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Original article

Chalkbrood *(Ascosphaera aggregata)* resistance in the leafcutting bee *(Megachile rotundata)*. I. Challenge of selected lines

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Summary — Twenty-nine cell series of the leafcutting bee, *Megachile rotundata* (Fabr), each containing 5 or more healthy larvae, no chalkbrood (*Ascosphaera aggregata* Skou) and no pollen masses were isolated from a population with over 36% chalkbrood. This selected subpopulation was split and isolated for 4 and 6 generations before being challenged by forcing the females to nest in heavily contaminated media, or by weekly dustings with approximately 175 x 10^8 *A aggregata* spores. The incidence of chalkbrood in both challenged lines was markedly lower than that of the wildtype, and comparable to that of the selected lines, suggesting a genetic component for resistance in both lines. The very low incidence of pollen masses (dead eggs or early instar larvae) in the selected lines throughout the 4 yrs of the study indicates that this trait may also be genetically mediated, either linked to, or independent of disease resistance.

chalkbrood / *Ascosphaera* / leafcutting bee / resistance / *Megachile*

INTRODUCTION

Few studies have been conducted on resistance in endemic insects to naturally occurring pathogens. Most data deal with susceptibility differences among laboratory populations of insect pest species to viral (Martignoni and Schmid, 1961; Klein and Podoler, 1978) and bacterial pathogens (Harvey and Howell, 1965; Hoage and Rothenbuhler, 1966; Kinsinger and McGaughey, 1979; Georghiou *et al*, 1983). Among non-pest species, extensive studies have been conducted on resistance to viral pathogens among strains of *Bombyx mori* L (Watanabe, 1967; Briese, 1981), and 3 reports describe resistance to the "hairless-black syndrome" in honey bees (Bailey, 1965; Kulincevic and Rothenbuhler, 1975; Rinderer and Green, 1976). Resistance to chalkbrood, *Ascosphaera apis*, among colonies of honey bees was suggested by DeJong (1976), and large differences in susceptibility among silkworm populations to *Beauveria bassiana* (Aratake, 1961) and *Aspergillus flavus* (Kawakami, 1975) have been noted. The only direct selection for resistance to an entomogenous fungal pathogen cited in current literature is the study by Vansulin

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(1974) in which the mortality in *Culex pipiens molestus* is halved after 8 generations of exposure to constant doses of *B bassiana*.

Megachile rotundata (Fabr) is a gregarious Eurasian bee first recorded from the eastern United States in the mid 1930's (Mitchell, 1937). It spread rapidly across temperate North America reaching the western states by the late 1950's (Daly, 1952; Hurd, 1954). In 1959, when the bee was first recognized as a domesticable oligolege on alfalfa (Stephen, 1961; Stephen and Torchio, 1961), it was apparently ubiquitous throughout the Pacific Northwest with its numbers limited by the availability of suitable nesting sites. Populations increased rapidly through trap nesting and, by 1962, large commercial operations in bee sales were established.

The leafcutting bee propagation industry has been centered in western Canada for more than a decade with annual commercial sales of 200 to 300 million cells, principally to alfalfa seed producers in the western United States. We suggest that these importations, extensive local and regional commerce, as well as the tendency of the species to disperse widely have prevented the development of locally adapted populations of the bee. The result is a near continuous array of highly adaptable, poorly adapted local populations throughout western America. The rate at which chalkbrood has spread tends to support this idea.

Chalkbrood is a fungal disease of leafcutting bee larvae (*Megachile rotundata* (Fabr) caused by *Ascosphaera aggregata* Skou, and was first identified from Nevada populations in 1973 (Thomas and Poinar, 1973). It spread rapidly throughout all areas of the western United States in which the bee was propagated and is now cosmopolitan (Stephen *et al,* 1981). This disease is the principal limiting factor to bee production, accounting for losses up to 75% in some areas of the Pacific Northwest (Stephen *et al,* 1981). Chalkbrood is spread by spores in contaminated nesting media and by spores carried on the body surface of adult bees (Vandenberg *et al,* 1980). Spores deposited in cell provisions are ingested by a developing larva and germinate in the gut. The mycelia penetrate the gut wall and rapidly fill the hemocoel (Vandenberg and Stephen, 1982; McManus and Youssef, 1984). At present, there is no effective control for the disease other then prophylactic measures directed at reduction of inoculum (Stephen and Undurraga, 1978; Kish *et al,* 1981).

Although various materials are used as nesting media for the bee, paper soda straws are the medium of choice for population monitoring. Selected soda straws can be removed from a box without disruption of the cell series and their contents can be evaluated through X-ray analysis (Stephen and Undurraga, 1976). The cells in a nesting tunnel (from 1–12) are usually those of a single female. We examined thousands of straws while monitoring bee populations in the western United States for chalkbrood. In heavily diseased populations, cadavers occur in a near-random distribution within and among straws, but occasionally an entire series of from 5–10 cells in a single straw will be found to be disease-free. As only 500 to 1000 spores constitute an LD_{50} (Fichter and Stephen, unpublished), and nearly 10^8 spores are carried by a single female (Vandenberg *et al,* 1980), it is highly improbable that an entire nesting tunnel in a heavily diseased population could escape inoculation.

Thus, the premise of these studies was that, if all the progeny of a single female were chalkbrood-free in a population with more than 35% diseased larvae, genetically–mediated factors for disease resistance may be present in the population. Our ob-

jectives were to demonstrate that intrapopulation differences in pathogen susceptibility exist and that through selection and challenge, disease resistance lines may be established.

MATERIALS

Oregon stock

A population of leafcutting bees near Adrian, Oregon with 36.1% chalkbrood was chosen as the wild type (WT) from which selections were made. Approximately 10 000 straws were X-rayed at the end of the 1977 season. Only straws containing 5 or more healthy larvae and no chalkbrood or pollen masses were selected: a total of 29 straws with from 5–9 live cells each formed the base population from which expansion was made.

Study sites

The straws were incubated and placed out for increase in 1978 at 2 sites near Los Molinos, CA. The sites were approximately 7 km apart with walnut and almond orchards intervening. Both sites were carefully surveyed and trap-nested in 1977 and no *M rotundata* were found. Approximately half of the selected material was placed at each site with new, brown paper soda straws provided for renesting. Cells from the 2 sites were designated Lines A and B. Los Molinos was selected as the principal experimental area because of its long, dry summer season during which at least 3 generations of the leafcutting bee could be reared and because of the relatively low endemic bee populations. All but 2 (original) replication sites had to be abandoned during the 4-year study period because our selected lines were swamped by in-flight from endemic bee populations. Thus, replications were confined to data gathered from individual cartons placed at different locations at the same site. Outbuildings at each site were sprayed with Dibrom in late May and/or early June of each year to eliminate any residual population of bees. A 2nd location near Nyssa, OR was utilized to test the protocol for the chalkbrood challenge studies.

Expansion and selection

All bees were reared in 9 cm long x 5.5 mm diameter brown, paper soda straws packed tightly in cartons with approximately 250 straws each. Tight packing permitted the removal of capped straws and replacement with new without altering the position of other straws. Each spring, bees were incubated at 30 °C until emergence of the first males after which they were placed into the field. Every 10 d after field placement, capped straws were color-coded and left *in situ* for an additional 20 d to mature. They were then removed from the carton and returned to the laboratory for X-ray analysis. Live larvae, chalkbrood, dead larvae, pollen masses (= provisioned cells with no eggs or with dead eggs or early instar larvae), and parasitism were recorded. Any straw containing a chalkbrood cadaver was removed and destroyed during the expansion phase. No spore challenges were made during the 1st 4 generations of expansion. Cells parasitized by *Tetrastichus* spp were removed from each straw to avert a build-up of parasites during the season. The remaining straws were returned to their original sites for emergence and renesting of the subsequent generation.

METHODS

Experiment I

To ascertain whether the selected lines were resistant to chalkbrood, the 4th expansion generation was forced to nest in soda straws heavily contaminated with chalkbrood. Six straw cartons, used for propagation of the Adrian wild type (= WT) population in 1977 and 1978 and heavily contaminated with chalkbrood, were selected for this test. Each carton contained approximately 220 straws, all of which were capped indicating that each had been occupied (and presumably provisioned) at some time during the 2 yr period. Two cartons were destruc-

tively sampled for 150 straws each and 30 capped straws were selected haphazardly from each of the remaining 4 cartons. All straws were X-rayed to determine live larvae, chalkbrood and pollen masses. Two of the latter cartons were laboratory incubated in March 1979 to remove all live bee larvae and parasitoids; and the remaining 2 were kept as Adrian 1979 WT Controls.

Incubation of the 2 WT cartons (above) resulted in emergence from fewer than 30% of the straws, *ie* the caps were not disrupted on the remaining straws suggesting that they contained only chalkbrood cadavers, pollen masses, and dead larvae. As nesting females will only accept tunnels which are uncapped or those in which the cap has been disrupted, and, as our objective was to expose the renesting selected population to a maximum amount of inoculum, the caps of all straws were pierced with a 3 mm x 10 cm reamer and the contents of the straw carefully disrupted along its entire length. Care was taken to minimize the loss of inoculum during the reaming process. Thirty-three and 34 straws containing 220 and 221 live larvae of the 4th expansion generation (Select Line B) were introduced into these 2 WT cartons and placed in a field south of Gerber, CA. Emergents were thus forced to accept contaminated nesting media.

Caps of all straws in the 2 1979 WT Control cartons were pierced and disrupted to a depth of approximately 1.25 cm so as to provide the emerging population with access to an adequate supply of tunnels. Such disruption was shallow enough to avoid damage to most if not all cells in each straw, yet deep enough to provide searching females with ready access to the tunnel. To maintain isolation it was necessary to locate the WT Controls in a field 24 km west of Los Molinos. Controls for Select B 5th expansion generation were re-established at the Los Molinos site.

Emergence began on July 23 and nesting was allowed to continue until August 31 (table I). Capped straws in each of the 6 cartons were painted on August 11 and August 31, permitted to develop for 2 weeks under field conditions, and then removed for X-ray analysis. Cells of all capped straws of each population were evaluated for live larvae, chalkbrood and pollen masses. The pollen mass category was included because it was suspected (and later confirmed [Fichter and Stephen, unpublished]) that early instar larvae could be killed after ingesting large amounts of *A aggregata* inoculum. Second (and often early third) instar larvae killed by the fungus are difficult and often impossible to identify on the X-ray plate. Thus, any abrupt decrease in the incidence of 5th instar chalkbrood cadavers which may be accompanied by a comparable increase in pollen masses could reflect a difference in the stage at which mortality occurred rather than a real diminution of the disease.

Experiment II

An evaluation of the efficacy of the chalkbrood challenging procedure under field conditions was undertaken in 1980 on 2 populations of leafcutting bees located north of Nyssa, Oregon.

The Nyssa site was selected because local Californian populations of the size necessary for these tests did not exist, and because we wished to avoid any introductions which would jeopardize the isolation of our selected lines. One population was derived from a 1979 chalkbrood-free Canadian source; the 2nd from regionally trapped bees with a low incidence of chalkbrood (7.2% at the end of 1979). Each population was housed in different domiciles and was propagated in drilled wooden boards from which cells could be removed.

Inoculum preparation

Inoculum was prepared using 1977 Adrian wild type (WT) paper soda straw nesting medium. X-ray analyses indicated that each carton contained approximately 700 chalkbrood cadavers (table I). All straws were removed from 1 carton and macerated in a blender. The residual paper was then removed, the contents passed through a 10-mesh screen to remove pieces of leaf matter and matted pollen, and the screenings thoroughly pulverized in a mortar. One quarter of the pulverized contents of each WT carton was dusted into each straw carton prior to field placement, and at weekly intervals thereafter. A hand-operated piston-type duster was used for spore inoculation. It is estimated that each cadaver produces from 10^8–10^9 spores (Stephen,

Table I. Selected line B of *M rotundata* challenged with contaminated nesting medium (Los Molinos, CA 1979).
* Total is less than 100% as cells parasitized and/or with dead larvae are not included.

Material	*Sample date*	*% Live larvae*	*% Chalk brood*	*Pollen mass %*	*Total cells*
Select line B 1979 4th expansion gen	6/02–21	92.3	2.3	3.6*	478
Adrian Wild Type (WT) Contaminated Stock	1977–78	14.6	55.1	30.3	2 320
Select B – renest in 1978 WT straws	7/23–8/31	77.7	12.1	2.9	1 268
Select B Control 5th expansion gen	7/23–8/31	91.6	3.4	3.1	1 537
Adrian WT control renest in WT 1979	7/23–8/31	33.1	33.4	26.0	329

unpublished) and each carton received approximately 175 x 10^8 spores weekly.

The experiments were initiated on July 18 1980, approximately 2 wks after each population had emerged and when the bees were actively nesting. One board from each of 4 field domiciles containing active nesting populations of either Canadian (2 domiciles) or Nyssa (2 domiciles) females was removed on the morning of July 18 and was replaced with 2 new straw cartons inoculated with 1977 Adrian WT inoculum prepared as described above. Two boards were removed from the far side (3–5 m distant) of each of the same domiciles and replaced with 4 straw cartons to serve as controls. Females from the displaced boards began provisioning the inoculated straws by late morning. Straws were dusted with Adrian WT inoculum at approximately weekly intervals (4 applications) until August 10, when flight had ceased. Test straws were removed from the field on August 20. Four cartons located approximately 3 m from the test cartons in each field domicile were removed on September 3 and served as controls for each population. Forty capped straws were removed from each of the Nyssa challenged cartons, 50 from each of the Canadian challenged cartons, and 50 from each carton serving as controls to the 2 populations to determine the effect of the challenging method on the incidence of chalkbrood, live larvae and pollen masses. Selection of capped straws was not random for the number of capped straws ranged from 42–177 per carton among the 24 cartons used in this test.

Experiment III

Chalkbrood resistance in the 6th expansion generation of selected line A was field evaluated by challenging with inoculum.

In June 1980, 136 straws containing 791 live larvae of the 6th expansion generation were divided with equal numbers placed in each of 4 cartons of new straws prior to emergence. Because of limited isolation sites, each carton was located on different out-buildings of adjacent farmyards, separated by distances of 150–750 m. Inoculum to challenge the selected line was prepared as described above. One-fourth of the pulverized contents of each 1977 Adrian WT

carton was dusted into each of the 4 cartons containing selected bees prior to their emergence. This procedure was repeated weekly for 8 wks with each application of inoculum made in the morning before females began foraging. Dusting was terminated on August 8 and cartons removed for analysis on August 19. An isolated population of the selected line served as a Select Line A 1980 control (7th expansion gen). Four cartons of straws were each supplied with 200 cells from the same susceptible Canadian bee population used in Exp II. In this experiment 2 cartons served as "challenge" controls (Canada, 1980) and were inoculated as described above. Two cartons were isolated in a field 11 km south of Corning as controls. Cartons were removed from the field between August 19 and 21. All capped straws from each treatment were X-ray analysed for live larvae, chalkbrood and pollen masses.

RESULTS

The progeny of selected line B females forced to nest in heavily contaminated straws (Exp I) showed an increase in chalkbrood from 2.3 to 12.1%, a decrease in live larvae from 92.3 to 77.7% and no change in the proportion of pollen masses. Chalkbrood increased 10% in the Select B line as a result of the challenge, but this was more than 21% less than the disease incidence in the unselected population. The controls for the selected line (Select B 5th expansion gen) were almost identical to the 1978 populations from which it was taken (Select B 4th expansion gen) (table I). Analysis of the Adrian WT contaminated straws 1978 was made prior to the laboratory emergence of bees and represents the status of the material used both as the source for inoculum and as the Adrian WT 1979 control for this experiment (table I). Analysis of the Adrian controls was confined to those straws in which females had nested during the 1979 season, as the inclusion of all previous cadavers and pollen masses in the calculations would have highly biased the data. This accounts for the apparent doubling of live larvae and sharp reduction in chalkbrood incidence in the Adrian WT 1979 control compared to those categories in the Adrian WT 1978 Stock. The latter data include all cells constructed during both the 1977 and 1978 seasons (table I).

Data in table II indicate that the method of field inoculation for chalkbrood was effective. Chalkbrood in the unchallenged endemic population increased from 7.6 to 15.7% during 1980, while that of the challenged rose to 27.1%. The incidence of disease in the challenged Canadian material was 26.4% whereas that of the unchallenged was 3.8%. Both challenged populations had fewer live larvae and fewer pollen masses than the controls (table II).

There was little difference between challenged and control populations on selected Line A in chalkbrood (5.0 *vs* 2.8%) or pollen masses (3.2 *vs* 2.8%) (table III). The proportion of live larvae remained high in both populations (90 and 93%) both higher than that of the populations from which they were derived. The spore challenges, however, resulted in 27.8% chalkbrood in the chalkbrood-free Canadian material used as the challenge control (table III). Chalkbrood incidence in subsamples of the Canadian population challenged in Oregon (Exp II) and in California (Exp III) was nearly identical (26.4 *vs* 27.8%) even though the latter was given 2 more treatments of inoculum (the population had died off by the 1st wk in August). The Canada (80) challenged population suffered bird damage during the 1st 2 wks of flight and thus reproduction was lower than expected. The Canadian 1980 controls were swamped by inflight of endemic *M rotundata* beginning during the 2nd wk (table III).

Table II. Unselected lines of *M rotundata* challenged with chalkbrood inoculum weekly for 5 wks. (Nyssa, OR 1980).
* Total is less than 100% as cells parasitized and/or with dead larvae are not included. ** Overwintering population from which samples were drawn for 1980 tests.

Material	*Sample date*	*% Live larvae*	*% Chalk brood*	*Pollen mass*	*Total % cells*
Nyssa ('79) Wild type**	1979	60.8	7.6	20.6*	992
Nyssa ('80) Challenge	18 July–20 Aug	50.0	27.1	17.6	642
Nyssa ('80) Control	18 July–03 Sept	57.6	15.7	21.2	827
Canada (79) Wild type**	1979	89.3	0	6.7	1 234
Canada (80) Challenge	18 July–20 Aug	54.0	26.4	16.2	893
Canada (80) Control	02 July–03 Sept	62.4	3.8	21.2	911

Table III. Selected line A of *M rotundata* challenged with chalkbrood inoculum weekly for 8 wks. (Los Molinos, CA 1980).
* Total is less than 100% as cells parasitized and/or with dead larvae are not included.

Material	*Sample date*	*% Live larvae*	*% Chalk brood*	*Pollen mass*	*Total % cells*
Select line A 1979 6th expansion gen	1979	86.2	4.9	3.8*	917
Select line A 1980 challenged	19 Aug 1980	90.3	5.0	3.2	3 448
Select A control 7th expansion gen	19 Aug 1980	93.1	2.8	2.8	1 175
Canada Wild type	1979	89.3	–0–	6.7	1 234
Canada 1980 challenged	19 Aug 1980	64.8	27.8	5.5	907
Canada 1980 control	(population swamped by inflight)				

DISCUSSION

The method used to challenge nesting female bees with inoculum appears to the effective; the incidence of chalkbrood in both the Nyssa and Canadian lines, treated identically over 4 wks, increased from 7.6 to 27.1% and 0 to 26.4% respectively (table II). The chalkbrood increase from 7.6 to 15.7% in the endemic line is consistent with the expected rate of disease increase in an isolated population (Stephen unpublished), but the 3.8% chalkbrood in the unchallenged Canadian population after 1 yr of expansion is considerably less than expected. We assume that inflight of contaminated endemic adults to this site was minimal. The efficacy of the method is further supported by the increase in chalkbrook from 0 to 27.8% as a result of challenging a subsample of the same chalkbrood-free Canadian population (above) in California (table III).

Progeny of Line A females which were dusted with spores from the equivalent of 179 cadavers each wk for 8 wks, did not differ appreciably in chalkbrood, live larvae or pollen masses from those of the control or the 1979 stock from which they were derived (table III). This strongly suggests that a genetic component for disease resistance is present.

Chalkbrood was much higher in the progeny of females renesting in heavily contaminated soda straws (Exp I) than in those of females challenged with chalkbrood spores by dusting (Exp III) (12.1 *vs* 5.0%). This may suggest that : Line A was more resistant to the disease than Line B as Line A was in its 6th expansion generation and Line B in its 4th when challenged; or, the 2 methods of challenging differ in their efficacy – spores were applied at weekly intervals in the latter and were present continuously from the onset of nesting in the former (Line B). Although both lines A and B were derived from the same population, each was expanded from a limited gene pool, the progeny of 14 and 15 females respectively. It is improbable that factors conferring disease resistance exhibit any form of dominance, for there is no evidence of the rapid spread of the trait during the 10 years chalkbrood has been a problem. If we assume that resistance in these two lines is recessive and polygenic, it is probable that allelic components for resistance in the 2 lines differed at the time of testing.

Chalkbrood in the challenged chalkbrood–free Canadian population was comparable (26.4%) to that of the challenged endemic bees (27.1%) (table II). There is a widely held belief among leafcutting bee consumers in the USA that Canadian bees are far more susceptible to chalkbrood than endemic US populations. While Exp II was not directed towards answering that question, the data suggest that if differences in susceptibility exist, they are minor, at least in the Canadian population used in this study.

An interesting observation in these experiments relates to pollen masses. As indicated above, pollen masses result from the absence or death of an egg or early instar larva, leaving the unconsumed provision in the cell. In most areas of the Pacific Northwest, pollen masses rank second only to chalkbrood as identifiable mortality in leafcutting bee populations, commonly 15 to 35% (see tables I and II). Nest exposure to direct sunlight is known to cause early instar mortality (Undurraga, 1975) and various other factors such as pesticides and nectar dearth have been suggested as causes. In the original selections from the Adrian WT population, we chose only straws containing 5 or more live larvae, and concomitantly, no cells with pollen masses. During the entire period of selection and expansion, the incidence of

pollen masses never exceeded 4.4%. The pollen mass trait may also be genetically mediated, either linked to, or independent of, disease resistance.

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Résumé — Résistance au couvain plâtré *(Ascosphaera aggregata)* chez la mégachile *(Megachile rotundata)*. I. Test de lignées sélectionnées. Vingt neuf séries de cellules de l'abeille *Megachile rotundata* (Fabr) ont été sélectionnées à partir d'une population présentant un taux de couvain plâtré, ou ascosphériose *(Ascosphaera aggregata* Skou), de 36,1%. Seules les séries comprenant 5 larves saines ou plus, pas de couvain plâtré ni de masses polliniques ont été retenues (la présence de masses polliniques indique que l'œuf, ou la jeune larve, est mort). La moitié de la population s'est reproduite pendant 4 générations puis nous avons testé sa résistance en forçant les femelles à nidifier dans des matériaux fortement contaminés. Le taux de couvain plâtré parmi la descendance est passé de 2,3 à 12,1% (tableau I) mais cela représente une baisse de 60% par rapport à la population sauvage d'origine. Lors d'une série de tests menés pour déterminer l'efficacité de l'induction du couvain plâtré parmi des lignées de mégachiles non sélectionnées, nous avons fait macérer des pailles à boire fortement contaminées. Le produit obtenu a été tamisé, pulvérisé et saupoudré dans les matériaux de nidification à intervalles d'une semaine. Cette méthode s'est montrée efficace : l'incidence du couvain plâtré est passée de 7 à 27% dans les populations testées (tableau II).

La seconde moitié des séries sélectionnées s'est multipliée durant 6 générations puis a été testée avec environ 175 x 10^8 spores chaque semaine pendant 8 semaines. La différence a été faible entre les populations testées et les témoins (5,0 contre 2,8%) (tableau III). Les données suggèrent qu'une composante génétique de résistance au couvain plâtré était présente dans les 2 lignées d'abeilles. La très faible fréquence des masses polliniques dans les 2 lignées tout au long des 4 années d'étude (2,8 et 3,8%) contraste fortement avec celui présent chez le type sauvage (20%), suggérant que cette caractéristique peut être également déterminée génétiquement, soit en liaison avec, soit indépendamment de la résistance à la maladie.

***Megachile rotundata* / *Ascosphaera* / couvain plâtré / résistance**

Zusammenfassung — Kalkbrut- *(Ascosphaera aggregata)* Resistenz bei der Blattschneidebiene *(Megachile rotundata)*. I. Prüfung selektierter Linien unter harten Bedingungen. Aus einer Population der Blattschneidebiene *(Megachile rotundata* Fabr) mit einem Befall von 36,1% Kalkbrut *(Ascosphaera aggregata* Skou) wurden 29 Zellserien ausgewählt. Es wurden nur Serien ausgesucht, die fünf oder mehr gesunde Larven und keine Kalkbrut oder Pollenmassen enthielten (Pollenmassen sind Anzeichen dafür, daß das Ei oder die junge Larve abgestorben ist). Die Hälfte der Population wurde vier Generationen hindurch vermehrt und dann auf ihre Resistenz streng geprüft, indem man die Weibchen zwang, in schwer verseuchtem Material zu nisten.

Die Kalkbrut stieg in diesem Test bei der Nachkommenschaft von 2,3 auf 12,1% an, aber das war um 60% weniger als bei

der Wildpopulation, von der sie herstammte. In einer Versuchsserie, in der die Wirksamkeit der künstlichen Kalkbrutauslösung bei nicht selektierten Linien geprüft werden sollte, wurden schwer kontaminierte Getränke-Halme mazeriert, dann gesiebt und pulverisiert; schließlich wurde das Nestmaterial mit den Sporen in Intervallen von je einer Woche bestäubt. Diese Methode erwies sich als wirksam, da sie den Kalkbrut-Befall in den Testpopulationen von 7 auf 27% erhöhte. Die zweite Hälfte der Population wurde 6 Generationen hindurch vermehrt und anschließend durch Bestäuben mit etwa 175 x 10^8 Sporen wöchentlich für 8 Wochen geprüft. Es bestand ein nur geringer Unterschied zwischen den geprüften Zuchten und den selektierten Kontrollen (5,0 gegen 2,8%). Diese Daten lassen vermuten, daß in beiden Linien eine genetische Komponente für Krankheitsresistenz bestand. Das auffallend niedrige Vorkommen von Pollenmassen in beiden Linien während der vier Jahre der Untersuchung (2,8 bis 3,8%) stand in starkem Gegensatz zu der Häufigkeit von Pollenmassen in der Wildpopulation (über 20%). Dies legt die Vermutung nahe, daß auch dieses Merkmal genetisch übertragen wird, entweder gekoppelt oder unabhängig von der Kalkbrutresistenz.

***Megachile rotundata* / *Ascosphaera* / Kalkbrut / Resistenz**

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