Boll. Ist. Ent. "G. Grandi" Univ. Bologna, 48: 195-202, 1994.

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Mould Control by the Harvester Ant *Messor structor* (Latr.) (Hymenoptera Formicidae) on Stored Seeds. (*)

(Research supported by MURST 60%)

Introduction

Seed conservation by harvester ants poses two main questions: why do the seeds not germinate and why do they not turn mouldy? Moggridge (1873) postulated that harvester ants let the seeds germinate, so as to enable the transformation of starch into sugar, and then detach the rootlet and place the seeds in the sun. In contrast with him, Emery (1915) reported that while conservation was ensured by keeping the seeds in dry conditions, the ants would store the seeds for immediate consumption in wet chambers so as to facilitate breaking them up into small pieces.

Doflein (1920), cited by Forel (1923), reported that *Messor meridionalis* Krausse uses whitish, wax-like secretions to water-proof the chamber walls, thereby keeping the seeds in a dry environment. Delage (1968) maintains that the ants act essentially in a preventive way: they nest in dry, well-drained soils, collect only dry seeds and use galleries to aerate the storage chambers, eventually exposing any wet seed to the sun. Cerdan (1989) notes that the removal of the highly hygroscopical integument is a way to conserve the seeds. However, according to Schildknecht (1976), the metapleural gland in some Myrmicinae species, including *M.barbarus* L., produces a group of substances involved in inhibiting the germination of both fungal spores and seeds: phenylacetic acid, indolacetic acid, and β-hydroxydecanoic acid (Maschwitz *et al.*, 1970; Schildknecht and Koob, 1970, 1971).

Preliminary observations of a *M.structor* (Latr.) colony reared in an artificial nest indicate that the ants do not necessarily store the seeds in dry chambers but often use those where the humidity is such as to allow mould development and germination. The present study reports and discusses the ants' ability to control mould development, leaving aside for the moment the question of seed germination.

Materials and methods

The *M.structor* colony was collected in a meadow in the WWF's wildlife preserve at Orbetello (near Grosseto in central Italy) on 3 March 1989. The original

(*) Accepted for publication February 26, 1994.

group of 4 queens, 1,349 workers and 32 larvae was housed in an artificial nest, constructed of glass, chalk and plastic, featuring 12 chambers marked by different humidity and temperature levels; the nest was connected to a dry feeding area through a seven-meter gallery. The ants, which were fed on seeds of various plant species as well as on insects, adapted immediately to the nest, producing numerous workers and sexuals the very first year; the population by 1992 was estimated at about 15,000 individuals. The plant species used in the test was the sunflower *Helianthus annuus* L. (Asteraceae); the seeds employed were not subjected to any chemical treatment for storage.

1. Fungi species identification on seed integument.

All the seeds used in this test were first dipped for 8 minutes in a 1.4% sodium hypochlorite water solution to remove any surface contamination and to prevent the likely development of *Rhyzopus nigricans* Ehrenb, a very common and fast-growing species that can impede the determination of other species present. The seeds were then divided into groups and placed under different growth conditions: 100 seeds in moist chamber at 20-23°C (5 chambers each with 20 seeds); 100 seeds in potato dextrose agar medium (PDA) at 25°C (10 dishes each with 10 seeds); 100 seeds in moist chamber at 30°C (5 chambers each with 20 seeds).

2. Determination of the integument spore contamination.

From a stock of seeds stored in dry environment (initial seeds) four samples of 25 seeds each were placed in 25 ml of water and stirred for 3 minutes to suspend spores in solution. The evaluation of spore number per ml was calculated after the Celle Toma method. The same method has been applied to evaluate the sporal contamination of seeds stored in wet environment, with and without ants, after Stage 1 of the seed storage experiments (see the following paragraph).

3. Seed storage.

These tests employed an experimental granary (Fig. I): a plastic box measuring 10x25x7 cm with 10 chambers aligned in two rows and featuring chalk flooring and a brass net set into the side walls to ensure the uniformity of microclimatic conditions and to prevent the ants' moving from one chamber to the other. An infrared lamp warmed the granary up to the temperatures set for the two experiments: 25±1°C and 30±1°C; the RH levels were kept close to saturation (98-100%) by adding water to the chalk at the beginning and sealing the plastic cover with silicon. A transparent red screen was put on the cover. A psychrometer for temperature and RH monitoring was placed in the two central chambers. Sixty *H. annuus* seeds were placed on the moist chalk in each of the eight remaining chambers. A hole was drilled in the side wall of each chamber to connect it to the nest through a plastic tube; an adjustable tap provided the ants with an opening wide enough to let them move to and from the nest, yet narrow enough to prevent removal of the sunflower seeds.

Two tests were run in two stages at the two temperature regimes.

Stage 1.

For 8 days the ants were permitted access to the 4 chambers of one row and excluded from the 4 chambers of the other. At the end of this time, the infrared lamp was switched off to induce the ants to exit the chambers. The granary was then carefully opened, first uncovering the chambers with the ants, and the following data recorded: (i) the number of seeds eaten, (ii) the number of seeds covered by mould, (iii) the presence of any foreign material introduced by the ants, and (iv) the integument spore contamination on samples of 25 seeds taken from each of the 8 chambers (4 with ants and 4 without ants), as described above for the initial seeds.

Stage 2.

When all the ants had left the chambers, the granary was sealed and the connection to the nest reversed, so that the ants had access only to the other 4 chambers for 8 days. The data for the first three sets in Stage 1 were recorded.

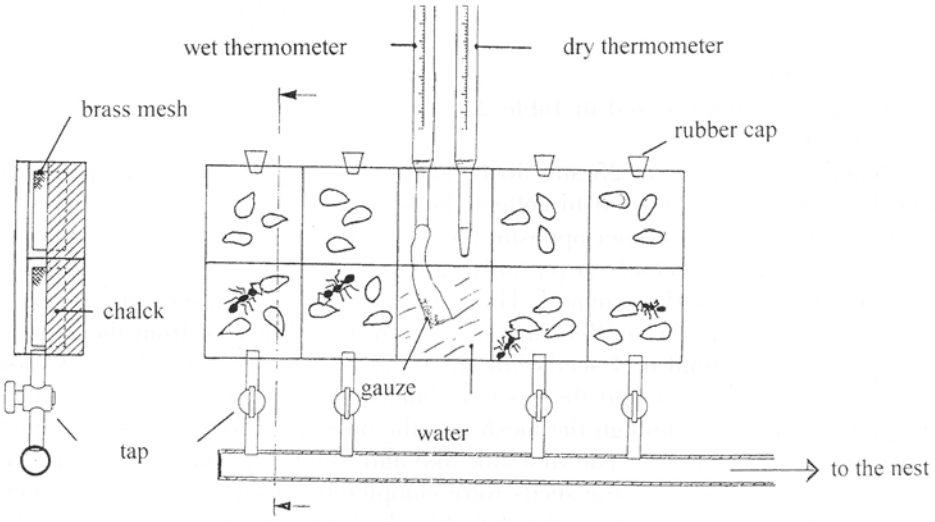

Fig. I: Scheme of the experimental granary.

4. Statistical analysis.

A factorial analysis of variance of the percentage of seeds eaten by the ants in the two stages of the experiments was performed after angular conversion of the data (arcsin x). Temperature (25°C and 30°C) and test stage, i.e. the state of the seeds given to ants (presence or absence of mould on the integument), were taken as the main factors. The same analysis was applied to the number of spores/ml, the main factors being the presence or absence of ants and the temperature (25°C and 30°C).

Results

1. Fungi species identification on seed integument.

The following genera and species were determined on seed integument: *Alter-*

naria alternata (Fr.) Keissl, *Pennicillium* spp., *Sclerotinia sclerotiorum* (Lib.) De By, *Aspergillus flavus* Link. and *R.nigricans* Ehnreb. *R.nigricans* and *A.alternata*, both mainly saprophytic species, were by far the most predominant.

2. Determination of the integument spore contamination.

The spore contamination calculated on the integument of the seeds stored in a dry environment (initial seeds) was found to be of 0.2±0.1 spores/ml. The spore counting data, recorded after Stage 1 of both experiments, are summarised in Table 1.

Table 1 - Count of spores on seed integument (spores/ml x 10^4) (X±SD).

temperature (°C)	without ants	with ants
25	42.8 ± 6.2	6.4 ± 1.3
30	71.3 ± 5.8	49.6 ± 3.5

3. Seed storage.

The data are summarised in Table 2.

Stage 1.

Chambers with ants: at 25 and 30°C many ants visited the seeds. Upon entering the chambers, they tried to collect the seeds and then, being unsuccessful, stored the seeds in the corner opposite the entrance. In both tests, a certain number of seeds were completely or partially eaten (Table 1), the remainder appearing well conserved and without mould. The ants introduced into the chambers larvae and small size seeds (i.e. millet), taken either from the nest or from the external feeding area, for a total of 4 seeds introduced at 25°C and 13 at 30°C. No waste material was introduced into the visited chambers, although the ants did throw integument fragments through the mesh into the opposite unoccupiable chambers.

Chambers without ants: The first silk-like and filamentary mycelium appeared by day 3, and by day 8 all the seeds were completely covered; and in the experiment at the mycelium was more developed in the 30°C than in the 25°C test. The identification of the microscopic fungi revealed the same species as those determined under the culture test.

Stage 2.

Chambers with ants: with the nest now connected to the four chambers cut off in Stage 1, many ants entered them on day 1 and started to remove the mycelium from the seed integument with their legs and mandibles. Next day, all the seeds, as well as the floor and the side walls, were clean and the waste materials thrown through the mesh during Stage 1 removed. By day 8 of Stage 2, the seeds in both the 25 and 30°C tests were still clean of mycelium, although the integument appeared more opaque and softer. The ants ate part of the mould-cleaned seeds. In the 30°C test, the chambers were kept clean, while by day 3 in the 25°C one the ants began accumulating in the chambers bits of integument from the eaten seeds. The ants did not introduce into the chambers any small-sized seed but, as in Stage 1, did introduce larvae.

Chambers without ants: the seeds kept from contact with the ants were cove-

red by mould by day 4, one day later than in Stage 1, and the filamentary mycelium was less developed.

Table 2 - Seed fate at the end of the two Stages of the seed storage experiments.

	STAGE 1 (8 days)				STAGE 2 (8 days)			
	N initial seeds	% of mouldy seeds	eaten seeds	sampled seeds	N seeds left	% of mouldy seeds	eaten seeds	
with ants 25 °C	240	0	52 (21.6%)	100	88	100	–	without ants 25 °C
with ants 30 °C	240	0	30 (12.5%)	100	110	100	–	without ants 30 °C
without ants 25 °C	240	100	–	100	140	0	32 (22.9%)	with ants 25 °C
without ants 30 °C	240	100	–	100	140	0	25 (17.9%)	with ants 30 °C

Discussion and conclusions

As evinced by the percentage of seeds eaten in the two stages of both the experiments (Table 2), temperature influenced the number of seeds eaten by the ants, being significantly higher ($P<0.01$) at 25 than at 30°C. The state of the seeds did not affect their desirability, there being no difference found between the consumption of initial seeds (Stage 1) and of the mould-cleaned ones (Stage 2). No correlation was evinced between the two main factors.

The count of spores/ml showed a significantly lower contamination of the initial seeds than those taken from the chambers, with or without ants, in both experiments. However, the results in Table 1 indicate that ant presence reduces significantly ($P<0.001$) the number of spores/ml and that this effect is dependent on temperature, being greater at 30 than at 25°C, both in the chambers with and without ants ($P<0.001$). No interaction between these two factors (temperature and ant presence) was found ($P=0.14$).

The absence at both 25 and 30°C of mycelium and fruiting bodies and the significantly lower spore contamination in the chambers with ants indicate that the ants prevented the development on the seeds of moulds, despite the favourable microclimatic conditions for the latter. However, the spore count/ml for the seeds stored by the ants (at 25 and 30°C) was significantly higher than for the suspension-tested seeds stored in a dry environment. As no mycelium growth was detected in the chambers with ants, it is assumed that the high spore count is due to a continuous contamination via wire mesh by spores from the chambers without ants. If this proves correct, it would strengthen the assumption that the ants play an active role in seed conservation.

The higher contamination registered at 30 than at 25°C is more likely attributable to the rapid growth of *R.nigricans*, the former being its optimum temperature, than to a poorer effectiveness of the ants. Indeed, some of the data suggest that the ants prefer the warmer chambers: they introduced more small-sized seeds in the chambers at 30 than at 25°C; in both Stages 1 and 2 they kept the 30°C chambers cleaner and free of refuse (once the mycelium was removed, they accumulated waste in the chambers at 25°C in Stage 2); they ate more seeds at 25 than at 30°C at both Stage 1 (initial seeds) and Stage 2 (mould-cleaned seeds). These findings agree with the preliminary monitoring conducted in the granaries of the artificial nest, where the ants were repeatedly observed bringing the seeds to the warm and moist chambers in response to the shifting beam of the infrared lamp.

The ants were also able to remove the mould from the seed integument and the chamber walls. They ate these seeds, and the number of 'mouldy and cleaned' seeds eaten was not significantly different from the number of initial seeds eaten. These observations do not confirm Delage's report (1968) that *Messor* refuse the mouldy seeds. Moreover, the ants were able to stop a mould development that was well under way.

This ability to remove the mycelium could suggest that the ants exert their control over mould development through the removal of every newly developed fungine filament. Yet, preliminary observations of visibly moist nest granaries, where small-sized seeds were accumulated in multiple layers, seem to indicate that this action is not performed often enough to explain the absence of mould on the seeds at the bottom layers. The delay of mould growth on the seeds removed from ant control (Stage 2) and the less developed mycelium near the mesh seem to imply instead an inhibiting chemical substance. Such a compound would have to be but slightly, if at all, volatile, given the mould growth covering all the seeds in the excluded chambers, and readily degradable, given the rapid mould formation on the seeds removed from contact with ants.

Thus, even if it is still unclear what kind of action is performed by these ants in seed preservation, the role played by *M.structor* in an artificial nest is not simply one of prevention (seed storage in a dry environment). Then, too, the optimum microclimatic conditions sought by *M.structor* for seed conservation remain to be determined. In effect, if the ants prove to exert an active control on mould growth and development, they are likely to choose places with a given RH that would result in a certain softening of the integument, thereby making the seeds easier to break up.

Acknowledgements

We wish to thank Prof. N. D'Ercole, University of Bologna, for the scientific support in setting up the methodology for evaluating the sporal contamination and for the determination of fungi species, and Prof. E. Pacini, University of Siena, for the discussion about the ants-seeds relationships.

SUMMARY

The aim of this research is to test ants' ability to conserve seeds in the extreme conditions of humidity saturation.

An experimental granary was prepared. It was made of ten chambers, arranged in two rows, divided by a brass net to prevent the ants' passing from one chamber to another. An instrument measuring humidity and temperature was placed in the two central chambers. The plaster bottom was moistened to mantain relative humidity at 98-100%. An infrared lamp kept the temperature at the values set for the experiments. Sixty sunflower (*Helianthus annuus* L.) seeds were allocated to each chamber. Two experiments were conducted, at 25±1°C and 30±1°C, divided in two stages: in Stage 1, for 8 days, the workers were allowed to enter only the 4 chambers of one side, through a tap whose opening did not allow the passage of the sunflower seeds. In Stage 2 the connection with the nest was inverted, and the ants had access, for 8 days, to the 4 chambers previously excluded, while the seeds till that moment in contact with the ants were isolated. At the end of Stage 1, all the seeds without ants were completely covered by a layer of mould, while all the seeds in contact with the ants were well-preserved. A certain number of seeds were eaten: 13.0±4.1 seeds at 25°C (21.6%) and 7.5±2.6 (12.5%) at 30°C. The contamination analysis of the seed integuments showed a significantly lower number of spores on those with ants. In Stage 2 of both the experiments, the ants entered the chambers with the mouldy seeds. The ants removed the mould layer using legs and mandibles, thereby preventing further fungi development; they ate a number of seeds comparable with Stage 1: 8.0±1.4 (22.9%) at 25°C; 6.3±1.3 (17.9%) at 30°C. The seeds cut off from their action were covered by mycelium one day later than in Stage 1. The recognition of the fungi species showes that they are mainly saprophitic. The chemical substances eventuallly involved in seed conservation, as Schildknecht (1976) hypothesized, are slightly volatile and readily decomposable, if at all, and they could be eventually used in combination with the removal of every newly emerged mycelium.

Attività micostatica di *Messor structor* (Latr.) (Hymenoptera Formicidae) sui semi conservati nei granai.

RIASSUNTO

Su una colonia di *Messor structor* (Latr.) sono stati condotti esperimenti per saggiare la loro capacità di conservare i semi in condizioni di umidità relativa prossima alla saturazione. E' stato realizzato un granaio sperimentale costituito da 10 camere, allineate su due file, divise da una fitta rete di ottone per impedire il passaggio diretto delle formiche da una all'altra. Nelle due camere centrali è stato collocato uno psicrometro per la misurazione della temperatura e dell'UR. In ciascuna delle restanti 8 camere sono stati collocati 60 semi di girasole (*Helianthus annuus* L.). Il fondo di gesso è stato bagnato per mantenere l'umidità relativa al 98-100%, mentre una lampada a raggi infrarossi manteneva la temperatura sui valori prescelti per i due esperimenti: 25±1°C e 30±1°C.

Entrambi gli esperimenti sono stati suddivisi in due fasi: nella Fase 1, per 8 giorni, le formiche hanno avuto accesso solo alle 4 camere di una fila. Nella Fase 2 i collegamenti sono stati invertiti e le formiche hanno avuto accesso solo alle 4 camere precedentemente escluse. Al termine della Fase 1 le formiche avevano consumato 13,0±4,1 semi a 25°C (21,6%) e 7,5±2,6 semi a 30°C (12,5%), mantenendo i restanti in buono stato, mentre i semi esclusi dal contatto con le formiche sono stati coperti da un fitto strato di micelio fungino; il calcolo della presenza di micospore sul tegumento dei semi ha mostrato un numero di spore significativamente inferiore sui semi prelevati dalle camere visitate dalle formiche, rispetto a quelli esclusi. Le specie fungine sviluppatesi sono risultate essere prevalentemente saprofite. Nella Fase 2 le formiche, entrate nelle camere con i semi ammuffiti, li hanno completamente ripuliti dalle muffe - impedendone ogni ulteriore sviluppo - e hanno consumato un numero di semi comparabile a quello della Fase 1: 8,0±1,4 a 25°C (22,9%) e 6,3±1,3 a 30°C (17,9%); i semi delle quattro camere private della presenza delle formiche sono stati ricoperti di micelio fungino.

Viene avvalorata l'ipotesi dell'impiego di sostanze chimiche per la conservazione dei semi, già avanzata da Schildknecht (1976), che risulterebbero comunque poco volatili e velocemente degradabili, e potrebbero essere usate in combinazione con la rimozione meccanica dei miceli fungini.

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