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Chalk Brood Disease in the Leafcutting Bee

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Chalk brood is a fungal disease, believed to be caused by *Ascosphaera proliperda* Skou (Thomas and Poinar, 1973), which has become a severe problem in the alfalfa leafcutting bee, *Megachile pacifica,* in the last 4 years. It is most severe in the western United States and in a population introduced into Argentina where losses range from 20 to 65 percent. The disease is now recorded in most areas of the U.S. and Canada where the bee is produced commercially for pollination or sale.

Attempts to infect developing leafcutting bee larvae with spores from laboratory cultures of the fungus have been unsuccessful (Hackett *et al.,* 1977). This has led some workers to believe that the fungus may be a secondary rather than a primary pathogen. Current evidence, however, strongly supports the contention that the fungus *Ascosphaera* is the cause of the larval mortality.

Chalk brood was described from infected honeybee colonies in Germany in 1911 (Claussen, 1921). In 1969, the disease was recorded from honeybees in California (Thomas and Luce, 1972) and since that time has been found in most states and provinces of the U.S. and Canada (DeJong and Morse, 1976; DeJong, 1977 unpublished thesis). There have been several reports of chalk brood from various species of solitary bees in Europe (Melville and Dade, 1944, Clout, 1956; Bailey, 1963) and the U.S. (Baker and Torchio, 1968; Batra *et al.,* 1973) and in 1973, the disease was isolated from the leafcutting bee, *M. pacifica* (Thomas and Poinar, 1973). Current knowledge of chalk brood suggests that the species attacking the honey bee (*A. apis)* is different from that attacking the leafcutting bee and that the species are not cross-infective. It must be emphasized that the entire group of fungi-causing chalk brood in bees is poorly known and the developmental patterns not understood.

How to recognize the disease. The infected larvae die before they reach maturity, harden and turn cream colored, then gray, black or at times mottled with both colors. It is these hard, chalky, dead larvae which gave rise to the common name of the disease. Usually the larva succumbs to the disease in the last instar before it has had time to complete its cocoon (Fig. 1). Cells containing dead last instars have the appearance of a normal cell except for an opening in the top where the "nipple" of a normal cell is found (Fig. 2). The walls of a cell containing a dead chalk brood larva are fragile because of the absence of the cocoon and collapse when rolled between one's fingers. During the last two years, increasing numbers of younger bee larva

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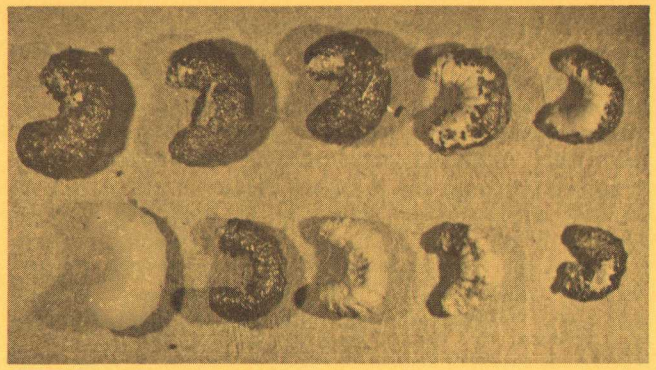

Figure 1. Exposed live prepupa and cadavers of leafcutting bees. Top row: chalk brood cadavers of last instar bee larvae. Bottom row: (left) healthy prepupa; (right) chalk brood cadavers of younger bee larvae.

cadavers (Fig. 1) have been found as have others which have completed the cocoon before dying. In the former, the hard dark larvae rest on partially eaten provisions and readily fall from the cells when they are removed from the nesting medium. There is no regular pattern in the occurrence of diseased larvae in a cell series. Dead larvae are randomly dispersed among live larvae (Fig. 3). Rarely, in heavily infected

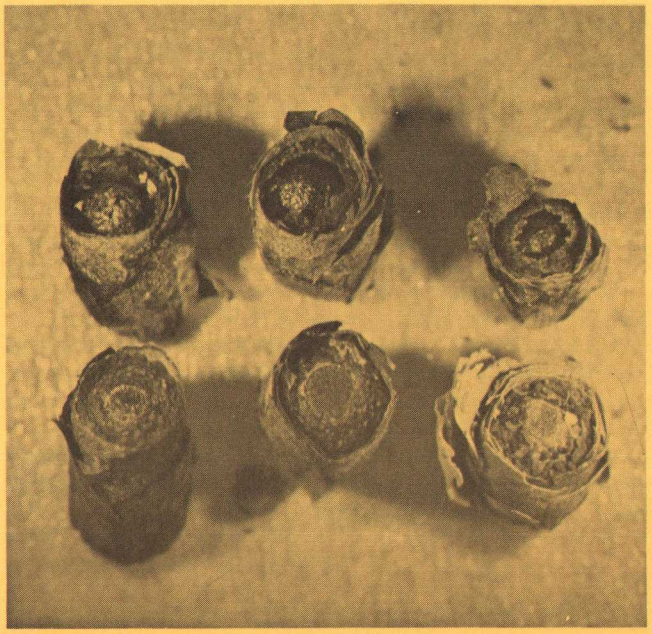

Figure 2. Leafcutting bee cells. Top row: cells with chalk brood cadavers of mature larvae. Bottom row: normal cells with live prepupae. Top of cell sealed by flattened nipple of the cocoon.

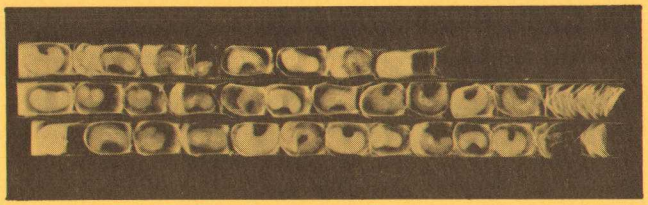

Figure 3. Photo reproduction from X-ray of three straws containing leafcutting bee cell series. Top: (reading top to bottom of straw) cells 3 and 8 with live prepupae; cells 2, 4, 5, 6, and 7 with chalk brood cadavers. Middle: cells 2, 6 and 11 with live prepupae; cells 1, 3, 4, 5, 7, 8, 9 and 10 with chalk brood cadavers. Bottom: cells 1, 4, 5 and 7 with live prepupae; cells 2, 3, 6, 8, 9 and 10 with chalk brood cadavers. Note the near random distribution of diseased larvae and the difference in size of affected larvae.

aggretations of the bee, an entire cell series of from 6 to 12 live larvae may be found suggesting the possibility of a resistance factor in some individuals. In heavily contaminated bee populations, chalk brood has resulted in the death of a high percentage of *Sapyga* larvae, one of the bee parasites.

Spread of the disease. Until the life history of the fungus (and/or other organism) is known, we can only speculate as to the manner in which the disease is spread. The chalky cadavers are laden with spores. As live adult bees emerge from tunnels they chew through the cadavers and become heavily contaminated with spores. The mascerated larvae are pushed to the base of the tunnel during bee emergence and continue to serve as a source of spore innoculum to females which ultimately accept that tunnel for renesting. It is likely that this nesting medium also is heavily contaminated with spores and serves as a secondary source of inoculum. However, it is our opinion that the disease is disseminated principally by the adult bee.

After completion of this manuscript, K. Hackett, University of California reported four additional species of *Ascosphaera* from leafcutting bee larvae, one of which is *A. aggregata* Skou and the other three undescribed. He was able to infect *Megachile* larvae with a cyst-less culture of *aggregata*, as well as with a spore mixture of *proliperda* plus two of the undescribed species. Hackett also implicates two honey bee viruses as debilitating factors which predispose the leafcutting bee larva to fungus attack. This does not alter the control recommendations which follow.

DISEASE CONTROL

Off all the control measures attempted during the last three years only those involving prophylaxis have yielded promising results. That is, any management method which reduces the spore count to which the bees and developing larvae are exposed helps reduce the incidence of the disease. In each of the tests which have given promise, the use of the loose-cell system of management has been an integral part.

Loose-cell system. The program involves the use of nesting material from which the cells can be removed (punch-out boards and wood laminates). Once the cells have been freed from the nesting medium, emerging adults do not have to chew through cells containing chalk brood cadavers thus reducing the degree of contamination.

We used the following management in 1977 to reduce the incidence of chalk brood from 48 percent to 11.8 percent in two generations of the bee. The reduction in chalk brood plus the parasite and predator control afforded by the system resulted in an increase in live bee larvae from 25 percent at the beginning of the season to 64 percent by the season's end.

1) Remove the cells from the nesting materials in the fall and pass them through a cell tumbler 2 to 3 times on successive days. This helps remove most of the smaller chalk brood cadavers and excess leafy material. Thorough tumbling is one of the most effective ways to control nest destroyers including flour beetles (*Tribolium* spp., *Trogoderma* spp.), *Trichodes, Nemognatha* and the Indian meal moth (*Plodia).*

2) Refrigerate cells for the winter at 2 to 5°C (35 to 40°F).

3) In the spring, incubate loose cells at 30°C (86°F) for 5 to 7 days. This temperature induces activity in larvae and adults of nest destroyers not eliminated in the fall tumbling and causes them to leave the cells. The cells should then be removed in batches and dipped in a 4 to 6 percent hypochlorite[1] solution for three minutes (Fig. 4). The hypochlorite solution acts as a surface decontaminant of fungal spores and mycelium, reducing the possibility of contamination of emerging adult bees.

4) Spread the cells to dry overnight. Once dry, the cells are placed in trays, covered with one-half inch of coarse sawdust and replaced in the incubator.

5) Field domiciles should be cleansed thoroughly with a 4 to 6 percent hypochlorite solution or with live steam each spring prior to their use.

6) Prior to bee emergence, the domicile should be stocked with nesting material which has been fumigated or sterilized. Fumigation can be ac-

[1]Hypochlorite solutions are available as sodium hypochlorite (household bleach) in all supermarkets and grocery stores. The strength ranges from 4 to 6 percent depending upon the brand.

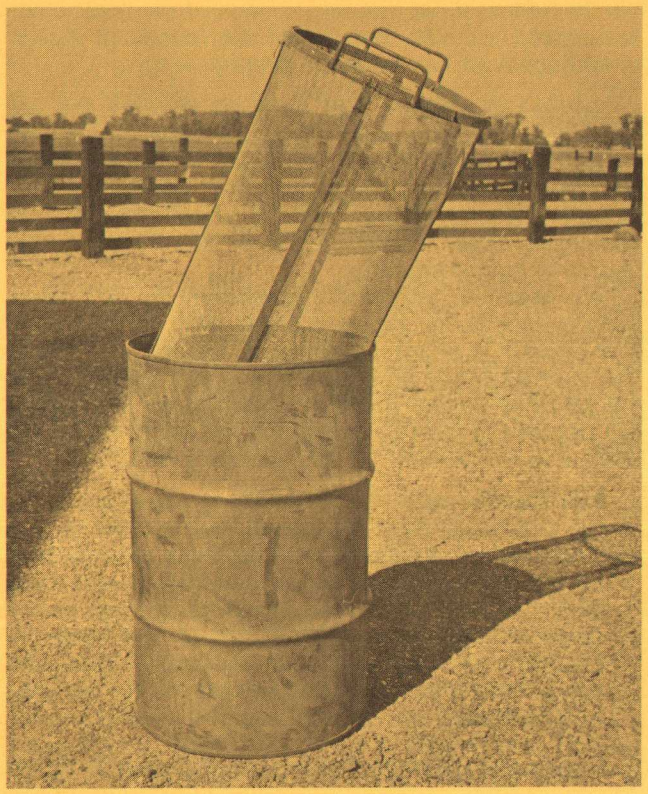

Figure 4. A 20-gallon drum fitted with a screen insert for hypochlorite dipping of loose cells.

complished with chloropicrin[2] (2 pounds per 1000 cubic feet at 90 to 95°F for 24 hours). Heat sterilization of the medium at 100°C (212°F) under 10 psi pressure with steam for four hours can also reduce effectively the spore count in used nesting materials.

7) Field domiciles should provide protection to the nesting materials from the direct sun. Excessive heat resulting from direct exposure to morning or afternoon sun, or from poor ventilation, can stress the developing larvae, causing them to be more susceptible to the disease. Well ventilated domiciles minimize the relative humidity around the nesting medium. High humidity, like high temperature, enhances the development of the disease. Locate the domiciles in a bare area within or at the edge of the field to be pollinated. This minimizes in-domicile humidity resulting from field irrigation and plant respiration. A floor in the domicile, which is elevated above plant level, will further reduce humidity.

8) Cleanse the outer walls, floors and inner walls of the domicile with a 4 to 6 percent hypochlorite solution during the evening at weekly intervals for the first four weeks of nesting activity. Care should be taken to avoid application of the hypochlorite solution to the nesting medium or the bee therein. Excess solution on the floors should be wiped up to minimize in-domicile humidity.

Use of solid boards and straws. Once chalk brood has become established in a population nesting in solid boards or straws it is difficult to keep the disease at a low level. This is because adult bees of each generation must chew through chalk brood cadavers on emergence thus becoming contaminated with spores in the process. Under these conditions the incidence of the disease soon reaches epidemic proportions. Attempts to control chalk brood by phasing out diseased populations from solid boards into clean solid boards have met with limited success. However, if a producer is committed to solid nesting media his only recourse is to use new or sterilized materials every year in an attempt to limit the sources of contamination and to reduce the population of nest destroyers which also act as spore carriers.

Use of redrilled boards. Solid boards from which a contaminated population of bees has emerged can be redrilled to remove the refuse and reused if they are steam sterilized. However, rapid contamination can be expected after sterilization if the boards are repopulated with bees emerging from contaminated material. Such boards could be used in trapping or in a phase-out program by growers committed to this medium.

Dipping redrilled boards in a 1 percent hypochlorite solution for three minutes has been recommended as a way to decontaminate. This method should not be used on punch-out boards or wood laminates because it causes warping and checking of the wood which enable small wasp parasites to enter and attack bee larvae. In addition, the holes often become fuzzy with erect wood fibers, making them unacceptable to female bees. Steam heat sterilization should be used to decontaminate redrilled boards.

Fungicides for disease control. The following fungicides were applied directly to the nesting medium at weekly intervals during the flight season: Bravo (chlorthalonil) 1000 ppm; benlate (benomyl) 500 ppm; anthium dioxide (chlorine dioxode) 50,000 ppm; Cap-

[2]As the fungus cannot be effectively cultured, fumigation with chloropicrin or heat sterilization cannot be objectively evaluated. These treatments are recommended as they, in combination with steps 3 and 5 reduced the incidence of chalk brood significantly. Heat sterilization at 212°F for 4 hours is included as a means of decontamination as there is no known Ascomycete which can survive this time-temperature exposure.

tan 500 ppm; Dithane 2-78 (Zineb) 500 ppm; Fumadil B (fumagillin) 1000; Fore 1000 ppm; Manzate (maneb) 500 ppm; Nipagin (methyl p-hydroxybenzoate) 1000 ppm; phaltan (folpet) 500 ppm; terramycin 200 ppm + thiabendazole 500 ppm; thibenzole (thiabendazole) 1000 ppm; tolnaftate 1% powder; undecylenic acid 10% solution and 2% powder. None of the materials showed a consistent response in reducing the incidence of the disease and we do not recommend their use. Surface decontaminants such as bleach (hypochlorite), benzalkonium chlorite and anthium dioxide also were ineffective when the nesting medium was tested.

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