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TREE CRICKETS AS CARRIERS OF *Leptosphaeria coniothyrium* (Fckl.) Sacc. AND OTHER FUNGI.

W. O. GLOYER AND B. B. FULTON.

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TREE CRICKETS AS CARRIERS OF *Leptosphaeria coniothyrium* (Fckl.)* Sacc. AND OTHER FUNGI.

W. O. GLOYER AND B. B. FULTON.

SUMMARY.

The name tree-cricket canker is proposed for a disease of apple branches in which areas of dead bark infested with ***Coniothyrium fuckelii*** Sacc. surround the oviposition punctures of tree crickets (***Œcanthus*** spp.). The constant association of ***C. fuckelii*** with such cankers aroused the suspicion that tree crickets act as carriers of the fungus. Through investigations herein described this suspicion has become an established fact. It has been shown that they may carry ***C. fuckelii*** from raspberries to apple trees and infect them. Also, that they carry within the digestive tract and on the outside of their bodies spores of many other kinds of fungi. Spores and fragments of mycelium of many kinds of fungi have been found in the excrement of tree crickets captured in the field and in excrement covering the oviposition punctures of the tree crickets. Many of these spores were viable,— germinating readily in drops of water.

In feeding experiments, spores of ***Ustilago zeae*** (Beckm.) Ung., ***Coprinus micaceus*** (Bull.) Fr., ***Coniothyrium fuckelii*** Sacc., ***Nummularia discreta*** Tul. and ***Sphaeropsis malorum*** Pk. passed through the digestive tract of tree crickets without loss of viability.

Typical cankers resulted when tree crickets fed on ***Coniothyrium fuckelii*** were permitted to oviposit on apple branches. The percentage of oviposition punctures resulting in cankers was considerably greater when the insects were fed with ***C. fuckelii*** on raspberry canes than when they were fed with pure cultures of ***C. fuckelii*** isolated from apple branches. The oviposition punctures of ***Œcanthus niveus*** gave a higher percentage of infection than did those of ***Œ. angustipennis;*** and for both species the percentage of infection was considerably higher when the punctures were covered with grafting wax than when they were left unprotected.

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* In this paper *Coniothyrium fuckelii* Sacc., the name given to the pycnidial stage of *Leptosphaeria coniothyrium* (Fckl.) Sacc., is frequently used as a matter of convenience when reference is made to the pycnidial stage of the fungus.

Typical cankers have been produced artificially, also, by inserting pellets of tree-cricket excrement into punctures made in apple branches with a sterile instrument and covering them with grafting wax.

Clean cultivation and the use of arsenical sprays as for codling moth appear to be the only remedial measures required for the control of the tree crickets.

REVIEW OF LITERATURE.

That the various species of tree crickets (*Œcanthus* spp.) disseminate plant diseases has been suggested by previous writers, but in all cases the evidence adduced was only circumstantial. During the last two years the writers have studied more closely some of the habits of these insects and their relation to certain fungus diseases. The data here presented positively convict them of disseminating one parasitic fungus, *Leptosphaeria coniothyrium*, and strongly suggest the possibility of their carrying others.

The present paper is to be considered as supplemental to Bulletin No. 388 and Technical Bulletin No. 42 of this Station rather than as a summary of these publications. Some of the things mentioned are not considered in detail when such observations have already been discussed in the previous writings.

It is impossible to consider here the work of the vast number of writers (9, 27) who have discussed the dissemination of human and animal diseases. It will be necessary to confine ourselves chiefly to the literature of the agricultural phase of the subject. In considering agents of the transmission of bacterial diseases of plants E. F. Smith (38, 39) states that man, domestic animals (through the agency of the dung heap), birds, insects, and worms may distribute the bacteria. Waite (46), Stewart (43), Jones (23), and Burrill (3) have proved that insects carry the bacterium causing the fire blight of fruit trees. Others who have made observations on this disease have associated many other insects as possible carriers. Brenner (2) and Smith (38) have observed that aphides and the larvæ of the cabbage butterfly may disseminate the bacterium causing the black-rot of cabbage. Smith (37) has shown that the striped cucumber beetle (*Diabrotica vittata*) disseminates the organism causing the wilt of cucurbits; and, recently, Rand (33) has demonstrated that these insects may carry the disease over winter. Küster (24), working with pathogenic bacteria, has shown experimentally that bacteria remain at least 24 hours in the intestinal tract of cockroaches. He was able to find some of the bacteria in the excrement a month after the insects were fed cultures. These organisms were still viable, and without any effect upon the insect. Wheeler (48) suggests that ants may disseminate the fire blight of fruit trees because they feed on the liquid, saccharin excrement (honeydew) of sucking insects such as plant lice, scale insects, psyllids, cicadas, etc., which may have fed previously on diseased tissue.

Considering the fact that insects normally act as carriers of pollen, it appears but natural that smaller organisms such as fungus spores could be carried with greater ease. Heald (18) and Stewart and Hodgkiss (42) found a mite associated with the bud-rot of carnations. Grossenbacher and Duggar (17) noticed that the American currant borer, *Psenocerus supernotatus*, fed on the sclerotia and stromata of *Botryosphaeria ribis*, and that in some cases infection had taken place about some of the oviposition punctures. Clinton (5) and Burrill (4) have shown that the fungus of the apple bitter-rot is carried by insects. Ducloux (10) believes that the woolly aphis is the cause of the distribution of the European canker fungus, *Nectria ditissima*.

The dissemination of the chestnut bark disease has been much considered by recent writers. Heald and Studhalter (19) have shown that birds may carry the causal organism. Studhalter (44) and Studhalter and Ruggles (45) ascertained the number of Endothia or other spores found on the bodies of ants and other insects, and have shown that these spores may become dislodged by the movements of the insects. Craighead (8) observed that certain insects, especially *Leptostylus macula*, eat the pustules and stromata of *Endothia parasitica*. He made plate cultures of the stomach contents and excrement of this species but failed to obtain growth of Endothia. Because of this fact he concluded that the insect plays an important role in checking the chestnut bark disease.

Whether or not the spores of fungi are acted upon by the digestive juices in their passage through the intestinal tract of animals has often been discussed, but less often proven. *Ascobulus furfuraceus* (22) and *Onygena equina* (31) were found to germinate only after they had been acted upon by the digestive juices of animals. Honcamp and Zimmerman (20) while working on the passage of stinking smut through the digestive tract found that the spores were not viable after passing through chickens or other higher animals. Arzberger (1), in some of his unpublished work on corn, has shown that the spores of *Coniosporium gecevi* and *Fusarium* spp. found on the kernels of corn, would pass through the alimentary tract of chickens uninjured. In fact, spores had germinated and produced mycelium that readily grew on the culture medium. That insects are attracted to stinkhorns has long been known (since 1575) and that their spores are found in insect excrement was shown by Fulton (14). Cobb (7) showed that the spores of the stinkhorn, *Ithyphallus coralloides* (said to cause a root disease of the sugar cane), are eaten by flies, beetles, ants, cockroaches, earwigs, wood-lice or sow-bugs. Such spores passed through the digestive tract unchanged. On the other hand, the spores when eaten by a mule did not germinate, having apparently been acted upon by the digestive juices. Lewton-

Brain (25) also, working with sugar cane diseases, found that the red stem-rot due to *Colletotrichum falcatum*, usually enters through wounds made by the cane borer. Grassi (16) fed flies Lycopodium spores, *Oidium lactis* from cream, and the spores of Botrytis taken from silkworms. Both the Oidium and Botrytis were found in the flies' dejections. Mercier (26) found the excrement of *Sciara thomae* composed mostly of the spores of Claviceps. Wheeler (47) in discussing the fungus-growing ants of North America describes ants collecting caterpillar excrement and portions of leaves in order to make the so called "fungus gardens." He (48) also suggests that the ants may distribute spores by dropping hypopharyngeal pellets or by dropping their germ-laden feces. Petch (30) observed that the termites used their own excrement in making the fungus gardens and that on this material various species of fungi were usually found more or less constantly. The ambrosia beetles (47) are known to have fungi growing upon their feces and that these fungi are also used as food. Schneider and Orelli (36) found this fungus generally to be *Monilia candida*, and that the female *Xyleborus dispar* carries in her gizzard a supply of the spores which are not digested. Forbes (12) studied the feeding habits of tree crickets and noted that spores of various fungi could be found in their excrement.

Several species of Coniothyrium have been suspected of being carried by insects and higher animals. Prillieux (32) describes the white rot of the grape due to *C. diplodiella*, states that it is a wound parasite, and that it is found about injuries made by the Cochylis. Stewart and Eustace (41) observed lesions produced by *Coniothyrium fuckelii* about oviposition punctures made by the tree cricket* on raspberry canes. Clinton (6) found a fruit-rot of the raspberry due to this fungus, and states that the spores may have been carried by bees or insects that had previously crawled over stems coated with spores. Hopkins (21) noted a blighted or cankered area about tree-cricket punctures, but did not ascertain the cause. Parrott and Fulton (28) have called attention to *Leptosphaeria coniothyrium* as being present in such cankers and suggested that tree crickets were the possible carriers. Engler and Prantl (11) record *C. coprophilum* as occurring on rabbit dung in Argentina, indicating that this fungus may have entered the alimentary tract of the animal and was uninjured in the digestive processes.†

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* Reported as snowy tree cricket, *Œcanthus niveus;* but, from the description of the manner of oviposition, evidently *Œ. nigricornis.*

† That fungi are carried in and develop on the dung of herbivorous animals has long been known. Fresh horse dung placed under proper conditions will develop many species of fungi that appear and disappear in rapid succession. Some fungi are to be found only on the dung of certain species of animals due to their peculiar feeding habits. This subject has been considered by many writers amongst whom are Brefeld (Unters. Gesammtgeb. Mycol. 14: 29, 1908), Arthur (Ind. Sta. Rpt. 1899, p. 125), Morse (*Phytopath* 2: 147, 1912), Massee and Salmon (*Ann. Bot.* 15: 313-357. 1901), and Buller (Researches on fungi, pp. 224-230. 1909).

DESCRIPTION OF TREE-CRICKET CANKERS.

The prevalence of the cankers formed by *Leptosphaeria coniothyrium* (Fckl.) Sacc. (*Coniothyrium fuckelii* Sacc.) has been generally overlooked in the orchard due to the fact that they are seldom recognized as such. More often they are mistaken for the early stages of other cankers such as those caused by *Bacillus amylovorus* (Burr.) De Toni, *Sphaeropsis malorum* Pk., or *Glomerella cingulata* (Stonem.) S. & v. S. Previous writers at this Station (28, 13) have described the interesting egg-laying habits of the various species of the tree crickets. From the latter part of August until the insects are killed by frost, the females deposit their eggs in the various host plants. As *Œcanthus niveus* De Geer and *Œ. angustipennis* Fitch are of economic importance to apple trees these species were given special study with reference to their ability to carry fungi. The essential difference between these two species, insofar as dissemination of diseases is concerned, lies in the fact that the former plugs the opening of the oviposition puncture with a pellet of excrement, while the latter uses portions of chewed bark that it has gathered promiscuously. *Angustipennis* prefers branches one-third to one-half inch in diameter while *niveus* generally selects succulent branches from one to three inches in diameter. *Œ. exclamationis* Davis also uses a pellet of excrement, and it is believed that this species, although much less common and localized in its distribution, may also be a disseminator of plant diseases.

If, in the fall, before the leaves drop, one examines the tree-cricket punctures made on apple trees, the early stages of this canker may be found. The slightly-sunken, circular or elliptical areas of bark may be as large as 2.5 cm. (one inch) in diameter with the oviposition puncture in the center or slightly below the center. When first attacked the bark becomes water soaked and, upon drying slightly, turns brownish in color. Upon complete desiccation the color changes to a dull red. The parasite not only kills the bark but invades the underlying wood and gives it a brown color. The desiccation of the diseased bark, combined with continued growth of the healthy bark, produces a line of demarcation between these tissues. In the warm days preceding the blooming period these cankers may enlarge or new ones which had not shown any activity before may become aggressive. Usually, this period is more favorable for the development of the natural cankers than the fall of the year. Plate I, fig. 1, shows Leptosphaeria cankers about oviposition punctures. In this case the punctures were covered with grafting wax as soon as made. The cankers enlarge by degrees through periods of growth alternating with periods of quiescence. This results in the formation of rings of light- and dark-colored bark. In some cases eight or nine such rings have been observed. Plate I, fig. 2, is the upper canker shown in Fig. 1 with the grafting wax removed. It shows a newly-formed ring of secondary growth which has not changed color and no crack has formed between the first and second periods of fungus activity. In the Spring the cankers may be as much as 7.5 cm. (3 inches) in length or may be merely a puncture with some dead tissue about it. Sections made through a puncture and the surrounding dead tissue will show strands of mycelium passing from the excrement to the tissue. Cultures have been made of the cankered bark and from the tissue about the punctures which showed but little disease, and the Coniothyrium was readily isolated. Saprophytes, also, are found in these tissues, but these are considered as secondary, having attacked the tissue after it was killed by the parasite. In this bulletin we have considered all lesions larger than 5 mm. ($\frac{3}{16}$ of an inch) as produced by the organism while those smaller than this are attributed to mechanical injury made by the insect's ovipositor.

There is always a tendency on the part of the host to exclude the parasite by the production of a callus at the boundary between the healthy and diseased bark. This may consist, in some cases, of a few bark parenchyma cells formed at right angles to the line of fungus attack. The parasite is unable to attack the growing tissue and in some seasons the entire wound may be healed. Often the callus tends to pry the dead bark loose from the wood, thus producing a concave dead area of bark. Usually this bark is separated from the wood and is held in position at the margin by fragments of bark. In but few cases does the fungus again become aggressive in the autumn after a callus has formed; but when this happens the callus is killed back and concentric rings of dead tissue resembling minature Nectria cankers are formed. Plate II, fig. 1, shows a tree-cricket canker that made a growth in the autumn of 1915. Fig. 2 is a longitudinal section of the same. The wood beneath the bark lesion is dark brown in color with tissue strands of still darker color in the 1914 wood. Mycelium of the parasite was seen in this wood and portions of the wood transferred to culture media gave rise to the pycnidial stage of *Leptosphaeria coniothyrium.* These figures also show how the callus pries off the diseased bark, forming cavities in which woolly aphides and other insects like to hide.

In some cases the callus, in the healing process, tends to bunch at the center of the wound. This tender tissue, when irritated by the presence of the woolly aphides, may produce outgrowths called flap tumors by Reed and Crabill (34). The largest outgrowth of this kind observed in the orchards about Geneva was 6 mm. (one-fourth inch) in diameter and was produced in 1915 from the center of a healed canker estimated to be more than three years old. Upon this dome-shaped outgrowth, as well as on other smaller ones, woolly aphides were found. It is believed that the flap tumor, as described by Reed and Crabill, is not due to the tree crickets or the parasite

Coniothyrium; but that the woolly aphides, by their presence, stimulate the growth of the tissues in the same manner as in the formation of galls produced on roots on branches infested by this insect.

An examination of the cankers a year after the punctures were made by the insects will show but few fungus fruit-bodies developed. In some cases, especially on the large cankers, the pycnidial form, *Coniothyrium fuckelii*, may be found as black dots in a gray-tan background of dead bark at the edge of the lesion. In other cases, the papery epidermis may become loosened and expose the pycnidia resting on the bark. Should the bark have become sloughed off early in the summer the pycnidia may be found resting on the surface of the wood. On the loose bark which has been dead for two or more years the pycnidia can be readily seen hidden underneath the epidermis and resting upon the surface of the bark. When moistened with water these can be seen even with the naked eye. On such tissue, *Sphaeropsis malorum*, *Valsa leucostoma* or lesser parasites, and saprophytes also, can be found. In some orchards the superficial form of Sphaeropsis canker may be found on the larger apple branches about the tree-cricket punctures. These areas are from 5 to 10 cm. (2 to 4 inches) in diameter and differ from the Leptosphaeria cankers in that in the former only the outer layers of bark are attacked.

On young trees of *Ulmus americana* 10 to 12.5 cm. (4 to 5 inches) in diameter, and growing among tall weeds, *Coniothyrium fuckelii* was found producing superficial cankers about the oviposition punctures. Associated with this fungus was found *Pestalozzia insidens* (49). On older trees, grown in a nearby pasture, cankers were not found.

As the canker caused by *Leptosphaeria coniothyrium* is so often found associated with tree-cricket punctures it is proposed that the common name tree-cricket canker be applied to it. It is admitted by the writers that this insect is capable of carrying, also, other canker-forming fungi, but the cankers produced by them can be readily distinguished from the Leptosphaeria cankers. Moreover, they are of infrequent occurrence.

Considering the general habits of the tree crickets, there are several possible ways in which the spores of fungi may gain entrance to oviposition wounds and, later, form cankers. Spores may be introduced: (1) With excrement used in plugging the punctures; (2) with chewed vegetable tissue used in plugging the punctures; (3) on the ovipositor; (4) by being washed into the punctures by rain. These different methods will now be considered in detail.

PASSAGE OF FUNGI THROUGH THE DIGESTIVE TRACT OF TREE CRICKETS.

If bacteria or fungi are still viable after passage through insects, this fact in itself would indicate that the insects are disseminators of diseases. Before making feeding tests it was thought advisable first to ascertain what organisms are found in the excreta of the tree crickets found in the field. The nature of the excreta naturally depends upon the material that the insect eats. Forbes (12) states that the food of Œcanthus is highly miscellaneous, consisting largely of floral organs of grasses and other plants, pollen, leaf tissue, various fungi, plant lice and other insects. To this list may be added dead leaves, diseased bark, and fruit such as pears, peaches, plums, etc. In order to obtain excrement, 100 tree crickets were captured in a somewhat neglected orchard and placed in a clean cage for 24 hours. No food was given them during this period. Excrement was also collected from the openings of oviposition punctures. In some cases the feces were forced from the body and collected under sterile conditions. These pellets were examined under the microscope and in no case were any found free from fungus spores or mycelium. Spores of all sizes and descriptions were found, most of which it was impossible to classify. Besides numerous bacteria and yeasts, there were fungus spores which might belong to the following genera: Penicillium, Aspergillus, Mucor, Phoma, Cladosporium, Fusicladium, Cephalothecium, Pestalozzia, Fusarium, Septoria, Ascochyta, Sphaeropsis, Coniothyrium, Coryneum, Puccinia (uredo and teleutospores of *P. graminis*), Oidium, Fumago, etc. The spores and adults of *Gregarina* sp., an intestinal protozoan, were also very common. Many of the fungus spores germinated when placed in drops of water and kept in a damp chamber. Poured-plate cultures were made of the pellets, but in most cases the medium was soon overrun with the more rapid-growing fungi. That *Sphaeropsis malorum* may also be found in the excrement is seen in that six out of eight pellets examined on one occasion showed these spores present and, when plated out, colonies of this fungus were recognized.

Preliminary feeding tests were made to determine if spores will pass through the digestive tract uninjured. For this purpose 14 feeding experiments were made with three tree crickets in each experiment. One of these tests has already been reported (29). The insects were placed in cages and permitted to feed on raspberry leaves which had been sprayed with a 2 to 5 per ct. sugar solution containing spores of corn smut (*Ustilago zeae*), or spores of a mushroom (*Coprinus micaceus*). The tree crickets were observed for several days, and the excrement collected and placed in drops of water. In no way was the germination of the spores impaired in going through the digestive tract, the percentage of germination being the same as in the checks.

The preliminary experiments having proved satisfactory, it was thought advisable to ascertain if the various fungi causing apple-tree cankers would pass through the digestive tract uninjured. For this purpose a single tree cricket (*niveus*) was chosen. It was placed under a soil percolator which was properly ventilated, and fed upon a ripe pear (variety Vermont Beauty). It was allowed to feed also on the following fungi, only one being present at a time: (1) A stroma of *Nummularia discreta* and its surrounding bark, upon whose surface there were estimated to be from three to five million spores*; (2) a dead branch of an apple tree bearing a large number of pycnidia of *Sphaeropsis malorum;* (3) a portion of a Cuthbert raspberry cane attacked by *Coniothyrium fuckelii,* the spores of which were so plentiful as to give the cane a dusty brown coating. In order to provide drink for the insects and facilitate mastication the portions of diseased tissue were moistened with water every other day.

The test was carried on from October 28 to November 25, 1915, and in no case was the germination of the spores affected in any manner. From six to twelve pellets of gray-black excrement measuring 1.5 x .75 mm. are given off in 24 hours. Tree crickets starved for 40 hours and then fed spores showed these same spores in the excrement within 6½ hours. Generally, the spores remain at least 24 hours in the digestive tract. It was observed that when the branches were moistened with water very few pellets were collected the following day. Instead, the wall of the glass soil percolator was spattered with soft pellets ejected by the insect. In one case the excreta were thrown 7 cm. (2¾ inches) from the branch and on a level with the top of the branch. In other cases pellets were found 7.5 cm. (3 inches) from the nearest portion of the branch and 17.5 cm. (7 inches) from the top of the branch from which point they were ejected. This would indicate that in some conditions the excrement is not under control of the insect. Under such conditions few plugs would be found in the oviposition punctures.

The feeding experiments made with *Nummularia discreta* showed some interesting facts. A drop of water was placed on the stroma to induce the insect to drink. The cricket was seen to walk through this liquid, and soon thereafter preen its legs with its mouth-parts. Only after 44 hours were a few pellets collected that showed 10 to 15 spores that had been taken into the digestive tract during the preening. After 8 days the insect finally found the spores on the stroma and made a good meal of them. An examination of the stroma showed but few spores where previously there were millions. The pellets collected the next day were black and made up mostly of spores. Five days later the last of these spores were expelled by the insect. Pellets, made up mostly of spores, showed in one case about 2 per ct. of the spores chewed while in other pellets none were observed to be ruptured. Pellets, as well as spores taken from a stroma, were placed in drops of water to compare the germination. In both cases there was a retarded and low percentage

* Based on counts made by a hemacytometer on similar stromata.

(2 per ct.) of germination. The experiment was then repeated, with a few hand sections of apple wood placed in some of the drops of water. The spores became attached to the wood sections and those from both the pellets and checks showed a rapid germination of over 95 per ct. in 24 hours. Those placed in distilled and tap water again showed the low percentage of germination. These tests were repeated many times (using vials as well as drops of water), and it was established that in the fall of the year the spores of *N. discreta* readily germinate in the presence of apple wood and that these spores pass uninjured through the intestinal tract of the tree cricket.

When apple branches bearing Sphaeropsis or raspberry canes bearing Coniothyrium were used in the feeding tests the same general results were noted. On the apple branch the tree cricket would chew the pycnidium of *S. malorum* as well as the surrounding bark producing a cavity as large as a pin head. The papery epidermis of the raspberry canes was also eaten. As the insect has a tendency to climb as high as it can, these chewed portions of the bark were most often observed near the tops of the canes or branches. The first spores of *S. malorum* found in the pellets were few and did not germinate even when placed in the presence of sections of apple wood. Later, spores were more abundant; also pycnidial tissue from which mycelium would grow. These later spores germinated readily in water, and quite as well in the absence of wood sections as in their presence. The spores of *Coniothyrium fuckelii* were readily eaten and were plentiful in the excrement. In water, the spores from the pellets and also those taken directly from the canes germinated slowly, producing short hyphæ. When placed in the presence of sections of apple wood the percentage of germination increased from 25 to 100 per ct., and the growth of mycelium was five to six times as rapid. Spores of *Sphaerella rubina* also passed through the digestive tract uninjured.

Since the tree cricket used in the above experiments lived about a month on a diet of pear and diseased apple bark and raspberry canes it may be of interest to consider the source of its nutrition. It is clear that no nourishment was obtained in eating the spores, for they were not digested. Perhaps some of the bark eaten was predigested by the fungi; but this would not explain why the large mass of Nummularia spores was eaten. It is possible that the spores may act as roughage and were eaten for that purpose; but it appears more plausible that the spores still retain on their surface some of the protoplasm of the ascus or pycnidium which makes them palatable. In some pellets most of the material was made up of spores, mycelium or bark, while in others the greater portion consisted of digested pear tissue. In the pear, the insect would first chew holes about 2 mm. in diameter — just large enough to permit the entrance of its head. In subsequent feedings the holes would be enlarged. It was observed that the insect rejected small portions of pear tissue .5 to .8 mm. in size. Upon examination these were found to consist mostly of stone cells. However, all of the stone cells were not rejected as being too hard to chew for some were also found in the dejections. That the toughness of the skin of the fruit is important in resisting the insect is shown by the fact that when tough-skinned grapes or apples were placed in the cage they were not attacked. These observations confirm those made by Garman (15) who noted that tree crickets rather than bees injure plums, peaches and grapes. About the holes made on the pear, decay would set in and Penicillium, Alternaria, *Sphaeropsis malorum* and *Coniothyrium fuckelii* were found, indicating that spores of these fungi may have been distributed through the mouth-parts of the insect or carried on its body and deposited on the injured tissue.

That *Œ. nigricornis* may disseminate fungi can be seen in the examination of its oviposition punctures made on raspberry. Hard sections of the tissues about these wounds showed the presence of ramifying strands of mycelium arising from the masses of chewed tissue inserted into the holes. In this manner may be explained the numerous lesions of cane blight that develop about tree-cricket punctures on raspberry canes.

After the death of the specimen of *niveus* used in the above experiments, it was dismembered and the portions examined under the microscope. As the insect was last feeding on a branch attacked by *Sphaeropsis malorum* it was covered with the dark-colored Sphaeropsis spores which could be readily seen and counted. On the hind legs were counted 456 spores of which the greater number were found at the proximal end of the tarsus near the tibial spurs, which assist the insect in clinging to the bark. Plate IV, fig. 1, is a photomicrograph of the tarsus showing the numerous spores clinging to the spurs, hairs and surface of the leg. On the second pair, and on the fore legs, 87 and 18 spores respectively were counted. On the head, mouth-parts and antennae, 30 spores; on the dorsal and ventral surfaces of the body 25 spores; and on the wings 39 spores were counted, making a total of 655 Sphaeropsis spores found on the tree cricket. Six Nummularia spores were found — two on the wings and four on the tibia of one of the hind legs. As the insect had not been in contact with the spores of this fungus for 20 days this further demonstrates how long the spores may cling to the body of these insects. The small size of the Coniothyrium spores and their similarity in color to that of the insect made it difficult, by direct observation, to obtain data in regard to the number of spores present. In examining the wings we were surprised to find germinated spores of unidentified fungi which had gotten on the wings before the insect was introduced into the feeding cage. Plate IV, figs. 2 and 3, are photomicrographs of portions of the wings. Figue 2 shows the mycelium arising from a portion of dead bark, and on one side are seen Sphaeropsis spores that had not germinated. In Fig. 3 the mycelium arose from some spiny spores of an unidentified fungus. In general, those portions of the body most difficult to preen had the most spores on their surfaces, and it appears evident that the insect can disseminate fungus spores not only by means of its excrement, but also by dropping spores that become attached to its body.

ATTEMPTS TO INDUCE CANKER FORMATION BY MEANS OF THE EXCREMENT.

The evidence that spores pass through the digestive tract of the tree cricket uninjured, a fact in itself sufficient to convict the insect of being a disseminator of diseases, led the writers to inquire if, under controlled conditions, apple cankers could be formed by them. For this purpose a large number of tree crickets were collected in 1914, placed in cages and allowed to feed on raspberry leaves, which they readily ate. Mycelium from pure cultures of *Coniothyrium fuckelii*, isolated from an apple canker, was ground in a sterile mortar, placed in prune juice, 2 per ct. sugar solution or in distilled water, and then spread upon the raspberry leaves. In other cages, were placed raspberry canes infested with *Coniothyrium fuckelii* showing an abundance of the pycnospores, and the insects allowed to feed on them for at least 24 hours. The two species, *Œ. niveus* and *angustipennis*, were then separated and placed in cages about apple branches.* For *niveus*, branches at least 2 cm. ($\frac{3}{4}$ inch) in diameter were selected while for *angustipennis* smaller branches were used. These tests were begun September 9, and ended October 13, when the cold weather made it impracticable to continue them longer.

* The cages used in these experiments proved highly satisfactory. They are made in the following manner: Stretch tightly two parallel wires A and B, 21 inches long and ten inches apart. Commencing three inches from one end of these wires, lay across them, at intervals of five inches, three pieces of wire 22 inches long and one piece, C, 30 inches long. Let the long wire project about four inches beyond the shorter ones at each end. At the points of intersection fasten the cross-wires securely to A and B. This is best done by means of U-shaped pieces of wire which are first placed astride the cross-wire, then bent under the intersecting wire and upward on the opposite side where the ends are finally twisted together over the cross-wire. In making the fastening, both wires must be bent a little to prevent slipping. If the wire used is not too coarse this is readily accomplished by twisting the ends of the fastener with a pair of pliers. Iron wire, No. 12 gage, is very satisfactory for this purpose. Up to this point the construction resembles closely that of a small wire fence. Time may be economized by making several frames at one time on two long pieces of wire.

The frame is now ready to attach to the branch. Place the long wire, C, parallel with the branch. Bend the wires A and B around the branch in such a manner as to make a four-sided figure and fasten together their free ends by means of a hook-and-eye attachment. At the upper and lower ends of the frame bend inward the free ends of the three shorter wires, place a ring of cotton between them and the branch, and fasten all securely to the branch by winding closely around them the projecting ends of the long wire C. (See Plate III.) Over this frame wrap cheesecloth closely and tie the ends above and below with string.

The insects are introduced just before tying the upper end.

It was observed that when the insects were caged on the branches for two nights the weaker ones (usually males) were killed and partly eaten by their stronger companions. The mortality being high it was decided to supply them with aphides (several species) found on weeds and grain. After this was done but few crickets were killed. At the end of the second day the cages were examined and the number of punctures counted. Some of them were covered with grafting wax, to exclude outside contamination, while others were left untreated, but their position was marked with white lead paint. This last method was necessary in order to distinguish cankers formed under controlled conditions from those that might be formed later in the natural manner. The growth of the cankers was observed from time to time the final examination being made November 18, 1915. The results, as given in Table I, include all cankers larger than 5 mm. ($\frac{3}{16}$ inch) in diameter. Cultures were not made of all the cankers formed, but whenever such cultures were made (especially in the early stages) no difficulty was found in obtaining the Coniothyrium. Other fungi (saprophytes) were also found, but they were not constant.

Table I.—Summary of Data Pertaining to Cankers Formed About the Oviposition Punctures of Tree Crickets Kept Under Controlled Conditions.

Number of cages.	Species of tree cricket	Number of punctures made.	Treatment of punctures.	Total number of cankers formed.	Percentage of cankers formed.
	Fed pure cultures of *C. fuckelii* from apple canker.				
17	*Œcanthus angustipennis*.....	118	Waxed...	9	7.6
23	"	210	Unwaxed.	4	1.9
4	"	0	....	0	0
31	*Œ. niveus*..................	275	Waxed...	17	6.1
37	"	414	Unwaxed.	8	1.9
6	"	0	....	0	0
	Fed raspberry canes infested with *C. fuckelii*.				
13	*Œ. angustipennis*..........	118	Waxed...	24	20.3
6	"	59	Unwaxed.	5	8.4
5	"	0	....	0	0
19	*Œ. niveus*..................	177	Waxed...	44	24.8
6	"	62	Unwaxed.	11	17.3
2	"	0	....	0	0
	Checks: fed raspberry leaves only.				
3	*Œ. angustipennis*..........	10	Waxed...	0	0
1	"	3	Unwaxed.	0	0
2	"	0	....	0	0
11	*Œ. niveus*..................	97	Waxed...	3	3.
11	"	88	Unwaxed.	0	0
5	"	0	....	0	0
202		1,631	....	125	7.6

EXPLANATION OF PLATES.

PLATE I.— FIG. 1. Cankers about oviposition punctures of *Œcanthus niveus* on an apple branch. Tree crickets were permitted to feed on raspberry canes infested with *Coniothyrium fuckelii* and then caged on an apple branch during two nights. Seventeen oviposition punctures were made each of which was covered with grafting wax. Four cankers, *a*, *b*, *c*, *d*, were formed. From *a* and *d* pure cultures of *Coniothyrium fuckelii* were obtained; while *b* gave rise to rapid-growing saprophytes which overran the slower-growing Coniothyrium. Cultures of *c* remained sterile owing to too long treatment of the tissue with mercury bichlorid. Natural size.

FIG. 2. Canker *a* of *fig. 1* twice enlarged. The grafting wax has been removed to show the relation of the puncture to the canker. This figure shows, also, the zonation of the dead tissues due to periods of varying activity of the fungus. The second zone was formed five days after the first. It was characterized by a slight reddening of the bark while the color of the older or central portion of the canker was reddish brown.

PLATE II.— FIG. 1. Canker formed about an oviposition puncture of *Œcanthus niveus* on an apple branch. Tree crickets were permitted to feed on raspberry canes infested with *Coniothyrium fuckelii* then caged on a Baldwin apple branch September 29, 1914. The oviposition punctures were not covered with grafting wax, but their location was marked by dabs of white paint. Eighteen punctures were made, but only one canker resulted. This canker, not in evidence as late as April 3, 1915, had attained a diameter of 2.5 cm. (one inch) by May 8, 1915. During the following summer a callus formed and the growth of the canker was arrested; but in the autumn of 1915 the fungus again became aggressive and produced an enlargement of the lesion at the upper end. The oviposition puncture from which the canker originated may be seen a few millimeters below the paint spot. Enlarged one and one-half diameters.

FIG. 2. A longitudal section through the canker shown in fig. 1. Microscopic examination of the discolored (brown) wood revealed the presence of fungus hyphae in it. Cultures were made and *Coniothyrium fuckelii* obtained. This figure shows how the formation of the callus causes the dead bark to separate from the wood. In the newer portion (upper part) of the canker the bark is dead but the wood underneath is not yet discolored. Enlarged one and one-half diameters.

PLATE III.— Frame of cage for confining tree crickets on apple branches. For description see footnote on page 14. About one-fourth natural size.

PLATE IV.— FIG. 1. Photomicrograph of the proximal portion of the tarsus of a hind leg of *Œ. niveus* showing spores of *Sphaeropsis malorum* clinging to spurs, hairs and surface of the leg. The spurs serve as holdfasts to assist the tree cricket in walking on upright surfaces.

FIG. 2. Photomicrograph of a mass of mycelium found on the wing of *Œ. niveus*. Spores of *Sphaeropsis malorum*, although present, have not germinated.

FIG. 3. Photomicrograph of germinated spores on the wings of *Œ. niveus*. In this case the spores (unidentified) are large and spiny. They became attached to the wings while the insect was in the field.

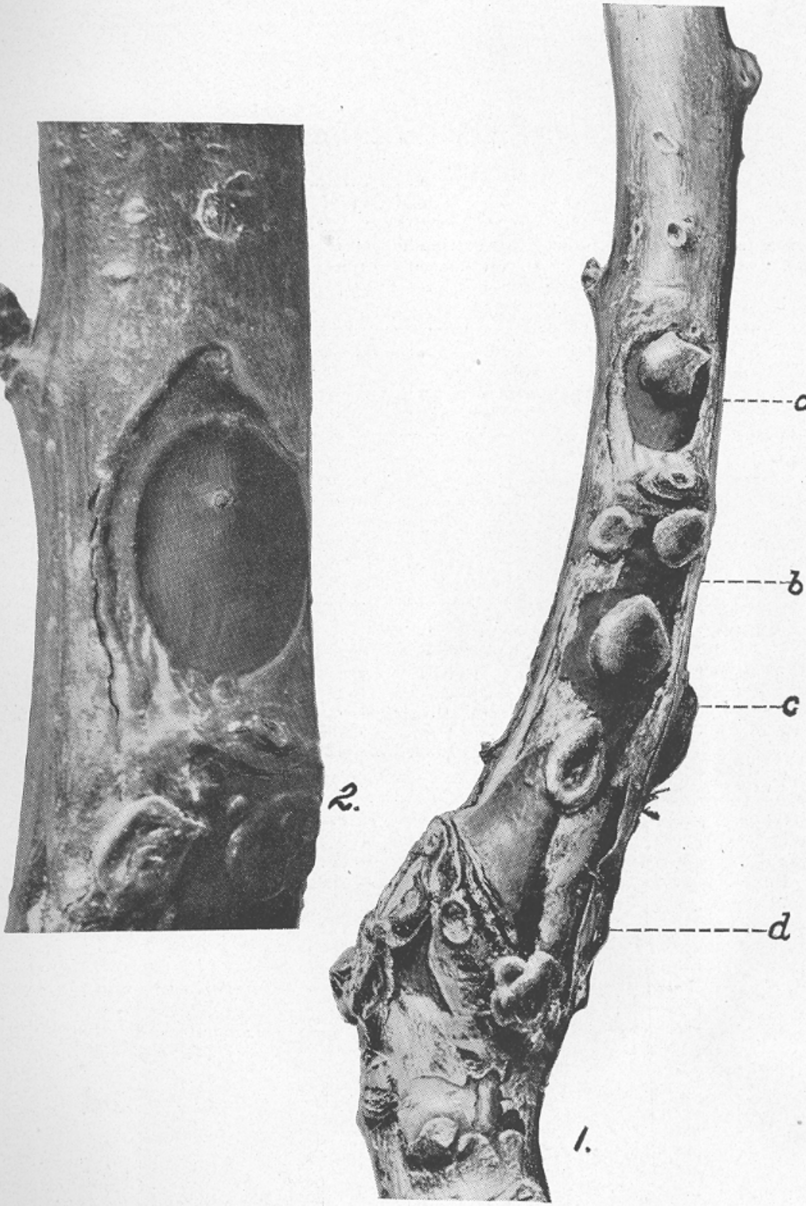

PLATE I.—TREE-CRICKET CANKERS ON APPLE BRANCHES.

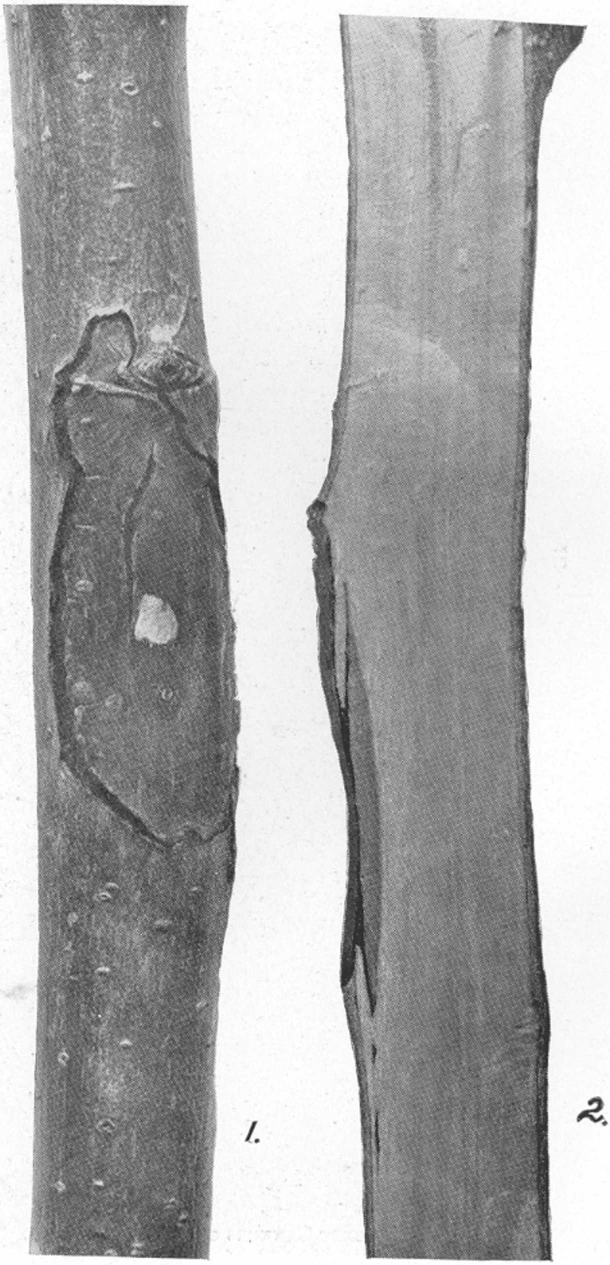

PLATE II.—CANKER AROUND OVIPOSITION PUNCTURE OF A TREE CRICKET ON AN APPLE BRANCH.

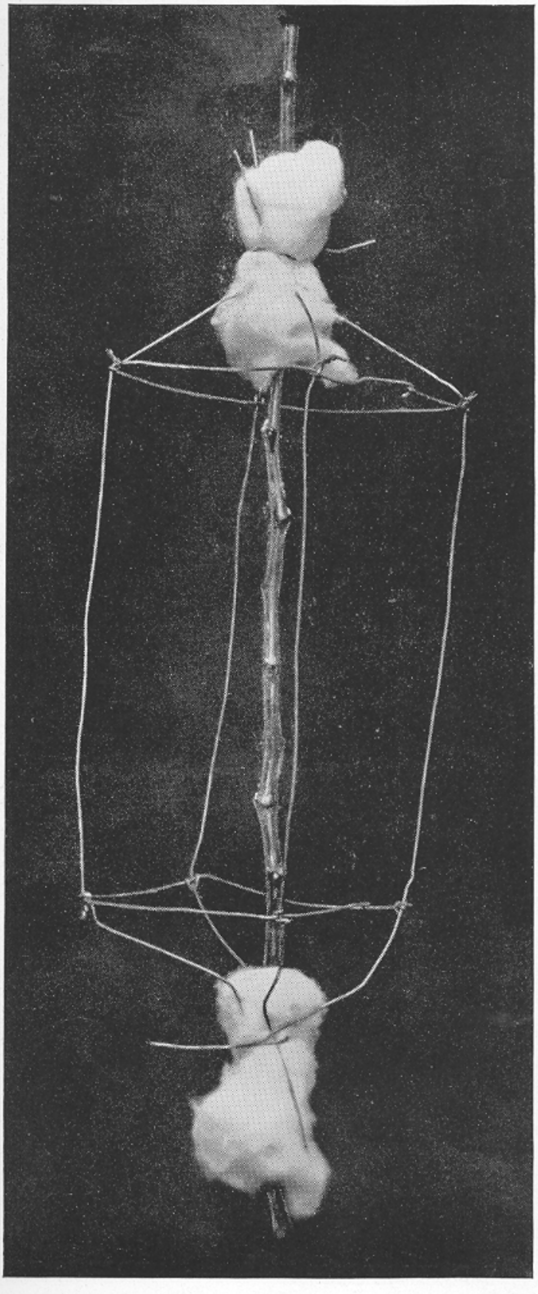

Plate III.—Frame of Cage for Confining Tree Crickets on Apple Branches.

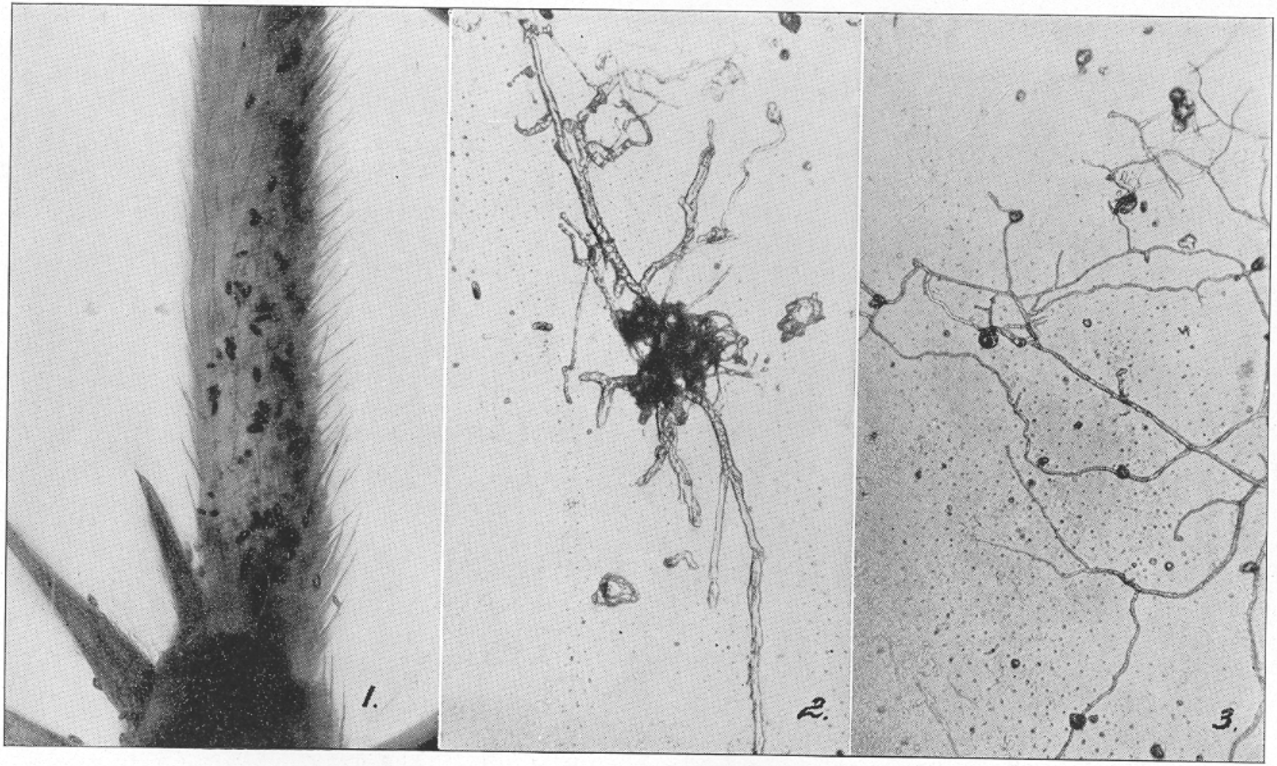

Plate IV.—Fungus Spores and Mycelium on Leg and Wings of a Tree Cricket.

Experiments were made to determine if cankers would form when the crickets were eliminated thus removing the possibility of infection from spores carried on their bodies. For this purpose pellets of excrement were collected from the feeding cages, and inserted into apple branches using the usual precautions to keep the tissues and instruments sterile. The wound was made by punching a hole in the bark with a sterile forceps thus imitating, in a way, the oviposition process. Into the wound a pellet was inserted and then covered with warm grafting wax. It was found that the wax would not readily adhere, due to a copious flow of sap. In most cases sufficient sap would be present to soften the pellet and disintegrate it; but upon drying it would reform as a neat cap covering the opening of the wound. On succulent branches enough sap would exude to wash all the excrement from the wound. In the early part of the egg-laying season pellets are often seen as eliptical masses forced into the punctures. Subsequently, caps form over the wounds, indicating that the exudation of sap may take place later when the condition of the host is more favorable for the process. Under ordinary conditions the insect (*niveus*) may be seen packing the excrement in position, but it may be assisted by the softening of the excrement by the sap. In the Spring of the year when the Leptosphaeria is about to become active, and continuing for some time, a brown, sweet, sap may be observed exuding from the oviposition punctures or from the cankers formed in the preceding Autumn. The results of attempts to form cankers with the insects eliminated are summarized in Table II. The experiments were made at the end of the egg-laying season, and the excrement used was that which had accumulated in the feeding cages for various periods, the number of days being indicated by figures in the column under "age of excrement" in Table II.

TABLE II.— SUMMARY OF DATA FROM EXPERIMENTS ON THE PRODUCTION OF CANKER BY ARTIFICIAL INOCULATION OF APPLE BRANCHES WITH TREE-CRICKET EXCREMENT.

DATE.	Number of punctures.	Age of excrement (days).	Number of cankers formed.	Percentage of cankers formed.
Fed raspberry canes infested with *C. fuckelii.*				
Oct. 22	39	5	5	13
Nov. 2	29	8	2	7
Nov. 2	183	30	5	3
Checks: fed raspberry leaves only.				
Nov. 2	22	5	0	0
Checks: punctures only.				
Nov. 2	77	0	0	0

A few attempts were made to produce cankers about oviposition punctures by the use of other organisms. Tree crickets were allowed to feed on peaches attacked by the brown-rot fungus, *Sclerotinia fructigena*, and then were placed on peach trees. Oviposition punctures were made, but in all cases the egg, as well as the excrement, was forced out of the puncture by the formation of gum. Experiments were made, also, with tree crickets fed pure cultures of the fire blight organism, *Bacillus amylovorus*, but in all cases the results were negative. These experiments were made September 9, 1914, and the season was not favorable for the activity of the organism.* While under ordinary conditions it is hardly probable that the blight organism is disseminated by tree crickets, yet, under exceptional conditions, they may be a factor in spreading it.

The parasitism of *Leptosphaeria coniothyrium* has been proven by making inoculations with pure cultures isolated from various hosts. That *Coniothyrium fuckelii* is genetically related to *Leptosphaeria coniothyrium* was suggested by Saccardo (35) and later proven by Stewart (40). It may be stated that variations in virulence and cultural characters exist among the various strains of Coniothyrium isolated from different hosts; but as more time is required for the completion of our studies along this line the discussion of the subject will be reserved for a future publication.

PREVENTIVE MEASURES.

In considering methods for the control of tree crickets in apple orchards we have observed that the ordinary practices of spraying and cultivation will control the insects. Dormant sprays do not affect the hatching of the eggs. It is the sprays containing arsenical poisons that are effective. An examination of the bark from unsprayed trees will show it to be very much pitted; while the bark of trees of the same age but well sprayed will be smooth and free from tree-cricket scars. As the nymphs appear early in June it is likely that they are killed by the ordinary arsenical sprays applied at that time for codling moth.

DISCUSSION.

It has here been shown that the tree crickets not only carry fungus spores on their bodies, but that they eat mycelium and spores, which are unaffected by the intestinal juices. It may be supposed that since *Œ. niveus* plugs the oviposition puncture with excrement

* That *B. amylovorus* may be active in September has been observed by one of the writers. In 1911, at Wooster, O., an attempt was made to keep a 25-year-old Tolman apple tree free from blighted twigs and branches. The last pruning was made early in September. Then the attempt was abandoned, for blight continued to appear, killing the twigs. The season was a very moist one, and the epidemic was closely associated with an infestation of leaf hoppers.

every wound ought to have produced a canker. There are several factors that may reduce the percentage of cankers formed: (1) The organism necessary for the production of the canker may not be present in the particular pellet inserted into the puncture; (2) the insect may have been disturbed in the act of oviposition and the pellet not inserted into the puncture (often punctures were observed uncovered and in some cases the pellets were found clinging to the bark 2 to 3 mm. from the wound); (3) when the insect has access to an abundance of water the dejections may not be under the control of the insect and thus no pellet would be found in the puncture; (4) the germination of the spores may have been inhibited by desiccation of the pellet; or the exudation of the sap, which under the stimulating influence of the wood tissue makes the spores germinate, may not have taken place. (5) Slow-growing branches may not be favorable for the formation of cankers; (6) the temperature, combined with the dormant condition of the tree, may not have been favorable for the activity of the fungus. Judging from inoculations made at the same time that the crickets were placed on the trees, it appears that numbers 1, 2, 3 and 4 were the prime factors in reducing the percentage of the cankers formed. That desiccation is important is seen whenever the punctures were not covered with grafting wax, for in all cases the percentage was lower than when they were covered. It is believed that but few spores are washed into the punctures by the rain, for, if such were the case, a higher percentage of cankers would have been formed where the punctures were left uncovered. The feeding of aphides to the tree crickets, while they were in the cages about the trees, had no effect in increasing or decreasing the percentage of cankers formed. That more cankers were formed when the insects were permitted to feed on raspberry canes infested with *C. fuckelii* than when they were fed pure cultures of this same fungus isolated from an apple canker can be explained by the greater virulence of the former. Also, in the case of the former the spores eaten were more resistant to desiccation than was the mycelium eaten in the latter case. That freezing of the tissues is not necessary for the formation of the canker is shown by the results of inoculation experiments, and also by the fact that cankers developed in the greenhouse.

It has been shown that spores of *Sphaeropsis malorum*, the causal organism of black-rot canker, may be found in the excreta of tree crickets captured in orchards, and that such spores often produce superficial cankers. It is possible, though not proven, that tree crickets disseminate, also, other canker-producing fungi such as *Nummularia discreta*, *Myxosporium corticolum* and *Glomerella cingulata*. It is not believed that the insects prefer to eat the spores of one fungus more than those of another. The intimate relation existing betweem tree crickets and the canker induced by *Leptosphaeria coniothyrium* is the result of certain concomitant factors.

In the light of the data here presented the conclusions of certain other writers require corroboration. Because certain spores pass through the digestive tract of the tree cricket uninjured it does not necessarily follow that all kinds of spores and bacteria will do so nor does it follow that when eaten by other insects these same or other spores will not be injured. In each instance actual experiments must be made to determine these facts. Craighead (8) regards *Leptostylus macula* as being of assistance in destroying the chestnut blight fungus *Endothia parasitica* because spores of the fungus eaten by the insect failed to germinate in his experiments. However, this subject has not been considered in all of its phases. It would be well to have the germination tests repeated. That tree crickets carry the chestnut blight is not on record, but that they oviposit on the chestnut has been reported.

In conclusion, the writers offer the suggestion that the fungi found in the "fungus gardens" of ants and termites and on the food (*fromage*) of the ambrosia beetles occur there because of favorable conditions for their development; and that the insects, in utilizing the fungi for food, unwittingly disseminate the spores with their excrement.

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