SASKATCHEWAN
ALFALFA SEED
PRODUCERS
ASSOCIATION

MICROORGANISMS ASSOCIATED WITH THE ALFALFA LEAFCUTTING BEE AND THEIR POTENTIAL IMPACT ON PRODUCER HEALTH

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The alfalfa leafcutting bee is an important domesticated pollinator of alfalfa and is widely utilized for alfalfa seed production in western Canada. Concerns relating to the presence of a fungal disease known as chalkbrood in alfalfa leafcutting bee populations in the northwestern United States and southern Alberta have led to extensive surveying of Saskatchewan bee populations. While chalkbrood has been detected only sporadically and at very low levels in the province, numerous other microorganisms have been isolated from leafcutting bee populations. The impact of these fungi and bacteria on bee populations has been discussed in previous extension bulletins. The purpose of this publication is to outline the potential for these microorganisms to impact negatively on the health of alfalfa seed producers.

RESEARCH METHODS

Isolates of microorganisms associated with alfalfa leafcutting bee populations were obtained by sampling adult bees, larval cadavers, spoiled cell provisions (i.e. stored pollen and nectar), bee cell surfaces, and tunnel surfaces in wood and polystyrene bee nest material.

In order to detect microorganisms on adult bees, adult females were captured in the field as they entered or exited nest tunnels and were placed in glass vials containing sterile distilled water. Individuals were released following hand-agitation and samples were transferred to the laboratory for culture. As well, newly emerged adults from cells incubated in the laboratory were individually placed in test tubes containing sterile distilled water, agitated in a vortex mixer, and then removed prior to sample culture.

To evaluate microorganisms on the exterior of leafcutting bee cells, groups of cells from various bee populations were placed in crystallite vials and washed with sterile distilled water. Tunnels in leafcutting bee nest material were sampled with sterile cotton-tipped wood applicators wetted with sterile distilled water. Applicators were stored in sterile glass culture tubes for transfer to the laboratory and rehydrated with sterile distilled water prior to culture of samples.

All samples collected from adult bees, cell surfaces, and nest tunnels were plated in the laboratory on potato dextrose agar (PDA), nutrient agar, or Sabouraud's dextrose agar, incubated, and assessed at regular intervals.

Cells containing larval cadavers and spoiled cell provisions were identified from samples of leafcutting bee cells examined in winter surveys of leafcutting bee populations and from samples of cells collected in summer trapnest surveys. Microflora associated with these specimens was plated directly on media and incubated in the laboratory.

Purified isolates from all sources were subcultured on PDA slants and refrigerated. Isolates were examined using lactophenol cotton blue mounts for preliminary identification; bacterial isolates were Gram stained. Fungal isolates were submitted to the Centre for Land and Biological Resources Research (Ottawa) for identification; selected larval cadaver and cell provision specimens were also sent to Ottawa. Bacterial isolates were submitted to Agriculture and Agri-Food Canada (Lethbridge Research Station) for identification.

RESEARCH RESULTS

The presence and relative abundance of microorganisms isolated from leafcutting bee adults, larval cadavers, spoiled cell provisions, cell surfaces, and nest material are given in Table 1. Prevalent microorganisms associated with field-collected adult females included *Alternaria alternata*, *Aspergillus niger*, *Enterobacter agglomerans*, *Penicillium* spp., *Pseudomonas solanacearum*, *Rhizopus arrhizus*, and *Trichosporonoides megachiliensis*. Likely sources of these microorganisms were alfalfa plant surfaces and the leaves of other plants commonly utilized for cell construction. The relative abundance of fungal and bacterial species fluctuated with temperature and humidity changes during the field season.

Male and female leafcutting bees emerging from laboratory-incubated cells were found to be carrying primarily *A. alternata*, *Aspergillus niger*, *Enterobacter agglomerans*, *Eurotium chevalieri*, and *T. megachiliensis*. All of these species were isolated

frequently from the leaf surfaces of cells and likely picked up by individuals through contact during emergence.

Fungi commonly associated with larval cadavers included *A. niger*, *E. chevalieri*, *Penicillium purpurogenum*, *P. simplicissimum*, other *Penicillium* spp., and *T. megachiliensis*, while dominant microorganisms found in conjunction with spoiled cell provisions included *Ascosphaera pollenicola*, *A. variegata*, *Aspergillus glaucus*, *A. niger*, *Bacillus circulans*, *Enterobacter agglomerans*, *Eurotium chevalieri*, *Penicillium* spp., *Pseudomonas* spp., and *T. megachiliensis*.

Surfaces of leafcutting bee cells were inhabited by a complex of microorganisms similar to that found in leafcutting bee nest material, with predominance of *Alternaria alternata*, *Aspergillus niger*, *Enterobacter agglomerans*, *Eurotium chevalieri*, *Mucor* sp., *Penicillium* spp., *Rhizopus* spp., and *T. megachiliensis*. Other species commonly isolated from nest material were *Bacillus circulans*, *B. mycoides*, *Corynebacterium* sp., *Pseudomonas* spp., and *Trichoderma citrinoviride*. The yeast *Saccharomyces* sp. was frequently found in cultures from wood nest material but rarely isolated from polystyrene nest material.

DISCUSSION

The presence of this diverse group of fungi and bacteria associated with alfalfa leafcutting bee populations in Saskatchewan probably has a broad range of effects on these populations. Some of the microorganisms may contribute to the production of beneficial compounds such as antibiotics, vitamins, amino acids, and anti-spoilage agents. However, many of the species listed may also harm leafcutting bee populations by interfering with larval development and spoiling cell provisions. *Aspergillus* spp. and *Trichothecium roseum* are known to be pathogenic on wild bees.

In laboratory studies several bacteria and yeasts were observed to cause larval mortality through fermentation of cell provisions. The spoilage of cell provisions often led to subsequent overgrowth of cell contents by numerous mould species. In addition to exhibiting antibacterial and fungistatic activity, toxins associated with some fungal species isolated here have been shown to elicit responses including feeding aversion and inhibition of protein synthesis. Any of these processes could contribute to mortality in developing alfalfa leafcutting bee larvae.

Many of the mould species found in association with the alfalfa leafcutting bee may be potentially harmful to the health of alfalfa seed producers as well. *Alternaria*, *Aspergillus*, *Penicillium*, and *Rhizopus* species are among those fungi which are considered most important medically. These species have been implicated in allergic reactions and bronchopulmonary disease and may cause conditions ranging from pulmonary hypersensitivity disease (e.g. allergy, asthma) to life-threatening infection.

During leafcutting bee incubation and cell-harvesting operations, large numbers of emerging bees or loose cells concentrated in confined areas may lead to high levels of airborne spores of these fungal species. Several less frequently isolated mould species reported here, including *Cylindrocarpon*, *Trichoderma*, and *Ulocladium* species, are not ordinarily associated with human diseases but under certain conditions may act as opportunistic pathogens.

Identification of the microorganisms associated with the alfalfa leafcutting bee is important. An awareness of the presence of mould species which have been implicated in human allergic reactions will allow alfalfa seed producers to take measures to reduce contact with potentially harmful spores and to incorporate control techniques which will reduce levels of microorganisms in leafcutting bee populations. A reduction in high levels of fungal spores will be beneficial to producer health and will also assist in increasing the quality of alfalfa leafcutting bee populations.

RECOMMENDATIONS TO PRODUCERS

Individuals working with alfalfa leafcutting bees should observe basic safety precautions, particularly during leafcutting bee incubation and cell harvesting operations. At these times, large numbers of bee cells 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 fungal spores exists. Eating, smoking, and drinking should be done away from the work area, and hands should be washed and work clothes removed when leaving the area.

Table 1. Microorganisms isolated from alfalfa leafcutting bee populations in Saskatchewan.[a]

Microorganism	Adult Bee	Larval Cadaver	Cell Provision	Cell Surface	Nest Material
FUNGI					
Alternaria alternata (Fr.: Fr.) Keissler	++			++	++
Ascosphaera aggregata Skou		+			
Ascosphaera atra Skou & Hackett			+		
Ascosphaera larvis Bissett		+	+		
Ascosphaera pollenicola Bissett			++		
Ascosphaera variegata Bissett		+	++		
Ascosphaera sp.				+	
Aspergillus glaucus Link: Fr.		+	++		
Aspergillus niger Van Tiegham	++	++	++	+++	+++
Cylindrocarpon sp.				+	+
Eurotium chevalieri L. Mangin		++	++	+++	+++
Eurotium sp.				+	+
Mucor sp.				++	++
Penicillium purpurogenum O. Stoll	++				
Penicillium simplicissimum (Oudem.) Thom	++				
Penicillium spinulosum Thom					++
Penicillium spp.	++	++	++	++	++
Rhizopus arrhizus A. Fischer	++		+	+++	+++
Rhizopus sp.			+	+	+
Saccharomyces sp.			++		++
Trichoderma citrinoviride Bissett					++
Trichosporonoides megachiliensis Inglis & Sigler	+++	+++	+++	+++	+++
Trichothecium roseum (Pers.: Fr.) Link	+			+	
Ulocladium atrum G. Preuss			+		
Yeast-like sp. NM-K (DAOM 212-057)					+
Yeast-like sp. CE-I (DAOM 212-058)				+	+
Unidentified yeast-like sp. Y01	+			+	
Unidentified yeast-like sp. Y02	+			+	
BACTERIA					
Bacillus circulans Jordan			++		++
Bacillus mycoides Flugge					++
Bacillus sp.	+				+
Corynebacterium sp.	+				+
Enterobacter agglomerans Ewing & Fife	+++		++	+++	+++
Flavobacterium breve (Lustig) Bergey					+
Pseudomonas solanacearum (Smith) Smith	++				
Pseudomonas spp.	++		++		++
Unidentified sp. B01	+				
Unidentified sp. B02				+	

[a] Isolation of microorganisms from alfalfa leafcutting bee populations is rated as occasional (+), frequent (++), or abundant (+++).

Equipment and facilities used for leafcutting bee cell incubation and cell harvesting should be cleaned and disinfected following use. Incorporation of decontamination techniques such as paraformaldehyde fumigation, bleach dipping, or heat treatment of nest material is highly effective in reducing levels of moulds, yeasts, and bacteria. The microorganisms which contaminate bee cell surfaces may be controlled using either paraformaldehyde fumigation or bleach dipping techniques.

SUMMARY

Isolates of fungi and bacteria associated with alfalfa leafcutting bee populations were obtained by sampling adult bees, larval cadavers, spoiled cell provisions, bee cell surfaces, and tunnel surfaces in bee nest material.

The presence and relative abundance of microorganisms isolated from alfalfa leafcutting bee populations are given in Table 1. Dominant microorganisms included the fungi *Alternaria alternata*, *Aspergillus niger*, *Eurotium chevalieri*, *Mucor* sp., *Penicillium* spp., *Rhizopus arrhizus*, *Saccharomyces* sp., *Trichoderma citrinoviride*, and *Trichosporonoides megachiliensis*; bacteria isolated included *Bacillus circulans*, *B. mycoides*, *Enterobacter agglomerans*, and *Pseudomonas* spp. Many of the fungal species are saprophytic, growing on material such as cell provisions and larval cadavers. Fungi may also be important for their activity in production of enzymes, antibiotics, and mycotoxins.

The microorganisms isolated from alfalfa leafcutting bee populations likely have a range of beneficial and deleterious effects. While many fungal and bacterial species may contribute to the production of useful compounds, others may interfere with larval development and spoil cell provisions. *Alternaria*, *Aspergillus*, *Penicillium*, and *Rhizopus* species may also be potentially harmful to the health of leafcutting bee producers. During bee incubation and harvesting operations, large numbers of bees or bee cells in confined areas may lead to high levels of airborne spores from these medically important moulds, which have been implicated in allergic reactions and bronchopulmonary disease.

A reduction in high levels of fungal spores will increase the quality of alfalfa leafcutting bee populations and be beneficial to the health of alfalfa seed producers. Individuals working with leafcutting bees should observe basic safety precautions, particularly during cell incubation and harvesting operations. The use of efficient ventilation systems and appropriate safety equipment (i.e. dust masks, gloves) is recommended to minimize contact with potentially harmful fungal spores. A respirator or positive pressure air filtration system may be used if known sensitivity to fungal spores exists. Leafcutting bee equipment and facilities utilized for bee cell incubation and harvesting should be decontaminated following use.

OTHER REFERENCES

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