CHALKBROOD DISEASE[1]

L.P. Kish, Department of Plant, Soil, and Entomological Sciences,
University of Idaho, Moscow, Idaho

W.P. Stephen, Department of Entomology,
Oregon State University, Corvallis, Oregon

Chalkbrood has been an increasing problem of the alfalfa leafcutting bee. The bee larva consumes spore-contaminated pollen and nectar in the cell and is attacked by the fungus. Sanitation of domiciles, nesting materials, and loose cells provides some control. Managing the bees to avoid heat and pesticide stress helps bees resist the fungus.

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ONE OF THE most serious diseases of honey bees and alfalfa leafcutting bees is chalkbrood, so called because larvae killed by the disease have the consistency of chalk. Only the larval stage is affected. Cadavers may be white, brown, green, or metallic black with gradations between these colors or they may be mottled in appearance.

All nine species of chalkbrood fungi (three genera) produce spores in sac-like structures called cysts. The species are separated by traits related to reproductive structures and form. The host is important in species identification but at least two species occur only on pollen. Generally speaking, all species are associated with bees; several occur on pollen, some infect both honey bees and wild bees and some have only been observed on wild bees. At least four of nine recognized species (plus several as yet undescribed species) are known to occur in the Pacific Northwest.

LIFE CYCLE OF CHALKBROOD FUNGI

Research studies have outlined a general picture of the mode of action of the chalkbrood pathogen and its development through the infection cycle. The following discussion pertains to work with the alfalfa leafcutting bee.

The spores, which are present in the pollen and nectar placed in the cell by the adult female, are eaten by the newly hatched bee larva during its first feedings. Within hours, these spores germinate in the gut.

The germ tube grows rapidly and within 12–24 hours it penetrates the gut wall and enters the blood. Once in the blood, the fungus multiplies by budding and maintains a free drifting yeast-like growth form for the first few days as its numbers increase. During this phase of the infection, the pathogen secretes enzymes which attack the blood and body tissues, breaking them down into more basic organic molecules which can be absorbed by the fungal cells and utilized for

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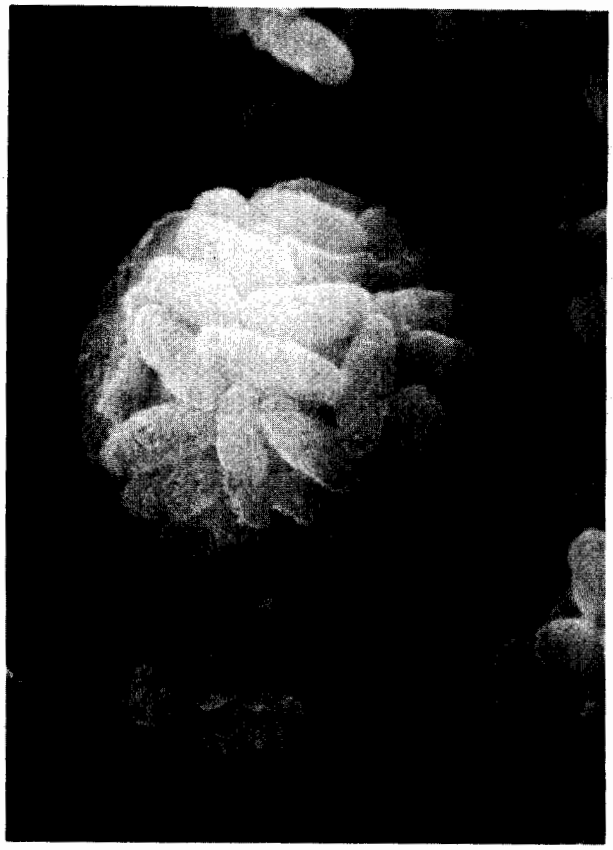

Fig. 1—Spore ball magnified 5,000 times.

growth and nutrition. In a weakened state, the larva begins to show definite symptoms which include moderate swelling and a somewhat uniform discoloration from healthy white to off-white or light brown, progressively, after five days.

The fungal cells continue to multiply rapidly in the blood, turning it into a slurry of fungal cells. Approximately eight days after penetration of the gut, the fungal cells in the blood are so dense that they begin to lodge and attack other tissues directly. Between the eighth and fourteenth day the larva dies.

During the final days of the disease, certain fungal cells in the area of the integument (outer skin) initiate a spore production cycle. Normally a molting fluid, secreted beneath the integument, promotes its breakdown and eventual shedding. Some of the breakdown products are then used in the formation of the new integument which grows simultaneously, inside the old one. In the infected larva, certain fungal cells become established between the old and newly formed integument. These cells expand rapidly in cavities that form as the old integument layers are dissolved. The expanding fungal cells soon become tightly packed in a single layer beneath what remains of the old integument.

Inside the large cells, spore balls are produced (Fig. 1). The walls of the large cells turn a metallic gray and become recognizable as spore bearing cysts. Produced in massive numbers beneath the cuticle, they give the classical chalkbrood appearance to the dead larva.

Unmolested in a hole, the spore-bearing cadavers do not break up quickly. Spores may not be liberated for quite some time unless another insect tries to utilize the nesting hole—or unless healthy bees beneath an infected cadaver must chew through it in order to leave the hole after pupation. Young adults exiting such holes, females renesting in such holes, and visitors such as predators, nest destroyers, and parasites all become heavily contaminated with spores on the external surfaces of their body. Adult bees have been found to carry as many as 300 million spores. Spores that remain in the holes have the potential to infect the next generation which may be reared there. Adults spread spores to other nesting holes, to the fields, and throughout the immediate nesting locality or domicile.

Relatively speaking, the small spores die quickly if they do not come into contact with a bee and manage to infect it. However, some spores have been known to remain alive even after three years. Even though 99% may lose viability, not get dispersed, or fail to come into contact with a susceptible bee, what remains may represent thousands of spores per nesting hole.

CHALKBROOD CONTROL IN THE ALFALFA LEAFCUTTING BEE

The alfalfa leafcutting bee became the dominant bee managed for pollination in the production of alfalfa seed in Nevada and in most of the Pacific Northwest by 1965. The species was easily maintained and manipulated by the seed grower and the problems with bee management that did

arise were resolved through cooperative efforts of growers and industry and university personnel. Until 1973, the presence of chalkbrood in leafcutting bee populations was acknowledged, but its low incidence did not concern anyone. In 1974, however, a 5–15% loss in the bee population in some parts of Nevada was attributed to chalkbrood; and by 1976, losses were in excess of 30%. Control of the disease is especially difficult because of the mode of infection, the population density and reproductive biology of the bee, and the constant potential for reinfection of nesting materials by the unmanaged (wild) bees which intermix and nest with those under management.

Infection via the gut of the larva presents special problems for control of the disease. The ungerminated spore appears extremely resistant to most chemicals with fungicidal properties. After germination, which occurs only in the gut, the fungus is much more sensitive to fungicides and other chemicals. The problem is that chemicals that kill germinated spores in the gut may also kill the larva. Consequently, control efforts have been developed which kill the ungerminated spores before they are eaten or reduce or eliminate spores in domiciles and nesting materials through a rigid program of sanitation. Sanitation includes direct exposure of domiciles, nesting materials, and bee cells to chemical and/or physical treatments. In the "loose cell" management system, extracted cells can be dipped in a 1% calcium hypochlorite solution (swimming pool/spa treatment) for 3 minutes (Figs. 2–4). Cells dipped in this manner must not be dried in excessive heat or in direct sunlight. Nesting materials can be treated before being reused. Dip cleaned wood laminates in a 1% calcium hypochlorite solution for 3 minutes and allow to dry (follow label directions). Other nest materials that cannot be dipped or heat treated for at least 4 hours at 250°F. or more (Figs. 5–6) must be discarded and replaced with new materials.

Spray field shelters with a 1% sodium hypochlorite solution (household bleach) before bees are placed in them in the spring. Thoroughly wet the inside, outside, and surrounding ground

Fig. 2—Mixing calcium hypochlorite in plastic vat.

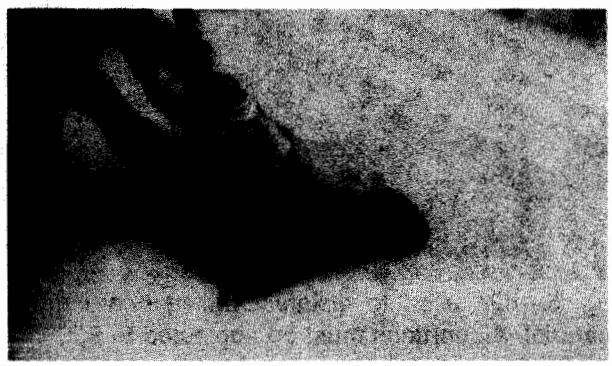

Fig. 3—Dipping loose cells in calcium hypochlorite solution.

Fig. 4—Draining excess solution from dipped cells.

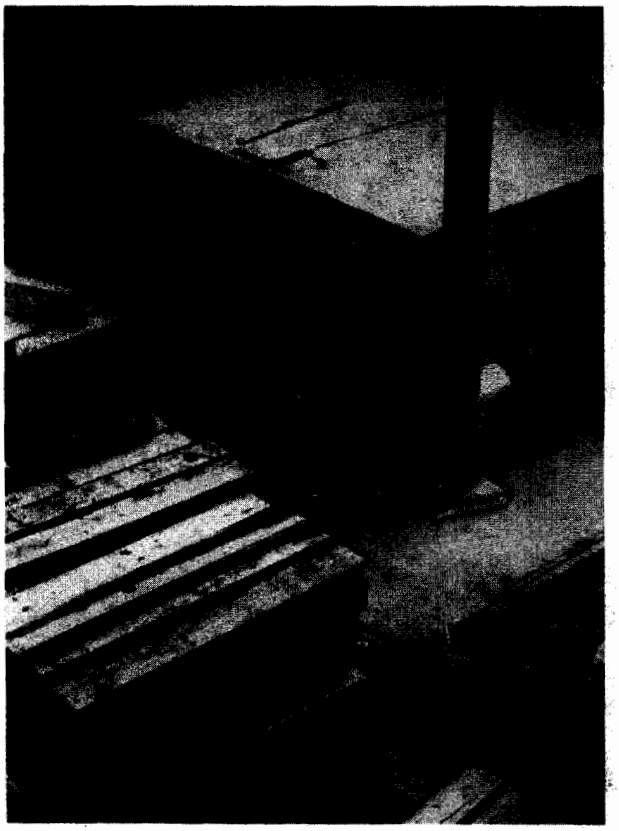

Fig. 5—Kiln used to disinfect drilled board nest material. All portions must be subjected to 250°F. for at least 4 hours.

where bees may warm themselves in the morning and evening.

Use only domiciles that have a floor. Sweep or vacuum litter off the floors at least three times a week during the season.

MANAGEMENT FOR REDUCED SUSCEPTIBILITY AND STRESS REDUCTION

In addition to sanitation, bee management can be effectively manipulated to minimize stress and other factors which predispose the bee population to chalkbrood and other diseases. Seed producers can maintain very high numbers of bees by constantly replacing those lost. Bees in the seed growing region, since they are constantly exposed to chalkbrood, always seem to dwindle in number so there are fewer than the grower needs for maximum seed production. The grower makes up the difference each year by importing bees from outside the seed growing area where disease incidence is low. Bees with little or no exposure to chalkbrood disease show little or no resistance to infection. Canadian or Montana bees exposed to the disease in the fields, in domiciles, and through intermixing with unmanaged bees are quite often devastated by chalkbrood disease in the second generation of the first year. Natural selection at work allows the more disease-hardy to survive; however, not enough of them survive to effectively pollinate the crop after the first season and the grower must buy more bees to maintain the desired population. This sequence of activity has been particularly necessary over the past 20 years, initially as seed acreage was increased, and then as the disease became established. As long as these pollinators are artificially maintained in numbers much higher than would exist naturally, the grower-producer must expect some increased costs and effort and, of course, the risks.

In addition to innate (genetic) susceptibility to disease, alfalfa leafcutting bees may become stressed or weakened by being exposed to environmental factors which are less than optimum or even unfavorable to their good health. In such condition, the bees may become even more susceptible to chalkbrood and other diseases much

Fig. 6—Rack used to space bee boards and load and unload kiln.

as humans are more likely to become ill when they are "run down." Such factors as domicile construction and positioning and pesticide exposure may result in increased mortality due to chalkbrood as well as direct mortality.

Potential stress can be reduced by putting bee nesting materials in field shelters where they are protected from weather. Design and position domiciles so they will be warmed by sunlight early in the morning and shaded in late morning and in the afternoon. Arrange ventilation to prevent heat buildup inside the domicile. A dead air space or insulation should be provided between the nesting material and the outside walls of the domicile that receive direct sunlight. In order to prevent bees from being exposed to a buildup of dead larvae and fungal spores in the immediate nesting area, domiciles should be placed in different locations each year.

Move portable domiciles out of fields that are about to be sprayed with pesticides and other chemicals harmful to bees. Plan all pesticide applications carefully. Avoid spray drift, pesticide applications when temperatures are low, potential chemical residues during seed set, or moving bees into fields too soon after application of a pesticide. Avoid killing or damaging your neighbors' bees, since they may also be working in your fields.

In summary, several measures can be taken to decrease the chances of spores being eaten by developing larvae and to reduce stresses which can make the developing larvae susceptible to infection. Good management practices can reduce direct stress factors such as unfavorable weather and pesticide poisoning and indirect stress factors such as adult competition for food and shelter.

A FIELD KEY TO SPECIES OF CHALKBROOD FUNGI THAT MAY OCCUR IN THE WESTERN UNITED STATES

Field identification is not always possible and certainly not 100% accurate; however, the following key based on current observation and research may be useful. A reminder is in order here that bee diseases other than chalkbrood occur in the Pacific Northwest, such as foulbrood, caused by a bacterium, and Nosema disease, caused by a protozoan. Bees killed by these diseases will not be mumified and chalky (cheese consistency in fresh cadavers) but will be limp and watery or dried and shrunken to brown or black scales. Symptoms of all larval diseases may be similar in early stages of infection so only the appearance of the cadaver is useful for field identification.

1a. Chalkbrood of honeybees (*Ascosphaera apis, Ascosphaera major*).....2
1b. Chalkbrood of wild bees (*Ascosphaera aggregata, Ascosphaera proliperda*).....3
1c. Growing on pollen (*Bettsia alvei* or *Ascosphaera atra*).....4
2. Distinguishing between *A. apis* and *A. major* in the field is impossible. Both produce a cottony growth, which is white and spore cysts which appear as dark green or black granules smaller than the head of a pin, outside the body of the cadaver.
3a. Mycelium and/or cysts on pollen or outside the cadaver body.....*Ascosphaera proliperda*
3b. No external mycelium or cysts apparent; cadaver hard, brown, or metallic black.....*Ascosphaera aggregata*
4a. Cysts large, round, shining black, interspersed with loose serial mycelium ..*Ascosphaera atra*
4b. Mycelium and cysts more tightly associated with pollen.....*Bettsia alvei*

In addition to these species, several other as yet undescribed chalkbrood fungi indistinguishable from *A. aggregata* in the field have been observed on ground-dwelling bees. Keep in mind that any fungus that grows on and thus "spoils" the pollen/nectar provision may kill the larva by causing it to starve.

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