CHALKBROOD CONTROL IN THE ALFALFA LEAFCUTTING BEE

Rosalind James and Junhuan Xu[1]

Chalkbrood is a disease of bees that kills the developing larvae. It is the most serious disease found in the alfalfa leafcutting bee, killing, on average about 15% of the larvae, but sometimes as many as 45% or more. It is more of a problem for some growers than others, and some areas are more affected than others (Figure 1). This disease is most commonly caused by a fungus (mold) called *Ascosphaera aggregata*. Twenty-two different species of *Ascosphaera* have been discovered and identified. Some of these only grow on pollen provisions, causing moldy pollen balls, but others are pathogens. We found that only one species, *A. aggregata*, causes the majority of all infections found in the alfalfa leafcutting bees from alfalfa seed fields. We initially thought that this pathogen could only infect alfalfa leafcutting bees because we have never seen it occur in other bees. However, this is not true. The disease probably originated in Europe and migrated into North America with its host, the alfalfa leafcutting bee. But in Europe, this pathogen can occur on other bees such as *Megachile centuncularis* and *Osmia rufa* (the red mason bee). This means that the pathogen potentially infects other wild bees in North America, although it has never been reported. Laboratory tests have demonstrated that this pathogen will not infect honey bees. Chalkbrood does occur in honey bees, but it is caused by another pathogen called *A. apis*.

Chalkbrood in the alfalfa leafcutting bee spreads when spores contaminate the body of nesting females, who in turn then accidentally deposit the spores in the pollen they collect to feed their young (the "pollen provision") (Fig. 2). When adult bees emerge from either drilled boards or loose cells, they become contaminated with spores from the larvae that died the previous year. This contamination is much greater when the bees emerge from drilled boards, as shown in Figure 2, but loose cell management provides adequate contamination levels for disease spread as well. This is unfortunate because the loose cell method was developed to prevent the spread of chalkbrood.

An interesting thing about chalkbrood is that it occurs at much lower levels in Canada than in the U.S. It is unusual for the disease to reach 5% in any Canadian population, and is generally less than 1%. The reasons why the disease occurs so much less frequently in Canada than the U.S. is unknown, but several hypotheses exists, and we summarize them here:

Canadian bee producers manage their bees differently than U. S. seed growers. Growers sometimes say that different farm practices are needed to produce high bee production than to produce high seed yields. And it is true that, on average, Canadian growers produce more bees, and U.S. growers produce more seed per acre. However, it is not clear exactly which practices

[1] R. James (Rosalind.James@ars.usda.gov) Research Leader, USDA-ARS Pollinating Insects—Biology, Management and Systematics Research, Utah State University, Logan, UT 84322. J. Xu (xu@biology.usu.edu) Research Associate, Department Biology, Utah State University, Logan, UT 84322. **In:** Proceedings, 2008 California Alfalfa & Forage Symposium and Western Seed Conference, San Diego, CA, 2-4 December, 2008. UC Cooperative Extension, Plant Sciences Department, University of California, Davis, CA 95616. (See http://alfalfa. ucdavis.edu for this and other Alfalfa Symposium Proceedings.)

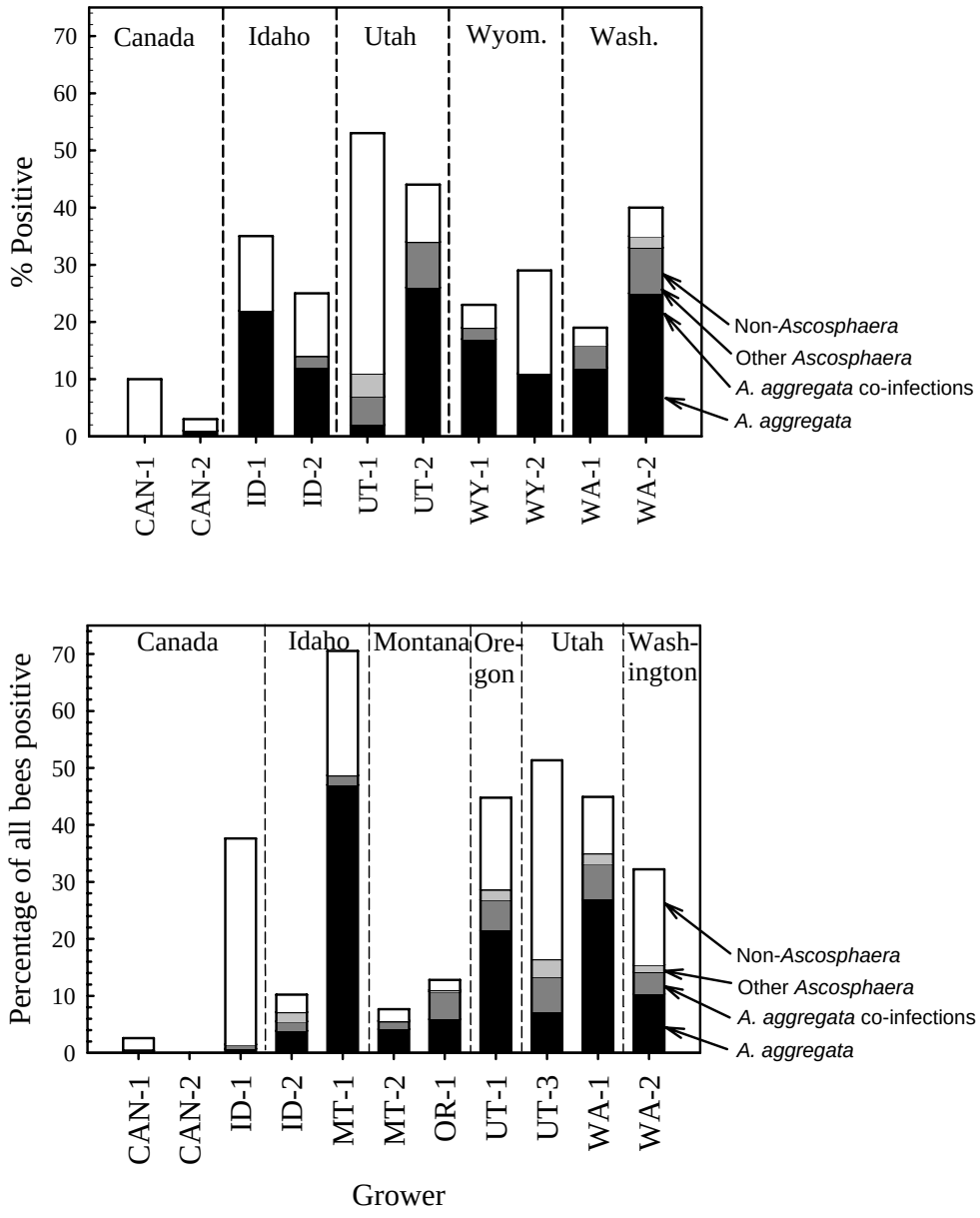

Figure 1. Proportion of alfalfa leafcutting bee larvae killed by diseases. Cells were collected from alfalfa seed growers throughout the western U.S. and Canada. Top graph is for 2004 and bottom graph is 2005. The proportions of larvae infected with chalkbrood are colored grey or black. The majority of chalkbrood infections were caused by *Ascosphaera aggregata* alone, or co-infecting with other *Ascosphaera* species. Non-*Ascosphaera* diseases are unknown at this time.

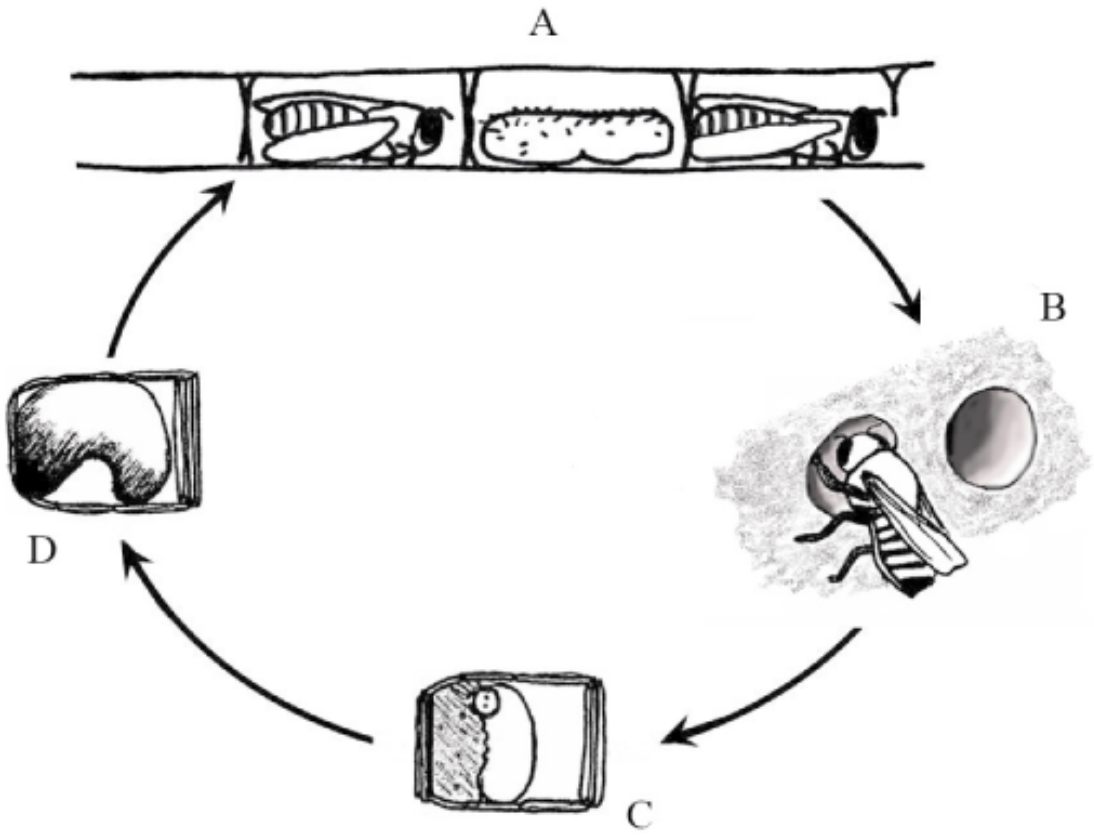

Figure 2. The life cycle of chalkbrood in the alfalfa leafcutting bee. (A) A cross section of a nest showing a dead, infected larvae between two adult bees who are ready to emerge in the spring. The adult bee to the left must chew through the dead larvae to get out of the nest, and in doing so, becomes contaminated with spores. (B) This contaminated bee now carries spores to her own nests as she builds them. (C) A larva contracts the disease after eating a pollen provision containing spores. (D) Larvae typically die after they have reached full size, and then the fungus sporulates. These spores will survive the winter to continue the cycle next spring.

lead to the higher bee yields, and whether these practices actually reduce seed yields. One hypothesis has been that the use of lower numbers of bees per acre in Canada may be one such factor. However, it may also be that farms with lower seed yields in Canada are not using irrigation and occur in locations with shorter, cooler summers, factors that also may affect yield. If the plants are producing fewer flowers, they may require fewer bees. Thus, the idea of differences in farm practices leading to different prevalence of disease may actually be an accumulation of several causes that include weather, planting rates and timing, irrigation, bee release methods, etc. However, despite the complexity of this hypothesis, we are not discarding it, but rather trying to dissect it into manageable pieces. For example, Dr. Pitts-Singer is finding that some of the higher bee release rates used today can have detrimental effects on bee health. Also, Dr. Guedot has demonstrated that chalkbrood increases as bees become more crowded at a shelter.

Treating loose cells with paraformaldehyde gas is used in Canada as a disinfectant and may reduce the spread of the disease. U.S. growers sometimes use paraformaldehyde to disinfect nesting boards, but they rarely use it to disinfect the bees themselves. Great care must be taken in doing this because the bees can easily be killed, as well. However, this practice probably does reduce disease spread significantly. We have been looking for other, safer, compounds for treating loose cells to kill spores. The performance of this method has been variable between growers, and for this reason, we are still looking for more effective compounds.

Alfalfa leafcutting bees that have gone wild may act as a disease reservoir, but in Canada, the extremely cold winters prevent the establishment of wild bees there. This theory could well be true, but at this point we have no evidence about the extent to which wild bees act as a disease reservoir, or whether wild bee populations have established in Canada. Furthermore, the winters in Montana and Wyoming seed growing areas can also be extremely cold, yet the disease persists there. We are currently working to determine how much the disease is spread over the course of the summer, and whether spores can be found outside the commercial nesting areas.

Canadian summers are colder, inhibiting chalkbrood development. In general, the Canadian summers are cooler than most seed growing regions of the U.S. However, we have shown that cooler temperatures actually make the bees more susceptible to chalkbrood disease. Furthermore, the effect of chilling bee larvae on chalkbrood incidence has been seen in other bees as well, including honey bees and the blue orchard bee. Thus, this factor can be ruled out.

The bees have only one generation a year in Canada, and disease spread is increased with multiple generations in a year. This correlation between latitude and the occurrence of second generation has been observed by bee producers, but to our knowledge, no one has actually tried to quantify it. We assume it is true. However, it is questionable whether second generation bees increase the incidence of chalkbrood. We have found that chalkbrood occurs most frequently in the first bees of the season, and decreases over time. However, we have observed a second smaller peak in chalkbrood during the middle of the summer, and are now working to determine whether this occurs during the second generation emergence, and whether this is a common phenomenon. In any case, this second peak is still much smaller than the initial peak that occurs at the beginning of the season, when the nesting females have the greatest spore contamination levels and air temperatures are occasionally cool.

Bees from Canada are highly susceptible to chalkbrood because resistance is not selected for, due to low chalkbrood levels. These bees are then brought into the U.S., where they are exposed to the disease, and have very high infection rates. In the early 1990's, the susceptibility of Canadian and U.S. bees was compared, and no difference were seen. However, this result does not exclude this hypothesis because a very large number of Canadian bees are brought into the U.S. every year, effectively causing the populations in the U.S. to be genetically similar that what occurs in Canada. This year, we used molecular DNA methods to identify potential immunity genes in the alfalfa leafcutting bees, and we discovered several immune response genes known in other insects. Next, we will begin an investigation to determine how these genes are regulated. In more simplified terms, we will determine the extent to which immunity is controlled genetically (nature versus nurture). We are beginning to investigate whether these immunity genes occur in different populations of the bees, or whether their expression is only affected by environmental factors such as temperature and nutrition. We will take bees from populations in the U.S. where Canadian bees have not been used and compare these to Canadian populations. We have been using molecular biology techniques to determine which genes are important to investigate. These methods tend to be very expensive, but they can yield results much faster than breeding experiments, which would require 5-10 years of breeding before experiments could be conducted.

The question as to why Canadian growers have healthier bees than U.S. growers is an important one. Some of the potential factors may be outside our control, such as the effects of weather. If disease outbreaks are primarily due to such factors, we may at least be able to control the disease in some regions of the U.S. where climatic conditions are similar to Canada. If the differences in disease levels are due to some of the other factors, such as disinfecting the loose cells, low bee release rates, or low incidence of second generations, then this knowledge can be used to develop more effective control strategies for U.S. growers. On the other hand, if genetics plays an important role in disease resistance, we will need to seriously consider the implications of importing large numbers of susceptible bees every year, or work to develop more disease resistant stocks. In addition to this, a better understanding of the disease process could lead to some new drugs for controlling the disease – although a delivery system for such drugs would also need to be developed.