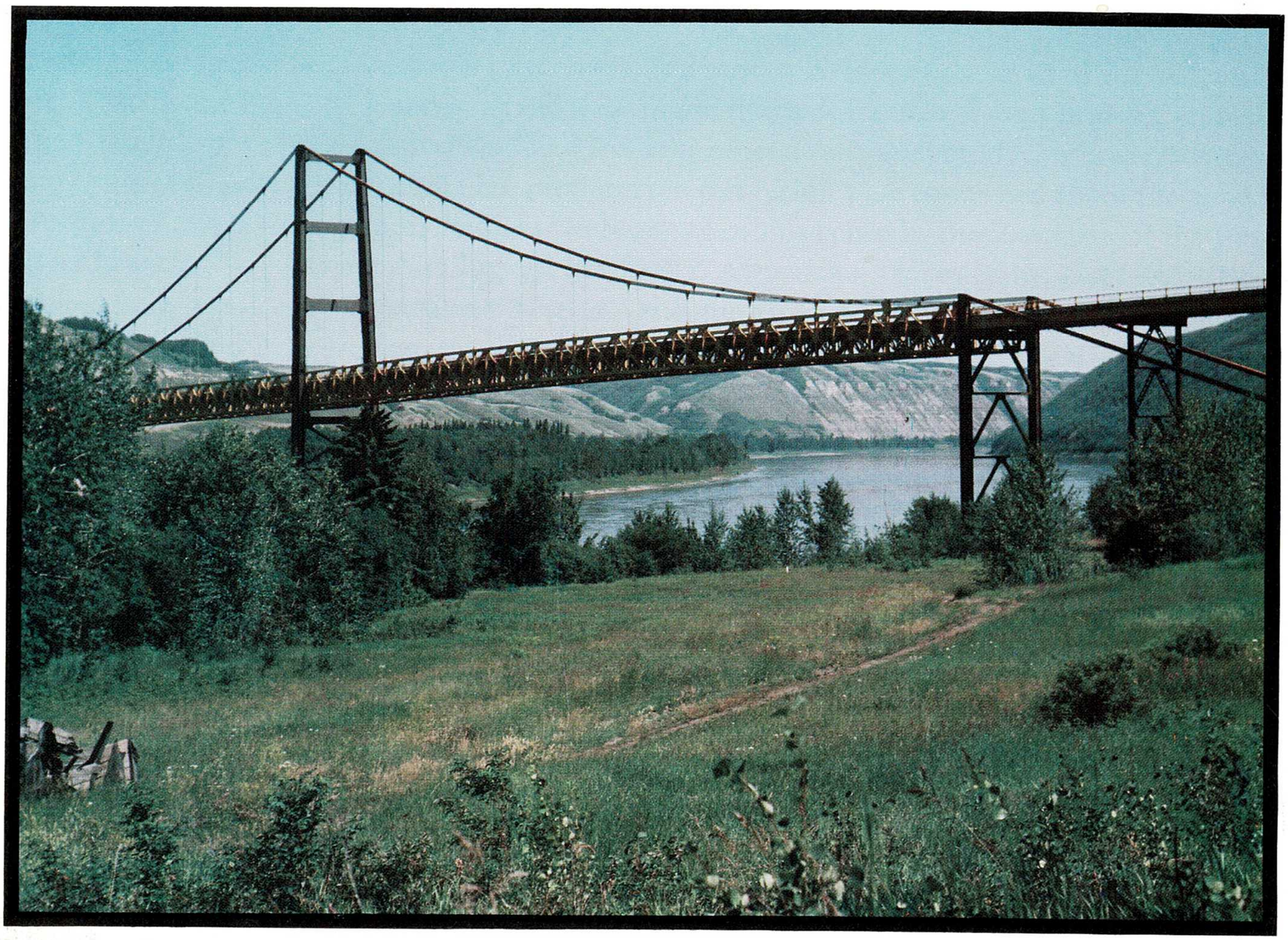
Detection of Chalkbrood in Leafcutting Bee Cells In the Peace River Region



Peace River Branch
Alberta Alfalfa Seed Producers' Association
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FROM THE PRESIDENT —

Chalkbrood, caused by ***Ascosphaera aggregata,*** is a serious disease of the larva of the leafcutting bee. This disease has been found in all the western provinces of Canada, except British Columbia. The Peace River region is relatively isolated from the other alfalfa seed producing areas in Alberta and British Columbia. Leafcutting bee producers in this region were concerned about the possible presence of this disease in local colonies. In response to this concern, the Executive of the Peace River Branch of the Alberta Alfalfa Seed Producers' Association requested the assistance of personnel from Agriculture Canada and Alberta Agriculture to conduct a survey to detect the presence of chalkbrood in cells produced in the 1985 growing season. CHALKBROOD CAUSED BY *Ascosphaera aggregata* WAS NOT FOUND IN ANY OF THE SAMPLES SURVEYED. This report outlines the results of this co-operative effort. Publication of these results was made possible by a grant from the Northern Alberta Development Council.

Raymond Wood

Executive of the Peace River Branch Seed Producers' Association: 1985-86

President: Mr. Raymond Wood

Vice President: Dr. Peter Pankiw

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Recording Secretary: Mr. David Wong, D.A. Alberta Agriculture

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Healthy larva

Chalkbrood-infected larva

Chalkbrood and Other Quality Indices of Leafcutting Bee Cells Produced in the Peace River Region: 1985-86 Survey

INTRODUCTION

The leafcutting bee is used extensively for pollination of alfalfa. Chalkbrood, caused by fungi, is a disease that infects the larva of the bee and can result in heavy bee mortality. One species of chalkbrood, ***Ascosphaera aggregata*** has been identified as the major casual agent of chalkbrood in the leafcutting bee. To date, chalkbrood has been found in the alfalfa seed producing areas in both the United States and all the provinces in western Canada except British Columbia (Stephen et al., 1981).

It has been speculated that there are isolated areas where this disease may not occur. In this survey, this possibility was investigated in the alfalfa seed producing areas in the Peace River region of Alberta and British Columbia. This region is geographically isolated from the other alfalfa seed producing areas in Alberta and British Columbia. Also, the quality and number of cells produced and some of the management practices that could curtail the incidence and spread of fungal diseases are documented.

MATERIAL AND METHODS

One 250 g sample of every batch of 1 million cells or less that was produced in the 1985 growing season was obtained from producers in the region. In addition, producers were asked to supply the following information: the area or areas where these cells were produced, the total number of cells produced, the type of nesting materials used, whether these nesting materials were disinfected with a solution of chlorine bleach - a treatment recommended for the control of chalkbrood, and whether cell removing equipment was shared.

Approximately 500 cells from each sample were examined by taking X-ray photographs. The number of larvae with chalkbrood symptoms, live and dead prepupae, immature larvae, dead larvea, pollen balls, second generation cells (adults already emerged), parasites and cells with mechanical damage were counted. The relative percentages of each of these categories were then calculated. In instances where larvae with chalkbrood symptoms were identified in the x-ray photographs, the cells with these symptoms were examined under a microscope for the presence of fungi and then cultured to verify whether ***Ascosphaera aggregata*** and/or other ***Ascosphaera*** were present.

RESULTS

Table 1. Quality of leafcutting bee cells in the Peace River region (1985 production)

Cell Contents	Mean %	Range %
Chalkbrood-infected larvae	Absent in sampled population	
Live prepupae	77.3	19.1 - 95.5
Dead prepupae	2.3	0 - 30.9
Immature larvae	0.4	0 - 3.1
Dead larvae	2.5	0 - 10.5
Pollen balls	16.3	2.7 - 61.0
Second-generation cells	0.4	0 - 5.9
Parasites	0.3	0 - 5.3
Damaged with cell-removing equipment	0.4	0 - 7.8

- Twenty-nine of the 32 producers in the region (91%), responded to the survey. Representative cell samples were taken from an estimated 82 million cells produced in a zone from Beaverlodge at lat. 55°12' to Fort Vermilion at lat. 58°23'.

- Chalkbrood caused by ***Ascosphaera aggregata*** was not found in any of these samples.

- On average, live prepupae accounted for 77.3% of the total cells (Table 1) and a high proportion of good quality cells were produced (Fig. 1).

- Pollen balls (cells provisioned with nectar and pollen but not containing viable larvae) accounted for 16.3% of the sampled cells (Table 1; Fig. 2). The reason for this nesting attribute is little understood. However, a comparison with data for the same year from the other prairie provinces showed pollen ball levels of 18.22%, 12.85% and 15.87% in samples from Alberta, Saskatchewan and Manitoba, respectively (Anon. 1986).

- There was a low level of parasite infestation (Table 1), and parasites were absent in a number of samples (Fig. 3). Vapona strips for parasite control are used in a majority of operations in the region.

- Wood boards and polystyrene boards and blocks were the only nesting materials used in the region. Polystyrene was used by 25 producers (86%). Only 2 producers (7%) used wood exclusively and the remaining 2 producers (7%) used a combination of wood and polystyrene.

- All the producers who participated in the survey were aware of the benefits of chlorine bleach for the control of chalkbrood and other fungi. However, chlorine bleach was used only when fungi were a visible problem.

- Only 14 producers (48%) used chlorine bleach as a routine part of their management, and the nesting material used by these producers was exclusively polystyrene.

- Visible fungal growth did not appear to be a problem with wood nesting material and this material was rarely, if ever, treated with sodium hypochlorite.

- In the 1985-86 season 11 producers (38%) shared cell removing equipment.

There is a relative abundance of wild bees in the Peace River region, and whereas these bees could be endemic reservoirs for chalkbrood disease, there are some factors that could contribute to keeping this region free from chalkbrood or at least keeping the disease at a low level. In recent years, the production of cells within the region has been adequate to meet local demands as well as demands for the export market. Consequently, there has been little or no importation of cells that could carry the disease. Also, Parker (1986) has suggested that high density bee populations in large shelters may be a primary factor contributing to the spread of this disease. In the Peace River region, small polyethylene shelters (1.2 x 2.4 m) with 40,000 cells are used by most producers. These small shelters with reduced bee populations may therefore promote disease control.

Acknowledgements

This survey was initiated at the request of the alfalfa seed producers in the Peace River region, and made possible by their continued co-operation. A grant to this producer organization from the Northern Alberta Development Council enabled the publication of these results.

We would like to thank the following. Mr. J.A.C. Lieverse, Agriculture Canada, Research Station, Beaverlodge, Alberta for assistance in collecting and collating the data. Mrs. P. Tognet, Canadian Cocoon Testing Centre, Brooks, Alberta, for X-ray photographs. The District Agriculturists, Alberta Agriculture, Peace River region, for assisting in sample collection.

Fig. 1. Percent Live Prepupae

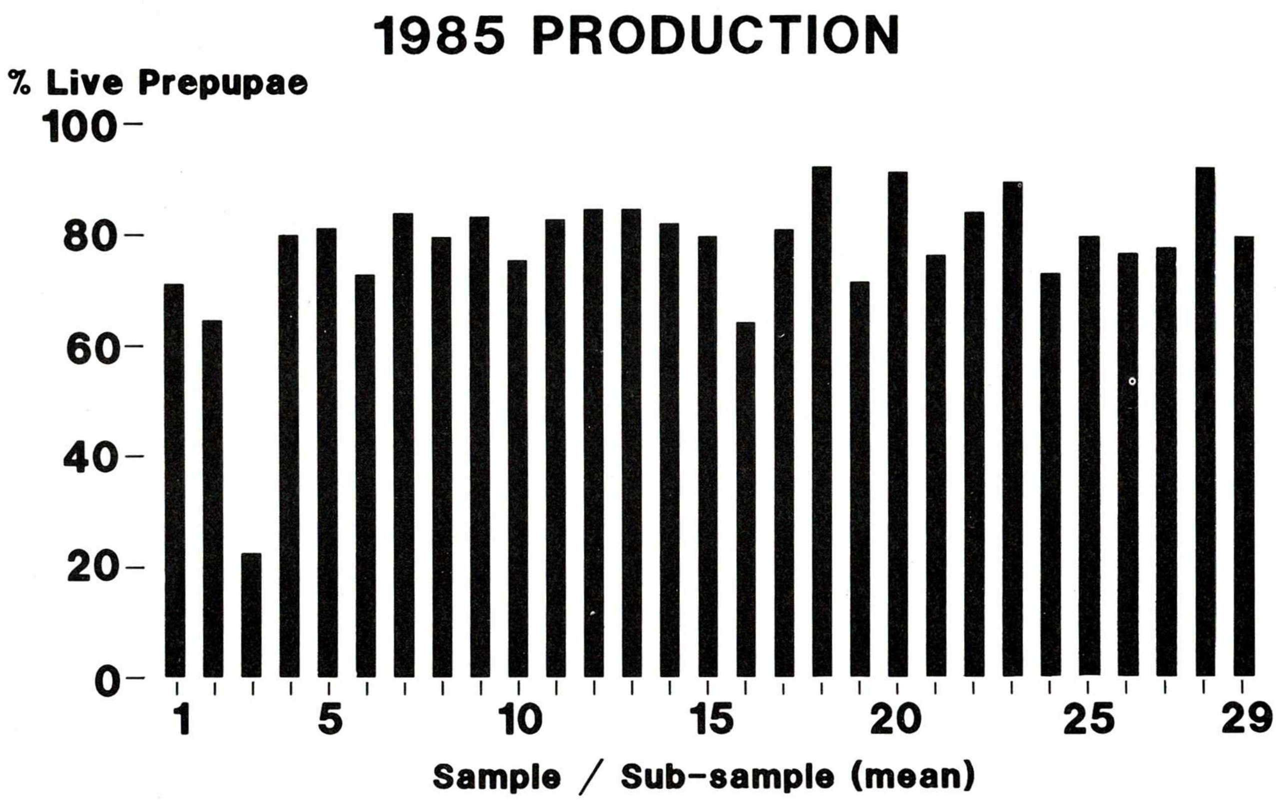


Fig. 2. Percent Pollen Balls

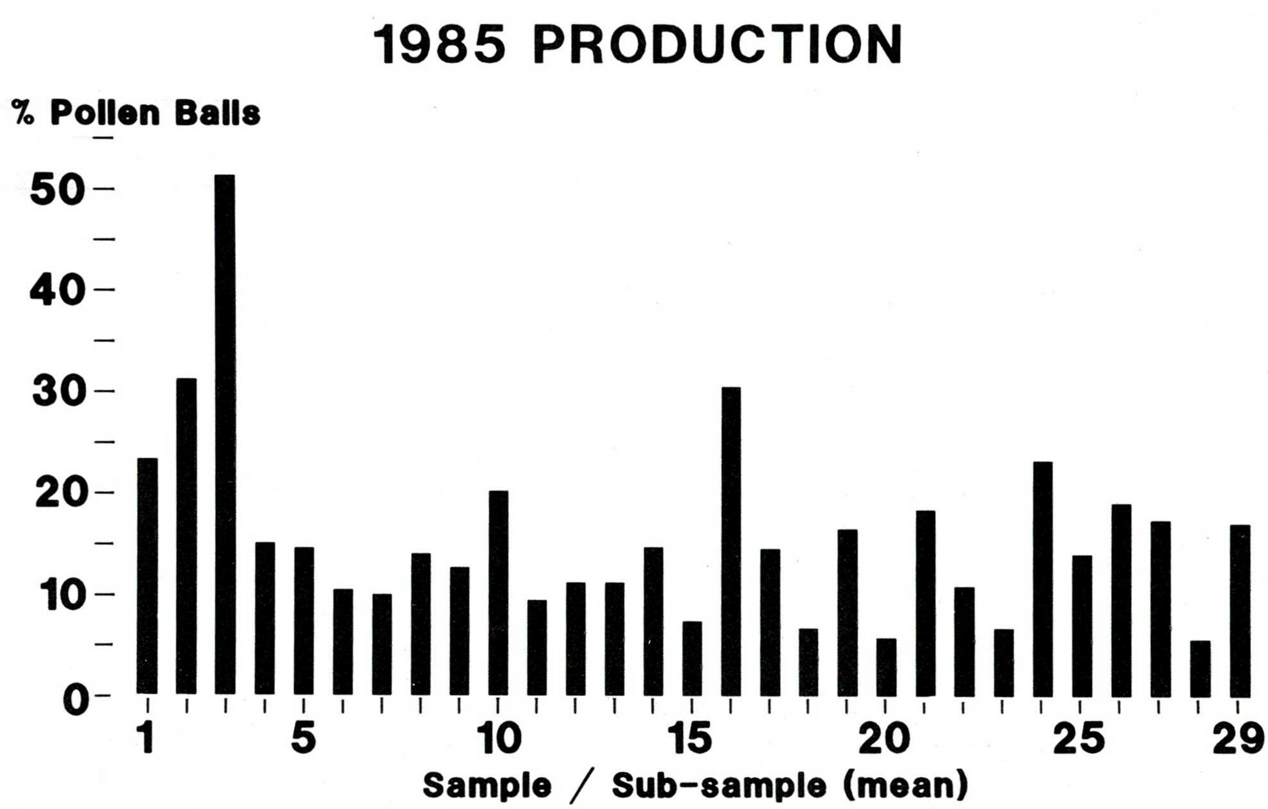
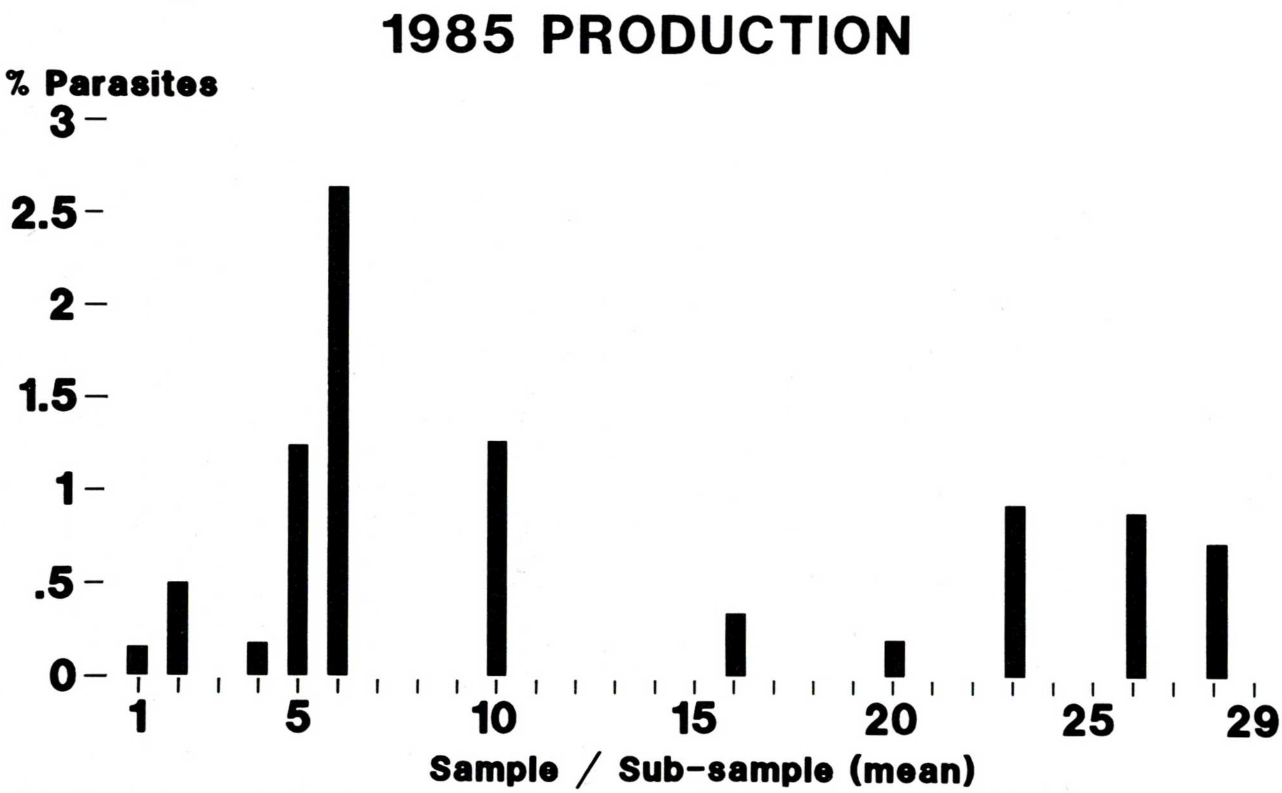


Fig 3. Percent Parasites



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A Different Bee Story

Forage seed production is big business in the Peace region and alfalfa seed production is an important part of this business. Alfalfa must be cross pollinated to produce seed. Cross pollination or the transfer of pollen from the anthers of one plant to the stigma of another plant, is done by bees. The abundance of bumble bees and other native pollinators assured a good alfalfa seed crop for a number of years. Between 1935 and 1955 about 1,500 tonnes of alfalfa seed were produced annually. Alfalfa seed fields in the Peace were traditionally surrounded by uncleared land - the natural habitat of native pollinators. Large acreages of bush were cleared for cultivation in the mid 1950s thus resulting in a depletion of the natural habitat for insect pollinators. This coincided with a decrease in alfalfa seed production. Average annual production between 1955 - 1958 was 50 tonnes.

Honey bees are not reliable pollinators of alfalfa in the Peace River region and a reliable insect pollinator had to be found. The leafcutting bee, ***Megachile rotundata*** (Fab.) appeared to be the best bet. There are a number of native leafcutting bees that are good pollinators of alfalfa. However, they nest in the wild and cannot be reared as a colony to provide a reliable pollinating service. The species ***Megachile rotundata*** (Fab.) imported from Europe into the United States, quite by accident in World War II crates, differs from the North American leafcutting bee in one important aspect - they are gregarious and can be raised as a pollinating colony if suitable nesting materials are provided. Between 1963-67, leafcutting bees were imported into the Peace from Idaho.

Leafcutting bees are provided with nesting tunnels. These tunnels are aggregated into nesting boxes that are in turn placed inside a shelter. In the United States, nesting boxes were placed in wood shelters and this practice was continued in the Peace. There was no bee activity below 23°C and reproduction rates were low. It appeared that the successful performance of the bee in the United States could not be duplicated in the Peace, at least not with the same management procedures. Nevertheless, work at Agriculture Canada's Research Station, Beaverlodge and the Experimental Farm, Fort Vermilion continued with these small experimental colonies, and by 1967, it was found that the bees had gradually adapted to the area, and could forage at lower temperatures. However, the major breakthrough came in 1971 when wood shelters were replaced with polyethylene shelters (Fig. 1). The greenhouse effect in polyethylene shelters raised the temperature and stimulated the bee into activity. Average bee reproduction rates doubled and the Peace River region was on its way to big business in leafcutting bees!

Native pollinators still contribute to pollination. However, up to six-fold increases in alfalfa seed yields have been obtained in experimental plots provided with leafcutting bees as compared to those where fluctuating populations of natural bees were the only pollinators (Fig. 2).

Alfalfa flowers are pollinated when bees forage for pollen and nectar. The nectar is at the bottom of the flower, and the bee has to poke its proboscis down to the bottom of the flower tube to get it. When the bee alights on the keel petal of the flower, to forage for nectar, the cluster of stamens (male organs) and pistil (female organ) spring forward with a jerk, i.e., the flower is tripped. Three things then happen:

- The pistil surface is ruptured and it is now ready to receive pollen.
- The pistil protrudes a little beyond the stamens and it usually hits the body of the bee before the stamens do. Thus it is more likely to come in contact with the pollen from other plants already deposited on the body of the bee than the pollen from its own flower.
- The stamens then hit the body of the bee and powder it with pollen.

Fig. 1 A Polyethylene Shelter for Leafcutting Bees

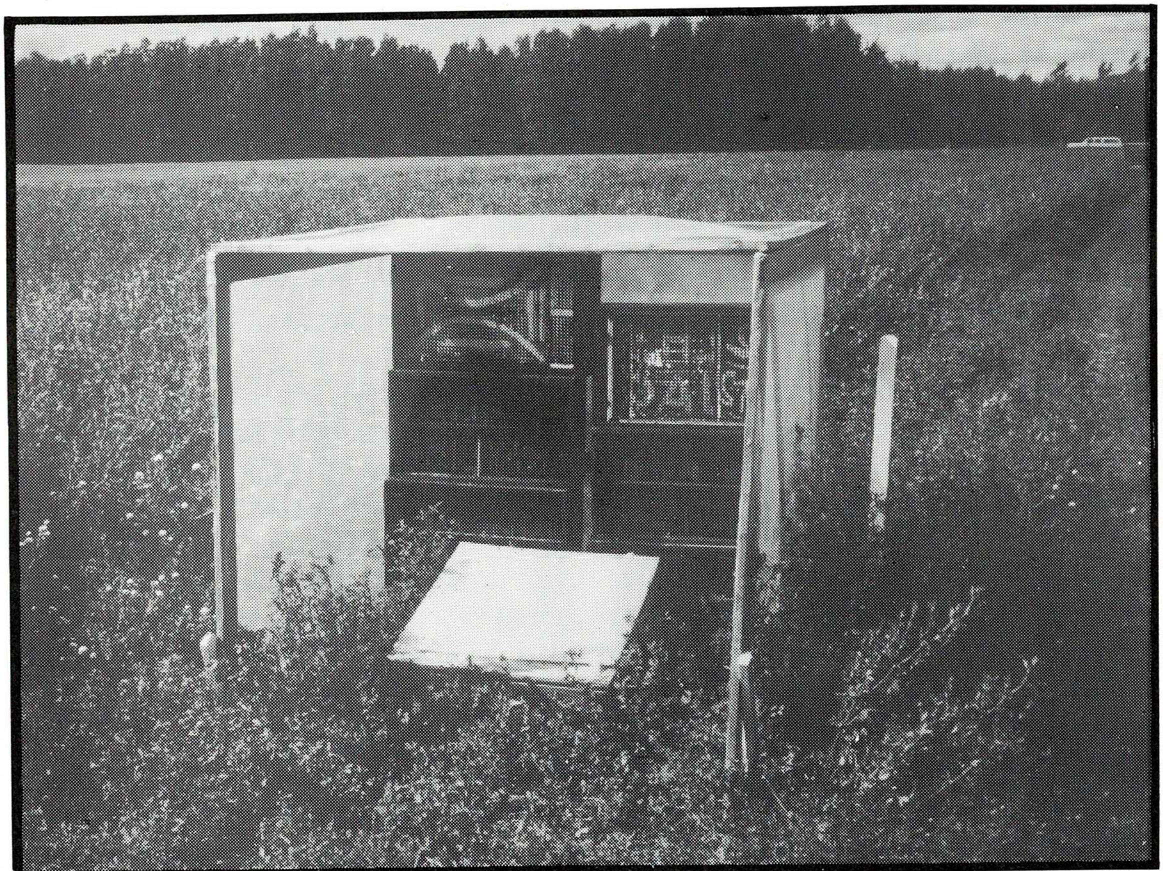
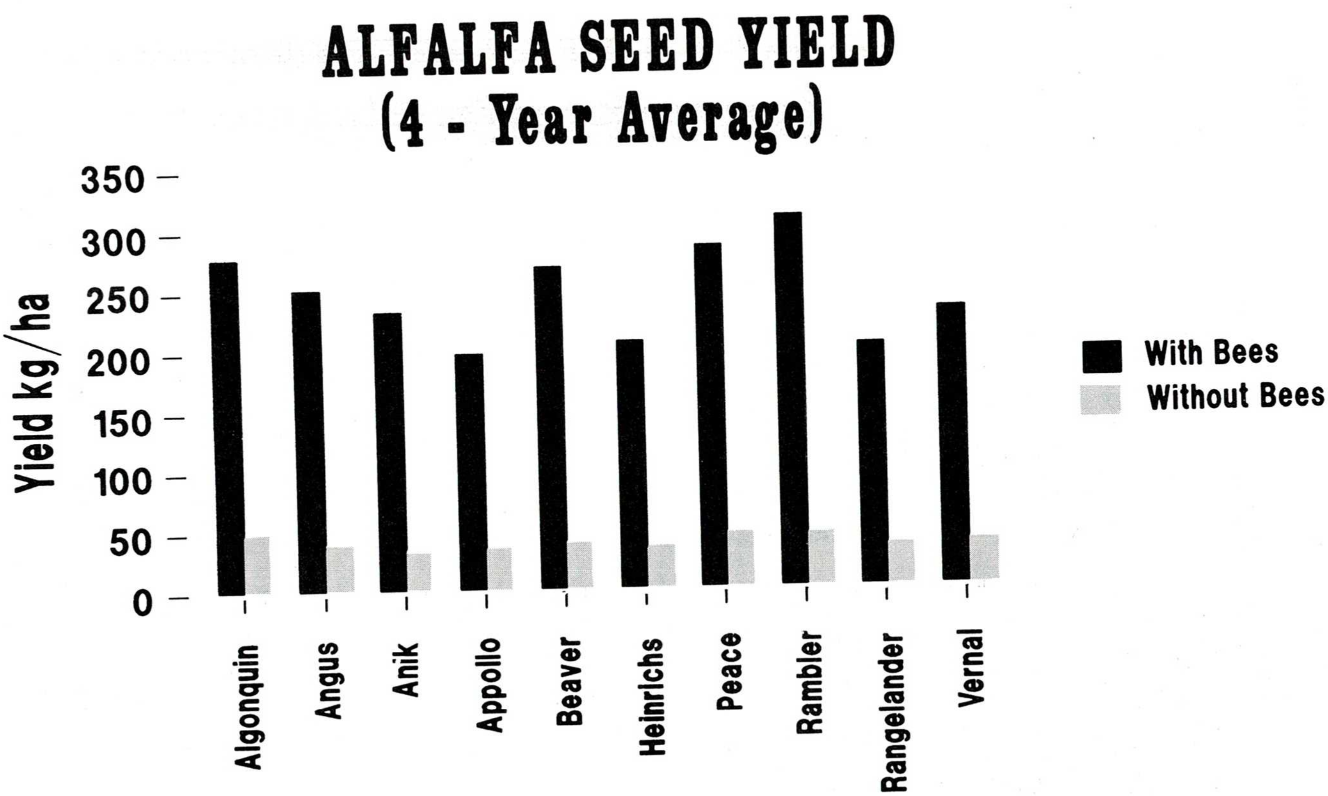


Fig. 2. Alfalfa Seed Production



Some attempts have been made to substitute the pollinating efficiency of bees with a man-made machine. To date this has not been successful and it would appear that the flower structure of alfalfa does not lend itself to pollination by mechanical means for the following reasons.

- Alfalfa has an indeterminate inflorescence and new flowers are developing all the time. These flowers are in a prime condition for fertilization for only one to three days. To trip them at the right time would necessitate going over the field a number of times. Attempting to do this with a machine would probably result in excessive damage to the crop.
- The more easily tripped older flowers are usually tripped by mechanical means. However, pod fertility decreases with increasing flower age and this has been cited as one of the reasons for reduced seed yields in mechanically-tripped racemes.
- Even if it is assumed that tripping can be accomplished mechanically, this does not necessarily ensure cross pollination. It is most unlikely that large quantities of pollen can be generated because economy in pollen production and sticky heavy pollen are major features of legumes. Even if it is assumed that enough free pollen could be generated it is not likely to land on the stigma of another flower. This small quantity of sticky pollen will probably land in close proximity to where it was generated - the stigma of the same flower. Thus self fertilization would be effected.

The deleterious effects of self fertilization on seed yield and plant vigour of alfalfa have been amply demonstrated. It would thus appear that the job of cross pollination, done with such meticulous efficiency by an insect cannot as yet be duplicated by a machine!

Where do we go from here? Studies are underway on the use of leafcutting bees as pollinators of other legume crops. The bee has been found to be very adaptable to the severe climates in the north and the performance of adapted colonies is being documented. Management procedures are being updated and modified to meet current needs and preventative measurers to maintain parasite and disease-free colonies are being investigated.

The first experimental colonies of leafcutting bees were introduced into the Peace River region in 1963, and the investment in research has paid off handsome dividends - the alfalfa seed industry has been re-established in the region and bee cells from the area have found their way to domestic and international markets.

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What is Chalkbrood and What You Can Do About It

Chalkbrood is a disease of leafcutting bee larvae, caused by the fungus ***Ascosphaera.*** One species, ***Ascosphaera aggregata,*** can result in heavy losses of larvae in a relatively short time. At present, there is no cure for this disease. The symptoms and measures for control or possible eradication are detailed below.

Spread

Chalkbrood is spread by the spores of the fungus, which can remain infective for many years. They may be introduced into an area on contaminated equipment or nesting material or by infected bee cells and leaf material.

Symptoms of infected larvae

Infected larvae shrink and harden. Their interiors turn chalk white from the fungal growth and their outer surfaces become glossy and cellophane-like. Some of the dead larvae remain white, but most turn dark grey to black. Cells containing such larvae are fragile and collapse easily releasing millions of spores.

Methods of infection

Only larvae become infected by chalkbrood. A healthy larva is infected when it eats pollen that is contaminated with chalkbrood spores. When these spores germinate, the fungus grows inside the gut of the larva, and later moves through the wall of the gut into the body cavity. The fungus eventually forms black spore cysts under the skin. These mature, shatter and disperse a large number of new spores. The continued growth of the fungus eventually kills the larva.

Contamination of pollen

If emerging bees crawl through infected leaf material or chew through infected dead larva, they become covered with massive numbers of spores that adhere to their body hairs. These new adults then contaminate their mates, eggs and pollen provisions.

Preventing entry of infected material

- Ensure that each consignment of cells purchased has been screened for chalkbrood. If possible, buy bees from areas known to be chalkbrood free.
- If cells are brought into an area from an outside location, run the consignment separately and isolate the offspring until they are screened for chalkbrood.
- Disinfect all nesting material, equipment and storage facilities annually.
- Use the loose-cell management system. Tumble groups of cells to break them into individual cells. Chalkbrood cadavers are light and a large percentage can be removed during tumbling. Breaking up the groupings will also ensure that the new adults do not have to chew through chalkbrood cadavers to emerge; an emerging bee can become dusted with up to 300 million spores when it chews through such a cadaver.
- If equipment has to be shared, ensure that it is properly disinfected before and after use. Avoid sharing equipment whenever possible.
- After bee emergence is complete, incinerate all leaf debris from the incubation trays.

- Relocate field shelters each year to prevent buildup of fungal spores around them. Spray shelters and surrounding ground with a 5% sodium hypochlorite solution (household bleach) after nesting boxes are taken indoors.

Control and eradication

For control and possible eradication of chalkbrood, use the following in conjunction with prevention methods.

- Remove infected cells, nesting material and incubation trays from area.
- Disinfect storage facilities and all equipment that cannot be moved from area.
- In the early spring, burn top growth in any alfalfa field where infected bees were used for pollination in the previous growing season.
- If possible, do not use leafcutting bees or other pollinators in the above field during the following growing season. Then place trap nests in the field to determine whether bees are still in the area. If so, screen samples of their larvae for chalkbrood.
- If 'clean' leafcutting bees have to be introduced into a field where chalkbrood-infected cells were found in the previous growing season, follow procedures recommended for preventing entry of infected material.
- If the offspring of the 'clean' bees are disease-free, surface sterilize cells before use as an added precaution. If offspring are infected, then remove them from the area.

Disinfection of bee cells and equipment

Any practice used to control fungus or mold will also help control chalkbrood.

LEAFCUTTING BEE CELLS - Cells can be surface-sterilized by immersing them in a 3% sodium hypochlorite (diluted household bleach) solution for 1-2 minutes. The cells should then be dried away from direct sunlight or excessive heat. Do this **before** incubation.

NESTING MATERIAL - It is preferable to incinerate all nesting material that is known to have contained chalkbrood-infected cells. If this is not possible, disinfect it.

Wood nesting material can be disinfected by placing in an oven at 100°C for 24 hours. Both wood and polystyrene nesting boards can be dipped in a 5% sodium hypochlorite solution, or a 3-5% solution of stabilized dry chlorine to which a wetting agent has been added. Disinfect the boards in the spring and dry them completely before use. Some loss of nesting material due to cracking or warping is inevitable.

SHELTERS, EQUIPMENT AND STORAGE FACILITIES - To disinfect shelters, equipment (e.g., strippers and tumblers) and storage facilities, use a mist spray of a 5% sodium hypochlorite solution.

Dr. Daphne Fairey[1], Dr. John Bissett[2], and Mr. John Lieverse[1]

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Quality of Leafcutting Bee Cells
Peace River Region Directory
1985-86

Producers were encouraged to publish the data on the quality of their leafcutting bee cells. Some decided in the affirmative, and the following list was compiled by the Association.
Please note: Chalkbrood caused by ***Ascosphaera aggregate*** was **NOT PRESENT** in any of the samples that were screened for quality.

Serial Number	Name and Address	Live Prepupae Per Kologram	Percent Parasites
1	Roger Andreiuk, Box 312, Spirit River, AB., T0H 3G0	9320	0.19
2	Harold Boissy, Gen. Del., Ft. Vermilion, AB, T0H 1N0	8820	0.00
3	Frank Breault, Box 464, Dawson Creek, B.C., V1G 4G3	9260	1.22
4	Dave Buck, Box 1784, Fairview, AB., T0H 1L0	8500	0.00
5	Doug Colter, Box 415, Falher, AB., T0H 1M0	8440	2.58
6	Eugene Dextrase, Box 134, High Level, AB., T0H 1Z0	8420	0.00
7	Sam Drader, 9103 - 101 St., Grande Prairie, AB., T8V 2S3	9500	0.00
8	Bob Gartley, Box 101, Eaglesham, AB., T0H 1H0	9280	0.00
9	Peter Gruys, Gen. Del., Spirit River, AB., T0H 3G0	9700	0.92
10	Rick Klippenstein, Box 462, Ft. Vermilion, AB., T0H 1N0	8840	0.00
11	Nick Konopelka, Box 162, Rycroft, AB., T0H 3A0	8100	0.00
12	Peter Pankiw, Box 395, Beaverlodge, AB., T0H 0C0	9520	0.00
13	Don Pederson, Box 785, Dawson Creek, B.C., V1G 4H8	9260	1.22
14	Gilbert Poirier, Box 71, Jean Cote, AB, T0H 2E0	9100	0.00
15	John Reinders, Box 99, Deadwood, AB. T0H 1A0	7700	0.50
16	Howard Rumball, Box 2337, Peace River, AB., T0H 2X0	8380	1.25
17	Al Toews, Gen. Del., Ft. Vermilion, AB, T0H 1N0	8840	0.00
18	Stewart Watson, Box 1888, High Level, AB., T0H 1Z0	7620	0.34
19	David Wong, Box 359, Lamont, AB., T0B 2R0	8880	0.00
20	Raymond Wood, Box 368, Peace River, AB., T0H 2X0	8380	0.17

For further information contact the 1986 Executive:

Name	**Telephone**
Raymond Wood	403-624-1438
Robert Gartly	403-359-3952
Gilbert Poirier	403-322-2151
Don Pedersen	604-782-2419

(Addresses for these individuals can be found in the Quality Directory)