Seasonal and spatial patterns of mortality and sex ratio in the alfalfa leafcutting bee, Megachile rotundata (F.)
by Ruth Pettinga ONeil

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology
Montana State University

Abstract:
Nests from five seed alfalfa sites of the alfalfa leafcutting bee Megachile rotundata (F.) were monitored over the duration of the nesting season in 2000 and 2001, from early July through late August. Cells containing progeny of known age and known position within the nest were subsequently analyzed for five commonly encountered categories of pre-diapause mortality in this species. Chalkbrood and pollen ball had the strongest seasonal relationships of mortality factors studied. Chalkbrood incidence was highest in early-produced cells. Pollen ball was higher in late-season cells. Chalkbrood, parasitism by the chalcid Pteromalus venustus, and death of older larvae and prepupae , due to unknown source(s) exhibited the strongest cell-position relationships. Both chalkbrood and parasitoid incidence were highest in the inner portions of nests. The “unknown” category of mortality was highest in outer portions of nests. Sex ratio was determined for a subset of progeny reared to adulthood. The ratio of females to males is highest in cells in inner nest positions. Sex ratio is female-biased very early in the nesting season, when all cells being provisioned are the inner cells of nests, due to the strong positional effect on sex ratio.

SEASONAL AND SPATIAL PATTERNS OF MORTALITY AND SEX RATIO IN THE ALFALFA LEAFCUTTING BEE, *Megachile rotundata* (F.)

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Ruth Pettinga O'Neill

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APPROVAL

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Ruth Pettinga O'Neill

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Nests from five seed alfalfa sites of the alfalfa leafcutting bee *Megachile rotundata* (F.) were monitored over the duration of the nesting season in 2000 and 2001, from early July through late August. Cells containing progeny of known age and known position within the nest were subsequently analyzed for five commonly encountered categories of pre-diapause mortality in this species. Chalkbrood and pollen ball had the strongest seasonal relationships of mortality factors studied. Chalkbrood incidence was highest in early-produced cells. Pollen ball was higher in late-season cells. Chalkbrood, parasitism by the chalcid *Pteromalus venustus*, and death of older larvae and prepupae due to unknown source(s) exhibited the strongest cell-position relationships. Both chalkbrood and parasitoid incidence were highest in the inner portions of nests. The "unknown" category of mortality was highest in outer portions of nests. Sex ratio was determined for a subset of progeny reared to adulthood. The ratio of females to males is highest in cells in inner nest positions. Sex ratio is female-biased very early in the nesting season, when all cells being provisioned are the inner cells of nests, due to the strong positional effect on sex ratio.

INTRODUCTION

History of Alfalfa Seed Industry in the U.S.

Alfalfa seed growers have benefitted from the presence of a Eurasian solitary bee, the alfalfa leafcutting bee *Megachile rotundata* (F.), for pollination since 1947 (Krombein, 1948), when they were first accidentally introduced into the United States. Alfalfa leafcutting bee females nest readily in many types of artificial nesting material, and can be easily managed and propagated for additional economic gain in their own right (Richards, 1984).

The first "localized population" of alfalfa leafcutting bees was found in 1958 by W. P. Stephen nesting in nail holes in a vacant outbuilding (Stephen, 1961). Soon after their introduction to the U. S, alfalfa leafcutting bees demonstrated their superiority over honey bees for alfalfa pollination, chiefly for two reasons: 1) honey bees are averse to the tripping mechanism of the alfalfa flower, which they can learn to avoid by chewing the corolla open from the outside, a method that circumvents cross-pollination; and 2) honeybees do not like alfalfa pollen, though they readily accept alfalfa nectar, and will not stay in alfalfa fields where alternatives exist. The only other commercial pollinator used in seed alfalfa fields is the alkali bee, *Nomia melanderi* Cockerell, a ground-nesting species that requires careful management of soil conditions to thrive, and cannot be transported between fields during the floral season (Baird et al., 1991).

Alfalfa leafcutting bees, on the other hand, have demonstrated many desirable traits for alfalfa pollination. They are polylectic, but exhibit a strong affinity for alfalfa (Stephen, 1961). Unlike alkali bees, they are easy to transport and maintain. They do not

circumvent the tripping mechanism of alfalfa flowers as honey bees do. They nest gregariously, and their seasonal activity synchronizes well with the timing of alfalfa bloom. Females reproduce readily with good management, adapting easily to artificial shelters (Bohart, 1972). In the early 1960's many alfalfa seed growers, in recognition of the pollination service provided by leafcutting bees, began providing materials such as drilled boards and paper soda straws for nesting females, as well as rudimentary protective winter storage in cool cellars or sheds (Stephen and Osgood, 1965). Since that time, management has become much more sophisticated, in part because of the appearances of disease organisms like chalkbrood *(Ascosphaera* spp), various nests predators, parasitoid wasps, and brood parasites. As a result, modern management is now based upon providing a high degree of care at every stage of the bee's life cycle to maximize bee productivity.

Female alfalfa leafcutting bees are roughly 7-9 mm in length, with a dorsoventrally flattened abdomen and chewing-lapping mouthparts characteristic of the genus (Michener, 2000). They are black with some areas of white on the body, with black eyes and a pale yellow v-shaped mark on the frons. Like most females of the Megachilidae, *M. rotundata* females have a pollen brush, or scopa, on the underside of the abdomen, consisting of long straight and rather unspecialized whitish-gray hairs. Males, which are smaller and have green eyes, lack the scopa and have dense yellow setae on the body and head (Gerber and Akre, 1969). In nature, females usually nest in abandoned burrows of wood-boring beetles (Rothschild, 1979), hollow plant stems, or old wasp nests (Gerber and Klostermeyer, 1972). Females do not construct their own

tunnels, although they will clean out and use the canes of pithy-stemmed plants such as raspberries.

Pollination of Alfalfa Flowers

The Tripping Mechanism

Alfalfa must be tripped in order to achieve cross-pollination and high-quality seed set (Viands et al., 1988), although seed set without tripping does occur (Brink and Cooper, 1936). The alfalfa tripping mechanism of a mature alfalfa flower is comprised of three interacting parts: a set of two fused keel petals, a set of ovarial and stamineal filaments (the sexual column) that the keel petals enclose, and the opposing banner petal. The sexual column is held back under tension in a fresh, untripped flower. Yet any disturbance, such as a strong breeze, rain, or hot sunshine, can cause the keel petals to peel apart. As they separate, the freed sexual column flips forward and strikes against the banner petal, breaking the stigmatic membrane separating the stigma from anthers. Fertilization then commences (Teuber and Brick, 1988). When foraging alfalfa leafcutting bee females attempt to access the basal nectary, the freed sexual column strikes the foraging bee's prosternum and the underside of her head. Heterogamous pollen from previous provisioning forays is distributed onto the stigma, and cross-fertilization occurs (Michener, 2000). A tripped alfalfa flower wilts within four hours; if left untripped, flowers last five to seven days though this varies with ambient temperature conditions.

Alternative Crops Pollinated by Alfalfa Leafcutting Bees.

Many investigations have been made into the feasibility of alfalfa leafcutting bees to pollinate other crops, mostly leguminous. These include diploid red clover *(Trifolium pratense* L.*)* (Fairey et al., 1989); arrowleaf clover *(T. vesiculosum)*, berseem clover *(T. alexandrinum)*, crimson clover *(T. incarnatum* L.*)*, and Persian clover (Richards, 1991); cicer milkvetch *(Astragalus cicer* L.*)* (Richards, 1986; Horne, 1995); sainfoin *(Onobrychis viciaefolia* Scop.*)* (Richards and Edwards, 1988; Horne, 1995); bird's-foot trefoil *(Lotus corniculatus* L.*)*, anik alfalfa *(Medicago falcata* L.*)*, white clover *(T. repens* L.*)*, alsike clover *(T. hybridum* L.*)*, and zigzag clover *(T. medium)* (Horne, 1995); canola *(Brassica napus* L.*)* (Fairey and Lefkovitch, 1991); and lowbush blueberry *(Vaccinium angustifolium* Ait.*)* (Stubbs and Drummond, 1997). Because alfalfa leafcutting bees are not strictly oligolectic, they will forage on a variety of leguminous forage crops. However, their progeny tend to survive less well on alternative legumes, due either to pollen quality, nectar availability or, to a lesser extent, leaf availability (Horne, 1995).

Seasonal Activity of the Alfalfa Leafcutting Bee

Emergence

Cells containing male progeny tend to be located toward the nest entrance. Alfalfa leafcutting bees are protandrous, with females emerging approximately one to three days later than the first males to emerge. Males use specialized teeth on their mandibles to chew through the cell cap and thick entrance plug (Michener, 2000).

Mating and Ovarian Development

Females spend the first 3-5 days feeding on nectar and pollen, mating, and investigating potential nest holes. Richards (1984) found that 100% of the females had mated within three days of emergence. Females mate only once (Gerber and Klostermeyer, 1972).

While little is known about the details of male mating behavior, it is clear that mate-seeking males interfere with the efficiency of female nesting activities, particularly while males are still present in high numbers early in the nesting season. Males pounce on females as they are sunning on the shelter floor or walls early in the morning, or as they are resting briefly with leaf pieces or provision loads prior to re-entering their nests. Leaf pieces are often dropped and abandoned in these interactions. Males also intercept flying females that are hesitating momentarily at nest entrances, knocking them to the shelter floor. Pollen-laden females in this situation seem unable to negotiate the steep trajectory back up to their nest holes, and must instead fly a circuitous loop back to the shelter to gain sufficient altitude to enter the nest (personal observation). Males tend to sleep earlier in the afternoon than females do; if they select an active nest for sleeping, the female is unable to continue nesting activities for the remainder of the day (Gerber and Klostermeyer, 1972).

Nesting

Megachile rotundata makes a type of sequential nest that is common in cavity-nesting bees and wasps (Krombein, 1967). Though variations routinely occur, most nests consist of a linear series of contiguous cells finished with a nest "plug" of varying length

that tends to be flush with the surface of the nesting tunnel. In this study, the terminal cell was commonly followed by a "vestibular cell", an empty space of variable length.

Under natural conditions, females typically make use of the same nest from which they emerged. Under managed conditions, females must search out a new nest site, investigating numerous nest holes using feedback from leg position to evaluate nest geometry and make a selection. Hole depth, as well as diameter relative to female size appear to be important qualities evaluated by females (Gerber and Klostermeyer, 1972).

For cell construction alfalfa leaves are commonly used, but alfalfa leafcutting bees also readily use other types of leaves and flower petals when these are available, including clover *(Trifolium* spp), buckwheat *(Fagopyrum esculentum* Moench*)*, lamb's-quarters *(Chenopodium album* L.*)* (Richards, 1984), Russian olive *(Elaeagnus angustifolia* L.*)*, canola *(Brassica* spp*)* flower petals (personal observation), and sometimes alfalfa *(M. sativa* L.*)* flower petals. When Gerber and Klostermeyer (1972) offered rose *(Rosa* spp) petals, females flew over 300 feet from the shelter to retrieve them.

To cut a leaf piece, the female bee straddles one edge of the leaf or petal, folding the margin over as the cutting proceeds so that as the excised section separates from the plant. She grasps the edges, with the central fold appressed to her sternum and the leading edge secured in her mandibles, as she flies to her nest. Oval pieces are used for the cups; round pieces are used for the individual cell caps; while the entrance plug is made of a combination of round and irregular pieces. Often the 2-4 mm of a nest are

filled with loosely packed cup pieces, perhaps to correct any gapping the female perceives at the rear of the nesting board (personal observation).

Nine to 27 leaf pieces are required for each cup. The propleura is used in conjunction with the mandibles to press leaf pieces into position. Pieces are then tacked into place and sealed with secretions of the salivary glands. Time estimates for completion of a cell, including cup construction, provisioning, egg-laying, and closure vary from as little as 2.5 hours under "ideal foraging conditions" (Richards, 1984) to 7.5 hours (Gerber and Klostermeyer, 1972).

Cell Provisioning

Nesting females can begin provisioning flights as soon as ambient temperature reaches 20.5 °C (Richards, 1996). While in flight between floral visits, females scrape the pollen that has collected on their legs backward onto the scopa (the brush of pollen-collecting setae on the venter of the abdomen) (Gerber and Klostermeyer, 1972). *M. rotundata* females have simple setae on the scopa and therefore rely on "muscular flexing of the abdomen" rather than specialized setal characteristics to retain the pollen (Roberts and Vallespir, 1978). Females are rapid fliers, visiting eight flowers per minute on average. For each cell, approximately 15-27 pollen or nectar collecting forays are completed. The ratio of pollen to nectar collected is approximately 1:2 by mass. Pollen and nectar loads are carried simultaneously, with the texture and moisture content of the growing provision mass adjusted during assembly. Initial trips are relatively high in pollen; subsequent trips tend to be more nectar-rich, with the final several loads being

entirely nectar. Females can carry slightly less than a fourth of their total body weight (Richards, 1984).

Oviposition

The first eggs are laid within seven days of female emergence (Richards, 1994). When the last nectar load is worked into the pollen mass, the female turns around in the nest and deposits one egg on the center of the mass. Like all Hymenoptera, alfalfa leafcutting bees have haplo-diploid sex allocation. There is therefore a simple mechanism by which females can select the sex of individual progeny: eggs that are placed in the first several cells at the back of the nest are coated with stored sperm as they are being extruded (Gerber and Klostermeyer, 1970); these eggs develop into females. In the remaining cells, unfertilized eggs are produced, which develop into males. Males typically outnumber females by roughly 2:1.

Many attempts have been made to decrease the ratio of males to females. Large numbers of males are undesirable mainly because their value as pollinators is negligible, being incidental to nectar feeding. Maternal investment in males is therefore unproductive from the alfalfa seed producer's point of view. In addition, males appear to interfere substantially with nesting females (see above), reducing their efficiency. Selective nest removals (Jay and Mohr, 1987) and selective variation in nest hole dimensions (Stephen and Osgood, 1965) to manipulate sex ratio have been attempted. Wider-diameter holes can produce a higher ratio of females, perhaps by attracting larger females to nest. Gerber and Klostermeyer (1972) found the optimum to be 0.5 cm, when

compared with 0.4 and 0.6 cm. However, sex ratio appears to be very refractory to any improvement.

Larval Development

Eggs hatch within about 2-15 days, depending upon temperature. Healthy larvae completely consume all provisions, leaving the nest cup thoroughly cleaned out. If temperatures are favorable, larvae complete development in approximately 10 to 35 days (Richards, 1984). They spin a thin, papery cinnamon-colored cocoon after first eliminating fecal pellets at the outside end of the cell, and then remain, head-out, in pre-pupal diapause.

Ideally, emergence will not occur until a lengthy period of winter storage. However, an estimated 10-20% of all prepupae avert diapause, and pupate immediately after spinning the cocoon. Second-generation adults emerge in about 30 days (Gerber and Klostermeyer, 1972; Kemp and Bosch, 2001). This is an unfortunate characteristic from a seed production perspective, especially in Montana and other northern-latitude regions, where the predominant bloom occurs fairly early in the season. Thus, females active later in the season (August and later) have fewer flowers to forage on, and forage quality may be lower. Pollination after July may be unprofitable in any case because late flowers may not have time to produce mature pods. Under poor forage conditions, second-generation females may not replace themselves in the nest boards by fall.

Alfalfa Leafcutting Bee Management

Nesting Materials and Shelters

Alfalfa leafcutting bees are managed in large numbers in shelters that can house up to 400,000 nest holes. Shelter styles vary, but all should be large enough to provide orientation for females, and should be raised high enough off of the ground to prevent moisture buildup from the ground, avoid the high temperature boundary layer at the ground, provide adequate cross-ventilation, provide adequate surface area for sunning bees, and provide shade and weather protection (Stephen, 1981). Arrays of nesting tunnels are typically made of an expanded polystyrene material, a material that is relatively inexpensive in addition to being lightweight and easy to sterilize. These boards are 0.7 X 1.4 m^2, containing over 7,000 nest holes. They are all a standard gray color, but are painted or stenciled by seed producers with orientation marks. These designs facilitate nest location by alfalfa leafcutting bee females. Boards are typically fitted side-by-side within shelters in book-fashion, with sufficient spacing to allow females nest access.

Winter Storage and the Loose-cell System

As problems with insect pests and diseases have increased over the past 40 years, growers have shifted towards the "loose-cell" system of management as opposed to allowing bees to emerge from undisturbed nests naturally. Under this system, entire nests are punched out of the nesting boards in the fall, as soon as they have dried down enough to permit removal (Richards, 1984). They are then tumbled in screened barrels to separate individual cells and remove debris, excess leaf pieces, and some pest insects.

Cells that contain no cocoon due to larval death or failure to develop tend to fall apart at this stage without the cocoon to help bind the leaf pieces together, and these tend to be screened out with the rest of the debris. Tumbled cells are stored for six or seven months at 1-10°C while prepupae are in diapause (Richards et al., 1987).

There are many advantages to the loose cell system. Nest removal allows growers to sample cells from the current production year to make estimates of mortality. Screening allows removal of nest parasites and predators. More efficient use is made of storage and incubation space because bulk nesting material is separated and stored elsewhere. Emergence is more synchronized, and bees may emerge in better condition, having bypassed the hazards of chewin and moving through nest detritus to emerge from the nest.

Incubation and Field Release

When loose cells are removed from winter storage, they are warmed to ambient temperature for a few days and then incubated for roughly three weeks at 29-32°C. During this time, pupation and adult development is completed. The lower temperature threshold for development is 15-19°C; at temperatures in excess of 38°C, prepupal death occurs (Richards, 1984; Kemp and Bosch, 2001).

In Montana, alfalfa leafcutting bees generally emerge in early July. One or two days after the first females begin to emerge, emergence trays are placed in bee shelters in the field. There, emergence of both sexes continues for several more days. Open emergence trays are placed on shelter floors in the evening or very early morning, ideally

when conditions are expected to be warm and calm. Most growers in Montana use three gallons of bee cells for each acre of seed alfalfa (1 gallon = ~10,000 bees).

Managing Bee Density

There is a trade-off between managing for bees and managing for alfalfa seed. A short pollination period with high bee density benefits the alfalfa plant by reducing the flowering period, during which the plant is most vulnerable to attack by plant-sucking insects such as *Lygus* and several aphid species. When bee density is low relative to flower density, bees may move less between plants, causing more self-pollination with fewer seeds and reduced viability (Strickler, 1999). For the bees, however, relatively less crowding is advantageous, as there is both less competition for floral resources and less confusion at the nesting boards (Stephen, 1981).

Pests and Diseases

Chalkbrood

Chalkbrood is a fungal mycosis of alfalfa leafcutting bee larvae and overwintering pupae caused by the genus *Ascosphaera*, the principal species being *A. aggregata* Skou. Bee larvae and prepupae infected with chalkbrood initially develop a grayish-yellow coloration with a slack, sunken-appearing integument. Cadavers subsequently harden, turning a darker gray or black as the fungus sporulates under the integument.

Chalkbrood spores are primarily introduced to the provisions placed in the cells by adult female leafcutters dusted with spores on the outer body surface. Larvae ingest the spores when they hatch and feed on the pollen provision. Spores then germinate in the gut and send germination tubes into the hemocoele. If successful, the fungus

multiplies by budding, breaking down larval tissues and blood for absorption. At this time the larvae begin to take on the characteristic appearance of infection (McManus and Youssef, 1984). Chalkbrood-infected larvae die between day eight and day fourteen (Kish and Stephen, 1980). Chalkbrood can cause mortality in excess of 65% if not managed properly (Stephen et al., 1981). Goettel and others (1991) report that it takes ingestion of as much 1,000,000 spores for 50% mortality to occur. However, temperature (Stephens, 1981; Tepedino and Parker, 1986) and larval diet (Goettel et al., 1993; Vandenberg, 1994) are known to affect susceptibility levels. In general, there is superior resistance to chalkbrood in larvae reared on a natural diet as compared to those given artificial provisions (Goettel et al., 1992; Vandenberg, 1994). This may be due to the fact that natural provisions contain microorganisms (Goerzen, 1997) that suppress the chalkbrood fungus. Inglis and others (1992) report increased mortality in larvae that were fed sterilized natural provisions compared to a natural diet that was not sterilized.

Provision Thieves

Several stored product beetles of the genus *Trogoderma, T. glabrum* Herbst (smooth carpet beetle) and *T. variabilis* Ballion (warehouse beetle) feed on cell provisions, killing *M. rotundata* larvae incidentally as they chew through the nest. Beetle eggs are deposited in dark areas at the rear of nests, so anywhere that there are gaps or crevices at the back of nesting boards progeny are vulnerable to attack. Beetle larvae feed from the back of the tunnel first, incidentally destroying bee larvae, of which a disproportionate number are female, as they feed consecutively on several pollen masses in each nest (Eves et al., 1980).

Nest Predators

Checkered flower beetle *(Trichodes ornatus* (Say)*)* larvae feed on both pollen stores and bee larvae. Beetle eggs hatch on or within the nest plug located at the nest entrance. The beetle larvae chew into the cells, easily consuming the contents of one or more entire nests. Losses exceeding 90% of developing bee larvae may be realized if a *T. ornatus* infestation is left unchecked. Checkered flower beetle larvae are successfully screened out of cells and destroyed prior to winter storage (Eves et al., 1980).

Brood Parasites

Cuckoo bees *(Coelioxys* spp) are brood parasites. Although this term is not confined to the field of entomology, with insects it refers to the introduction of progeny into the nests of another insect species, whose offspring are deprived of provision stores (O'Neill, 2001). Most cuckoo bees specialize on *Megachile* spp. There are several species of cuckoo bees of the Megachilid genus *Coelioxys* that parasitize the provisioned cells of alfalfa leafcutting bees. All cuckoo bee females look very similar to the alfalfa leafcutting bee female in body shape and coloration, but the tip of the abdomen is shiny black and pointed, and the abdominal sternum is devoid of a scopa. While mature larvae of *Coelioxys* spp. strongly resemble *M. rotundata* larvae, second and third-instar *Coelioxys* look radically different in having sclerotized head capsules and large powerful mandibles, with which they kill the bee larvae (Michener, 2000). There is no control for cuckoo bees, but losses in the US and Canada due to *Coelioxys* have been negligible.

Parasitoid Wasps

Three species of parasitoid wasps are persistent pests of *M. rotundata*. The Canadian chalcid, *Pteromalus venustus* Walker, is about 2.5 mm long with a shiny, pointed abdomen; males are slightly smaller. Both sexes have mostly black coloration, with some metallic green primarily on the legs. Females insert their stings through *M. rotundata* nest cells and inject paralyzing venom into the larvae. Numerous eggs are laid on the surface of each larva, from which as many as 50 adult Canadian chalcids will emerge. During spring incubation adult Canadian chalcids emerge in 14 days, emerging while developing leafcutters are in the pupal stage. Without adequate control, re-infestation of the same bee generation is likely. The imported chalcid, *Monodontomerus obscurus* Westwood, is larger than the Canadian chalcid. Females are about 3.5 mm long, males about 2.5 mm. Adults are metallic blue-green and are easily recognized by their red eyes. Females also have a characteristic long slender ovipositor, used to penetrate the bee cell and inject paralyzing venom. Roughly 10 larvae develop from each bee cell, emerging in approximately 10 days from onset of incubation. Like the Canadian chalcid, they are likely to re-infest remaining cells if not controlled. *Sapyga pumila* Cresson, a native parasitoid wasp, can destroy almost 75% of *M. rotundata* nests if left uncontrolled (Eves et al., 1980). *S. pumilla* oviposits through the cap end of the bee cell. Only one wasp larva develops within each cell. The overwintering cocoon is the same size as the bee cocoon. If returning foragers intercept *S. pumila* in a nest, the wasps are attacked aggressively and often have their antennae damaged (Torchio, 1972).

Pollen Ball

In many cases, dissection of non-viable cells discloses an unconsumed pollen mass, often with a desiccated egg or very young larva on the surface. Cells that have ceased development during any of these stages are collectively referred to as "pollen ball". In some cases, the cause of cell failure can be determined to be chalkbrood because visible black spore balls pervade the provision mass and/or young larva. In many cases, however, no known cause for termination of development is known. Pollen ball differs from other sources of alfalfa leafcutting bee mortality in that it is not a distinct diagnosis, but rather a catch-all term for mortality that occurs at the earliest stages of development, and for any number of unknown reasons.

Pollen ball has variously been attributed to advanced maternal age, overcrowding, pesticides, undiagnosed chalkbrood, low pollen or nectar availability, poor weather conditions, or extreme temperatures (Mayer, 1992; Kemp and Bosch, 1998). Pollen ball may be the outcome of any or all of these factors, and others yet to be determined. There are variations in pollen ball morphology and content that may turn out to be linked with some of these predictors. For example, there is variation in moisture content of the provision mass, and perhaps sugar and protein content as well. Rarely, a cell is found that is normally capped, but the entire cell is only about 2-3 mm long, with an abnormally small amount of provision. In these cases, female bees may be responding to challenges in the environment that affect provision quality or quantity, or limit foraging ability, by abandoning some cells in mid-construction. In other cases, pollen balls appear to be abnormally long.

Pollen ball losses of can exceed 60% (Kemp and Bosch, 1998). Reliable estimates of percent pollen ball are difficult to obtain from processed samples of loose cells. The difficulty arises because cells without reinforcing cocoons tend to disintegrate during machine processing in the fall and are screened out along with excess debris, resulting in underestimation of pollen-ball frequency.

Current Status of Alfalfa Seed Production in Montana

In Montana in 2003 there were 6,200 harvested acres of alfalfa seed, less than one third the acreage harvested in 2000 (Montana Agricultural Statistics, 2003). The shrinkage in the alfalfa seed industry in Montana and elsewhere in the U.S. has several underlying causes. Imports, particularly of uncertified alfalfa seed, appear to have created a widespread price depression (Alfalfa Seed Growers News, Fall 2002, page 2). In addition, an important seed contractor throughout the northwest, AgriBioTech, Inc. (Henderson, Nevada), dropped out of the market in 2001, failing to meet its full financial obligations to producers.

As a result of lower alfalfa seed production, bee prices have plummeted from a high of several hundred dollars per gallon early in the history of the industry to the current price of approximately $10.00/gallon or less. For this reason, many growers have shifted their efforts away from managing for the benefit of bees, focusing instead on maximizing seed yield. This approach often entails crowding bees onto acreage for rapid seed set, with less concern for bee health and productivity. However, there is a widespread expectation that the seed industry will recover to profitable levels in the future, albeit with fewer producers. In anticipation of this recovery, care should be taken to maintain

healthy bee stocks and improve management protocols to ensure an adequate supply of pollinators in the future,.

OBJECTIVES

The overall goal of this study is to examine within-nest seasonal and spatial patterns of alfalfa leafcutting bee progeny mortality and progeny sex ratio.

Specific objectives are:

1) To determine seasonal patterns in the extent and causes of alfalfa leafcutting bee progeny mortality within nests in southwestern Montana.

2) To determine spatial patterns in the extent and causes of alfalfa leafcutting bee progeny mortality within nests in southwestern Montana.

3) To determine seasonal and spatial patterns of progeny sex ratio within alfalfa leafcutting bee shelters in southwest Montana.

MATERIALS AND METHODS

Shelters

Four bee shelters were constructed following a design created specifically for this study. These shelters were smaller than conventional shelters and were collapsible, allowing them to fit into the back of a field vehicle for transport. However, care was taken to provide cross-ventilation, weather protection, and adequate height above ground, all of which are considerations of larger-scale commercial shelters. Interior shelter dimensions were approximately 0.7 x 1.0 x 1.7 m^3. Sides, back, and floor were constructed of wood and plywood, with a roof of resin corrugate. Ten-centimeter gaps in the upper back of each shelter provided cross-ventilation, and nest boards were suspended above the shelter floor on corner blocks to further encourage adequate ventilation. Shelter floors were raised about 0.5 m above ground to prevent moisture build-up, and when necessary support legs were coated with Tanglefoot to discourage ants and rodents.

Each shelter housed one single-sided nest board, comprised of vertically stacked sheets of expanded polystyrene nestboard laminate (Beaver Plastics, Edmonton, Alberta: techsupport@beaverplastics.com). Nest boards faced outward to facilitate data collection. Nest tunnels were 9.5 cm deep, and hole diameter was either 5.5 mm (60% of nest holes) or 6.5 mm, a manufacturing decision intended to provide a range of nest hole diameters for female bees.

Site Descriptions

Studies were completed at three field sites in 2000 (Toston 1, Amsterdam, and Post Farm) and four sites in 2001 (Toston 1, Toston 2, Amsterdam, and Flikkema).

Amsterdam (45°48'00"N 111°23'00"W), Post Farm (45°40'00"N 111°09'00"W), and Flikkema (45°45'00"N 111°06'00"W) were located within a 25-km radius of Montana State University, near Bozeman (Gallatin County, MT). Toston 1 and Toston 2 were located on a large scale alfalfa seed farm 97-km northwest of Bozeman, in Toston (Broadwater County, MT) (46°11'00"N 111°26'00"W). Overall climatic conditions were very similar for the two Toston sites, as they were only about 0.5-km apart. However, the two sites differed in some important ways. Toston 1 is located on a west-facing slope of well-spaced alfalfa plantings on very rocky well-drained soil. Toston 2 is located <0.5 km west of Toston 1 on the Missouri River in a level field of river sediments, with more humus-rich soil, and a denser cover of alfalfa. Both Toston sites were irrigated, and planted primarily with *Medicago sativa* (cultivar Ladak). The Amsterdam site is a an irrigated Montana State University alfalfa seed trial plot located between the towns of Amsterdam and Manhattan, Montana, on a level 2-acre plot bordered on three sides by seed potatoes in 2000, and wheat in 2001. Seed alfalfa varieties at Amsterdam are part of a series of varietal trials being conducted by Dr. Raymond Ditterline, and cultivar names are not yet released. Post Farm is a level 0.5-acre irrigated experimental alfalfa forage plot on the A. Post an experimental farm located near the MSU campus in Bozeman, Montana; this plot was left uncut and allowed to develop seed for the purposes of this project. Flikkema is a dryland forage alfalfa field, of which 0.5 acres was fenced off from the surrounding alfalfa forage and left uncut for the season. *M. sativa* cultivars at the latter two sites are not known.

Two of the sites, Toston 1 and Amsterdam, were used in both years of the study. To avoid confusion, these sites are treated as separate sites between years, generating seven total site-years: Toston 1 2000, Amsterdam 2000, Post Farm 2000, Toston 1 2001, Toston 2 2001, Amsterdam 2001, and Flikkema 2001.

Bee Release

Bees for this study were obtained from Alberta, Canada (JWM Leafcutters, Inc., Nampa, Idaho). At arrival, these bees were diapausing prepupae. The prepupae were incubated for ~3 weeks at Thomas Helm's alfalfa seed farm in Toston, Montana. In 2001, only local bees were used. These were provided by Thomas Helm.

Shelters were set up during the first week of July in both years, just prior to bee release. Shelter openings were oriented at 135° SE, to receive morning sun and afternoon shade. Bees were released before dawn on 2 July (Amsterdam 2000 and Post Farm 2000), 3 July (Toston 1 2000), 5 July (Toston 1 2001 and Toston 2 2001), and 6 July (Amsterdam 2001 and Flikkema 2001). These releases coincided with typical timing of bee release in Montana, and were concurrent with grower bee releases at all Toston sites in both years. Rate of bee release was 1.5 gallons (5.7 liters) per shelter, sufficient to pollinate 0.5 acres of seed alfalfa. This allowed for an estimated two to three tunnels for each nesting female over the course of the nesting season.

Nest Completion Survey

Field Protocol

Field sites were visited on a rotational basis every one to four days in 2000 and every three to four days in 2001, dependent upon weather and local chemical

applications adjacent to field sites. Sites were generally visited between 7:30 am and 1:30 pm.

All 7,000+ nest tunnels at each site were examined for evidence that the construction of the nest plug either had been completed or was under construction. These completed or nearly-completed nests were marked with a streak of enamel paint color-coded by date. In 2000, only nests that were fully plugged with the plug flush with the exterior surface of the nest board were marked with paint, in an attempt to ensure that the nest was completed on the indicated date. However, in 2001, nests were examined more closely and, if the nest plug was in the process of being completed, the nest marked on the same day, a change in protocol that resulted in more accurate estimates of final cell completion for each nest.

In the first half of September 2000, shelters at Toston and Amsterdam were dismantled and the boards were stored in the field with the shelter at Post Farm outside until early October, to allow any remaining immature larvae to spin cocoons. The following year the same procedure was followed, with the boards being stored outside at a location in Bozeman.

Winter Storage and Nest Handling

In early October of both years, nesting boards were transferred to a cold wet storage room located at the MSU Plant Growth Center, where diapause took place at a constant 8°C and 85% relative humidity for seven months.

During the winters of 2000-2001 and 2001-2002 small blocks of 100 nest tunnels were removed one at a time from winter storage. These were dismantled and cells from

each nest (excluding focal nests, see below) were sorted based upon 1) the date of nest completion, indicated by the color-code on the nest plug, and 2) the relative cell position within each nest. Cells were assigned position numbers consecutively from the rear of the nest forward, so that "cell 1" was the first cell constructed in the nest. In this manner, both an estimate of nest completion date and the relative position of each cell within its nest was preserved. Sorted cells were then returned to cold storage in ventilated containers to continue overwintering. Cells were held out of cold storage for a maximum of six hours during the sorting process.

Diagnostic Determinations

From March through July of both years, cells were dissected and sorted into one of six diagnostic categories:1) live prepupa, 2) parasitized by *Pteromalus venustus*, 3) chalkbrood, 4) unknown, 5) pollen ball. The few cells that did not fit into the preceding categories were not analyzed (see Discussion). Cells that contained a dead larva were placed in the pollen ball category if the larva was judged to be less than 2X the length of an egg. Larvae at this stage of growth are in the first instar of development, and have generally not yet defecated. Larvae older than this were placed in the unknown category, along with dead prepupae for which cause of death was unknown. A maximum of 50 cells was dissected from each date/position category. Dates at either end of the season (the first date in July , and the last several dates in August) as well as some of the later-constructed cell positions towards the fronts of nests sometimes contained fewer than 50 cells.

Focal Nests Survey

Field Protocol

Field sites were visited on a rotational basis every one to four days in 2000 and every three to four days in 2001, dependent upon weather and local agricultural chemical applications on field crops adjacent to research sites. Sites were generally visited between 7:30 am and 1:30 pm.

On each site visit, focal surveys were carried out on 200 nests in each shelter. Selected nests were monitored throughout the season, providing a continuous record of development for each nest. Monitoring consisted of measuring the depth of the nesting materials to the nearest millimeter using a probe that was inserted into the facing edge of each nest. Measurements ranged from 9.5 cm (empty nests) to 0.0 cm (fully plugged nests with the plug flush with the nest entrance). Probes were sterilized in 95% ethanol between nest measurements to prevent nest cross-contamination by pathogens. Washed probes were thoroughly air-dried before re-use to prevent damage to bees and nest contents from alcohol residue.

Winter Storage and Nest Handling

In early October of both years, nesting boards were transferred to a cold wet storage unit located at the Plant Growth Center on the campus of Montana State University, where diapause took place at a constant 8°C and 85% relative humidity for seven months.

During the winters of 2000-2001 and 2001-2002 small blocks of 100 nest tunnels containing both date-coded nests (see Nest Completion survey) and 20 focal nests were

briefly removed one at a time from winter storage. Nests that had been designated for focal measurements during the summer were separated from the date-coded nests described above. After the polystyrene laminate was pulled apart, each focal nest was carefully measured and drawn prior to pulling the nest from the nesting material. Individual cell dimensions and precise position were recorded, as were the dimensions of the vestibule (if present) and the nest plug. Cells were assigned position numbers consecutively from the rear of the nest forward, so that "cell 1" was the first cell constructed in the nest. Measurements were made to the nearest millimeter.

Recorded cells were placed individually in gelatin capsules for overwintering. Gelatin capsules were size 00, with one (2000) or two (2001) small perforations for gas exchange. Each capsule contained a label with the site, year, and individual nest number and ordinal position occupied by each cell. These cells were returned to cold storage and maintained in the same environment as indicated above for cells from the date-coded nests.

Data from the focal nest drawings were compared with data collected via nest probes, to determine the range of dates (from one focal survey to the next) during which each individual cell was estimated to have been constructed. If the cell was at least halfway completed in terms of total cell length, then the cell was judged to have been complete by the end of the sampling day. Because most of the labor and time involved in cell construction entails the assembly of the leaf cup and the collection of the provision mass, the bulk of which occupies the first half of each cell, this assumption was considered justified. Cells that have reached the halfway mark in length are generally

close to being fully provisioned, and require only oviposition and cell closure for completion.

Cells in gelatin capsules from the focal study were removed from cold storage in mid-June in both years and were allowed to rest at room temperature for three days. They were then incubated at a constant 32°C in a VWR Scientific Products Controlled Temperature Cabinet, with a broad shallow pan of water to maintain humidity. Gelatin capsules containing either parasitoid wasp adults or newly emerged *M. rotundata* adults were removed from the incubator as they emerged on a daily basis and frozen. When *M. rotundata* emergence was complete, the remaining cells were frozen for later dissection and analysis.

Diagnostic Determinations

Cells were dissected if necessary and sorted into one of six diagnostic categories:1) post-diapause (pupa or adult), 2) parasitized by *Pteromalus venustus*, 3) chalkbrood, 4) unknown, 5) pollen ball, and 6) "other". Cells that contained a dead larva were placed in the pollen ball category if the larva was judged to be less than 2X the length of an egg. Larvae at this stage of growth are in the first instar of development, and have generally not yet defecated. Larvae older than this were placed in the unknown category, along with dead prepupae for which cause of death was unknown.

Sex Ratio Survey

Sex ratio analyses used adults from the focal study (see above), for which cell construction dates as well as ordinal cell position had been established. Live emergent

adults, as well as those found fully developed but dead in their cells upon dissection were used in the sex ratio analyses.

Statistical Analyses

Logistic regression analyses were performed on SAS Version 8E for windows, using the PROC LOGISTIC function, where the probability of observing mortality in each category as a function of the predictors relied on the following relationship:

$$P_i = 1 / (1 + \exp(-\alpha - \beta_1 \chi_{i1} - \beta_2 \chi_{i2} - \ldots - \beta_k \chi_{ik}))$$

where α = intercept and β = maximum likelihood (ML) parameter estimate. For a discussion of categorical data analysis using logistic regression see Agresti (1996).

RESULTS

Nest Completion-date Survey 2001

Live Prepupae

At Amsterdam 2000 (Table 1) and Toston 1 2001 (Table 2), there was a positive maximum likelihood parameter (hereafter beta) estimate indicating an increase in the probability of prepupal survival from nests plugged progressively later in the season. At Toston 1 2000, Post Farm 2000, and Toston 2 2001 respective beta estimates were negative, indicating that live prepupa production decreased significantly in later-plugged nests. There were no significant seasonal trends in the probability of live prepupae at Amsterdam 2001 and Flikkema 2001. There was a significant negative cell x date interaction for Toston 1 2001 and Amsterdam 2001 (Table 3), but no significant interactions for the 2000 site-years (Table 4).

When live prepupa production was analyzed by cell position, there were significant negative correlations at Amsterdam 2000 (Table 5) and Flikkema 2001 (Table 6), indicating that live prepupae numbers decreased in outer cell positions. In contrast, at Post Farm 2000, Toston 1 2001 and Amsterdam 2001 live prepupa production improved in outer cell positions while Toston 1 2000 and Toston 2 2001 were non-significant.

Chalkbrood

At all four 2001 sites, chalkbrood showed a significant seasonal decline, but there were no differences in the probability of chalkbrood incidence across season in 2000 (Tables 1 & 2).

Table 1. Seasonal trends, logistic regression results from nest completion study for the binomial response of all diagnostic categories (2000).

		chalkbrood	parasitoids	pollen ball	unknown	live prepupae
Toston 1 2000	Maximum Likelihood Estimate (β_i)		0.0199	0.0260		-0.0263
	standard error		0.00587	0.0111		0.00335
	Wald chi-square		11.44	5.45		61.85
	p-value	ns[1]	0.0007	0.0196	ns	<0.0001
Amsterdam 2000	Maximum Likelihood Estimate (β_i)				-0.0334	0.0138
	standard error				0.00567	0.00481
	Wald chi-square				34.61	8.17
	p-value	ns	ns	ns	<0.0001	0.0043
Post Farm 2000	Maximum Likelihood Estimate (β_i)			0.0399		-0.0168
	standard error			0.00842		0.00557
	Wald chi-square			22.46		9.09
	p-value	ns	ns	<0.0001	ns	0.0026

[1]non-significant

Table 2. Seasonal trends, logistic regression results from nest completion study for the binomial response of all diagnostic categories (2001).

		chalkbrood	parasitoids	pollen ball	unknown	live prepupa
Toston 1 2001	Maximum Likelihood Estimate (β_i)	-0.0604		0.0252		0.0305
	standard error	0.00708		0.0120		0.0074
	Wald chi-square	72.74		4.41		15.53
	p-value	<0.0001	ns[1]	0.0358	(ns)	<0.0001
Toston 2 2001	Maximum Likelihood Estimate (β_i)	-0.1055	0.0256		0.0566	-0.0120
	standard error	0.0138	0.0115		0.0138	0.00375
	Wald chi-square	58.30	4.96		16.76	10.32
	p-value	<0.0001	0.0259	(ns)	<0.0001	0.0013
Amsterdam 2001	Maximum Likelihood Estimate (β_i)	-0.1569		0.0743		
	standard error	0.0380		0.00807		
	Wald chi-square	17.04		84.89		
	p-value	<0.0001	ns	<0.0001	ns	ns
Flikkema 2001	Maximum Likelihood Estimate (β_i)	-0.1855		-0.0457		
	standard error	0.0503		0.0161		
	Wald chi-square	13.60		8.03		
	p-value	0.0002	ns	0.0046	ns	ns

[1]non-significant

Table 3. Date x cell interaction trends, logistic regression results from nest-completion survey for the binomial response of all diagnostic categories (2001).

		chalkbrood	parasitoids	pollen ball	unknown	live prepupa
Toston 1 2001	Maximum Likelihood Estimate (β_i)			0.00725		-0.0118
	standard error			0.00229		0.00170
	Wald chi-square			9.99		48.46
	p-value	ns[1]	na[2]	0.0016	na	<0.0001
Toston 2 2001	Maximum Likelihood Estimate (β_i)	0.0125		0.00561	-0.00759	
	standard error	0.00421		0.00202	0.00237	
	Wald chi-square	8.88		7.73	10.29	
	p-value	0.0029	ns	0.0054	0.0013	na
Amsterdam 2001	Maximum Likelihood Estimate (β_i)					-0.00732
	standard error					0.00252
	Wald chi-square					8.44
	p-value	ns	na	ns	na	0.0037
Flikkema 2001	Maximum Likelihood Estimate (β_i)			0.0181		
	standard error			0.00334		
	Wald chi-square			29.27		
	p-value	ns	na	<0.0001		na

[1]non-significant; [2]not applicable

Table 4. Date x cell interaction trends, logistic regression results from nest-completion survey for the binomial response of all diagnostic categories (2000).

		chalkbrood	parasitoids	pollen ball	unknown	live prepupae
Toston 1 2000	Maximum Likelihood Estimate (β_i)			0.00658		
	standard error			0.00226		
	Wald chi-square			8.45		
	p-value	na[1]	ns[2]	0.0037	na	na
Amsterdam 2000	Maximum Likelihood Estimate (β_i)			0.00849		
	standard error			0.00368		
	Wald chi-square			5.31		
	p-value	na	na	0.0212	ns	ns
Post Farm 2000	Maximum Likelihood Estimate (β_i)					
	standard error					
	Wald chi-square					
	p-value	na	na	ns	na	ns

[1]not applicable; [2]non-significant

Table 5. Cell position trends, logistic regression results from nest-completion study for the binomial response of all diagnostic categories (2000). Cell position 1 = first cell completed in nest.

		chalkbrood	parasitoids	pollen ball	unknown	live prepupae
Toston 1 2000	Maximum Likelihood Estimate (β_i)	-0.2299	-0.8991	-1.3234	0.3299	
	standard error	0.0308	0.0466	0.4756	0.0185	
	Wald chi-square	55.83	372.3	7.7432	317.8	
	p-value	<0.0001	<0.0001	0.0054	<0.0001	ns
Amsterdam 2000	Maximum Likelihood Estimate (β_i)		-1.8230	-1.6058	0.4254	-0.3524
	standard error		0.4162	0.7737	0.0214	0.0178
	Wald chi-square		19.19	4.3077	394.2	392.6
	p-value	ns[1]	<0.0001	0.0379	<0.0001	<0.0001
Post Farm 2000	Maximum Likelihood Estimate (β_i)		-1.2987	0.0828	0.3061	0.0432
	standard error		0.087	0.0257	0.0259	0.0166
	Wald chi-square		246.6	10.36	139.6	6.74
	p-value	ns	<0.0001	0.0013	<0.0001	0.0094

[1]non-significant

Table 6. Cell position trends, logistic regression results from nest-completion study for the binomial response of all diagnostic categories (2001). Cell position 1 = first cell completed in nest.

		chalkbrood	parasitoids	pollen ball	unknown	live prepupa
Toston 1 2001	Maximum Likelihood Estimate (β_i)	-0.4298	-0.6745	-1.4773		2.7101
	standard error	0.0321	0.0482	0.4936		0.3634
	Wald chi-square	179.6	195.6	8.9584		55.62
	p-value	<0.0001	<0.0001	0.0028	ns[1]	<0.0001
Toston 2 2001	Maximum Likelihood Estimate (β_i)	-3.1223	-0.8854	-1.1159	1.8446	
	standard error	0.8863	0.0913	0.4314	0.5063	
	Wald chi-square	12.41	94.12	6.69	13.27	
	p-value	0.0004	<0.0001	0.0097	0.0003	ns
Amsterdam 2001	Maximum Likelihood Estimate (β_i)	-1.2073	-1.1687	0.1366	0.2087	1.5053
	standard error	0.2810	0.1120	0.0217	0.0235	0.5389
	Wald chi-square	18.46	109.0	39.66	78.68	7.80
	p-value	<0.0001	<0.0001	<0.0001	<0.0001	0.0052
Flikkema 2001	Maximum Likelihood Estimate (β_i)	-0.6895	-1.4232	-3.7387	0.3467	-0.1603
	standard error	0.2036	0.3567	0.7053	0.0290	0.0186
	Wald chi-square	11.47	15.92	28.10	142.7	74.44
	p-value	0.0007	<0.0001	<0.0001	<0.0001	<0.0001

[1]non-significant

Chalkbrood also showed a significant decline with cell position at all four 2001 sites (Table 6) as well as one 2000 site (Toston 1 2000), with the remaining two 2000 sites showing non-significance (Table 5). At Toston 2 2001, however, nest-completion date interacted with cell position (Table 3).

Parasitoids

All parasitoids encountered in the date-coded nests study were determined to be the Canadian chalcid, *Pteromalus venustus*. There were significant relationships between seasonal progression and incidence of parasitism at two of seven site-years surveyed. Seasonal trends were positive at Toston 1 2000 (Table 1) and Toston 2 2001 (Table 2); the remaining five site-years showed non-significance.

At all seven site-years, cell position was negatively related to parasitism. The highest parasitism was in all cases found to occur in the earliest-constructed cells in the nest, declining to nearly zero in the outermost cells (Tables 5 & 6). This was true even at Flikkema 2001, though parasitism was uncommon (N = 20 parasitized cells) relative to the other three sites that year and relative to all 2001 site-years.

Pollen Ball

Pollen ball increased significantly with nest-completion date in four of seven site-years; Toston 1 2000, Post Farm 2000 (Table 1), Toston 1 2001, and Amsterdam 2001 (Tables 1 & 2). Pollen ball declined with nest-completion date only at Flikkema 2001, with the remaining two sites indicating non-significance.

Pollen ball declined with cell position in five of seven site-years; Toston 2000, Amsterdam 2000, Toston 1 2001, Toston 2 2001, and Flikkema 2001 (Tables 5 & 6).

Pollen ball increased with cell position at the remaining two site-years, Post Farm 2000 and Amsterdam 2001. There were significant positive interactions between nest-completion date and cell position at five out of seven site-years (Tables 3 & 4).

Unknown

Mortality in this category was non-significant when analyzed for date variation at five out of seven site-years. However, at Amsterdam 2000 mortality in this category declined with nest-completion date (Table 1), and at Toston 2 2001 unknown mortality increased with nest-completion date (Table 2). For the latter site-year, nest-completion significantly interacted with cell position (Table 3).

Cell position results for this mortality category were more obvious, increasing significantly in six out of seven site-years with advancing cell position, with Toston 1 2001 non-significant (Tables 3 & 4).

"Other" cell conditions. Several cell conditions were diagnosed too infrequently to include in a meaningful analysis of seasonal or positional trends in mortality. These were excluded from analyses, but are mentioned briefly here. At Toston 2 2001, six larval *Melittobia chalibii* were found in cell position six; one *Trichodes* beetle larva in cell position four; and one adult red-marked sapygid adult *(Sapyga pumila)* in cell position one. At Flikemma 2001 one American black flour beetle adult was found dead on a pollen ball, and three fly puparia of unknown identity were found in separate mid- to and late-season nests. At Toston 2 2001 pollen balls sometimes contained glycophagid mites (see Focal Survey, below).

Focal Survey

Post-diapause (Adults and Pupae)

The predominant diagnostic category at all seven site/years analyzed was adults (Table 7), with the highest adult emergence rates at Flikemma 2001 (80.6%), and the lowest at Post Farm 2000 (40.1%). The latter low value was due to excess mortality during incubation and handling, and does not represent field mortality rates (see Discussion). The second lowest adult emergence was seen at Toston 2 2001 (45.4%). With the exception of Post Farm 2000, rates of death occurring at the pupal stage during incubation across all sites were less than 2%, and less than 1% for 2001 sites.

While the adult emergence rate for the Amsterdam site was similar between 2000 and 2001 (65.4% and 70.8% respectively), emergence improved markedly in the second year at Toston 1 (from 46.2% to 64.7%). Excluding Post Farm 2000, higher emergence rates were seen at the experimental seed plots (i.e., not the commercial Toston seed sites) Amsterdam 2000, Amsterdam 2001, and Flikkema 2001 (Table 7).

At Toston 1 2000 and Toston 1 2001, and at Flikkema 2001 positive beta estimtes indicate a positive relationship between seasonal progression and post-diapause survival (Tables 8 & 9). At the remaining four site-years, however, there was no seasonal trend. There were significant negative interactions between cell position and cell completion date both years at Toston 1 (Tables 10 & 11) while the remaining five site-years had no significant interactions.

There were significant cell position effects in five out of seven site-years. These results were split, with the three Toston site/years (Tables 12 & 13) showing that overall

Table 7. Total frequencies (and percentages) for each diagnostic category in focal nests, by site-year.

	Toston 1 2000	Amsterdam 2000	Post Farm 2000	Toston 1 2001	Toston 2 2001	Amsterdam 2001	Flikkema 2001	Mean % (± SE)
total no. cells	647	751	566	861	896	726	625	724.6 (±46.23)
chalkbrood (%)	37 (5.7)	4 (0.5)	2 (0.4)	50 (5.8)	105 (11.7)	5 (0.69)	5 (0.8)	3.66 (±1.63)
parasitism (%)	72 (11.1)	25 (3.3)	76 (13.4)	63 (7.3)	27 (3.0)	27 (3.72)	3 (0.5)	6.06 (±1.79)
pollen ball (%)	97 (15.0)	44 (5.9)	52 (9.2)	141 (16.4)	226 (25.2)	101 (13.91)	48 (7.7)	13.32 (±2.48)
unknown (%)	129 (19.9)	173 (23.0)	148 (26.2)	48 (5.6)	126 (14.1)	77 (10.61)	61 (9.8)	15.59 (±2.88)
dead pupae (%) *	9 (1.4)	7 (0.9)	43 (7.6)	2 (0.2)	5 (0.6)	2 (0.29)	4 (0.6)	1.66 (±1.00)
dead pupae (%) **	4 (0.6)	7 (0.9)	18 (3.2)	0 (0.0)	0 (0.0)	0 (0.00)	0 (0.0)	0.68 (±1.17)
adults (%)	299 (46.2)	491 (65.4)	227 (40.1)	557 (64.7)	407 (45.4)	514 (70.80)	504 (80.6)	59.05 (±5.74)

* not parasitized; **parasitized (2nd generation)