

TRANSMISSION OF FIRE BLIGHT BY BEES AND ITS RELATION TO NECTAR CONCENTRATION OF APPLE AND PEAR BLOSSOMS¹

By G. W. KEITT, *professor of plant pathology*, and S. S. IVANOFF, *formerly research associate in plant pathology, Wisconsin Agricultural Experiment Station*²

INTRODUCTION

The pioneer investigations of Waite (17, 18, 19)³ and later experiments and observations by many others have established beyond doubt that the honeybee and some other insects are capable of transmitting the blossom blight of apple (*Malus sylvestris* Mill.) and pear (*Pyrus communis* L.) incited by *Erwinia amylovora* (Burr.) Winslow et al. For many years after this discovery insects were generally thought to be the only important agents for transmission of blossom blight. Later investigations (e. g., 2, 3, 4, 7, 10, 16), however, have shown that meteoric water is an important factor in its spread and that under some conditions minute aerial strands of bacterial exudate may be disseminated by wind. The relative importance of insect and water transmission, which seems to vary greatly with conditions, is subject to considerable difference of opinion. Since blossom blight is one of the most important phases of the fire blight problem, a better understanding of its epidemiology is highly desirable.

Though it is generally accepted that bees can transmit blossom blight, comparatively little experimental work has been done on the details of this transmission or on the factors that favor or limit it. It has been the purpose of the present work to contribute to a re-examination of these aspects of the problem in the light of recent information, especially in their relation to nectar concentration. These studies, which were pursued in the spring of 1936 and 1937, were unavoidably interrupted when one of the authors was called to another post. The available results are reported herein. A companion study on nectar concentration in relation to the growth of *Erwinia amylovora* and infection of blossoms following artificial inoculation is reported elsewhere in this Journal (8).

Literature on the transmission of fire blight has been reviewed by Parker (11) and others. Consequently, only papers that seem especially pertinent to the present work are cited herein.

DIRECT TRANSMISSION FROM CONTAMINATED TO UNCONTAMINATED BLOSSOMS

An objective of the following experiments was development of methods whereby transmission of fire blight from one blossom to another by honeybees (*Apis mellifera* L.) could be studied experimentally under adequate control. Some needs for specific information

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³ Italic numbers in parentheses refer to Literature Cited, p. 752.

concerning such transmission in relation to the epidemiology of blossom blight have been discussed elsewhere (8).

Experiments were performed in three ways: (1) Individual marked bees of an uncontaminated nucleus hive in a large cloth cage in the greenhouse successively visited inoculated⁴ and uncontaminated blossoms of potted trees; (2) bees of an uncontaminated nucleus hive in a large cloth cage in the greenhouse freely visited two potted trees, one with inoculated blossoms and the other with uncontaminated ones; and (3) an uncontaminated bee, handled in a specially designed wire cage, visited inoculated blossoms and then uncontaminated ones in the greenhouse or the orchard.

Evidence that the bees were not contaminated with the fire blight bacteria at the beginning of the experiments is based on the following precautions and tests. The bees of the nucleus hives were brought from Louisiana in early spring before they had opportunity to leave the hive. If the hive had been contaminated in the preceding year, the evidence is strongly against the possibility that the bacteria would have overwintered in it (6, 11, 12, 14, 15). When the hives had been placed in the cloth cages, the bees were allowed on 2 successive days to visit uncontaminated blossoms of potted apple and pear trees that had been held in a moist chamber to bring the nectar to a dilution favorable for infection. Each individual bee used in experiments with the small wire cage was allowed to work on a few uncontaminated blossoms before it was employed in the subsequent transmission experiments. In all these control experiments the bees were seen to introduce the glossa into the receptacle cup and remain in position long enough to indicate that they were sipping nectar. Throughout all the experiments on transmission, no indication was found that the bees were contaminated before they were permitted to visit the inoculated or diseased blossoms.

Some typical experiments on transmission are described below as illustrative of the methods and results.

About 50 blossoms of a 3-year-old dwarf Bartlett pear tree in the greenhouse were inoculated in the nectar by means of a small camel's hair inoculator at about 6 p. m. The tree had previously been held for a few hours in a moist chamber (9) with the curtains wet but spray not running, in order to bring the nectar to a concentration low enough to favor infection.⁵ After inoculation it was kept overnight in another moist chamber. On the following morning measurements of nectar by means of an Abbé refractometer showed that the sugar concentration varied from 3 to 5 percent, whether the blossoms had been inoculated or not. Platings were made from inoculated and uninoculated blossoms at 3 time intervals that day. Fire blight bacteria, which subsequently caused typical infection in inoculation tests, were isolated in all trials from inoculated blossoms, but in no case from uninoculated ones. On the morning after inoculation, this tree, with a like one that had received similar moist treatment but no inoculation, was placed in a cloth cage. Each tree was protected by mosquito netting, so that

⁴ Unless otherwise stated all inoculations were made on the preceding day by introducing a drop of about 1/400 cc. of bacterial suspension into the receptacle cup by means of a camel's hair inoculator, with care not to wound the host tissue.

⁵ Unless otherwise stated all potted trees, before and after being used in the transmission experiments, were placed overnight in the moist chamber at about 20° C., with the curtains wet but the water not running. The inoculated and the uncontaminated trees were kept in separate chambers. At the end of a moist treatment, the nectar in the blossoms was usually abundant, and contained 3 to 5 percent of sugars. On keeping the trees in the greenhouse for 1 hour at about 24°, the concentration of nectar sugars usually rose to 10 or 12 percent.

individual blossom clusters could be exposed to visitation by bees or covered at will. An uncontaminated nucleus hive of bees was then placed in the cage. An inoculated cluster was exposed, with the aim of having one bee sip nectar from the blossoms.⁶ Most of the bees flew about above the trees but only a few actually approached the exposed cluster. As soon as one alighted on an exposed blossom it was marked on the thorax with a droplet of specially prepared aniline dye and no other bees were permitted to touch this cluster. This bee was allowed to work on the inoculated blossoms until it had a good chance to become contaminated but not long enough to get its fill of nectar. The inoculated cluster was then covered and one on the uninoculated tree was exposed. The marked bee, without returning to the hive, alighted on the blossoms and worked on each of them, at times returning to a blossom it had already sipped from before going to another it had not yet visited. Each blossom the bee touched was marked, and the order of visitation was recorded. After the bee had visited all the blossoms, it was caught and the cluster was covered. The glossa was cut off with sterile instruments and its apical part dipped successively into the nectar of 10 blossoms of a third available tree having abundant nectar of a concentration favorable for infection. The glossa and the honey stomach were crushed and plated. The three trees were then incubated overnight in the moist chamber at about 20° C., with the curtains wet but the water shut off. On the following morning the blossoms of all the trees were found to contain abundant nectar. The trees were then further incubated in the greenhouse for 7 days at about 22° to 24°, after which the results were taken. The trees were kept under observation for another month.

The results of this experiment show that the bee transmitted the disease from the inoculated blossoms to 2 of the 4 uncontaminated blossoms it visited. No disease developed in the 10 blossoms that were touched with the bee's glossa. The glossa, however, yielded a few fire blight colonies that were subsequently shown by inoculation to be pathogenic. The honey stomach yielded a great number of micro-organisms, none of which resembled the fire blight pathogen. Most of the blossoms inoculated with the camel's hair inoculator showed macroscopic symptoms of blight within 4 days. Blossoms that were not inoculated did not show any disease. The experiment just described was performed four more times under similar conditions. Only one of these trials gave positive results.⁷

Transmission was also accomplished in two trials of another type, in which an inoculated and an uninoculated tree were kept together in a cloth cage for 5 hours, the bees of an uncontaminated hive freely visiting the blossoms of both trees. In the first trial 32 of the 61 blossoms of a dwarf Bartlett pear tree (uninoculated when placed in the cage) became diseased within a week after the bees' visitation, and in the second 46 of the 87 blossoms on a Seckel pear tree (likewise uninoculated when placed in the cage) blighted. The disease was therefore transmitted to 52 percent of the blossoms of these two trees.

⁶ Bees may visit blossoms to collect pollen or reconnoiter without sipping nectar. In these experiments they were regarded as sipping nectar when they extended the glossa into the receptacle cup and remained in position for a distinctly longer period than would be required for reconnoitering.

⁷ The amount and concentration of nectra changed rapidly after the trees were taken out of the moist chamber. For instance, in one case the concentration of nectar sugars rose from 3.5 percent to 11 percent in 50 minutes. In a few hours the volume of nectar was so diminished that measurable samples were not obtainable. These changes in the amount and concentration of nectar during the course of an experiment may have influenced the amount of infection (δ), notwithstanding the fact that the moist treatment of the plants after visitation by the bees induced nectar concentrations favorable for infection.

Most of the tests on direct transmission of blossom blight by selected individual bees were made with the use of a small wire bee cage or trap (fig. 1). The chief advantage of this cage is that it permits keeping a particular bee with known history through several operations in association with any selected clusters as long as the bee remains alive and active. The cage, made of 16-mesh galvanized wire screen, is about 10 inches long and 4½ inches in width and height. It consists of two detachable halves connected with hinges on one side and with a hook on the opposite side. Two sliding doors, each placed near the outer end of one of the hinged sections, cut off small compartments (fig. 1, *a* and *c*). A bee is easily caught with this cage and confined in the central large compartment (fig. 1, *b*). Then

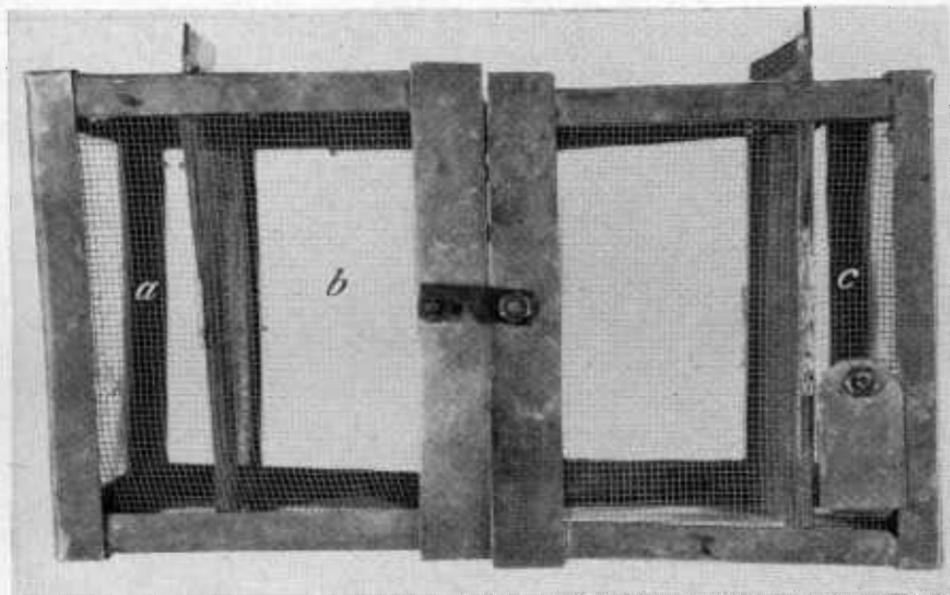


FIGURE 1.—Wire bee cage used in the inoculation experiments: *a* and *c*, Small outer compartments; *b*, large central compartment.

one of the trap doors is lifted and the insect is confined in the small outer compartment. Later the central compartment is opened, placed about a blossom cluster, then closed and hooked. The trap door is lifted, and the bee is allowed to visit the blossom cluster. After it has worked on the blossoms sufficiently, it is driven back into the small compartment and the sliding door is closed. The same bee is then used again on other blossom clusters, carried safely from one orchard to another, kept overnight, or handled otherwise according to the requirements of the experiment.

Transmission experiments with the wire bee cage included the following steps:

1. In an orchard in which no naturally occurring fire blight had been found, 10 blossom clusters of apple or pear were bagged separately, 2 of which were inoculated with the fire blight organism suspended in pure water or in artificial nectar⁸ of various concentrations, as desired.

⁸ The artificial nectar used contained invert sugars and sucrose in the proportions reported by Beutler (1) for apple nectar—6.4 parts by weight of dextrose, 6.4 of levulose, and 8.5 of sucrose being dissolved in the following weak nutrient solution: Asparagine, 0.1 percent; sodium chloride, 0.01 percent; dibasic potassium phosphate, 0.05 percent; magnesium sulfate, 0.02 percent; calcium chloride, a trace. The solution was adjusted to approximately pH 7.0. The details of preparation are reported elsewhere (8).

2. About 1 to 3 days later, a bee was caught in the sterilized cage and allowed to sip nectar from two of the uninoculated clusters. The total number of blossoms worked on was noted.

3. As soon as the cage could be shifted into position, the same bee was allowed to sip nectar from an inoculated cluster.

4. After a similar brief interval, the same bee was allowed to work on four of the uninoculated blossom clusters.

5. The bee then was decapitated, its glossa and honey stomach plated, and the pathogenicity of the recovered bacteria tested.

In some special trials the concentration of the nectar in the blossoms was measured just before or just after the bee's visit.

By using the technique just described, in some cases omitting steps 1 and 5, 32 transmission tests were made in the greenhouse or the orchard at Madison in 1936. Ten of these gave positive results; i. e., the bee transmitted the disease from an inoculated to an uncontaminated blossom.

The transmission tests were continued during the same season at Sturgeon Bay, Wis., where the blooming season is later than at Madison. In all cases the transmission was attempted with single bees, trapped in the wire cage. Of the 26 individual tests, 7 gave positive results, 13 negative, and 6 doubtful. Of the tests that gave positive results, 5 were made when the receptacle cups were moist or wetted and only 2 when they were apparently dry. None of the 13 tests that gave negative results were made when the receptacle cups were moist or wetted.

LENGTH OF TIME AFTER INOCULATION THAT BLOSSOMS ATTRACT BEES

One experiment was performed to gain evidence on the length of time after inoculation that blossoms will attract bees and serve as sources of contamination. It consisted in placing with an uncontaminated hive in the cloth cage two different Bartlett pear trees each day, one inoculated and the other not. The inoculated tree introduced on the first day had been inoculated 5 days; that on the second, 4; the third, 3; the fourth, 2. The results showed that under the conditions of the experiment the bees could transmit the disease to healthy blossoms from diseased blossoms that had been inoculated for 5 days. The diseased blossoms on the tree introduced 5 days after inoculation were already wilted and light brown in color. Three bees on more than 5 occasions touched these diseased blossoms with the glossa, then moved to the healthy blossoms. It was evident, however, that the healthy blossoms attracted more bees than the diseased ones and that the bees lingered longer on healthy than on diseased blossoms. The tree that was uninoculated when placed in the cage with the tree inoculated for 5 days had 46 blossoms, 29 of which were found diseased 10 days after the bees' visit.

CONCENTRATION OF NECTAR IN BLOSSOMS AT THE TIME OF THE BEE'S VISIT IN RELATION TO TRANSMISSION

In a greenhouse trial (table 1) some potted Bartlett pear trees were given a treatment in the moist chamber that brought the sugar concentration of their nectar within a range of 3 to 8 percent. They were then placed in a cloth cage and subjected to visitation by contaminated bees with similar trees that, having received no moist treatment, had

nectar with a sugar concentration of 45 percent. After removal from the cage the trees with low nectar concentration received a second moist treatment, whereas the others did not. Thirty-eight percent of the visited blossoms with the lower nectar concentration and none of those with the higher blighted.

TABLE 1.—*Nectar concentration in blossoms of 2-year-old Bartlett pear trees in relation to transmission of fire blight by contaminated honeybees*¹

Bee No.	Moist treatment of blossoms before or after bee's visit ²	Concentration of sugars in nectar before bee's visit ³	Blossoms visited by bee	Blossoms diseased	
				Number	Percent
		<i>Percent</i>	<i>Number</i>	<i>Num-ber</i>	<i>Per-cent</i>
1	Treated before and after.....	3, 4, 8.....	3	2	} 38
1	do.....	3, 4, 8.....	5	2	
2	do.....	3, 4, 8.....	3	0	
2	do.....	2, 3.....	4	1	
2	do.....	2, 3.....	2	2	
3	do.....	4, 5.....	4	1	
4	No moist treatment.....	45 or higher.....	4	0	} 0
1	do.....	45 or higher.....	5	0	
4	do.....	45 or higher.....	5	0	
5	do.....	(4).....	3	0	
6	do.....	(4).....	4	0	

¹The bees had just been contaminated by sipping nectar from blossoms artificially inoculated on the preceding day. The experiments were performed in the greenhouse at 20° to 24° C.

²A bee visited from 2 to 5 blossoms of a cluster.

³Values are for individual samples. The concentration of nectar rose in some of the blossoms to 15 percent during the time the bee worked and before the tree was put back into the moist chamber.

⁴Receptacle cups dry.

In a field trial it was aimed to control in part the nectar concentration of the blossoms from which the bees obtained inoculum, as well as of those to which they were to carry it. In a pear orchard in which no naturally occurring blight had been found, blossoms were inoculated on various days with fire blight bacteria suspended in artificial nectar solutions with sugar concentrations varying from a trace to 40 percent. At the same time small drops of artificial nectar of the same range of concentration, but containing no bacteria, were placed in the receptacle cups of uncontaminated blossoms. Some of the treated blossoms were bagged in an attempt to check the rapid increase of nectar concentration. On the following day individual uncontaminated bees were allowed to sip nectar, first from some of the inoculated blossoms, then from uninoculated ones containing the artificial nectar drops. Shortly before or after the bees visited the blossoms, the concentration of the nectar in these and other blossoms was measured. It was found in most cases that the concentration had undergone changes. Some of the drops that originally had contained a trace or 1 percent of nectar sugars were later found to contain as high as 10 or 12 percent. Likewise, blossoms that originally contained 40 percent of nectar sugars were found to have 70 or 75 percent. The results of these trials, which are summarized in table 2, show that when a bee worked on inoculated blossoms with nectar containing 2 to 12 percent sugars and then on uncontaminated ones with nectar containing 0 to 35 percent sugars, 49 percent of the latter group blighted. A higher percentage of infection might have resulted if the concentration of nectar in some of the blossoms had not risen so high. On the other hand, no infection resulted when the bees first worked on blossoms with nectar containing, respectively, 10-14, 42-56, and 48-75 percent sugars and then on others with nectar containing, respectively, 10-18, 44-47, and 46-70 percent. Likewise, bees that

worked on inoculated blossoms with apparently dry receptacle cups and then on uncontaminated ones with dry receptacle cups did not transmit the disease.

TABLE 2.—Concentration of pear nectar in relation to transmission of blossom blight by honeybees, Sturgeon Bay, Wis., 1937¹

Range of concentration of sugars in nectar of inoculated blossoms from which bees sipped	Range of concentration of sugars in nectar of healthy blossoms from which contaminated bees sipped	Bagging of blossoms after bees' visit	Bees used	Blossoms	
				Used	Diseased
Percent	Percent		Number	Number	Percent
2-8	0-10	Bagged.....	12	50	56
3-12	0-35	Not bagged.....	8	42	40
10-14	10-18	Bagged.....	6	22	0
42-56	44-47	do.....	10	34	0
48-75	46-70	Not bagged.....	9	36	0
(2)	(2)	do.....	18	52	0

¹ Wire cages were used for controlling the bees and the experiments were performed on orchard trees.

² Receptacle cups dry.

DISCUSSION

It is recognized that some degree of artificiality may attend all experiments with bees handled in captivity, and that results from such work are reliable only in proportion to the adequacy with which they are observed and controlled.

Transmission of the disease from one blossom to another was demonstrated by each of the three methods tried, and each method may be useful. However, work with individual bees greatly facilitates adequate observations and controls. Use of the bee cage substantially increases the range and flexibility of experimentation with individual bees.

The large number of instances in which blossoms did not blight after visitation by a contaminated bee indicates that there are important limitations on the efficiency of this insect in transmitting the disease. Indeed, if this were not the case, it would be very difficult to understand how our apple and pear culture could continue, in view of the great number and activity of bees.

The results of these experiments on transmission of blossom blight by bees indicate that nectar concentration is a very important factor limiting this mode of transmission. They are in general accord with the results of studies (5, 8, 13, 14) of nectar concentration in relation to fire blight infection initiated by artificial inoculation. However, in many cases in which the nectar was at a favorable concentration, little or no infection occurred after contaminated bees had sipped from it. It is, therefore, apparent that other factors besides nectar concentration are important in limiting blossom-blight transmission by bees. An experimental study of such factors lies beyond the scope of the present paper.

The need for information on factors favoring or limiting blossom-blight infection under conditions of natural transmission has been discussed elsewhere (8). The present investigation was interrupted soon after experimental methods for such work had been developed. Further studies under various conditions are needed. It would seem especially desirable to perform additional greenhouse and orchard

experiments in which contaminated bees visit uncontaminated blossoms containing nectar too concentrated to permit infection. The time during which bacteria thus deposited will live and the range of conditions they will tolerate without losing the capability to infect when favorable conditions occur are vitally important considerations in relation to the epidemiology and control of the disease. While work with artificial inoculation is very valuable in helping to define and interpret problems relating to blossom-blight transmission by bees and other insects, further experimental work on transmission by the insects themselves seems essential to an adequate understanding of their role in disseminating the disease.

SUMMARY

Transmission by honeybees of fire blight of apple (*Malus sylvestris*) and pear (*Pyrus communis*), incited by *Erwinia amylovora*, was studied by three experimental methods. The most flexible and convenient one employed individual bees handled in a specially designed wire cage.

Bees were attracted to blighting blossoms that had been inoculated 5 days before, and transmitted the disease to healthy blossoms.

In greenhouse and orchard experiments contaminated bees freely transmitted blight to healthy blossoms when the sugar concentration of the nectar was in the lower range encountered in nature, but not when it was in the medium or higher range.

In the experiments reported herein, nectar concentration was an important factor in limiting blossom-blight transmission by bees. However, in many cases in which the nectar was at a favorable concentration, little or no infection occurred after contaminated bees had sipped from it. It is apparent that other factors in addition to nectar concentration are important in limiting blossom-blight transmission by bees.

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