ETIOLOGY AND EPIZOOTIOLOGY OF CHALKBROOD IN THE LEAFCUTTING BEE, *MEGACHILE ROTUNDATA (Fabricius)*, WITH NOTES ON *ASCOSPHAERA* SPECIES

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ABSTRACT

This bulletin provides the most recent information on the etiology of the fungal pathogen causing chalkbrood in the leafcutting bee, *Megachile rotundata*. Its symptomatology, routes of infection, pathogenicity, and means of dispersal are discussed. Brief descriptions and comparisons are made of all described species of *Ascosphaera* and preliminary studies on cross-infectivity are presented. The bulletin is prepared for both the lay and research audience.

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ETIOLOGY AND EPIZOOTIOLOGY OF CHALKBROOD IN THE LEAFCUTTING BEE, *MEGACHILE ROTUNDATA (Fabricius),* WITH NOTES ON *ASCOSPHAERA* SPECIES

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Chalkbrood is a fungal disease infecting the larvae of many species of solitary and social bees and is caused by several species of fungi in the genus *Ascosphaera* (Ascomycetes:Ascosphaerales). One of the species, *A. aggregata* Skou (Skou, 1975), has become a severe problem in the alfalfa leafcutting bee, *Megachile rotundata* (Fabr.). It was first reported from Lovelock, Nevada, in 1973 and subsequently spread rapidly to most areas of western America where the bee is propagated for pollination or commerce. The impact of the disease has been most severe in the western United States where many populations have experienced losses from chalkbrood of more than 65 percent. We have recorded the disease from leafcutting bee populations in all states from the western Great Plains to the Pacific Coast and from all western Canadian provinces except British Columbia. Not all populations in all these areas are diseased but there are few disease-free populations of the bee in North America and the incidence of the disease varies greatly from area to area. Chalkbrood also has been isolated from populations of *M. rotundata* in Argentina and Chile which originated from disease-free stocks from northern Saskatchewan. The original description of the disease was based in part on specimens from endemic populations of *M. rotundata* in Spain (Skou, 1975).

The organism causing chalkbrood in *M. rotundata* was first identified as *A. apis* (Maassen ex Claussen) Olive & Spiltoir (Thomas and Poinar, 1973) from California populations of the bee and subsequently was incorrectly referred to as *A. proliperda* Skou (Stephen and Undurraga, 1978). In 1975, Skou described *A. aggregata* as a probable pathogen of the alfalfa leafcutting bee, and of the European megachilids, *Megachile centuncularis* (L.) and *Osmia rufa* L. Although the taxonomy of this fungal genus is better understood now than it was when the regional chalkbrood program began in 1978, considerable descriptive and revisionary work remains. However, with the development of an aseptic technique for rearing the bee from egg through adult (Fichter et al., 1981) and the perfection of a method for germinating spores of the pathogen in the laboratory (Kish, 1980) we have proved *A. aggregata* to be the causative agent of chalkbrood in the alfalfa leafcutting bee (Vandenberg and Stephen, 1982). Earlier suggestions implicating one or more viruses in the chalkbrood syndrome are without foundation.

Chalkbrood was described from infected honey bee colonies in Germany in 1911 (Claussen, 1921). In 1969, the disease was first recorded from honey bees in California (Thomas and Luce, 1972) and since that time has been found in most states and provinces of the United States and Canada (DeJong and Morse, 1976; DeJong, 1977 unpublished thesis). There have been several reports of chalkbrood from various species of solitary bees in Europe (Melville and Dade, 1944; Clout, 1956; Bailey, 1963; Skou, 1972, 1975) and in the United States (Baker and Torchio, 1968; Batra et al., 1973), and in 1973, the disease was identified in California populations of the alfalfa leafcutting bee, *M. rotundata* (Thomas and Poinar, 1973). In extensive surveys made on trap nests of solitary bees during the last two years, Youssef and Parker (personal communication) have shown a diverse flora of chalkbrood-causing fungi to exist among solitary bees in western America. However, all work indicates those species of fungi which affect the honey bee, *(A. apis* and *A. major)* differ from those attacking the leafcutting bee and that the species probably are not cross-infective (see below).

HOW TO RECOGNIZE THE DISEASE

Infected larvae die before they reach maturity, harden, and turn cream colored, then gray, black, or at times become mottled with both gray and black. It is these hard, chalky cadavers which give rise to the common name of the disease. Usually the infected larvae succumb to the disease in their last instar before completing their cocoons (Figure 1). Cells containing dead last instars have the appearance of normal cells except that the tops of the cells are unsealed where the "nipples" of normal cells are found (Figure 2). The walls of a cell containing a chalkbrood cadaver are fragile because of the incomplete cocoon and often the cell collapses when rolled between fingers. During the last two years, increasing numbers of younger larval cadavers (Figure 1) have been found as have numbers of younger, completely sealed cells containing chalkbrood cadavers. In the former, the hard, dark larvae rest on partially eaten provisions and readily fall from the cells when cells are removed from the nesting medium.

There is no regular pattern in the occurrence of diseased larvae within a cell series. Dead larvae are randomly dispersed among live larvae (Figure 3). Occa-

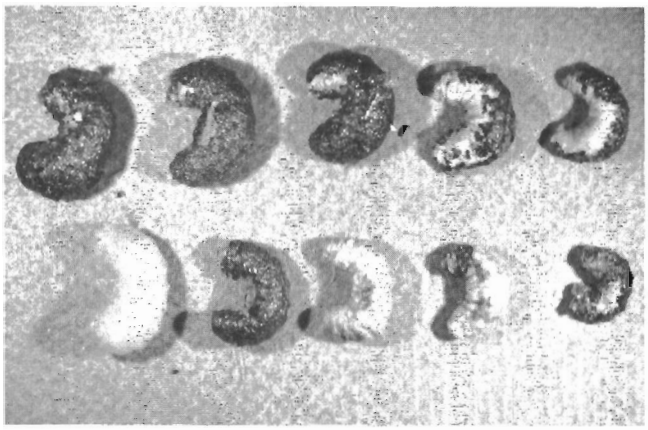

Figure 1. Exposed live prepupa and cadavers of leafcutting bees. top row: chalkbrood cadavers of last instar bee larvae. Bottom row: (left) healthy prepupa: (right) chalkbrood cadavers of younger bee larvae.

Figure 2. Leafcutting bee cells. Top row: cells with chalkbrood cadavers of mature larvae. Bottom row: normal cells with live prepupae. Top of cell sealed by flattened nipple of the cocoon.

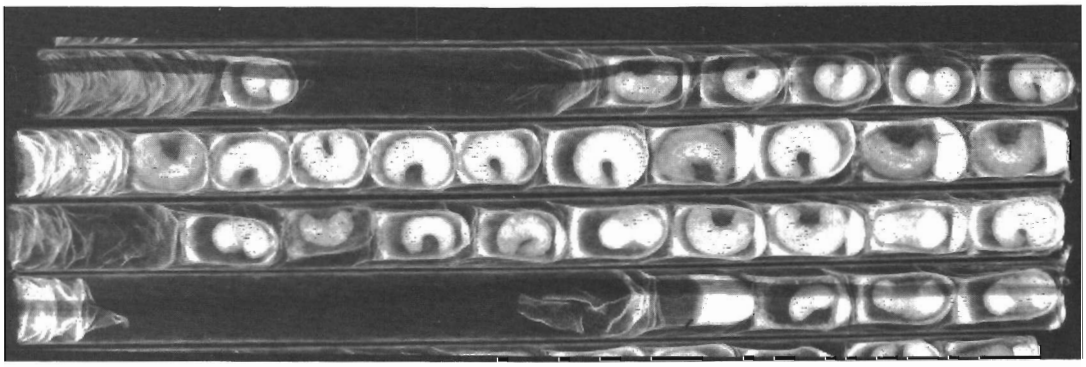

Figure 3. Photo reproduction from an X-ray of two straws containing cell series of the leaf cutting bee. Top (reading l to r): cells 1, 7, 9 and 10 with chalkbrood cadavers. Botton (reading l to r): cells 1, 3, 5 and 9 with live prepupae; cells 2, 4, 6, 7 and 8 with chalkbrood cadavers. Note: Cells containing chalkbrood cadavers are rarely sealed at their upper end, and usually have uneaten pollen residues at their bases.

sionally, in heavily infected populations of the bee, an entire cell series of from 6 to 12 live larvae may be found, suggesting the possibility of a resistance factor in some individuals.

SPREAD OF THE DISEASE

Earlier speculations as to the principal modes of pathogen dispersal (Stephen and Undurraga, 1978) have been substantiated by recent field studies (Vandenberg et al., 1980). The surface of each chalkbrood cadaver is covered with densely packed spore cysts, each containing numerous spores. The number of spores on a cadaver averaged about 100 million with a range of from 3 million to 500 million, the number being a function of the size of the larva when it died. As live adult bees emerge from nesting tunnels, they chew through any cadavers ahead of them and become heavily contaminated with spores. In laboratory studies, male and female bees were forced to emerge from heavily contaminated chalkbrood material, each chewing through from 0 to 9 chalkbrood cadavers. Counts of spores from body washes made of emerging bees showed that many adults carried more than 100 million spores (Vandenberg et al., 1980). The data suggest a direct relationship between the total spore load per adult and the number of cadavers through which it chewed. Females carried many more spores than did males. Also, the first bee to chew through one or more cadavers was more heavily contaminated than were bees which emerged through previously macerated cadavers. However, newly emergent males which had not chewed through any cadavers carried as many as 100,000 spores on their body surfaces. This indicates that the spores may have come from contaminiated nesting material. Scanning electron micrographs of a newly emerged female revealed spores on all body parts examined from the antennae to the ano-genital opening. Spores were most densely packed about the mouth parts and on the underside of the thorax at the base of the legs (Figures 4a-d).

Spores are readily transferred from adult to adult during mating and upon casual contact. Spores are also scraped or knocked from the body during nesting, while resting at or on the domicile, and while foraging. Vaseline-coated slides placed beneath emergence traps, on exposed floors of field domiciles, and directly beneath media in which bees were nesting were generously covered with

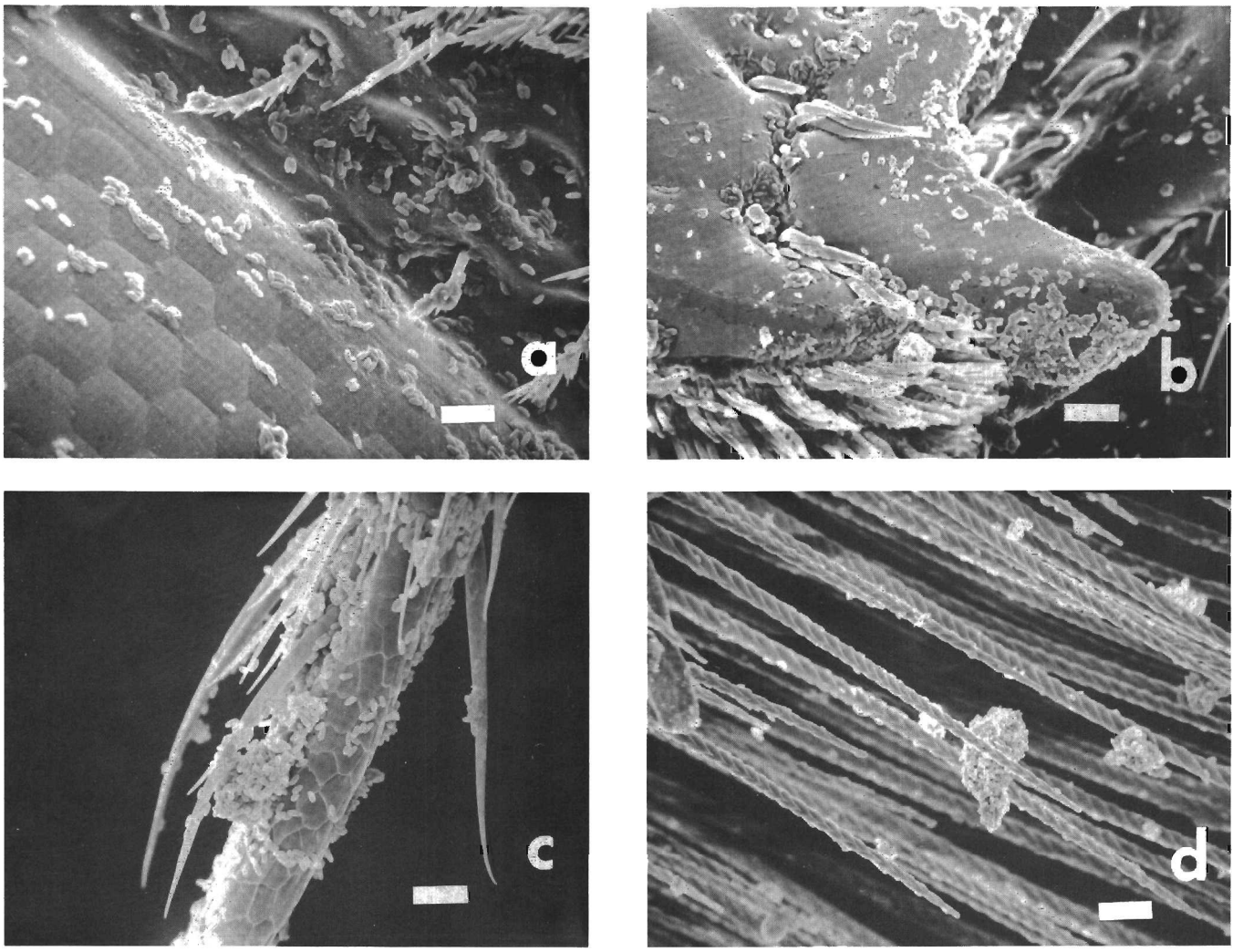

Figure 4. Scanning electron micrographs of a newly emergent female of *M. rotundata* showing chalkbrood *(A. aggregata)* spores on: a) eye and head, b) mandible, c) tarsus of front leg, d) pollen collecting hairs of abdominal scopa. Figures a and c: bar = 20 um, Figures b and d: bar = 35 um. (From Vandenberg et al., 1980).

spores within two hours (Table 1). As expected, there was a positive correlation between the incidence of chalkbrood and the number of spores observed on the slides (Site 2, Table 1, had the highest incidence of chalkbrood among all populations sampled). The area immediately under the emergence trap consistently had the highest airborne spore load, and swabs taken from the interior screens of the trap revealed massive numbers of spores—sufficient to contaminate any bee crawling over their surfaces. The relative number of spores indicated in Table 1 is at first rather unimpressive. However, if the counts made at site 2 on June 16 and 29 are averaged ($\pm$ 50 spores/36 mm^2/ 2 hr) we could expect to find a surface accumulation of approximately 6 million spores/square inch beneath the nesting medium by the end of the second week after emergence.

The number of spores at the trap and in the proximity of the incubator is surprising, for the incubator from which the samples were obtained contained only loose cells, all of which had been dipped in a 5 percent sodium hypochlorite solution. The source of the inoculum is believed to be partly from residual spores in the cells, spores in the leafy cell material concealed from the hypochlorite dip, and especially from cadavers in cell series which were not disrupted before dipping. Whereas bees emerging from isolated cells rarely chew into other loose cells adjacent to them, they will chew through cells immediately above them if the cells remain in an unbroken series. Thus, even in a loose-cell system, if series of cells are not separated before dipping and incubation, emerging bees will chew through cells above them, macerating chalkbrood cadavers in the process. Thus, spores washed from bees emerging from hypochlorite-dipped cells are both viable and non-viable; dipping may kill surface spores but not necessarily those from the cell and leafy material over which the bee must crawl.

Adult females were sampled from several populations at weekly intervals after emergence to ascertain if the spore loads carried on the body surface changed with time (Table 2). The inoculum load was found to decline

Table 1. Density of spores adhering to vaseline-coated slides exposed for 2 hours at various field locations near Corning, CA.

		Location of slides				
		under emergence trap	domicile			
			floor	under nesting medium		
Date	Site No.	1	1	2	3	4
16 June 1979		+ +	+ +	+ + +	+ +	+ +
		+ + +	+	+ + +	+ +	—
		+ +	—	+	—	—
		+ +	—	+		
				—		
				—		
29 June 1979		+ + +	—	+	+ +	—
		+ +	+	+ + +	+	—
		+ +	—	+	—	—
		+ +	—	+ +		—
				+		
				+ +		
17 July 1979		+ +	—		+	+ + +
		+ + +	—		+	+ + +
			+ +		+	+ + +
			+ +		+	
			—		—	

Relative number of spores on 36 mm² of slide.
— = no spores in sample
+ = a few spores ($\leq$ 10)
+ + = several spores ($10 \leq n \leq 50$)
+ + + = many spores (50)

Table 2. Age associated changes in spore loads on field collected adult female leafcutting bees

		Total spore load (x10⁴)	
Age (weeks)	# bees washed	Mean	Range
1st generation 0	10	120	26 - 199
1	10	13	1 - 34
3	10	23	6 - 37
5	10	12	6 - 22
2nd generation 0	10	51	15 - 121

rapidly from millions of spores at the time of emergence to tens of thousands three weeks later. It remained at the low level until the end of the first generation. However, the inoculum load carried by new or second generation females was heavy and airborne spore counts at the nesting site once again reached enormous numbers.

In contaminated nesting materials which are not phased out (solid boards or straws), macerated larval cadavers are pushed to the base of the nesting tunnel during emergence. Here they are a source of spore inoculum to females which accept that tunnel for renesting. In this case, the nesting medium serves as a secondary source of inoculum. However, it is evident that the disease agents are disseminated principally by the adult bee.

ETIOLOGY OF THE DISEASE

Only spores of the fungus are infective. Larvae fed on mycelia of *A. aggregata* did not contract the disease (Vandenberg and Stephen, 1982). Spores of the fungus are ingested by a developing larva and germinate in the gut in 12 to 24 hours. The mycelia penetrate the gut wall and pass into the body cavity where they proliferate, ultimately killing the larvae. Within 24 hours after death, larval hemolymph becomes milky from fungal mycelia and a pink, tan, or gray spot develops internally in one body area. The off-color cast spreads throughout the host in 24 to 48 hours and the body distends slightly. Sections made of larvae at this stage show dense mycelial mats just beneath the cuticle. Some mycelia appear to penetrate or be carried to the layer between the epidermal cells and the outer cuticular area. It is from this layer of mycelial cells that reproduction proceeds. The cell walls grow enormously to form the membranes of the spore cysts and the nuclei divide many times to fill the cysts with spores, clustered tightly together as spore balls (Kish, personal communication) (Fig. 5). The cuticle remains intact although distended by the spore cysts beneath, but occasionally the fungus erupts and grows to a limited extent on the pollen and fecal residues (Vandenberg and Stephen, 1982).

One- to nine-day old larvae were each fed doses of 10^4 to 10^6 spores to determine the stage of development at which they were susceptible to infection. Treated larvae and controls were surface sterilized as eggs and reared aseptically on sterile medium (Fichter et al., 1981). It had been assumed, because the spores can be germinated *in vitro* only in high CO_2 environments, that early instars of the bee (1 through 3) were the stages most readily susceptible to infection. This assumption was based on the existence of a blind gut in the first 3 larval instars (i.e.,there is no opening to the anus) in which the digestive processes in the gut result in a CO_2 build-up to stimulate germination. At the molt to the fourth instar (5-6 days from hatch at 30°C) the mid and hind guts join, defecation begins, and the aerobic condition of the gut is modified. Our data proved otherwise (Table 3).

Table 3. Effect of larval age of *M. rotundata* on susceptibility to infection with *A. aggregata* spores (from Vandenberg and Stephen, 1982)

		No. of Larvae			
Age in days	Treatment[a]	Treated	Surviving[b]	Chalk-brood	Other[c]
1	Control	11	9	0	2
	Fresh spores	14	0	14	0
4	Control	26	22	0	4
	Suspension	38	3	33	2
6	Control	8	7	0	1
	Suspension	17	2	14	1
	Fresh spores	9	0	8	1
9	Control	6	6	0	0
	Fresh spores	6	0	6	0

[a]Control larvae inoculated with buffer only. Suspended spores, 5-10 ul, applied at 10^4-10^5 spores/larva. Fresh spores applied at 10^5-10^6 spores/larva.
[b]Survival past the fourth instar, or the stage at which chalkbrood may be diagnosed.
[c]Undiagnosed mortality.

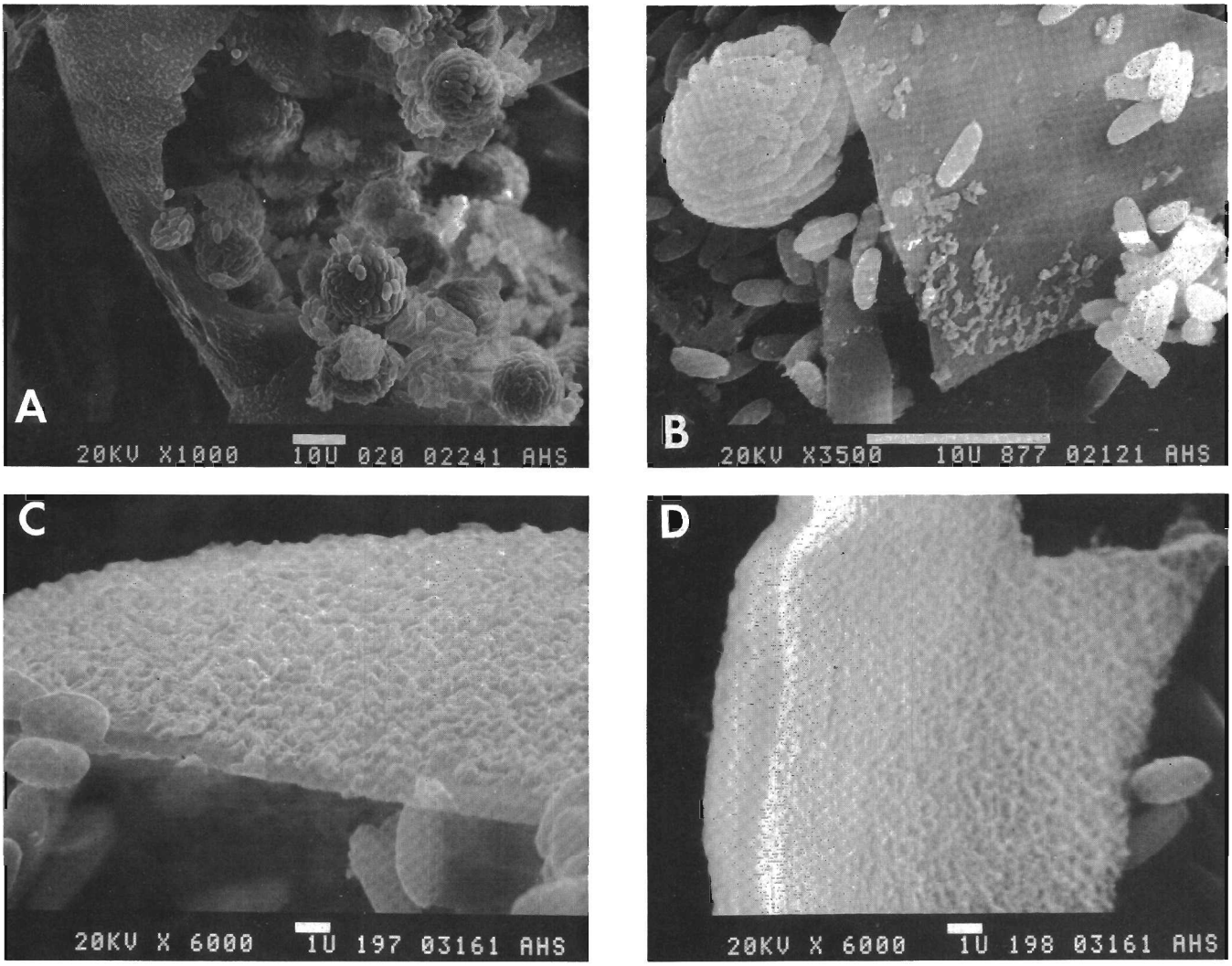

Figure 5. *A. aggregata.* A) portion of cyst showing marked outer wall and spore balls (WA 1980); B) portion of outer cyst wall showing smooth area and spore ball with mucoid covering on spores (ID 1979); C) heavily marked outer surface of cyst (OR 1980); D) ridged inner surface of cyst (OR 1980).

One-day-old *M. rotundata* larvae died approximately eight days after being fed spores, shortly before or after the molt to the fourth instar. No internal color change to pink, tan, or gray was observed in these younger larvae. Within 24 hours after cessation of movement, the hemolymph became cloudy and a cream colored mycelium was visible within the host. The larva retained the opaque color and hardened within a few days. The fungus failed to sporulate in any larvae infected during the first instar. Small, hard, cream-colored larvae have been noted frequently among field collected samples of leafcutting bee cells. All larvae from 4 (third instar) to 9 (mature fifth instar) days old were highly susceptible to infection (Vandenberg and Stephen, 1982).

OTHER *ASCOSPHAERA* SPECIES

As indicated earlier in this bulletin, the chalkbrood fungi are poorly known biologically and taxonomically. Only six species of *Ascosphaera* have been described, all isolated from honey bees or megachilids. Three have been recorded in North America; only two are known to be pathogenic. There is concern among beekeepers and leafcutting bee producers over the possibility of cross-infectivity among these fungi. Although preliminary data suggest that a low level of cross-infectivity may exist between the honey bee and *M. rotundata* pathogens, its extent has yet to be determined.

Ascosphaera apis—Principal causative organism of chalkbrood in honey bees; reported but unconfirmed pathogen of *Megachile* spp. in England (Melville and Dade, 1944) and from *Megachile inermis* in the United States (Baker and Torchio, 1968). The species is found throughout Europe, Canada, and the United States.

A. aggregata—Causative organism of chalkbrood in *Megachile rotundata, Osmia rufa,* and *M. centuncularis* (Skou, 1975). Found in North America, Europe, and Argentina.

A. atra—Saprophytic organism on pollen and feces in cells of *Megachile rotundata* (Skou and Hachett, 1979).

Known only from the western United States.

Three other species have been reported from Europe, but the known distribution and the number of described species simply reflect the limited study thus far accorded this genus.

A. major—Minor cause of chalkbrood in honey bees in Europe; a report of the species from honey bees in the United States was apparently an error (Gochnauer and Hughes, 1976). Taken from larval feces and between leaf pieces of cells of *M. centuncularis,* but considered to be a facultative parasite (Skou, 1972; Holm and Skou, 1972). Known only from Europe.

A. proliperda—Cause of chalkbrood in *M. centuncularis* (Skou, 1972; Holm and Skou, 1972). Known only from greenhouse cultured bee populations in Denmark.

A. fimicola—A saprophytic species taken from fecal pellets of *Osmia rufa.* Known only from Denmark (Skou, 1975).

All the described species except *fimicola* are in culture in our laboratory. Preliminary studies were made to determine the cross-infectivity of *A. apis, A. atra, A. major* and *A. proliperda* on *M. rotundata.* Quantities of spores of each species were placed directly on the surface of sterile medium in contact with aseptically reared early fourth instars. The results of the cross-infectivity studies are listed in Table 4.

Table 4. Response of *Megachile rotundata* larvae to inoculation with *Ascosphaera* spp.

	Number of Larvae				
Treatment	Treated	Prepupae	Chalk-brood	Over-grown[a]	Other[b]
Uninoculated control	8	7	0	0	1
A. aggregata	9	0	8	0	1
A. apis[c]	9	1	1	4	3
A. atra[c]	9	9	0	0	0
A. major[d]	9	6	0	2	1
A. proliperda[d]	9	0	2	6	1

[a]The fungus grew on the pollen provisions and the larvae died without becoming infected.
[b]Undiagnosed mortality.
[c]Oregon isolates. *A. apis* and *A. atra* subcultured on Sabouraud dextrose agar (SDA).
[d]Denmark isolates obtained from J. Rose, University of Wyoming, Laramie. Subcultured on SDA.

All the larvae fed with spores of *A. atra* matured to the prepupal stage confirming previous observations on the saprophytic status of this species. Eight of the nine larvae inoculated with *A. aggregata* died in the last instar with typical chalkbrood symptoms; the ninth died of undiagnosed causes. Although *A. major* is reported as a facultative parasite on the European *M. centuncularis,* none of the inoculated *M. rotundata* larvae developed chalkbrood symptoms. Rather, the fungus grew well on the diet and overgrew two larvae resulting in their death.

Inoculations of *M. rotundata* larvae with *A. apis* and *A. proliperda* both resulted in at least one larva succumbing with chalkbrood-like symptons. However, these symptoms were distinct from those produced by *A. aggregata.* The characteristic color changes associated with *aggregata* were not displayed; the larvae turned cloudy and white mycelium erupted through integumental membranes within 48 hours after death. Shortly thereafter, the larvae collapsed and the fungus sporulated on the outside surface of the host cuticle.

Characteristics used by Skou and others to distinguish the species of *Ascosphaera* from one another include spore length, diameter, the length/diameter ratio, spore ball and cyst diameter, and the architecture of the spore cyst membrane. Our observations of some of these characteristics are presented along with those of other workers in Tables 5 and 6. Scanning electron micrographs of five species of *Ascosphaera* are shown in Figures 5-9. Protoplasmic or mucoid coverings are evident on the spores of *A. aggregata, atra, major,* and *proliperda.* In addition, the spore balls of *A. major* are surrounded by material that does not appear to be a distinct membrane (Figure 8; cf. Gochnauer and Hughes, 1976).

We have examined the internal and external surfaces of cyst walls for these species of *Ascosphaera* (Figures 5-9). With the exception of *A. aggregata,* the outer cyst walls are smooth and the inner surfaces are variably marked with what appear to be crystalloid papillae. In *A. apis* the spots appear regularly arrayed, while those found on the inner cyst walls of *A. atra, major,* and *proliperda* are less regular in size and distribution. Both sides of the cyst walls of *A. aggregata* are marked. The outer wall may be heavily spotted with confluent large and small papillae, while in other isolates portions of the outer wall are smooth (Figure 5). The inner wall is characterized by many confluent ridges in an irregular array. Both *A. atra* and *proliperda* show evidence of a double wall structure (Figures 7 and 9; cf. Skou and Hackett, 1979; Hackett, 1980, p.13). These observations are tabulated along with those of other workers in Table 6. Because of the variable architecture of the cyst wall and the difficulty in describing its elements, we feel that more extensive studies are needed before this structure may be used as a taxonomic character (cf. Hackett, 1980, pg. 13).

While it may be possible to distinguish the species of *Ascosphaera* on the basis of spore size characteristics alone (Skou, 1972), our measurements show a considerable degree of variability and overlap in size distributions among species (Table 5). However, spore morphology data taken together with information on both habitat and cultural characteristics should allow a positive identification. Furthermore, host pathological signs may be diagnostic for certain species of *Ascosphaera.*

Bees indicated as hosts for species of *Ascosphaera* occur in 3 genera: *Apis, Megachile,* and *Osmia* (Table 5). *Ascosphaera major* was identified by Baker and Torchio (1968) on a species of *Anthophora* but this diagnosis is

regarded as dubious by Skou (1972). Other solitary bees are hosts for related fungi (Youssef and Parker, personal communication), so the overall picture has yet to be completed.

Table 5. Measurements of spore balls and spores of the *Ascosphaera* species

Fungus (host)	Source[a]	SPORE BALLS[b] n	$\bar{x}$ (s.d.)	range	SPORES[b] n	length $\bar{x}$ (s.d.)	length range	diameter $\bar{x}$ (s.d.)	diameter range	l/d[c]	References[d]
A. aggregata	OR 1979	—	—	—	50	4.5(0.4)	—	1.9(0.2)	—	(2.4)	1
(Megachile	Argentina	4	10.9(0.4)	10.4-11.5	30	4.0(0.3)	3.4-4.4	1.5(0.2)	1.4-1.7	2.7	2
rotundata)	ID 1979	4	11.8(0.8)	11.0-12.6	34	3.5(0.3)	2.9-4.1	1.3(0.2)	1.2-1.5	2.7	2
	OR 1980	—	—	—	65	3.8(0.4)	3.1-4.8	1.5(0.2)	1.4-1.7	2.5	2
	WA 1980	9	13.9(1.2)	12.0-15.5	35	3.8(0.4)	3.4-5.1	1.5(0.2)	1.4-1.7	2.5	2
(M. rotundata, M.centuncu-laris, Osmia rufa)	Spain and Denmark	—	17.1	10-25 (93%: 12-22)	—	5.2	3.8-6.8 (90%: 4.5-6.0)	2.0	1.3-2.6 (90%: 1.5-2.5)	2.6	3
A.apis	OR 1978	5	12.1(1.4)	10.0-13.4	37	2.5(0.2)	2.2-3.1	1.2(0.1)	1.0-1.3	2.2	2
(Apis mellifera,	OR 1980	10	12.1(3.3)	8.4-17.4	35	2.6(0.2)	2.8-2.9	1.2(0.1)	1.2-1.4	2.2	2
Megachile sp. ?)	Denmark	—	12.5	7.0-18.0	—	2.7	2.0-3.5	1.4	1.0-2.0	1.9	4
	Canada	—	16.0	13.0-19.4	—	2.6	2.3-2.8	1.9	1.7-2.1	(1.4)	5
	England	—	—	—	—	3.2	3.0-3.8	1.9	1.5-2.3	(1.7)	6
	Germany	—	—	—	—	—	3.0-3.5	—	1.5-1.9	(1.8-2.0)	7
A. atra	OR 1979	—	—	—	50	7.5(0.6)	—	3.5(0.7)	—	(2.1)	1
(M. rotundata Pollen)	OR 1980	9	10.7(1.4)	8.0-12.0	50	5.8(0.4)	5.0-6.5	2.6(0.2)	2.5-3.0	2.2	2
	NE 1974	—	12.7	9-18 (91.5%: 10-16)	—	7.3	4- 9 (77%: 6-8)	3.6	2 -5 (84%: 2-4)	2.0	8
A. fimicola (O. rufa feces)	Denmark	—	10.3	6.6-15.5 (94%: 8-14)	—	3.7	2.6-4.7 (90%: 3.0-4.5)	1.7	1.1-2.6 (90%: 1.5-2.0)	2.2	3
A. major	Denmark	—	16.4	9-24	—	3.4	3.0-4.0	1.3	1.0-1.5	2.6	4
(A. mellifera,	Denmark	—	14-18	11-20	—	—	3.2-4.0		1.3-1.4	(2.5-2.9)	5
M. centuncularis)	(WY 1978)[e]	11	14.7(1.6)	12.8-17.9	80	3.3(0.3)	2.7-4.1	1.2(0.2)	1.0-1.4	2.8	2
A. proliperda	Denmark	—	17.0	11-25	—	5.5	3.5-7.5	2.5	1.7-3.5	2.2	4
(M. centuncularis)	(WY 1978)[e]	14	13.2(1.9)	10.8-16.0	60	5.0(0.5)	4.1-6.2	2.0(0.2)	1.5-2.6	2.5	2

[a]All *A. aggregata* material and that of *A. apis* OR 1978 was taken from cadavers. All other material was taken from cultures except *A. fimicola* from feces. OR = Oregon, ID = Idaho, WA = Washington, NE = Nevada, WY = Wyoming, USA.

[b]All measurements in micrometers. n = number measured, $\bar{x}$ = mean, s.d. = standard deviation. All measurements for this study made from scanning electron micrographs.

[c]l/d = average length/average diameter. Numbers in parentheses indicate our own computations from previously published measurements.

[d]References: 1. Vandenberg et al., 1980. 2. this study. 3. Skou, 1975. 4. Skou, 1972. 5. Gochnauer and Hughes, 1976. 6. Spiltior and Olive, 1955; Spiltoir, 1955. 7. Prökschl, 1953. 8. Skou and Hackett, 1979.

[e]Cultures obtained from J. Rose, Univ. Wyoming, Laramie. These species have not been reported from North America (Gochnauer and Hughes, 1976; Skou, 1972).

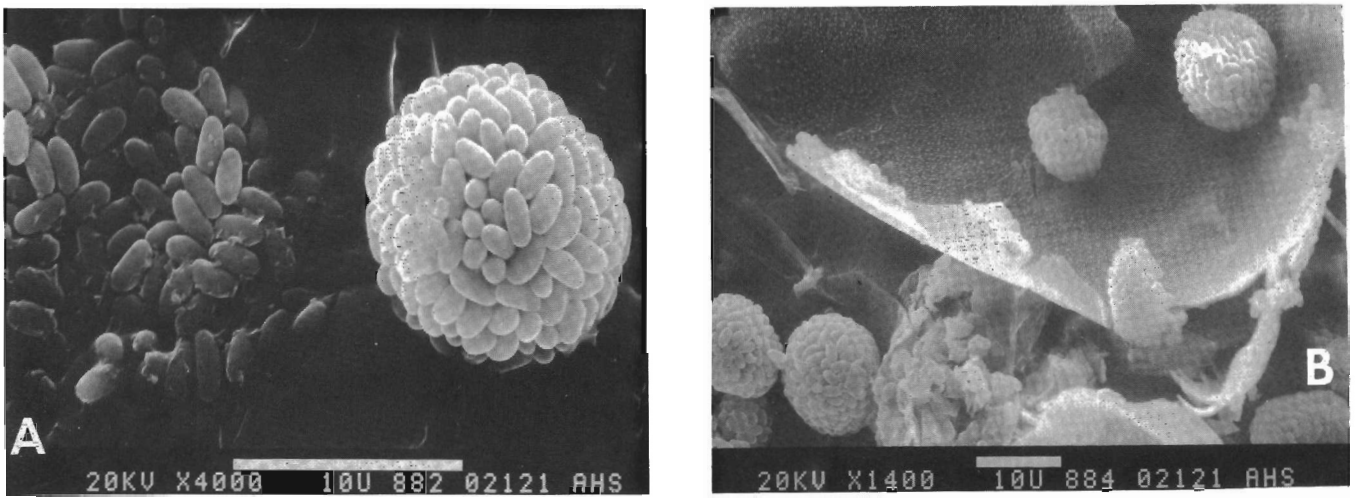

Figure 6. *A. apis.* A) spores and spore ball; B) portion of cyst showing smooth outer surface and regular array of papillae on the inner surface (OR 1978).

Table 6. Cyst Wall Characteristics for six species of *Ascosphaera*

Species	Characteristics	Reference
A. aggregata	inner: shallow ridges, irregular outer: large and small confluent papillae to smooth in parts	this study (Fig. 5)
	finely spotted	Skou, 1975
A. apis	inner: numerous papillae, regular outer: smooth	this study (Fig. 6)
	finely verrucous	Skou, 1972
	inner: numerous crystalloid papillae outer: smooth with slight rounded papillae	Gochnauer and Hughes, 1976
A. atra	inner: irregular, large papillae outer: smooth, double membrane apparent	this study (Fig. 7)
	double membrane with spots (crystalloid)	Skou and Hackett;1979
A. fimicola	distinctly verrucous	Skou, 1975
A. major	inner: irregular large and small spots to smooth in parts outer: smooth, with irregular ridges	this study (Fig. 8)
	indistinct spotting, hardly verrucous	Skou, 1972
A. proliperda	inner: irregular spotting, uniform size outer: smooth, double membrane apparent	this study (Fig. 9)
	verrucous with confluent large and small warts	Skou,1975

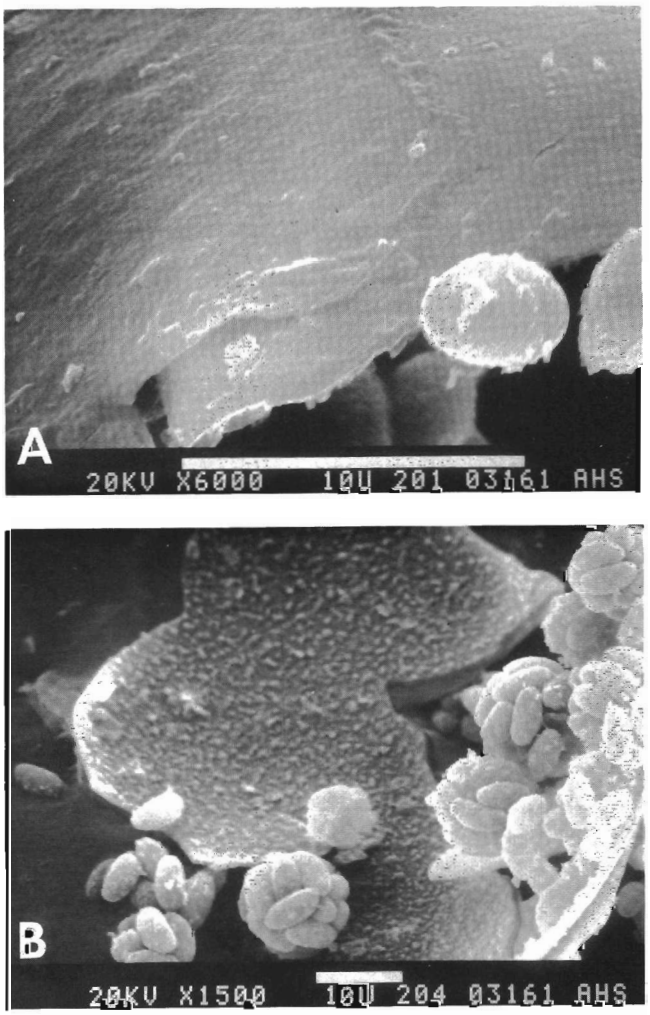

Figure 7. *A. atra.* A) smooth outer surface of cyst with double membrane apparent; B) portion of cyst with irregular papillae on inner surface and mucoid covering evident on spores (OR 1980).

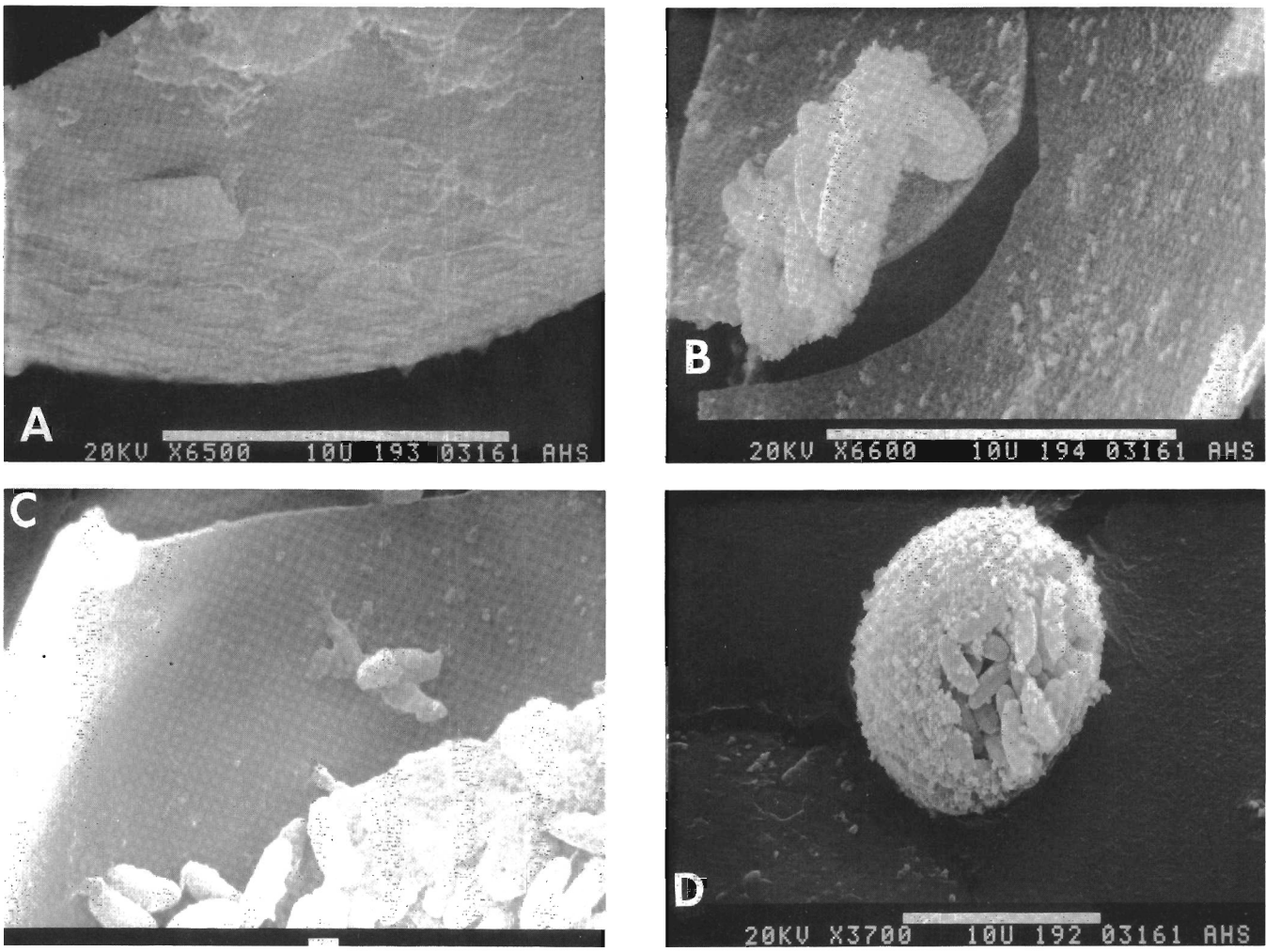

Figure 8. *A. major.* A) ridged outer surface of cyst wall; B) variably marked inner surface of the same cyst; C) smooth inner surface of another cyst; D) spore ball showing crust-like covering (WY 1978).

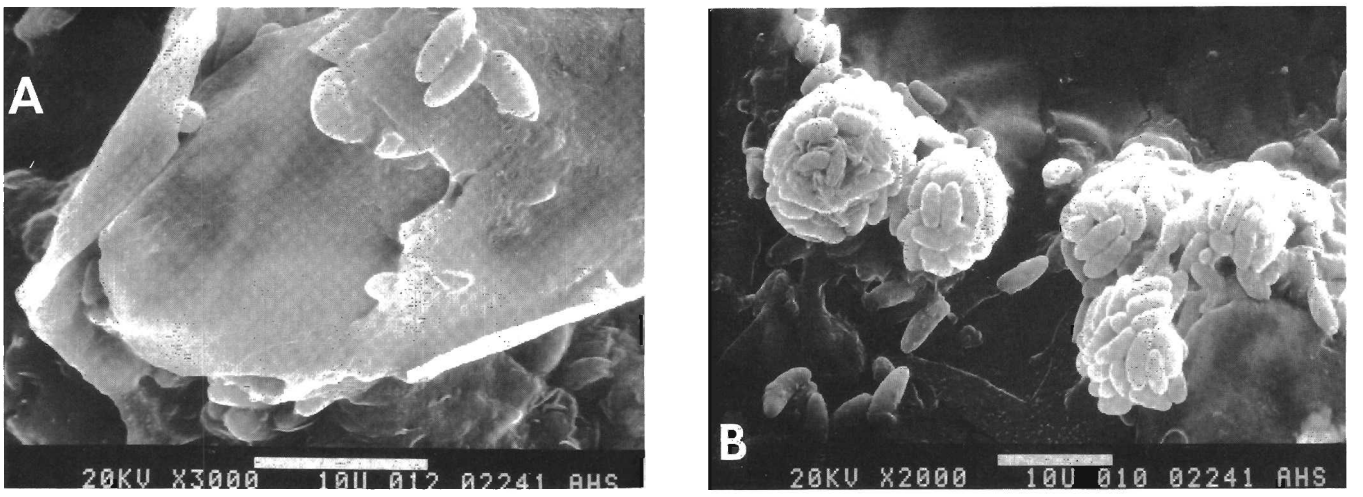

Figure 9. *A. proliperda.* A) portion of a cyst showing smooth outer surface with double membrane apparent; B) spore balls and portion of spotted inner cyst wall (bottom) (WY 1978).

LITERATURE CITED

Bailey, L. 1963. Infectious diseases of the honey bee. Land Books Hutchinson, London.

Baker, G. M. and P. F. Torchio. 1968. New records of *Ascosphaera apis* from North America. Mycologia 60:189-190.

Batra, L. R., S. W. T. Batra, and G. E. Bohart. 1973. The mycoflora of domesticated and wild bees (Apoidea). Mycopathologia et Mycologia Applicata 49:13-44.

Claussen, P. 1921. Entwicklungsgeschichtliche Untersuchungen über den Erreger der als "Kalkbrut" bezeichneten Krankheit der Bienen. Arbeiten aus der Biologie Reichsanstalt für Land-und Forstwirtschaft 10:467-521.

Clout, G. A. 1956. Chalkbrood and hunchback flies. Bee Craft 38:135.

DeJong, D., and R. A. Morse. 1976. Chalk brood: a new disease of honey bees in the U. S. Food and Life Sciences Quarterly N.Y. 9:12-14.

DeJong, D. 1977. A study of chalk brood disease of honey bees. M. S. Thesis, Cornell University (unpublished).

Fichter, B. L., W. P. Stephen, and J. D. Vandenberg. 1981. An aseptic technique for rearing larvae of the leafcutting bee, *Megachile rotundata* (Hymenoptera, Megachilidae). Journal of Apicultural Research 20(3):184-188.

Gochnauer, T. A. and S. J. Hughes. 1976. Detection of *Ascosphaera apis* in honey bee larvae from eastern Canada. Canadian Entomologist 108:985-988.

Hackett, K., D. Briggs, R. Thorp, and D. Pinnock. 1977. *Megachile* chalk brood research (1976). 8th Annual Interstate Alfalfa Seed School, Winnemucca, Nevada. pp. 14. 1-14.6. Extension Service, University of Nevada, Reno.

Hackett, K. J. 1980. A study of chalkbrood disease and viral infection of the alfalfa leafcutting bee. Ph. D. Dissertation, University of California, Berkeley.

Holm, S. N. and J. P. Skou. 1972. Studies on trappng, nesting, and rearing of some *Megachile* species and their parasites. Entomologica Scandinavica 3:169-180.

Kish, L. P. 1980. Spore germination of *Ascosphaera* spp. associated with the alfalfa leafcutting bee, *Megachile rotundata.* Journal of Invertebrate Pathology 36:125-128.

Melville, R. and H. A. Dade. 1944. Chalkbrood attacking a wild bee. Nature 153:112.

Prökschl, H. 1953. Beitrage zur Kenntnis der Entwicklungsgeschichte von *Pericystis apis* Maassen. Archiv für Mikrobiologie 18: 198-209.

Skou, J. P. 1975. Two new species of *Ascosphaera* and notes on the conidial state of *Bettsia alvei.* Friesia 11:62-74.

Skou, J. P. 1972. Ascosphaerales. Friesia 10:1-24.

Skou, J. P. and K. Hackett. 1979. A new homothallic species of *Ascosphaera.* Friesia 11: 265-271.

Spiltoir, C. F. 1955. Life cycle of *Ascosphaera apis.* American Journal of Botany 42: 501-508.

Spiltoir, C. F. and L. S. Olive. 1955. A reclassification of the genus *Pericystis* Betts. Mycologia 47: 238-244.

Stephen, W. P. and J. M. Undurraga. 1978. Chalk brood disease in the leafcutting bee. Oregon State University Agricultural Experiment Station Bulletin 630.

Thomas, G. M. and A. Luce. 1972. An epizootic of chalk brood, *Ascosphaera apis,* in the honey bee, *Apis mellifera* L., in California. American Bee Journal 112: 88-90.

Thomas, G. M. and G. O. Poinar. 1973. Report of diagnoses of diseased insects, 1962-1972. Hilgardia 42: 261-359.

Vandenberg, J. D. and W. P. Stephen. 1982. Etiology and symptomatology of chalkbrood in the alfalfa leafcutting bee, *Megachile rotundata.* Journal of Invertebrate Pathology (in press).

Vandenberg, J. D., B. L. Fichter, and W. P. Stephen. 1980. Spore load of *Ascosphaera* species on emerging adults of the alfalfa leafcutting bee, *Megachile rotundata.* Applied and Environmental Microbiology 39: 650-655.