Chalkbrood disease (*Ascosphaera aggregata*) is a serious limiting factor in the use of alfalfa leafcutting bees for alfalfa seed production. Chalkbrood-related alfalfa leafcutting bee losses in excess of 30% have been reported in some alfalfa seed production areas of the northwestern United States. Levels of chalkbrood disease vary widely in western Canadian alfalfa leafcutting bee populations; to date, the disease has occurred only sporadically and at very low levels in Saskatchewan alfalfa leafcutting bee populations.

Chalkbrood spores are ingested by alfalfa leafcutting bee larvae as they feed on contaminated nectar and pollen provisions placed in the cell by the adult female bee. These spores germinate and grow rapidly within the developing bee larvae. As the disease progresses, cysts containing spores are generally formed beneath the intact cuticle of infected larvae; in larval cadavers infected with chalkbrood, the fungus may or may not form spores. The result is two distinct chalkbrood cadaver phenotypes, commonly referred to as "sporulating" and "non-sporulating" chalkbrood.

Research undertaken in collaboration with scientists at the University of Saskatchewan demonstrated through DNA molecular analysis that despite the morphological differences between alfalfa leafcutting bee larval cadavers exhibiting the "sporulating" and "non-sporulating" forms of chalkbrood, both forms of the disease are caused by *A. aggregata*. Illustrations of chalkbrood cadavers are given in figures 1 - 4.

An individual "sporulating" chalkbrood cadaver may contain millions of chalkbrood spores. These spores are spread within alfalfa leafcutting bee populations as emerging adult bees chew through cells containing chalkbrood cadavers. Chalkbrood spores carried on the bodies of adult bees contaminate alfalfa leafcutting bee cell surfaces, nest material, and field shelters. Research carried out in order to prevent the disease from becoming a problem in Saskatchewan alfalfa leafcutting bee populations led to the development of the paraformaldehyde fumigation process which effectively decontaminates alfalfa leafcutting bee cells and nest material.

Several other *Ascosphaera* species may also occur in alfalfa leafcutting bee populations. Among these species is the fungus *Ascosphaera larvis*, which causes chalkbrood-like symptoms in developing alfalfa leafcutting bee larvae and eventually produces spores within the larval cadaver and on the cadaver surface as well (figures 5 - 7). Leafcutting bee cadavers infected by *A. larvis* can be easily distinguished from those infected by chalkbrood (*A. aggregata*) because *A. larvis* sporulates on the surface of the cadavers.

A final note on alfalfa leafcutting bee larval mortality concerns the occurrence of dead discoloured larvae, which are commonly found at levels in the 2.5 - 5.0% range in alfalfa leafcutting bee populations. There is usually no sign of fungal growth on the surface of these cadavers; their colour may vary from light brown through red or black (figures 8 - 10). The causes of dead discoloured larvae may be related to either fungal or bacterial infections, and may also include interaction with environmental and other factors.

Chalkbrood disease may be spread through movement of alfalfa leafcutting bee cells and nest material contaminated with chalkbrood spores. Methods available for decontamination of alfalfa leafcutting bee cells and nest material include paraformaldehyde fumigation and chlorine bleach treatment. Wood nest material may also be decontaminated through a heat treatment process.

Important considerations involved in maintaining chalkbrood-free alfalfa leafcutting bee populations include utilizing the loose cell management system, effectively decontaminating bee cells, nest material, and other equipment annually, and regularly sampling alfalfa leafcutting bee cell quality.

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Figure 1. Sporulating chalkbrood cadaver (*Ascosphaera aggregata*) - external view.
Figure 2. Sporulating chalkbrood cadaver (*Ascosphaera aggregata*) - cross section.
Figure 3. Non-sporulating chalkbrood cadaver (*Ascosphaera aggregata*) - external view.
Figure 4. Non-sporulating chalkbrood cadaver (*Ascosphaera aggregata*) - cross section.
Figure 5. Alfalfa leafcutting bee larval cadaver infected with *Ascosphaera larvis* (heavy external sporulation).
Figure 6. Alfalfa leafcutting bee larval cadaver infected with *Ascosphaera larvis* (moderate external sporulation).
Figure 7. Alfalfa leafcutting bee larval cadaver infected with *Ascosphaera larvis* (light external sporulation).
Figure 8. Dead discoloured alfalfa leafcutting bee larva.
Figure 9. Dead discoloured alfalfa leafcutting bee larva.
Figure 10. Dead discoloured alfalfa leafcutting bee larva.

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