Saskatchewan Agriculture Development Fund

ALFALFA LEAFCUTTING BEE MOULDS AND METHODS FOR THEIR CONTROL

ISBN 0-88656-586-3

Research on Saskatchewan alfalfa leafcutting bee populations has shown that a number of moulds, in conjunction with yeasts and bacteria, are commonly associated with leafcutting bees. These mould species are found in leafcutting bee nest material and on cocoon surfaces, pollen balls, working adult bees, and larval cadavers. While these moulds are generally saprophytic, that is, growing on any material which will provide them with nutrients (leaf debris, pollen provisions, dead bee larvae), some species of moulds may cause considerable damage to bee populations.

Many of these mould species may also be harmful to the health of alfalfa seed producers. Methods for controlling moulds in leafcutting bee populations include bleach dipping of nest material and bee cocoons, heat treatment of nest material, and paraformaldehyde fumigation of nest material.

FOLIAR MOULDS AND BEE POPULATIONS

Surveys of cocoon samples from numerous leafcutting bee populations throughout the province indicate that the most common moulds found on larval cadavers, pollen balls, and cocoon surfaces include **Aspergillus** species (Figures 1, 2, and 3), **Eurotium** species (Figures 4, 5, and 6), and **Rhizopus** species (Figure 7), The most common yeast, **Trichosporonoides** species (Figures 8 and 9), is associated with all leafcutting bee populations sampled. Bacteria isolated from leafcutting bee samples include **Bacillus** and **Enterobacter** species. The relative abundance of all these microorganisms fluctuates with changing temperature and moisture conditions.

The presence of moulds, yeasts, and bacteria in leafcutting bee populations can lead to problems in larval development. Bacteria and yeasts are capable of fermenting the nectar and pollen provision on which the bee egg is laid; this may lead directly to egg or larval mortality and subsequently allow moulds to overgrow the entire cell. The result is a mouldy pollen ball contaminated with a number of mould and yeast species (Figures 10 and 11). Larvae which die for other reasons may also become overgrown with moulds.

The table below shows levels of larval and pollen mould in Saskatchewan leafcutting bee surveys over the past seven years. The percentage of healthy larvae is significantly reduced in populations where foliar moulds are present at high levels on cocoon surfaces.

Mould levels in Sask. leafcutting bee populations

Year	% Larval Mould		% Pollen Mould	
	maximum	mean	maximum	mean
1985	4.3	0.5	16.4	2.4
1986	2.8	0.6	29.3	5.3
1987	2.7	0.6	34.3	6.4
1988	1.8	0.2	17.9	2.8
1989	3.4	0.3	27.1	3.4
1990	1.0	0.2	21.4	2.6
1991	1.4	0.3	25.5	5.1

OTHER FUNGI AFFECTING BEE POPULATIONS

Chalkbrood disease affects leafcutting bee larvae after they ingest spores of the fungus **Ascosphaera aggregata** while feeding on pollen. Once the larvae die, the fungus may or may not form spores in the cadavers. The resulting sporulating and nonsporulating cadavers are shown in Figures 12 to 15. The appearance of these infected larvae is unique and totally unlike that of larvae overgrown with other moulds.

Several native **Ascosphaera** species have also been isolated from Saskatchewan leafcutting bee populations. These include **Ascosphaera atra, Ascosphaera larvis, Ascosphaera pollenicola,** and **Ascosphaera variegata** (Figures 16 to 20). All of these species may occur on pollen balls or larval cadavers. The appearance of these native species of **Ascosphaera** on infected larvae is distinctly different from classic chalkbrood disease, **Ascosphaera aggregata**. These native species have been found at levels above 4% in samples of domestic leafcutting bees and above 15% in wild leafcutting bee species sampled.

Moulds and Yeasts on larval cadavers, pollen balls, and cell surfaces

Fig. 1 **Aspergillus** sp. (cadaver)

Fig. 2 **Aspergillus** sp. (pollen)

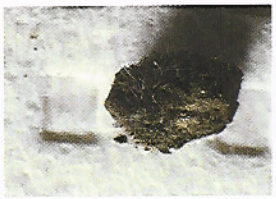

Fig. 3 **Aspergillus** sp. (pollen)

Fig. 4 **Eurotium** sp. (cadaver)

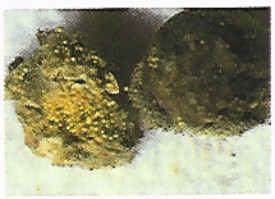

Fig. 5 **Eurotium** sp. (pollen)

Fig. 6 **Eurotium** sp. (cell surface)

Fig. 7 **Rhizopus** sp. (cell surface)

Fig. 8 **Trichosporonoides** sp. (cadaver)

Fig. 9 **Trichosporonoides** sp. (pollen)

Fig. 10 Numerous mould spp. (pollen)

Fig. 11 Numerous mould spp. (pollen)

OTHER BEE MORTALITY

A final note on leafcutting bee mortality concerns the occurrence of dead discoloured larvae, which have been found at levels of 3.1 to 6.0% in surveys over the past seven years. There is no obvious sign of mould growing on the surface of these cadavers; their colour may vary from light brown through red or black (Figures 21 to 23) and the internal organs of affected larvae are often discoloured as well (Figure 24). This problem is not apparently due to the presence of moulds; it may be related to invasive fungal or viral infections in some cases, interacting with environmental or other factors.

Chalkbrood - **Ascosphaera aggregata**

Fig. 12 **A. aggregata** (nonsporulating)

Fig. 13 **A. aggregata** (nonsporulating)

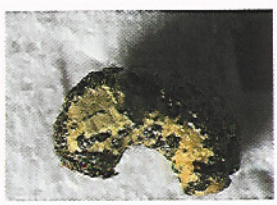

Fig. 14 **A. aggregata** (sporulating)

Fig. 15 **A. aggregata** (sporulating)

MOULDS AND PRODUCER HEALTH

Many of the moulds found in association with leafcutting bee populations are potentially harmful not only to the bees but to alfalfa seed producers as well. Alfalfa seed producers, working in close proximity to leafcutting bees and cocoons on a regular basis, are exposed to high levels of spores from bee-related fungi at a time when fungal disease is becoming recognized as a major medical problem. Moulds including **Aspergillus** and **Rhizopus** species have been implicated in allergic reactions and bronchopulmonary disease and are considered major fungal allergens.

Individuals working with bees should observe basic safety precautions, particularly during bee incubation and harvesting operations. At these times, large numbers of cocoons or emerging bees concentrated in confined areas may lead to high levels of airborne fungal spores in the workplace.

The use of efficient ventilation systems and appropriate protective equipment, combined with safe work habits, can minimize contact with potentially harmful spores. Work clothes, dust masks, and gloves should be worn to minimize movement of contamination into the living environment. A respirator or positive pressure air filtration system may be used if a known sensitivity to moulds exists. Eating, smoking, and drinking should be done away from the work area, and hands washed and work clothes removed when leaving the area. Facilities used for cocoon incubation and harvesting should be cleaned and disinfected following use.

CONTROL OF MOULDS

Incorporation of decontamination techniques such as bleach dipping, heat treatment, or paraformaldehyde fumigation of leafcutting bee nest material has been shown to be highly effective in reducing levels of moulds, as well as yeasts and bacteria.

Bleach Treatment of Nest Material

Dipping of nest material in sodium hypochlorite bleach is most compatible with those operations utilizing polystyrene and styrofoam nest materials, since wood materials may warp if not properly dried following wetting. Nest material should be dipped in a 3 to 5% active chlorine solution for 3 to 5 minutes. A bleach test kit should be used periodically to determine the active chlorine concentration of the dipping solution. A wetting compound (for example Triton X-100, Agral 90, or Amway LOC) can be added to the dipping solution at a rate of 0.1% to enhance penetration of bleach solution into nest tunnels. After nest material has been dipped it should be well drained and then either stacked to dry or placed in field shelters. Polystyrene and styrofoam nest material should be allowed to dry for several days prior to bee release.

Heat Treatment of Nest Material

Heat treatment within a temperature range of 80°C to 95°C for a 12 hour period is required in order to eliminate moulds, yeasts, and bacteria from wood laminate and wood block nest material. It is critical to maintain a uniform temperature within the treatment facility during the heat treatment procedure. Fans may be used to circulator hot air through the kiln or oven, and nest boxes should be stacked loosely to allow for movement of hot air through the material.

Native **Ascosphaera** species

Fig. 16 **A. atra** (pollen)

Fig. 17 **A. larvis** (cadaver)

Fig. 18 **A. larvis** (cadaver)

Fig. 19 **A. variegata** (pollen)

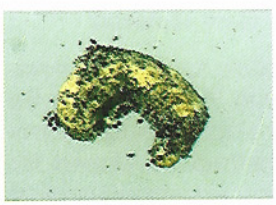

Fig. 20 **A. variegata** (cadaver)

Discoloured dead larvae

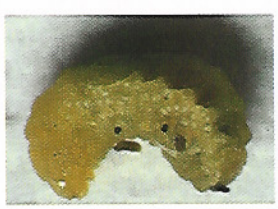

Fig. 21 Discoloured larva

Fig. 22 Discoloured larva

Fig. 23 Discoloured larva

Fig. 24 Discoloured larva

All figures courtesy of W. Goerzen, with R. Underwood and M. Erlandson.

Bleach Treatment of Cocoons

Cocoons may be dipped in a 3% bleach solution for 3 minutes just prior to incubation. After dipping, the cocoons should be well drained and spread out to dry in a ventilated area away from direct sunlight. Once they are completely dry, the cocoons may be incubated.

Dipping cocoons in bleach solution is an efficient way to eliminate moulds from cocoon surfaces. This reduction in foliar contamination has a significant impact on the number of spores subsequently found on emerging adult bees. Spore counts taken on female leafcutting bees emerging from trays of bleach-dipped cocoons have shown a 94% reduction in total spore numbers in comparison with spore counts taken on female bees emerging from trays of undipped cocoons.

Paraformaldehyde Fumigation of Nest Material

Paraformaldehyde is a white crystalline substance which gives off formaldehyde gas when heated. Paraformaldehyde fumigation of nest material is highly efficacious for control of mould in nest material. The method developed for fumigation is outlined below. Note that extreme care must be taken in handling and use of paraformaldehyde; adequate ventilation following its use is essential. Under no circumstances should paraformaldehyde be exposed to an open flame.

To fumigate with paraformaldehyde, place nest material in a well-sealed fumigation chamber. Because problems may occur with persistence of formaldehyde vapor under certain conditions, treatment of nest material should only be undertaken in a building set aside specifically for this purpose. Prior to fumigation, condition nest material in the chamber for 48 hours at 20-25°C with a relative humidity of 60-70%.

Fumigate with paraformaldehyde at a rate of 20 grams of product per cubic meter of fumigation chamber (1.1 lb./1000 cubic ft.), by placing the product in a heat generation unit (electric frying pan) attached to an electric timer. Paraformaldehyde prills should be handled with caution; the use of gloves, eye protection, and a dust mask or respirator is advised. Set the timer to heat the product for 4 hours at maximum heat setting in the sealed and locked chamber.

After a 24 hour period, begin continuous ventilation of chamber by exhausting from the top and ensuring an adequate incoming flow of fresh air. Ventilate for 48-72 hours. If an odor of formaldehyde is still detected, ventilate for an additional 24-48 hours. Open and re-enter chamber only after completion of adequate ventilation. Use a full-face NIOSH approved respirator with formaldehyde or acid gas cartridge and particulate filter; coveralls and gloves should also be worn. Following fumigation, deploy nest material directly into the field.

SUMMARY

Moulds, along with yeasts and bacteria, are commonly associated with all leafcutting bee populations. These microogranisms are found in nest material and on cocoon surfaces, pollen balls, adult bees, and larval cadavers. Their presence interferes with larval development by spoiling larval provisions, and lowers the quality of leafcutting bee populations by overgrowing pollen balls and cocoon surfaces.

Many mould species are also implicated in human allergic reactions and bronchopulmonary disease. To avoid these problems, alfalfa seed producers should take precautions to reduce contact with potentially harmful spores, particularly during cocoon incubation and harvesting operations. Incorporation of mould control techniques, including bleach dipping, heat treatment, and paraformaldehyde fumigation have been shown to be very effective in reducing levels of mould, yeasts, and bacteria in leafcutting bee nest material.

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March, 1992

This research was funded by the Saskatchewan Agriculture Development Fund (ADF).
