known to bind to erythrocytes. Thus it would be interesting to investigate the bioactivity and establish a bioassay of polyene antibiotics against yeasts in the presence of blood. The polyenes are thought to interact with sterols in sensitive cell membranes[19]. The sterol in membranes of erythrocytes is cholesterol and that in yeast cells is largely ergosterol. Such studies may indicate a more in vivo bioactivity and could contribute to our understanding of the rather severe side effects of polyene antibiotics (amphotericin B for example is nephrotoxic[20]).

Flow microcalorimetry in conjunction with standardized liquid nitrogen stored inocula may also prove useful for examining the effects of polyene antibiotics on yeast cells at different stages of growth. The effect of pharmaceutical formulations of the antibiotics may reveal the effects, if any, of pharmaceutical excipients on bioactivity of polyene antibiotics.

Finally the effects of combinations of antifungal compounds on yeast cells could be examined for possible inhibitory or potentiation effects by microcalorimetry.

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Influence of adult food on the reproduction of virgin females of an *Acanthoscelides obtectus* strain originating from Colombian altiplanos

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Summary. The reproductive activity of virgin females of an *Acanthoscelides obtectus* strain originating from the high Colombian plateau was variable. Some females did not synthesize vitellogenin during imaginal life while others produced mature oocytes. In the case of virgin females without reproductive activity, the supply of pollen in the adult diet did not stimulate synthesis and incorporation of vitellogenin. On the other hand, the supply of pollen always induced a higher vitellogenin titre in the haemolymph of females which produced oocytes, and stimulated ovarian production.

Previous research on *Acanthoscelides obtectus* has shown variations between the reproductive activity of virgin females from strains of different geographic origins.

In a population obtained from *Phaseolus vulgaris* seeds harvested on the Colombian altiplanos in the insect's zone of origin, 90% of the first generation females did not produce any mature oocytes when kept as virgin females in the absence of their host plant and fed with honeyed water[1,2]. Laboratory experiments showed that the introduction of *Phaseolus vulgaris* seeds and copulation can remove this reproductive quiescence after a latency period. This type of regulation would make possible a synchronization between the insect's and the host plant's reproductive cycles in nature. Production and prolonged oocyte retention in the female genital tract would thus be avoided outside the host plant's maturation period. This would be advantageous to the insect since prolonged retention of oocytes affects their subsequent developmental capacity after fertilization[3].

However, in another Bruchidae (*Bruchus pisorum*) which also shows a reproductive quiescence outside the host plant's maturation period, Pajni et al.[4] have shown that a diet containing pea-flower pollen is one of the factors inducing reproductive activity. We have therefore tried to determine whether a pollen diet does indeed induce the reproductive activity of *Acanthoscelides obtectus* females, or favour the action of other stimulating factors, and might thus be involved in the synchronisation of the insect and host plant's reproductive cycles.

Materials and methods. The *A. obtectus* strain was collected in the Buesaco area (Province of Nariño) on the Colombian altiplano. The insects were reared in climatic conditions approximating those found in their biotope (2° N latitude, altitude 1800 m); photoperiod 12 h D:12 h L; thermoperiod 21 °C (L)–13 °C (D); relative humidity 70%. Experiments were carried out on insects reared for 15 generations under these conditions. Only 20–30% of the virgin

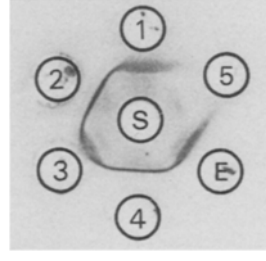

Figure 1. Demonstration of vitellogenin present in the haemolymph by the Ouchterlony technique in *A. obtectus* females. S, antiserum; E, egg; aqueous extracts of females 1, 2, 3, 4 contain vitellogenin, female 5 has not synthesized vitellogenin.

Table 1. Reproductive activity of virgin females fed with or without pollen in absence of bean seeds

Age of females	Diet	Number of females	% females which have synthesized vitellogenin	% females with mature oocytes (A)	Ovarian production* of females A
20 days	Honeyed water	69	88.4	76.8	18.8±2.0 (a)
	Honeyed water + pollen	68	88.2	77.9	22.1±2.1 (b)
50 days	Honeyed water	79	91.0	74.3	15.5±1.8 (a)
	Honeyed water + pollen	75	94.6	76.0	26.1±2.5 (b)
100 days	Honeyed water	88	79.5	72.7	17.6±2.1 (a)
	Honeyed water + pollen	84	97.6	97.6	23.0±2.6 (b)

Values (a) are not significantly different. They are different from values (b). Mean significative difference $p < 0.001$ in the 2 cases.
* Number of eggs oviposited plus number retained.

females in the population under study were reproductively inactive[5].

Throughout the experiments, virgin females, isolated immediately after emerging, were either fed on honeyed water or honeyed water and pollen (finely homogenized pollen pellets collected by bees on various plants). Depending on the groups, females were offered (or not) *Phaseolus vulgaris* seeds, which stimulate oogenesis[6]. For each group, fecundity (number of eggs laid) and ovarian production (number of eggs laid and number of oocytes in retention) were estimated for 3 different ages of imaginal life (20, 50 and 100 days). These ages correspond to well known stages in female reproductive activity[7]. For females which had not produced oocytes, possible vitellogenin presence[8] was detected by using the Ouchterlony immunodiffusion method after homogenizing each insect in 5 ml of NaCl at 9‰[9] (fig. 1). The specific antivitellogenin serum was obtained by a method described by Biemont[10]. Laurell's[11,12] technique was used to make a quantitative comparison of the haemolymph's vitellogenin titre for females fed (or not) which pollen and females having (or not) produced oocytes.

For each group, haemolymph was obtained by centrifuging 18 females whose legs had been cut off[10]. 5-µl samples were placed on agarose gel containing antivitellogenin. Electrophoresis was carried out at 4 °C for 12 h with an electric potential of 100 V. Rockets were coloured with amido black B. Estimates of the relative quantities of antigens (i.e. vitellogenin) were obtained by comparing rocket heights[13].

Results. The ovarian production of females fed with honeyed water or honeyed water and pollen was compared (table 1). a) In quiescent *A. obtectus* females aged between 20 and 30 days, pollen supply neither increased the percentage of females which synthesized vitellogenin (88–95%) nor the percentage of females producing oocytes (74–78%). However, in oocyte producing females, pollen supply significantly increased ovarian production but no oocytes were ever laid; they were retained in the lateral oviducts without being resorbed. This increase in ovarian production has previously been shown in other strains[7–14].

b) In 100-day-old *A. obtectus,* the pollen diet considerably increased the percentage of females synthesizing vitellogenin (80–97%) and incorporating it into oocytes (73–97%). Their ovarian production (23.0) was not significantly different from that of young ones.

c) In all cases, mature oocytes accumulated in the lateral oviducts and never released, as has been observed earlier[2,3].

In the presence of bean seeds, all females (whether pollen-fed or not) produced vitellogenin and formed oocytes[3]. Pollen supply increased ovarian production, as observed in European strains[5]. The increase in ovarian production was particularly high between the 50th and 100th day of age (table 2). In most of the females some of the matured oocytes were laid near the beans.

Table 2. Reproductive activity of virgin females fed with honeyed water and pollen, in the presence of bean seeds

Age of females	Number of females	Ovarian production
20 days	32	40.1±4.2
50 days	42	49.6±2.6
100 days	26	86.1±6.4

The comparative analysis of vitellogenin content in virgin females (figs 2 and 3) shows: a) Since some females could produce vitellogenin without incorporating it (table 1), variations in the haemolymph vitellogenin were compared between quiescent and active females. In all cases, regardless of female age or diet, the content of vitellogenin was higher in reproductively active females (fig. 2).

b) When females were fed on honeyed water and pollen in the absence of bean seeds, the heights of the rockets were always greater than those recorded for females of the same age fed on honeyed water only (figs 2 and 3). Pollen supply, because of its high protein content, favoured vitellogenin synthesis.

c) There were no significant differences between the heights of rockets obtained from haemolymph of 20-, 50- and 100-day-old females fed on pollen and kept without bean seeds. Permanent pollen availability does not, therefore, lead to an increase in vitellogenin content during imaginal life. This phenomenon parallels the relative stability of ovarian production (table 1).

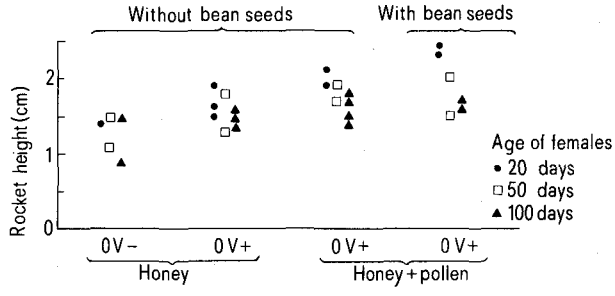

Figure 2. Variation of quantities of vitellogenin (heights of rockets) in haemolymph of *A. obtectus* in relation to adult diet in the presence or absence of bean seeds. OV−, females without mature oocytes (reproductive quiescence); OV+, females which have produced mature oocytes.

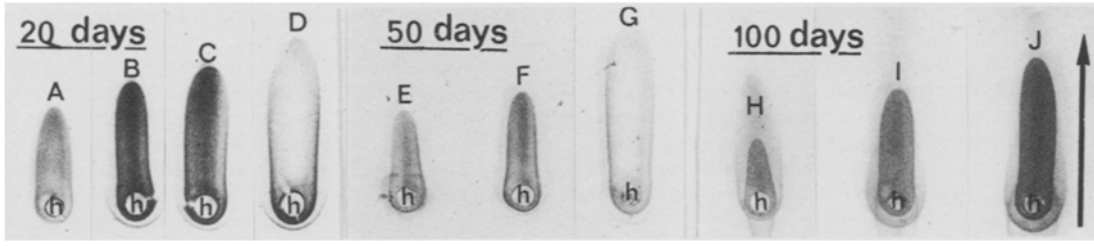

Figure 3. Rocket immunoelectrophoresis of *A. obtectus* haemolymph by Laurell's technique, h, haemolymph. A, E, H: virgin females without mature oocytes in the absence of bean seeds fed with honeyed water; B, F, I: virgin females with mature oocytes in absence of bean seeds fed with honeyed water; C: virgin females with mature oocytes in absence of bean seeds fed with honeyed water and pollen. D. G. J: virgin females with mature oocytes in presence of bean seeds fed with honeyed water and pollen.

d) Among females fed on pollen, rocket heights were similar to those found for controls (adults without bean seeds). High ovarian production, especially at 100 days, was not accompanied by a decrease in vitellogenin content. Therefore, vitellogenin content can only be maintained via synthesis from pollen constituents.

Discussion. In reproductively inactive females less than 100 days old, the presence of pollen in the adult diet was not an adequate stimulus to induce oocyte maturation, in marked contrast with what has been observed in *Bruchus pisorum*[4]. Vitellogenin (when synthesized) was maintained at a very low level. However, we emphasize that the pollen supplied to the bruchids in our experiments was not from the flowers of the host plant. On the other hand, for females aged 100 days or more, a diet containing this pollen allowed production of oocytes which accumulated in the lateral oviducts. In the absence of host-plant seeds, these oocytes were not laid.

Thanks to the reproductive quiescence of a fraction of the female population, *Acanthoscelides obtectus* would be able to wait for the maturation of *Phaseolus vulgaris* pods and seeds, neither producing nor releasing oocytes. Through this adaptation, eggs with small survival chances would probably not be laid[2,3]. If feeding was the stimulus inducing vitellogenesis in the early days of imaginal life, as in some Diptera and Lepidoptera[15], the insect's reproductive cycle might well be out of phase with that of the host plant.

In reproductively active females, provision of a protein diet increased both the haemolymph's protein content and ovarian production, as observed in *Musca domestica*[16] and *Oxya japonica*[17]. This food supply helped to maintain the vitellogenin content in the haemolymph even when oocytes were produced and then released in the presence of *Phaseolus vulgaris* seeds.

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Sporostasis between phylloplane microfungi and a foliar pathogen

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Summary. Interactions between germinating spores of phylloplane microfungi and *Pestalotiopsis funerea* Desm., a leaf spot pathogen of *Eucalyptus globulus* Labill., were studied in vitro. Findings suggest that sporostasis imposed on *P. funerea* by germinating spores of other nonparasitic microfungi was due to nutrient impoverishment or mycotoxins present in spore exudates.

Much work has been done on staling growth products leading to mycostasis between pathogenic and saprophytic fungi in a variety of microecological niches[3,4]. In our[5,6] previous studies on leaf inhabiting microfungi, we reported on the antimicrobial activity of staling growth substances of phylloplane fungi against *P. funerea* due to the presence of antibiotics as a major factor besides other minor causes like alteration in pH and nutrient impoverishment. Recently, Blakeman and Brodie[7] reviewed the literature and suggested that epiphytic bacteria inhibit the growth of foliar pathogens by creating a nutrient shortage during spore germination and successive disease development, analogous to the theory propounded for soil fungistasis by Ko and Lockwood[8]. However, it is evident from the literature[9,10] that nonparasitic fungi already growing on leaf surfaces or applied artificially were strongly antagonistic against developing pathogens, leading to biological control. Microscopic observations[11] showed that most of the microfungi remained on the leaf surfaces in the form of spores except for a few mycelial forms. Therefore, it is expected