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Host specificity and habitat preference of *Laboulbenia slackensis*

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Abstract: Experiments were carried out with *Laboulbenia slackensis* (Ascomycetes, Laboulbeniales), an ectoparasitic univorous fungus of *Pogonus chalceus* (Coleoptera, Carabidae). Relative growth of *Laboulbenia slackensis* populations was significantly affected by environmental conditions, i.e., the soil composition, under which the typical host was reared. Optimal conditions for the development of the fungus corresponded with the optimal habitat selected by the typical host, i.e., pure salt marsh clay. Artificial infections with *L. slackensis* showed that the fungus was potentially plurivorous on Carabidae and that its specificity was not fully accounted for by the host physiology or integumental characteristics. *L. slackensis* was successfully transferred to 19 atypical carabid host species, and the morphological characteristics used to differentiate it from related taxa are stable on these hosts. As with the typical host, successful establishment of *L. slackensis* on an atypical host also depended on the soil composition under which the atypical host was reared. The host was essential, but alone not sufficient, for the establishment of the fungus. It is postulated that the specificity of *L. slackensis* observed in nature depends both on its host specificity and environmental preferences. Specialization of *L. slackensis* probably was the result of reinforced ecological isolation caused by obligate ectoparasitism, host specificity, and dependence on a specific environment that was rigorously selected by a very restricted number of host species. Host populations of univorous Laboulbeniales probably are similar to islands in the model of island biogeography.

Key Words: Ascomycetes, Carabidae, ectoparasite, Laboulbeniales, specialization, specificity

INTRODUCTION

Laboulbeniales are obligate ectoparasitic Ascomycetes that are exclusively found on Arthropoda, mainly Hexapoda, and sometimes Acarina and Diplopoda. Laboulbeniales produce small thalli of determinate growth, usually bearing antheridia and perithecia on a receptacle with appendages. The thalli develop on the outside of the host integument and are attached to it by the lowermost part of the basal cell of the receptacle. The entire life cycle is spent on one host, there are no free-living stages and only sexual reproduction is known. The necessity of having a living host to complete the life cycle, was demonstrated by axenic culture experiments with *Herpomyces* from blattids (Richards and Smith, 1954) and *Fanniomyces* from flies (Whisler, 1968), that failed to produce mature thalli.

Most Laboulbeniales do not penetrate into the living tissue of their host but make contact through integument pores (Benjamin, 1971; Tavares, 1985). A few species are known to produce rhizoidal haustoria, that penetrate the integument and the body of the host (Benjamin, 1971; Meola and Tavares, 1982). In spite of their ectoparasitic nature, Laboulbeniales, especially those with superficial insertion, are considered harmless to their host (Scheloske, 1969; Benjamin, 1971; Majewski, 1994b).

The parasite-host lists given by several authors (Scheloske, 1969; Frank, 1982; Huldén, 1983, Santamaria, 1989; Santamaria et al., 1991; Majewski, 1994b) illustrate that Laboulbeniales are highly host specific. Based on the natural host ranges, the Laboulbeniales can be divided roughly into three groups. A large group consists of univorous species with one or rarely two related hosts. A second group comprises oligovorous species, such as *Laboulbenia flagellata* Peyritsch, *L. vulgaris* Peyritsch, and *L. pedicellata* Thaxter, that are usually found on related host species from genera of infrafamilial level. The last group consists of plurivorous species, such as *Misgomyces dyschirii* Thaxter and *Euzodiomyces lathrobii* Thaxter, that occur on phylogenetically more distantly related coleopteran families (Staphylinidae and Carabidae). Additional examples of Laboulbeniales infecting unrelated or atypical hosts are given by, among others, Blum (1924), Scheloske (1969), Benjamin (1971), Blackwell and Kimbrough (1978), Santamaria et al. (1991) and Majewski (1994b).

The host range of plurivorous Laboulbeniales usually consists of arthropods that share the same habitat or have a predator-prey, parasitic or commensal re-

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lationship (Blackwell and Rossi, 1986). Benjamin (1971) suggested that the plurivory of *L. ecitonis* Blum resulted from the similar 'body chemistry' its cohabitating hosts, members of Hymenoptera, Coleoptera and Acarina, acquired after long and intimate association. Scheloske (1969) assumed that plurivory in Laboulbeniales was due either to accidental infections of different hosts occupying the same habitat or to adaptation of the fungus in being able to exploit the nutrients available in the different cohabitating host species.

Experimental studies testing the potential host range of Laboulbeniales confirmed that these fungi are highly, but not entirely, host specific (Peyritsch, 1875; Cépède and Picard, 1908; Picard, 1913; Arwidsson, 1946; Lindroth, 1948; Richards and Smith, 1954; Boyer-Lefévre, 1966; Andersen and Skorping, 1991). Failure to infect a typical host has been assumed to be caused by the presence of different physiological strains of the fungus (Picard, 1913; Benjamin, 1971) or the host (Richards and Smith, 1954). Scheloske (1969) showed that Laboulbeniales are able to take up stain from the haemocoel of a host, and it is generally accepted that host specificity and nutrition of Laboulbeniales are linked through the physicochemical properties of the haemolymph and integument of the host (Benjamin, 1971; Tavares, 1979; Huldén, 1983; Santamaria, 1989). Scheloske (1976a,b) also indicated that specificity results from specific host-to-host contacts that mechanically liberate and transmit spores.

In most experimental studies dealing with host specificity of Laboulbeniales little attention has been paid to the possible direct impact of experimental conditions on the transmission and establishment of the fungus. All workers agree that the presence of Laboulbeniales in a natural habitat depends primarily on the occurrence of a suitable host. Since the absence of a fungus on a susceptible host might be caused by unfavorable characteristics of the habitat (Scheloske, 1969; Majewski, 1994a), it is evident that experimental conditions likewise apply to host specificity experiments.

In the present study, experiments were carried out to establish the optimal growth conditions of an extremely host specific species of *Laboulbenia.* In order to determine the factors involved in the specificity of this particular fungus, its ability to establish itself on a wide range of atypical and physiologically different Coleoptera was tested under optimal and nonoptimal environmental conditions. This new approach permits the drafting of a list of potential hosts and allows one to assess the importance of host quality and habitat quality on the specificity observed under natural conditions.

MATERIALS AND METHODS

Fungus and hosts.—Experiments were carried out with *Laboulbenia slackensis* Cépède and Picard, that is exclusively found on Pogoninae (Coleoptera, Carabidae) (Santamaria et al., 1991). In Belgium it occurs exclusively on the halophilic beetle *Pogonus chalceus* (Marsham) (De Kesel, 1989; De Kesel and Rammeloo, 1992).

Infected *P. chalceus* specimens were collected from April to July (1993 and 1994) using pitfall traps placed in coastal salt marshes of 'Het Zwin' nature reserve, near Knokke-Heist, Belgium. The captured *P. chalceus* specimens were divided into two age groups as described in De Kesel (1993). The first age group consisted of relatively young specimens, i.e., the new generation, with an intact exoskeleton and relatively low thallus density. The second age group consisted of old generation beetles, recognized by a weathered exoskeleton and relatively high fungal thallus density (De Kesel, 1993).

A number of atypical hosts, belonging to 31 coleopteran species (Table I), were caught from June until August (1993 and 1994) in different habitats.

Soil experiment.—The effect of the soil composition on the relative growth of *L. slackensis* populations was determined, under controlled conditions, by rearing moderately infected younger age group *P. chalceus* on a sand-salt marsh clay gradient. Salt marsh clay was obtained from the optimal microhabitat of *P. chalceus,* i.e., salt marsh *Salicornia-Halimione* zone (Desender, 1985; De Kesel, personal observations). The clay was crumbled using a wide-meshed sieve (5mm) and impurities were removed. The sand was washed with demineralised water and cleared from impurities by sieving (mesh:0.250mm).

The gradient was prepared by mixing and homogenizing sand with salt marsh clay. Six different soils, containing 0%, 20%, 40%, 60%, 80% and 100% salt marsh clay, were prepared. Viable spores of Laboulbeniales were eliminated by heating the soils at 120 C for 20 min. Decontaminated soils were transferred to transparent polyethylene containers (diam = 7.5cm, height = 5cm); soil thickness was kept at 1cm. The containers were closed, but aeration was provided through a ventilation hole of 1cm diam. The containers were placed in an acclimatized chamber with a constant temperature of 20 ± 0.5 C and with relative humidity close to saturation. The soils were kept moist by regularly adding autoclaved brackish water, that was obtained from a salt marsh rivulet in the sampling station. The photoperiodic regime was kept constant (L:D = 16h/8h).

A total of 236 infested *P. chalceus* (younger age group) were placed on the soils in groups of four

TABLE I. Artificial infections of *L. slackensis* on atypical hosts

Host (Coleoptera, Carabidea)	Rep[a]	Loc[b]	SMclay[c]	Sand[d]	Site[e]	Slide[f]
1. *Abax parallelepipedus* (Piller and Mitterpacher)	AS	1	4−			
2. *Agonum assimile* (Paykull)	S	2	2++, 3+++	2−, 1±, 1+	1, 4, 5	ADK906, ADK875a,b
		1	1++		7, 4	ADK921
3. *Agonum muelleri* (Herbst)	S	1	1+, 1++	1−	1, 2, 3, 4	ADK922, ADK919
4. *Agonum sexpunctatum* (L.)	S	1	1++	1−	4, 5	ADK926
5. *Amara spreta* Dejean	S	1	1+++		1	ADK894
6. *Asaphidion flavipes* (L.)	S	1	5−	2−		
7. *Bembidion minimum* F.	S	3	4−	2−		
8. *Bembidion normannum* Dejean	S	3	1++		4	ADK761b
9. *Bembidion tetracolum* Say	S	1	2++	1−	3, 4	ADK927, ADK918
10. *Calathus melanocephalus* (L.)	A	4	1+, 1++	1−, 1±	4	ADK883, ADK885
11. *Calathus piceus* (Marsham)	A	2	3−, 1±, 2++	2−, 1±	4	ADK893
12. *Carabus violaceus purpurascens* F.	A	1	1−, 1++		6	ADK914
13. *Dicheirotrichus gustavii* Crotch	A	3	1++	2−	4	ADK917
14. *Harpalus rufipes* (De Geer)	A	3	1−			
15. *Leistus fulvibarbis* Dejean	A	1	1±		4	
16. *Loricera pilicornis* (F.)	S	1	2−, 1±, 1+, 1++	3−, 1±	4	ADK925, ADK932
17. *Nebria brevicollis* (F.)	A	1	3±		4, 3	
		2	2−, 6±	6−	3, 4, 5, 7	
18. *Notiophilus biguttatus* (F.)	S	1	3−	1−		
		4	1±	2−	4	
		2	1++		4	ADK931
19. *Notiophilus rufipes* Curtis	S	4	1++	1−	4	ADK901
20. *Patrobus atrorufus* (Stroem)	A	1	1++		5, 4, 3	ADK886
21. *Pterostichus madidus* (F.)	A	1	2−, 1±, 1+	2−	2, 6	ADK895
22. *Pterostichus melanarius* (Illiger)	A	1	2++	1−	6, 2	ADK929
23. *Pterostichus oblongopunctatus* (F.)	S	2	1++, 1+++	1−, 1±, 1+	4, 6	ADK923, ADK879
24. *Pterostichus strenuus* (Panzer)	S	3	4−	2−		
25. *Pterostichus vernalis* (Panzer)	S	1	2++		4	ADK920, ADK928
26. *Trechus quadristriatus* (Schrank)	A	1	1+		5	
Noncarabid hosts (Coleoptera)						
1. *Blapstynus metallicus* (F.) (Tenebrionidae)		C	6−			
2. *Heterocerus hispidulus* Kiesw. (Heteroceridae)		3	1−			
3. *Thanatophilus sinuatus* (F.) (Silphidae)		1	1−			
4. *Philonthus* (s. str.) *cognatus* Stephens (Staphylinidae)		1	2−			
5. *Bledius tricornis* Herbst (Staphylinidae)		3	1−			

[a] Main host reproduction period (REP): S. during Spring; A. during Summer and Autumn; AS; mainly during Autumn.

[b] Locality (LOC): 1. Meise (Province Brabant), Domein van Bouchout, deciduous woodland with grasslands (park); 2. Hingene (Province Antwerpen), Domein d'Ursel: deciduous woodland (park); 3. Knokke-Heist (Province West-Vlaanderen), Het Zwin: nature reserve with salt marshes and dunes; 4. Lichtaart (Province Antwerpen), De Hoge Rielen: nature reserve with different habitats on dry sandy soil; C. species cultivated under laboratory conditions.

[c] Number of specimens reared on salt marsh clay (SM clay) and their degree of infection, with: (−) no infection; (±) only young thalli in pre-antheridial stage; (+) infection with low thallus density, thalli usually in trichogynic stage; (++) infection, with the occurrence of several fully developed thalli; (+++) infection with a large amount of adult thalli (>25 per beetle).

[d] As in [c], but reared on pure sand.

[e] Infection-site with, 1. on entire exoskeleton; 2. cephalon; 3. pronotum; 4. elytra; 5. legs; 6. mouth parts; 7. Abdomen.

[f] Reference numbers of the slides at BR.

specimens, with at least eight replicates per soil type. The sex ratio in each group was 1:1. The beetles were fed fresh chironomid larvae in excess.

Thallus density of each host specimen was determined at the beginning (TDI) and at the end of the experiment (TDF) following the method described by De Kesel (1993). Sex and other individual characteristics were noted in order to recognize the individuals in each group. All calculations and graphs were made using data obtained from beetles that were alive at the end of the experiment, i.e., 188 specimens. The relative growth (RG) of the fungus population on each host was calculated as follows: RG = (TDF-TDI)/TDI. The experiment lasted 12 wk after which all hosts were etherized and stored in 80% ethanol. Voucher specimens are deposited at BR.

Artificial infection.—Artificial infections with *L. slackensis* were induced by rearing atypical hosts with several heavily infested *P. chalceus*. Transmission of spores from the natural host to the atypical hosts took its own course. Environmental conditions were identical to those in the soil experiment. Two soil types, i.e., pure salt marsh clay and pure sand, were used to evaluate the effect of the soil on the success of infection of atypical hosts.

The inoculum consisted of heavily infested old generation *P. chalceus*. Thallus density of these beetles was artificially increased by rearing them at high density on salt marsh clay, in conditions similar to those described above.

The integuments of atypical hosts were scanned weekly for thalli under the dissecting microscope. Infection was considered successful when at least one fully developed thallus was present. Infection was considered unsuccessful when only spores or preantheridial stages of thalli were observed.

A number of thalli of each successfully infected atypical host were removed and slides were made following the method described by Benjamin (1971). Voucher specimens of artificially infected hosts and the microscope slide collection are stored at BR.

Statistical treatments.—Since TDI might affect TDF (De Kesel, 1993), the effect of soil composition on TDF was assessed using an analysis of covariance (ANCOVA) with TDI as covariate (Sokal and Rohlf, 1981). The significance of the regressions of TDF on TDI for the six soil types was tested using linear regression models (Sokal and Rohlf, 1981). The significance of the regression of the relative growth (RG) of *L. slackensis* on the soil type also was tested using a linear regression model (Sokal and Rohlf, 1981).

A G-test of independence, with Williams' correction (Sokal and Rohlf, 1981), was used to test whether frequency of successful artificial infection in salt marsh conditions was independent of the life cycle of the host. A second G-test of independence was used to test whether the frequency of successful artificial infection was independent of the soil type on which the atypical hosts were reared. In order to exclude possible host-species effects, this G-test was performed using only those host species that were tested on both soil types. Graphs and analysis were made using SYSTAT and SYGRAPH (Wilkinson 1988a,b).

Nomenclature and determinations.—Nomenclature of Laboulbeniales follows Santamaria et al. (1991); the Carabidae nomenclature is according to Coulon (1995); determinations of Carabidae were made using Freude et al. (1976) and Lindroth (1974). Data on the life cycles of the hosts are according to Lindroth (1945) and Desender (1986a,b,c,d).

RESULTS

Effect of soil composition.—The relation between TDI and TDF was highly significant in each of the six soils (FIGS. 1–6). The result of the one way ANCOVA indicated that there is heterogeneity in TDF among the six soils when TDI was kept constant ($F_{(5,181)}$ = 60.454, $p<0.01$). This indicated that the soil on which the host was reared significantly affected the TDF of *L. slackensis*. A significant relationship between RG and soil composition was found (RG = 0.276*%CLAY+12.571, $r^2 = 0.581$, $F_{(1,186)} = 258.52$, $p<0.001$) (FIG. 7). RG was particularly high on beetles reared on soils with high salt marsh clay content and lower at low salt marsh clay content.

Artificial infections.—Most atypical hosts were not adapted to the saline conditions and stayed alive for only about 4 wk, that was, however, long enough for the development of *L. slackensis*.

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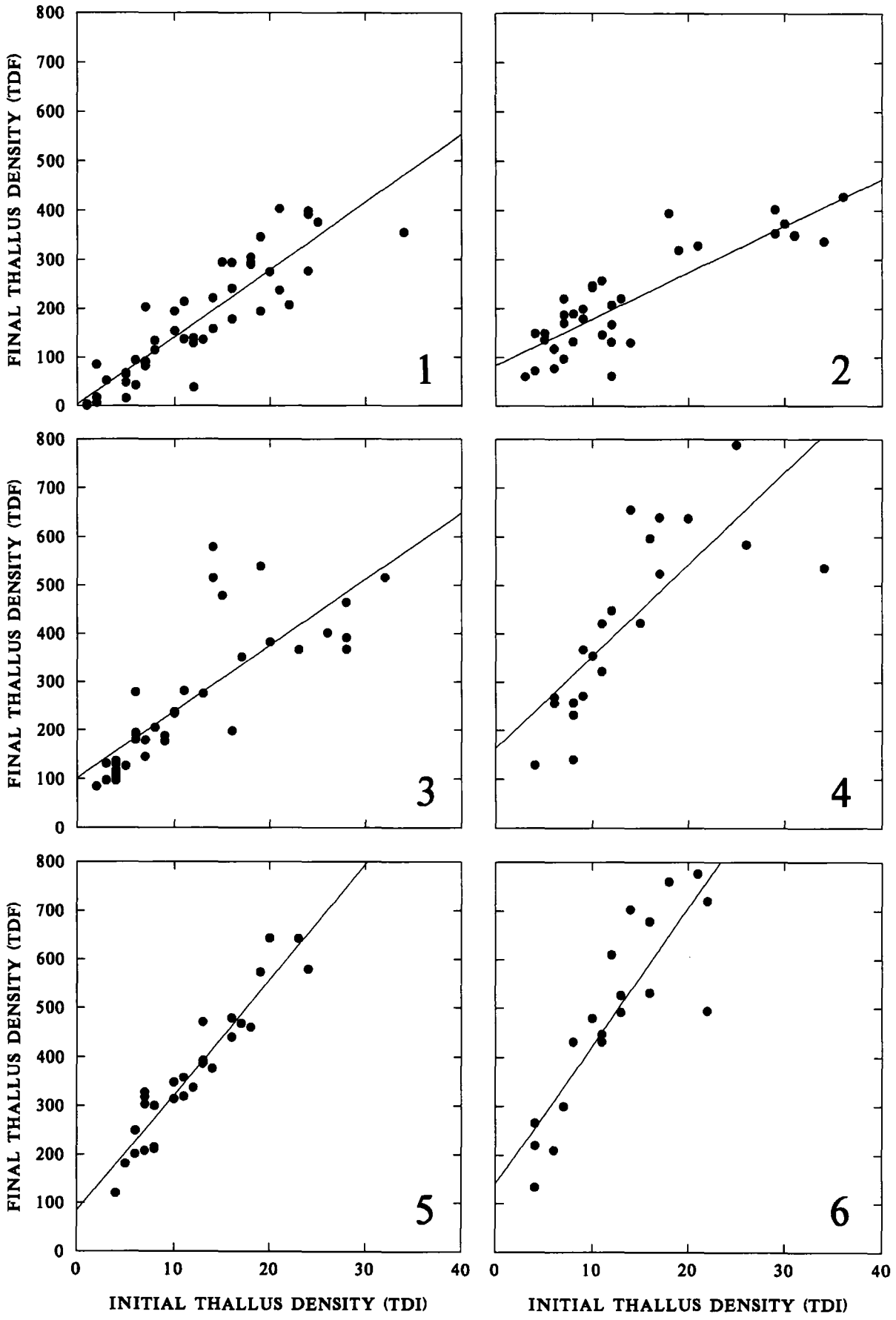
FIGS. 1–6. Data and regression lines from initial thallus density (TDI) and final thallus density (TDF) in six soils with different salt marsh clay content. 1, Salt marsh clay = 0%, TDF = 13.78841 * TDI + 2.485, $r^2 = 0.802$, $F_{(1,46)} = 190.78$, $p<0.001$); 2, Salt marsh clay = 20%, TDF = 9.506 * TDI + 82.729, $r^2 = 0.736$, $F_{(1,34)} = 98.510$, $p<0.001$); 3, Salt marsh clay = 40%, TDF = 13.695 * TDI + 100.824, $r^2 = 0.632$, $F_{(1,34)} = 61.112$, $p<0.001$); 4, Salt marsh clay = 60%, TDF = 19.877 * TDI + 163.027, $r^2 = 0.851$, $F_{(1,19)} = 28.733$, $p<0.001$); 5, Salt marsh clay = 80%, TDF = 23.664 * TDI + 83.678, $r^2 = 0.886$, $F_{(1,26)} = 210.75$, $p<0.001$); 6, Salt marsh clay = 100%, TDF = 28.184 * TDI + 140.805, $r^2 = 0.721$, $F_{(1,17)} = 47.567$, $p<0.001$).


FINAL THALLUS DENSITY (TDF)
INITIAL THALLUS DENSITY (TDI)
0
100
200
300
400
500
600
700
800
10
20
30
40
1
2
3
4
5
6

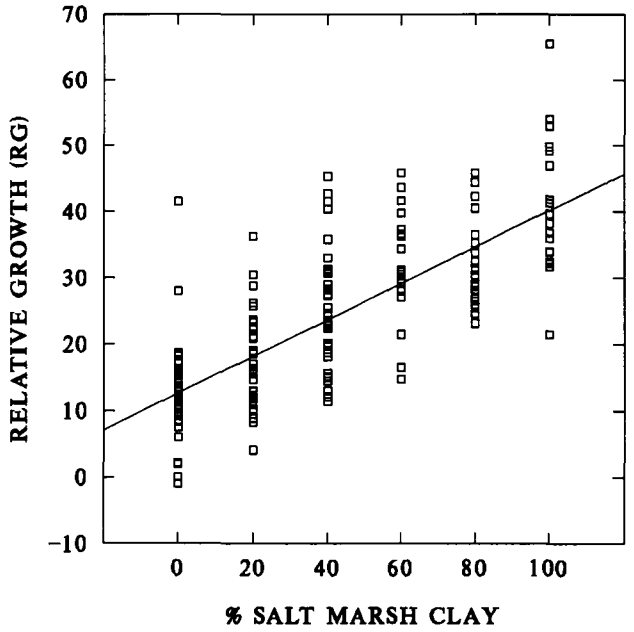


FIG. 7. Data and regression line from relative growth (RG) of *L. slackensis* populations in different treatments (RG = 0.276 * %CLAY + 12.571, r^2 = 0.581, $F_{(1,186)}$ = 258.52, $p<0.001$).

TABLE I shows that *L. slackensis* was able to develop successfully on 19 of the 26 tested carabid species when reared on pure salt marsh clay. All mature thalli, prepared from the integuments of atypical hosts, had fully developed ascospores within their perithecia.

The thalli on the atypical hosts occurred, in decreasing order of frequency, on the elytra (40%), pronotum (14.5%), cephalon (10.9%), legs (10.9%), abdomen (9.1%), pro-, meso-, and metathorax (7.4%) and mouth parts (7.2%). In larger carabid species, i.e. *Carabus violaceus purpurascens* F., *Pterostichus madidus* (F.), *P. melanarius* (Illiger) and *P. oblongopunctatus* (F.), the thalli were found solely on the mouth parts. Buccal infections were undoubtedly caused by direct transmission since all the heavily infested *P. chalceus* were predated upon within a week.

Seven carabid species were not infected successfully. Total absence of thallus development was observed in five carabid species and all noncarabid species. On two carabid species, i.e., *Leistus fulvibarbis* Dejean and *Nebria brevicollis* (F.), the thalli did not develop beyond the antheridial stage. In the case of *Calathus piceus* (Marsham), *Carabus violaceus purpurascens, Loricera pilicornis* (F.), *Notiophilus biguttatus* (F.) and *Pterostichus madidus* differential individual susceptibility to fungus infection was observed.

Establishment and host reproduction type.—Of 73 carabid specimens reared on pure salt marsh clay (excluding *Abax parallelepipedus* (Piller and Mitterpacher)), 21 out of the 41 spring-breeders and 11 out of 32 autumn-breeder hosts became infected successfully. The frequency of successful infection with *L. slackensis* was independent of the life cycle of the atypical hosts ($G_{adj.}$ = 2.046, p = 0.153).

Establishment and environmental conditions.—When the hosts were reared on pure sand, *L. slackensis* developed on two of the 40 tested carabid host specimens, i.e., *Agonum assimile* (Paykull) and *Pterostichus oblongopunctatus*. Under pure salt marsh clay conditions *L. slackensis* developed successfully on 23 of the 58 tested carabid host specimens, suggesting that the frequency of successful infection of atypical hosts was affected by the type of soil on which they were reared ($G_{adj.}$ = 17.127, $p<0.001$).

DISCUSSION

Effect of the soil.—The influence of the soil characteristics on Laboulbeniales is believed to be associated with substrate infection and spore survival (Lindroth, 1948; Meijer, 1971; Andersen and Skorping, 1991). The importance of substrate infection for *L. slackensis* is, however, extremely small compared to direct infection (De Kesel, 1993; 1996), and spore survival is extremely short and not significantly affected by the composition of the soil (De Kesel, 1996). The difference in spore transmission (direct or indirect) and spore survival among the tested soil types is therefore considered negligible.

Although it is obvious that the experimental conditions affect both the host and the fungus, the direct influence of the soil type on the hosts is extremely difficult to assess. Because development of *L. slackensis* is not affected by the host species, it is assumed that the *P. chalceus* specimens reared on the different soils were physiologically similar.

Since the thalli of *L. slackensis* develop on the outside of the host integument, I assume that environmental conditions may directly affect the growth and development of the thalli. The different soils form an extreme contrast in texture, pore space, water content and mineral composition. The relative humidity, however, was kept high in all treatments so that the effect of the water content of the soils probably was negligible. *L. slackensis* has a high water permeability that is, however, restricted to its appendages (De Kesel, unpublished data). Little is known about the function of the sterile appendages of *L. slackensis* but, assuming that they are of some importance to the water balance of the thallus, it is likely that the mineral composition (richness) and the osmotic pressure of soil solutions affect the fungus develop-

ment. More experiments should be carried out to test this hypothesis. The main objective of the soil experiment, however, was to illustrate that the optimal growth condition for *L. slackensis* on *P. chalceus,* i.e., the highest relative population growth, is reached only when the soil preferences of the typical host are fulfilled.

Morphological stability and fertility.—The morphological characteristics, used by taxonomists to differentiate *L. slackensis* from related taxa (Santamaria, 1989; Majewski, 1994b) were stable under all conditions in this study. Morphological stability of Laboulbeniales grown on atypical hosts was also observed in *Herpomyces* spp. (Richards and Smith, 1954).

Little is known about the fertility of Laboulbeniales grown on atypical hosts. Prof. Dr. H.W. Scheloske (personal communication) suspects that ascospores from *L. slackensis,* obtained from artificially infected atypical hosts may have lower fertility. Additional experiments testing the effect of the host type on spore fertility should be carried out.

Specificity.—The artificial infection of atypical hosts with *L. slackensis* indicates that the fungus is potentially plurivorous on many species of Carabidae. This contrasts with the classical concept of *L. slackensis* as being highly host specific. The group of successfully infected atypical carabid hosts consisted, considering their life cycles and habitat preferences, of physiologically different species (Thiele, 1977). Host specificity of Laboulbeniales has been considered to be linked with the physiological differences among hosts (Benjamin, 1971).

The extreme host specificity of *L. slackensis* observed in nature probably is not entirely governed by a direct physiological dependence towards *P. chalceus.* It is more likely that the nutritional resource, that *L. slackensis* derives from its typical host, is also present and accessible in a large number of other Carabidae. The failure to infect the noncarabid hosts suggests the presence of a recognition system or the absence of the necessary nutrients, although it must be kept in mind that the transfer of spores between inoculum and potential hosts was left to take its own course.

The effect of the soil type on the success of artificial infection of atypical carabid hosts indicates that the presence of a potential host is not the only factor required for the successful establishment of the fungus. The involvement of *L. slackensis* in an intimate association does not prevent it from having its own environmental preferences. The assumption made by Scheloske (1969) and Majewski (1994a), that the absence of a *Laboulbenia* on a potential host might be due to unfavorable environmental conditions, is probably correct.

Occasional jumps to a new potential host, that must have played an important role in the evolution of Laboulbeniales (Benjamin, 1968), theoretically involve some overlap between environmental tolerance of the fungus and the new environmental conditions provided by the host. The opportunities for the establishment on a new host, in natural situations, are determined by the probability of spore transmission to a new host, its suitability and all the factors that affect spore germination, thallus development and fungus reproduction. The opportunities for interspecific transmission of Laboulbeniales are probably very limited and only take place when beetles are in physical contact. This is mainly due to the well-known different habitat selections, microclimatological preferences, reproduction periods, generation overlaps and temporal ranges of carabid species (Thiele, 1977). Transmission may occasionally be extended by spores being deposited on the substrate, but this is negligible for *L. slackensis* (De Kesel, 1996).

This study has shown that establishment of *L. slackensis* on a new host is determined by several factors that include characteristics of the host integument, the availability of nutrients and the microclimate on the surface of the host. The adaptation of *L. slackensis* to the environmental conditions created by *P. chalceus* seems very strong since the fungus does not become established under natural conditions on other 'suitable' carabid hosts. The failure to infect the halophilic carabids *Dicheirotrichus gustavii* Crotch, *Bembidion minimum* F. and *B. normannum* Dejean under natural conditions in the same habitat as the typical host is probably due to differences in microhabitat selection and specific microclimatological preferences in time and space.

The data lead to formulation of the hypothesis that the specialization of *L. slackensis* results from its reinforced ecological isolation caused by obligate ectoparasitism, the lack of free-living stages, host specificity (nutrients) and dependence on a specific environment that is rigorously selected by a very restricted number of host species (Pogonini). In this context I believe that the host populations of extremely host specific Laboulbeniales are probably similar to islands in the model of island biogeography (MacArthur and Wilson, 1967). The large number of specific Laboulbeniales parasitizing carabid hosts is probably closely connected with the manifold modes of behavior and physiological requirements of carabid beetles.

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