch., to combat the beet weevil, *Cleonus punctiventris* Germ.; Rorer (1910) employed the same fungus in Trinidad to combat the sugar-cane frog-hopper; Forbes (1888), Snow (1889), and others have attempted to use *Sporotrichum globuliferum* Speg. to control the chinch bug; Rolfs (1907) and Fawcett (1908) have used *Sphaerostilbe coccophila* Tul. and *Ophionectra coccicola* E. and E. against the San Jose scale and the purple scale on the orange; and the last two investigators have also employed various other fungus diseases to combat the white fly in the citrus groves of Florida.

Very diverse opinions have been expressed by investigators who have worked upon this problem, concerning the success of their experiments and the promise of this method of insect control for practical work. Krassilschik claimed to have produced an epidemic of "green muscardine" among the weevils in the beet fields at Smelk, which destroyed from 50 to 80 per cent of these pests. Rorer reports the destruction of as many as 93 per cent of the sugar-cane frog-hopper in his field experiments in Trinidad, and most promising results have also been reported from work with the fungus diseases of scale insects and of the white fly in Florida. On the other hand, however, some workers have reported the complete failure of their experiments, and have expressed grave doubts concerning the practical utility of the method. Between these extremes, all degrees of success and failure have been reported, and all degrees of optimism and pessimism expressed by investigators who have studied the problem. On the whole, perhaps, a summation of these various reports and opinions tends rather to discredit than to recommend the method.

In spite of the disrepute into which the insecticidal use of fungus diseases has fallen in many quarters, the startling destructiveness of occasional natural epidemics of these diseases remains, and their importance as a constantly present natural check upon insect oscillations cannot be denied. These facts, therefore, together with the frank differences of opinion expressed by students of the subject, have seemed to justify reopening the question, and have led to the organization of a series of studies of which the present paper is a partial report.

The effectiveness of entomogenous fungi in both natural and artificially induced epidemics, appears to depend very largely upon climatic conditions. Indeed, those students of the subject who are most pessimistic concerning the practical utility of fungus diseases in insecticidal operations, recognize their great effectiveness under favorable weather conditions, but hold that sufficiently favorable conditions to insure success are encountered too rarely to justify the enthusiasm for the method which some of its advocates have expressed.

The procedure followed by former students of the subject has usually been to propagate some species of fungus diseased on artificial media, and to distribute the spores produced or the resulting culture of the organism, in fields or in other situations where the insect pest against which the operation was directed happened to be abundant. In such a case the results of the test and the conclusions of the operator are determined by the weather conditions which chance to prevail at the time. Laboratory studies generally have been little more than field tests in miniature, and usually with as little attention given to the precise relation between the results obtained and the conditions governing the test.

In organizing the present series of studies it was proposed to analyze the problem thoroughly and to study its various elements one by one: to proceed under carefully controlled conditions, and by changing certain of these conditions one at a time, to find how various degrees of temperatures, various percentages of humidity, and various other fators may influence the germination of the spores and the power of the fungus to penetrate the body of its insect host; to find how cultivation of the fungus on artificial media may influence its virulence or power to attack its proper insect host; to find how temperature, humidity and other factors may influence the power of the insect to resist infection when exposed to contamination with a fungus disease: and finally, in the light of the data thus accumualted, to canvass the meteorological records and to determine if practicable in what habitats of what localities during what months or seasons, fungus diseases may or may not be employed to combat outbreaks of noxious insects with a reasonable assurance that the operation will be successful. The present report relates to some of the effects of different degrees of temperature and different percentages of humidity upon the behavior of an entomogenous fungus toward one of its insect hosts.

The "green muscardine" of Metchnikoff, *Metarhizium anisopliae,* was chosen as the pathogenic agent in this series of studies because of its cosmopolitan distribution, because of its power to attack and destroy insects of many widely separated taxonomic groups, and because it has been employed in field operations with reputed success by several former investigators of the subject. For the insect host, pupae of the giant American silk worm, *Samia cecropia,* were chosen, because they are common and may easily be obtained in ample numbers, because they are large and easily handled and observed, because they are quiescent and more easily managed in the exposure cages than the active stages of any insect could possibly be, and finally, because they are available throughout the year if collected in the fall and kept in cold storage.

Constant temperatures of the various degrees indicated were maintained in a battery of six incubators, and in each incubator was provided a series of large dessicators, the atmosphere within each of which was maintained at a constant relative humidity by means of sulphuric acid in appropriate dilutions. This method for maintaining any desired degree of relative humidity is fully described and tables of dilutions are given by N. E. Stevens, Phytopathology, vol. 6, 1916, pp. 428-432. Thus, a predetermined series of humidity exposures could be made at the temperature maintained in each incubator, affording a temperature-humidity curve for each complete set of tests.

Spores both from pure cultures grown on potato and from infected cecropia pupae were employed in the course of the work, and in each test two lots of pupae were used, which were treated with these spores in two different ways. The pupae in one of these two series were simply dusted with the dry spores, while an emulsion of the spores in sterile physiological salt solution was prepared, and injected by means of a hpodermic syringe into the sub-hypodermal tissues of the pupae in the second series, each pupa in the inoculated series receiving many times the minimum fatal number of spores. That this method of inoculating the pupae is not injurious mechanically, is conclusively demonstrated by the fact that other pupae receiving injections of sterile physiological salt solution, similarly administered but several times greater in volume, survived the experience apparently uninjured and developed into normal adults.

The case history of this disease in the cecropia pupa presents several rather sharply defined stages which afford an excellent index to the effect of any combination of temperature and humidity conditions. The stages may be outlined briefly as follows: (1) The spores must germinate and penetrate the body wall of the pupa in sufficient numbers to overcome the natural resistance of the insect. This conquest of the host animal by the disease, and the development of the fungus at the expense of its tissues may be recognized by the characteristic hardening of the body of the insect commonly called mummification, and the two series of pupae just described afford an accurate check at this point, on those conditions which either entirely prevent the germination of the spores in contact with the pupa, or prevent their penetration of the body wall of the insect in numbers sufficient to infect the animal. (2) Having completed its development within the body of its host, the fungus must again penetrate the body wall to reach the exterior where the new crop of spores may be produced in a position most favorable for dissemination. This stage is characterized by the appearance of velvety-white masses of hyphae which appear first through the thinner portions of the integument between the abdominal segments, but which eventually may cover the entire surface. (3) The first evidence of spore formation consists of the appearance upon the velvety-white masses of aerial hyphae of small, irregular, light olive-green patches, which increase in size until they become confluent. (4) The final stage is the ripening of the spores, which are a dark, gray-olive in color, and which typically form a solid layer from one to two millimeters thick over the entire surface of the pupa.

Time will not permit a discussion of the details of individual experiments, but a summary of the general results presents the following facts which seem especially worthy of note: *A.* Even at optimum relative humidities, development of the fungus in these tests did not occur in either series of pupae at temperatures below 14° nor above 38° C. *B.* At 35° C. aerial hyphae may appear on the surface of a mature mummified specimen, but a new crop of spores will not be formed. At this temperature the injected spores may infect and kill the insect host, but the fungus can not propagate itself. *C.* The most rapid development of the fungus, or the shortest time observed between exposure to infection and the ripening of a new crop of spores (17 days for the injected, and 29 days for the dusted series), occurs at 30° to 31° C. *D.* Spores are produced in greater abundance at temperatures between 18° and 26° C., which appears to be the optimum temperature range for this species of entomogenous fungus. *E.* At optimum temperatures, constant relative humidities below 80 per cent appear to be prohibitive. At constant relative humidities between 80 per cent and 90 per cent infection does not occur in the dusted series of pupae, and while the disease in the inoculated series will progress to the mummification stage, the subsequent external development of the fungus and especially the formation of spores is very scanty. Relative humidities between 95 per cent and 100 per cent constitute the optimum range for this species.

It is needless to say that combinations of temperatures between 18° and 26° C. with relative humidities between 95 per cent and 100 per cent of any considerable duration are not common, especially during periods when injurious insects are most abundant, unless it be in tropical or sub-tropical regions or possibly in subterranean habitats. Further investigations are under way or projected which are designed to show what minimum exposure to optimum or favorable conditions may alternate daily with unfavorable or even prohibitive conditions and still permit a normal development of the fungus, and studies are being made of some of the problems relating to the behavior of this fungus under subterranean conditions upon which it is hoped a further report may be made in the near future.