Saskatchewan Agriculture and Food

CHALKBROOD CONTROL IN ALFALFA LEAFCUTTING BEES

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Chalkbrood disease of leafcutting bees was first identified in American bee populations in the early 1970's. It spread rapidly through wild and domestic leafcutting bee populations throughout the northwest United States, and within several years caused population losses as high as 60% in some states. Chalkbrood is now found at economically damaging levels in all major American alfalfa seed-growing areas where leafcutting bees are used for pollination.

From 1982 to 1984, chalkbrood disease was identified at low levels in Alberta, Saskatchewan and Manitoba. Since then it has become well established in southern Alberta, causing losses of up to 28%. For unknown reasons it has not reached similar proportions in Saskatchewan or Manitoba.

Chalkbrood, a fungal disease of bees, is caused by species of the genus **Ascosphaera**. Different species of **Ascosphaera** affect honey bees, leafcutting bees, and other solitary bees. The species which affects the alfalfa leafcutting bee is **Ascosphaera aggregata**.

LIFE CYCLE

Chalkbrood causes the death of leafcutting bee larvae. The dormant stage of chalkbrood is a spore which survives for many years under Canadian conditions. When an adult female leafcutting bee emerges from her cell, if she chews through a cocoon containing a chalkbrood cadaver she becomes dusted with spores, which stick to her body hairs. These spores then become mixed into the pollen balls which she prepares prior to laying her eggs.

The young leafcutting larva eats the pollen ball and ingests chalkbrood spores. Once in the larval gut, the spores germinate and the fungus grows, moving through the gut wall into the body cavity. Eventually the larval tissues are broken down and the larva dies. Death generally occurs in the final larval instar before pupation, so dead larvae are usually full-sized.

Once the larva is dead the fungus may begin to form spores in spore cysts between larval skin layers (Figure 1). When mature, these spore cysts are easily shattered allowing spore dispersal.

The fungus may not form spores (Figure 2), and these nonsporulating cadavers are not infective. Also, the fungus may only partially sporulate (Figure 3).

SYMPTOMS

Figure 1 illustrates a sporulating cadaver. Spore cysts under the skin give the larva a black, grey or dark tan appearance (Figure 1a), often with a honeycomb-like surface. The dry larval skin takes on a metallic sheen. Spore cysts are shiny black and oblong, and are packed closely together under the larval skin. In cross-section (Figure 1b), body tissues are dry and are surrounded by a ring of oblong spore cysts between the skin layers.

Figure 2 illustrates a nonsporulating cadaver. The cadaver is a tan colour (Figure 2a), and the dry skin imparts a metallic sheen to its surface. The cross-section (Figure 2b) shows dry larval tissues surrounded by a ring of slightly darker tissue.

Figure 3 illustrates the mottled appearance of a partially sporulating cadaver.

Cadavers are hard and dry in all cases.

Figure 4 illustrates a healthy leafcutting bee prepupa.

PREVENTION AND CONTROL OF CHALKBROOD DISEASE

1. PURCHASE OF BEES AND EQUIPMENT

THE MOST COMMON WAY OF SPREADING CHALKBROOD IS IN COCOONS AND USED NESTING EQUIPMENT. Ensure that chalkbrood is absent before buying leafcutting bee cocoons. Ask to see a quality certificate from the Canadian Cocoon Testing Centre (CCTC). Cut open a thousand cocoons and inspect them for symptoms of chalkbrood, or submit them yourself for analysis by the CCTC. Do not buy bees from regions known to have chalkbrood. It is illegal to import leafcutting bees or used equipment from the United States.

Before buying used leafcutting bee nests or other equipment, determine that chalkbrood has not been found in the beekeeping operation. Check the CCTC certificates for the bee population from that equipment. Cut open a sample of cocoons from the equipment and inspect for chalkbrood symptoms, or submit the sample to the CCTC.

Clean and sterilize all used equipment and nests before using them, and preferably before moving them to your farm. Keep newly purchased bees and equipment separate from the existing operation. Sample their offspring separately to determine disease status before incorporating them into the main operation.

Chalkbrood spores are known to occur in used wood nesting material in the U.S. We may not legally import used nesting equipment including bee boards, often called "redrills". While chalkbrood spores are probably not present in new wood nesting material bought from the U.S., it is a good idea to sterilize wood nesting material purchased from the United States before using it on your farm.

2. LOOSE CELL MANAGEMENT

Canadian leafcutting beekeeping was developed around the principle of loose cell management, where cocoons are removed from nests each year, stored and incubated as individual cocoons, rather than in tunnels. This principle is essential for control of diseases, molds and parasites, since it allows sterilization of nesting material and treatment of cocoons.

Remove cocoons from nesting material each year. Do not purchase solid block-type nesting material unless you can remove the cocoons from it. **IF YOU CAN'T STRIP IT, DON'T BUY IT!** Do not incubate cocoons in nesting material!

Cocoon groups can be broken into individual cocoons to prevent adults from chewing through other cocoons when emerging. Use a "cell-breaking" apparatus, BUT take care that the apparatus is adjusted properly and is not crushing cocoons.

Tumble the cocoons to remove debris, and burn the debris.

Sterilize the nesting material prior to use.

Surface sterilize the cocoons prior to incubation.

3. TREATMENT OF NESTING MATERIAL

Bleach Treatment of Nesting Material

Treat nesting material in the spring. Dip nests in a 3 to 5% solution of bleach for 3 to 5 minutes (Figure 5). Bleach, or sodium hypochlorite, is available in a 5% solution from grocery stores (eg. Javex) or in a 12% solution from agricultural chemical suppliers. Use the following formula to determine the quantity of 12% bleach required to make up the amount and the percentage desired.

$$\frac{\text{Volume of dilute solution desired} \times \text{Percent of dilute solution}}{12} = \text{Volume of 12\% liquid bleach required}$$

eg. $\frac{200 \text{ gallon tank} \times 5\%}{12} = 83.3$ gallons 12% liquid bleach required

Calcium hypochlorite may also be used. It is available as a powder containing 65% chlorine. It tends to leave a calcium sludge in the tank unless well mixed, and may leave a chalky residue on nest surfaces. Use the following formula to determine how much calcium hypochlorite to use:

$$\frac{\text{Volume (gal) dilute solution desired} \times 10 \times \text{Percent of dilute solution}}{65} = \text{Weight (lb) of 65\% dry bleach required}$$

eg. $\frac{200 \text{ gallon tank} \times 10 \times 5\%}{65} = 154$ lb of 65% dry bleach required

Add a surfactant at the rate of 0.1% to both of these solutions to ensure wetting of all surfaces.

Bleach solutions lose strength with time, with exposure to air, and when mixed with organic material such as leaf pieces. Test the solution every few hours using a bleach test kit. Test in the morning if the solution is used for more than one day. Add bleach as required.

Stack dipped nests in staggered fashion, holes down, or take them directly to the field shelters to dry. Allow several days for polystyrene and styrofoam nests to dry and two weeks for wood nests to dry before bees are released.

Bleach is extremely corrosive and harmful to skin, eyes and lungs. **READ THE LABEL!** Make sure that you are wearing eye protection, protective clothing and a respirator with a chlorine filter while using bleach. Do not tuck pant legs into boots.

Remove bleach-soaked clothing. If bleach contacts eyes, rinse for 15 minutes with water and seek medical attention. If bleach is swallowed, do not induce vomiting; drink as much water as possible to dilute the bleach, and seek medical attention.

Dispose of used bleach solution by spreading it over an area where soil sterilization will not be a concern, and where there is no possibility of contamination of water bodies or septic systems.

Heat Treatment of Wood Nesting Material

EXPOSURE TO TEMPERATURES OF 93°C (200°F) FOR 12 HOURS WILL KILL CHALKBROOD SPORES. A kiln or oven must be designed to heat equipment evenly, to avoid hot and cold spots. Use circulating fans, and stack nests to facilitate air circulation during heating. Set up the kiln in a separate shed to minimize the potential for fire damage to other buildings and equipment. The length of time nests are treated will depend on the time required to reach a temperature of 93°C.

Paraformaldehyde Fumigation of Nesting Material

Paraformaldehyde is a white crystalline substance which gives off formaldehyde gas when heated. Paraformaldehyde fumigation of nest material is very effective for control of chalkbrood and other molds. Extreme caution must be taken when handling and using paraformaldehyde. Adequate ventilation following its use is essential. **Under no circumstances should paraformaldehyde be exposed to an open flame. READ THE LABEL!**

To fumigate with paraformaldehyde, place the nesting material in cross-stacked piles in the fumigation chamber (Figure 8). Problems can occur with persistent formaldehyde vapour after fumigating, so treat nesting material in a room or building set aside for this purpose only. **The building or the room to be used for fumigation must have an air exhaust system with air intake capability rated to the capacity of the exhaust system. In addition, you must be able to seal and lock the room or building.**

Condition the nesting material in the chamber for 48 hours at 20-25°C with a relative humidity of 60-70%. Use circulation fans to ensure even humidity and temperature throughout the chamber.

Fumigate with paraformaldehyde at a rate of 20 grams of product per cubic metre of fumigation chamber (1.1 lb. of product per 1000 cubic feet). Place the product in one or more electric frying pans attached to electric timers (Figure 7). Paraformaldehyde prills should be loaded to a depth no higher than the sides of each frying pan. Use gloves, eye protection, and a dust mask or respirator. Set the timer to heat the paraformaldehyde for 4 hours at the maximum heat setting, then seal and lock the chamber. Do not re-enter the chamber once fumigation has begun.

After a 24 hour period, begin continuous ventilation of the chamber by exhausting air. Ensure an adequate incoming flow of fresh air. Ventilate for 48-72 hours. If you can still smell an odour of formaldehyde, or your eyes sting, ventilate for an additional 24-48 hours. Enter the chamber only after completion of adequate ventilation. Use a full-face NIOSH-approved respirator with formaldehyde or acid gas cartridge. Also, wear coveralls and gloves. Place nesting material directly into the field for aeration prior to bee release.

Figure 1A

Figure 1B

Figure 2A

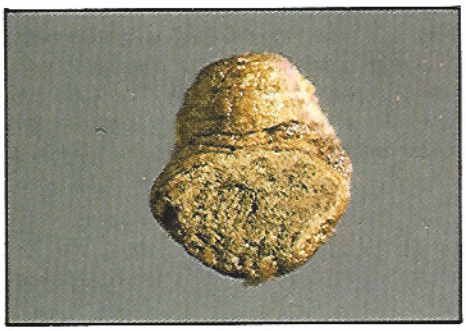

Figure 2B

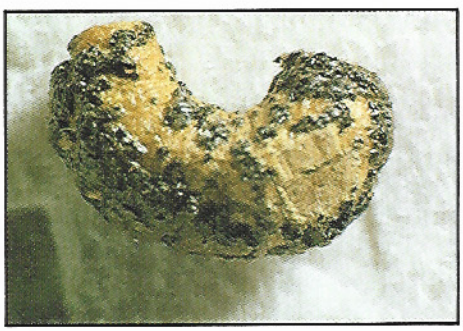

Figure 3

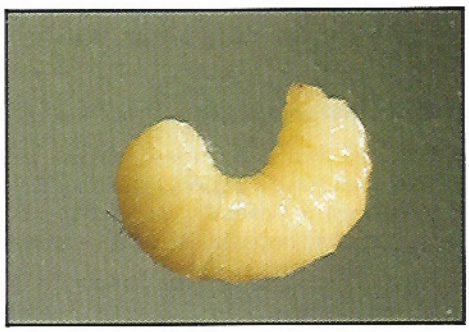

Figure 4

Figure 5

Figure 6

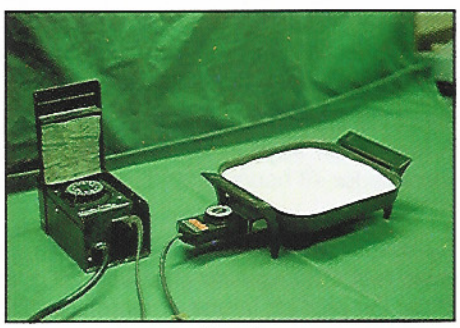

Figure 7

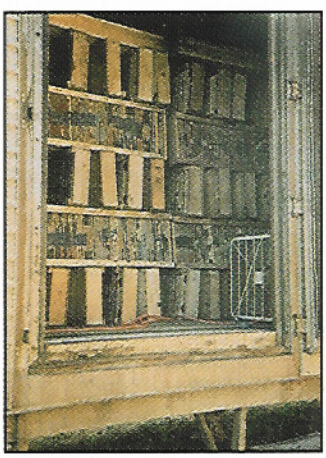

Figure 8

Figure 1. Classical sporulating chalkbrood cadaver **(Ascosphaera aggregata)**. A. Side view. B. Cross section. **Figure 2.** Classical nonsporulating chalkbrood cadaver **(Ascosphaera aggregata)**. A. Side view. B. Cross section. **Figure 3.** Partially sporulating chalkbrood cadaver **(Ascosphaera aggregata)**. **Figure 4.** Healthy leafcutting bee prepupa. **Figure 5**. Bleach treatment of leafcutting bee nests in a semicircular tank with a reel type rack. **Figure 6.** Cage to hold cocoons for bleach treatment. **Figure 7**. Paraformaldehyde in an electric frying pan with an electric timer, ready for fumigation. **Figure 8.** Leafcutting bee nests cross-stacked in a trailer ready for fumigation with paraformaldehyde.

4. SURFACE STERILIZATION OF BEE COCOONS

Both bleach treatment and paraformaldehyde fumigation can be used to surface sterilize bee cocoons, killing chalkbrood and other mold spores. Adult bees will emerge from incubation trays carrying fewer spores.

Bleach Treatment of Cocoons

Treat the cocoons just prior to incubation. Use the same dip tank as you use for nests. Use liquid bleach rather than dry bleach to make up the solution, otherwise the cocoons will become coated with a chalky residue. Construct mesh cages of similar dimensions to your nests (Figure 6). Fill them with cocoons and dip the cocoons for 3 minutes in a 3% bleach solution. **AIR DRY COCOONS THOROUGHLY, AWAY FROM HEAT AND DIRECT SUNLIGHT!** Then place them in incubation trays and begin incubation.

Paraformaldehyde Fumigation of Cocoons

Because problems may occur with persistence of paraformaldehyde vapour, treatment of cocoons should be undertaken in a facility set aside for paraformaldehyde fumigation and not used for other purposes.

If you plan to fumigate your leafcutting bee cocoons, you first must ensure that your incubator is equipped with a hooded exhaust fan in the 1600-2200 maximum cubic feet per minute (cfm) range. Air intake capability must be adequate for the exhaust capacity. A two-speed or variable speed exhaust fan will allow you to adjust your air exhaust rate. You will need air circulation fans within the incubator. You may also need to upgrade your heating capacity to maintain the incubation temperature of 30°C with continuous air exhaust during incubation. Without this equipment you should not proceed with fumigation of your cocoons.

Fumigate cocoons just prior to incubation. Place the cocoons in incubation trays in the fumigation chamber. Condition the cocoons for 48 hours at 20-25°C with a relative humidity of 60-70%.

Fumigate with paraformaldehyde at a rate of 1.1 lb. per 1000 cubic feet of fumigation chamber (20 grams per cubic metre). Place the product in one or more electric frying pans attached to electric timers. Handle paraformaldehyde prills with caution and do not load prills to a depth higher than the sides of the frying pan. Wear gloves, eye protection and a dust mask or respirator.

Set the frying pan to its maximum heat setting, set the timer to provide power for 4 hours, then seal and lock the chamber. After 24 hours, actively ventilate for 48-72 hours. Following ventilation, transfer incubation trays to the incubator. Use a full-face NIOSH-approved respirator with formaldehyde or acid gas cartridge, and wear coveralls and gloves.

Begin incubation at 30°C. Traces of formaldehyde gas will be released from cells and trays, requiring continuous ventilation. Leave the exhaust fans running at a high enough rate that you cannot detect formaldehyde in the incubator. On day 7 place dichlorvos strips in the incubator to control parasites. Turn the air exhaust system off for the parasite control period. On day 13 remove the dichlorvos strips, and turn the exhaust system on to remove both the dichlorvos and the formaldehyde gas which has accumulated. After ventilating for 24-48 hours, turn the exhaust fan down and run it as required, to remove any traces of formaldehyde gas.

5. FIELD PRACTICES

Do not share equipment, incubator or bees. Sharing is a good way to import disease problems. If you must, first ensure that chalkbrood is not present in any of the operations involved.

Do not dump old cocoons in the field. If chalkbrood cadavers are present in the old cocoons the adult leafcutter bees will come into contact with them, and will be exposed to chalkbrood spores. Collect and burn the old cocoons.

Keep Field Shelters Clean and Free of Debris. Spray the shelter interiors with a 3 to 5% bleach solution applied with a back pack sprayer, to help control chalkbrood and mold spores. Wear protective clothing and a respirator. Allow time for the shelters to dry before releasing bees.

Disinfect Cell Removers, Tumblers and Cell Breakers each year. Use a 3 to 5% bleach solution and a back pack sprayer. Rinse the equipment with water, dry thoroughly, and spray with a light oil to help prevent corrosion. Alternately, fumigate the bee handling equipment with paraformaldehyde, following the directions given above for fumigation of cocoons and nesting equipment.

Keep nests from each field as separate lots during stripping and sampling. If chalkbrood shows up it may be limited to one or more lots, allowing you to sell the affected lots and keep the clean lots.

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