Actes Coll. Insectes Sociaux, 5:25-30 (1989)

INHIBITION OF POLLEN GERMINATION BY ANT SECRETIONS

T. SANDERSON & P.J. WRIGHT

Department of Biology, Univ. of Keele, Keele, Staffordshire, ST5 5BG, England

RESUME

La pollinisation par les fourmis est un phénomène rare (Peakall *et al.* 1984), problement à cause de leur sécrétion antibiotique, qui entraîne la disfonction de la membrane du pollen (Beattie *et al.* 1986). On considère que ceci représente une conséquence de la défense des fourmis envers les champignons. Nous présentons ici une série d'expériences faites sur des ouvrières appartenant à trois espèces de fourmis couramment trouvées en Europe, *Lasius flavus*, *Myrmica scabrinodis* et *Formica fusca*, et nous étudions la conséquence de la sécrétion des fourmis sur la germination du pollen d'une variété de plantes à fleurs britanniques.

Les fourmis ont été placées pendant 30 mn dans des petits tubes a essai avec du pollen provenant des plantes. Le pollen a alors été transféré dans un milieu de germination approprié dans lequel on l'a laissé incuber entre 3 et 5 heures.

Dans 11 cas sur 16, la mise en contact du pollen avec les fourmis a provoqué une réduction sensible du taux de germination du pollen. Le degré d'inhibition s'est révélé être très différent selon les espèces de fourmis, *L.flavus* provoquant le plus d'inhibition, et *M.scabrinodis* le moins.

Le pollen extrait de certaines espèces de plantes était plus sensible à la sécrétion des fourmis que d'autres. Ceci est probablement dû aux caractéristiques variables de l'enveloppe du pollen selon les plantes.

Mots clés: Pollen, fourmis, sécrétions antibiotiques.

SUMMARY

Inhibition of pollen germination by ant secretions

Pollen exposed to the integument of 3 common European ant species showed reduced germination and pollen tube growth rates compared to controls. This effect was most marked in *Lasius flavus* and least in *Myrmica scabrinodis*. These results are in accordance with those of other workers on Australian and American ant species. Secretions from the metapleural glands have been implicated in this effect. Variation in the susceptibility of pollen of different plant species to these ant secretions was noted.

Key words; Pollen, ants, antibiotic secretions.

INTRODUCTION

Although ants are widespread and often abundant, there are very few verified accounts of ant pollination systems (Bates, 1979; Peakall *et al.*, 1984), perhaps surprising in view of the importance of other Hymenopteran groups in this area. Possible reasons for this have been investigated and the disruption of membranes of pollen grains exposed to the integument of several North American and Australian ant species has been demonstrated. This causes reduced pollen germination and pollen tube growth rates (Beattie *et al.*,1984, 1985; Hull and Beattie, 1987).

It is believed that this effect is due to antibiotic secretions from the ant's metapleural glands. The main function of these secretions appears to be the inhibition of fungal spore germination and reduction of fungal hyphal growth, thus reducing ant mortality due to fungal infections (Maschwitz, 1974; Maschwitz *et al.*, 1970).

Here we report on a series of experiments with workers of 3 common European ant species, *Lasius flavus* F., *Formica fusca* L. and *Myrmica scabrinodis* L., investigating their effects on germination of pollen of a variety of British flowering plants.

MATERIALS AND METHODS

All ants were collected from colonies in Staffordshire, England and were maintained in the laboratory. Pollen was collected daily, as required, from a variety of plant species as they came into flower. Pollen from freshly dehisced anthers was used at all times. Germination trials were carried out in various concentrations of sucrose, in order to establish the optimal germination medium for each plant species.

For each replicate a small quantity of ripe pollen was dusted into the bottoms of a pair of small round-bottomed tubes (6 x 23mm). An individual ant was allowed to walk into one of the tubes which was then sealed with a plug of laboratory tissue paper. This was then pushed down the

tube so that the ant was confined in the lower part of the tube and was in close contact with the pollen. Care was taken to avoid unduly irritating the ant. A paper plug was pushed down into the control tube in a similar manner.

After 30 minutes, the paper plugs were removed and the ant allowed to crawl out of the tube. It was then grasped by a second leg with fine forceps, causing it to curl up and attempt to bite and/or sting the forceps. This left the pollen-coated dorsal surface of the ant clear to be dipped into a drop of the germination mixture on a glass microscope slide, and reduced contamination of the sample with oral or anal secretions (Beattie *et al.*,1984). Pollen from the control tube was gently brushed off the sides of the tube into a separate drop of germination mixture.

The slides were placed in a chamber lined with moist filter paper to ensure high humidity, and incubated at room temperature for 3-4 hours depending on the plant species. The proportion of the pollen grains which had germinated in each sample was then assessed.

RESULTS

Pollen germination rates were sometimes low, indicating sub-optimal germination conditions, in which pollen germination could have been influenced by factors other than the ants. To obviate this, only results from trials in which more than 25% of the pollen grains from control samples germinated were included in the analysis, although the same trends were apparent even when the germination was very low.

All statistical analysis was carried out using arc-sine transformed data. G-tests (Sokal and Rohlf, 1981) were used to test for differences in mean germination rates between control and ant-treated pollen samples. The results are summarised in Table 1.

In 11 out of the 16 trials, exposure to ants caused a significant reduction in the pollen germination rate. In only one case was there a significant increase. The degree of inhibition was significantly affected by the ant species. *Lasius flavus* caused most inhibition, reducing germination in treated samples by approximately 37% compared to controls. Pollen exposed to *Formica fusca* was inhibited by an average of 19-20%, and that exposed to *Myrmica scabrinodis* by just over 10%.

Furthermore, pollen from some plant species was more vunerable to ant secretions than pollen from other species. Overall, foxglove (*Digitalis purpurea*) pollen was most affected by ant treatment, with wood sage (*Teucrium scorodonia*) and valerian (*Valeriana officinalis*) least affected.

Ant & plant species	Control pollen %	n	Treated pollen %	n	G-test p
Lasius flavus					
Endymion non-scriptus[1]	33.7	596	0.5	116	<0.001
	53.5	1108	11.1	676	<0.001
	62.3	300	11.0	36	<0.001
Valeriana officinalis[2]	27.5	418	20.3	285	NS
Teucrium scorodonia[3]	47.7	689	8.2	294	<0.001
Cheiranthus cheiri[4]	35.1	770	2.8	145	<0.001
	34.4	556	0.1	179	<0.001
Digitalis purpurea[4]	69.2	894	4.8	378	<0.001
Formica fusca					
E.non-scriptus	26.8	677	8.8	351	NS
V.officinalis	26.9	434	4.9	265	<0.001
	27.3	445	24.9	448	NS
T.scorodonia	36.3	672	21.4	402	<0.001
Myrmica scabrinodis					
V.officinalis	30.7	499	13.1	262	<0.025
T.scorodonia	51.8	703	48.9	525	NS
	51.4	683	65.5	776	<0.001*
	27.7	357	13.7	219	<0.001

* Significant increase in germination in ant-treated pollen compared to controls.
NS - no significant difference
[1] 0.1M sucrose, [2] 0.25M sucrose, [3] 0.125M sucrose, [4] 0.5M sucrose.

Table 1. Percentage germination of control and ant-treated pollen
Tableau 1. Pourcentage de germination du pollen dans des expériences témoins et après contact avec des fourmis.

DISCUSSION

The results clearly demonstrate that exposure of pollen to the integument of these ant species causes reduced germination in a range of plants. These findings are similar to those of Beattie *et al.*, 1984, 1985). Differences between the ant species are also apparent. Pollen exposed to the integument of *L.flavus* was 3 times

as inhibited as pollen exposed to *M.scabrinodis*.

L.flavus lives exclusively underground in warm and humid conditions, ideal for fungal growth. An effective system of preventing germination and growth of fungi would be necessary to stop the spread of infection in the colony. *M.scabrinodis* and *F.fusca* both forage above ground, they have small colonies, and are able to freely move nest site when necessary. *L.flavus*, however, has large, long-lived colonies and is usually confined to a small area around the permanent nest site. A fungal infection in such circumstances would possibly be more detrimental to this species than to *M.scabrinodis* or *F.fusca*, so selection pressure for some form of anti-fungal defence may have been stronger in *L.flavus*. Pollen inhibition appears to be a side-effect of the ants' fungal defence.

Several chemicals isolated from the metapleural glands of ants have been implicated in this effect (Schildknecht 1976). Among these, hydroxydecanoic acid (myrmicacin) is possibly the most important (Iwanami and Iwadare 1978, 1979). However, as yet, myrmicacin has not been isolated in any of the species tested here. Pollen inhibition has also been reported in ant species lacking metapleural glands (Beattie *et al.*, 1985), suggesting that antibiotic chemicals may be present in the cuticle, or in other glands. Further chemical analyses are being carried out to determine the source of the antibiosis in the 3 species studied here.

It was noted that ant-treated pollen sometimes failed to hydrate, an important step before pollen grains are capable of germinating (Stanley and Linskens 1974). Ultrastructural studies of pollen exposed to myrmicacin by Nakamura *et al.* (1982), have shown that this is due to the plasma membranes becoming porous and ineffective. It is possible that differences in pollen coat characteristics and pore size may affect the penetration of ant secretions into the pollen grains, and may account for the observed differences in pollen vunerability between the plant species.

Acknowledgements: We thank Mme. A. Mangeant for assistance with the French translation.

REFERENCES

BATES R., 1979- *Leporella fimbricata* and its ant pollinators. *Bull. Native Orchid Soc. S. Aust.*, *11*,9-10.

BEATTIE, A.J., TURNBULL, C., HOUGH T., JOBSON S., KNOX R.B., 1985.- The vunerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. *Am. J. Bot.*, *72*, 606-614.

BEATTIE A.J., TURNBULL C., KNOX R.B., WILLIAMS E.G., 1984.- Ant inhibition of pollen function: a possible reason why ant pollination is rare. *Am. J. Bot.*, *71*, 421-426.

HULL D.A., BEATTIE A.J., 1987.- Adverse effects on pollen exposed to *Atta texana* and other North American ants: implications for ant pollination. *Oecologia, 75,* 153-155.

IWANAMI Y., IWADARE T., 1978.-Inhibiting effect of myrmicacin on pollen growth and pollen tube mitosis. *Bot. Gaz., 139,* 42-45.

IWANAMI Y., IWADARE T., 1979.- Myrmic acids: a group of new inhibitors analagous to myrmicacin (β-hydroxy-decanoic acid). *Bot. Gaz., 140,* 1-4.

MASCHWITZ U., 1974.-Vergleichende Untersuchungen zur Funktion der Ameisenmetathorakaldruse. *Oecologia, 16,* 303-310.

MASCHWITZ U., KOOB K., SCHILDKNECHT H., 1970.- Ein Beitrag zur Funktion der Metapleuraldruse der Ameisen. *J. Ins. Physiol., 16,* 387-404.

NAKAMURA S.,MIKI-HIROSIGE H., IWANAMI Y., 1982.- Ultra-structural study of *Camellia japonica* pollen treated with myrmicacin - an ant-origin inhibitor. *Am. J. Bot., 69,* 538-545.

PEAKALL R., BEATTIE A.J., JAMES S.H., 1987.- Pseudo-copulation of an orchid by male ants: a test of two hypotheses accounting for the rarity of ant pollination. *Oecologia, 73,* 522-524.

SCHILDKNECHT H.,1976.- Chemical ecology - a chapter of modern natural products chemistry. *Angew. Chemie Int. Ed. Engl., 15,* 214-222.

SOKAL R.R., ROHLF F.J., 1981.- Biometry. 2nd Edition. *W.H.Freeman and Co.,* San Francisco.

STANLEY R.F., LINSKENS H.F., 1974.- Pollen: Biology, Biochemistry, Management. *Springer-Verlag,* Berlin.