Impatiens holstii pollen germination on an aqueous agar extract

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Summary. Impatiens holstii pollen grains germinate on a liquid medium consisting of an aqueous extract of agar but not to the quantitative extent manifested on a solid agar medium. However, the addition of calcium ions to the aqueous agar extract brings about germination percentages and extents of pollen tube growth similar to that observed on the solid agar medium.

Investigations concerning the effects of barbiturates on *Impatiens holstii* pollen germination have emphasized the ability of this species of pollen to germinate readily under completely endogenous nutrient conditions on a water-agar medium[2-4]. However, since pollen germination does not occur under completely endogenous nutrient conditions on the stigma in nature, it becomes questionable whether *Impatiens holstii* pollen germination actually occurred under completely endogenous nutrient conditions on the water-agar medium as claimed previously[2-4].

The very low percentages of pollen germination on glass-distilled water as a liquid medium (unpublished observations) and the presence of significant quantities of nutrient material in Oxoid Agar No.3 that are known to be essential for higher plant growth, especially Ca^{2+} and Mg^{2+} (see Oxoid Manual[5]), indicated that this species of pollen germinates readily on Oxoid Agar No.3 by virtue of this agar having supplied nutrient material(s) needed for pollen germination. That such is actually the case is reported here.

Materials and methods. 4-g-samples of Oxoid Agar No.3 taken from 5 different batches were each placed in non-borosilicate glass reagent bottles and to each of which were added 200 cm^3 of glass-distilled water. The bottles were stoppered, swirled briefly to disperse the agar in the water, and placed in the dark at 27 °C for 18 h. The supernatant liquid in each bottle was carefully drawn off without disturbing the agar layer that had formed on the bottom of the bottle and used for the pollen germination tests described below.

Pollen from mature open flowers of *Impatiens holstii* was gently dusted onto the surface of the following liquid media in thoroughly washed plastic petri dishes (50 mm diameter): (a) glass-distilled water; (b) agar extract; (c) 1:1 agar extract and glass-distilled water; (d) 1:1 agar extract and 1.00 mM $CaCl_2$; (e) 1:1 agar extract and 1.00 mM $MgCl_2$; (f) 1:1 glass-distilled water and 1.00 mM $CaCl_2$; (g) 1:1 glass-distilled water and 1.00 mM $MgCl_2$. Pollen plated out on 5 different batches of 2% autoclaved Oxoid Agar No.3 in plastic petri dishes as above (solid agar medium, h) served as germination controls.

Results and discussion. The germination behaviour of the pollen on the different media a–h is summarized in the table. The markedly lower germination percentages and extents of tube growth on media a and e–g compared with those on media b–d and h demonstrate the availability of unknown nutrient material(s) from the agar which promote the manifestation of these phenomena. The table also shows that the addition of 0.50 mM Ca^{2+} to the agar extract (medium d) brought about quantitative manifestations of germination and tube growth that were equivalent to those obtained on solid agar (medium h).

The marked promotive effect of added Ca^{2+} to the agar extract as regards both germination percentage and tube growth suggests: 1. that Ca^{2+} is one of the nutrient materials supplied by Oxoid Agar No.3 and which is essential for pollen germination and tube growth; and 2. that the Ca^{2+} level in the unsupplemented agar extract, if present, was below that available to the pollen grains that were in direct contact with the solid agar (medium h). However, the lower germination percentage and extent of tube growth on 0.50 mM Ca^{2+} alone (medium f) also suggest the presence of nutrient material(s) other than Ca^{2+} in the agar extract which also promote pollen germination and tube growth.

The presence of 0.50 mM Mg^{2+} in the agar extract (medium e) or on its own (medium g) had no apparent beneficial effect on germination percentage or tube growth. However, the use of $MgCl_2$ demonstrates that Ca^{2+} and not Cl^- was the agent responsible for the marked increase in germination percentage and extent of tube growth on medium d compared with the quantitative manifestations of these phenomena on the other liquid media. The importance of Ca^{2+} for germination and tube growth has been described for a wide range of pollen species[6].

Basal nutrient media consisting of sucrose or boron or both are considered to be necessary for angiosperm pollen germination[7,8]. Although boron was not added to any of the media used in previous investigations[2-4] or here, the possibility cannot be excluded that adequate amounts of boron may have been supplied to the solid agar media from the Pyrex flasks used for putting the agar into solution during boiling or autoclaving. However, the agar extraction procedure at 27 °C in non-borosilicate glassware described here removes the possibility of having supplied significant amounts of boron to any of the liquid media used here. Thus, neither exogenously supplied boron or sucrose is needed for *Impatiens holstii* pollen germination or tube growth.

Statements made previously to the effect that *Impatiens holstii* pollen grains germinate readily under completely endogenous nutrient conditions on a water-agar medium[2-4] implied that barbiturate inhibition of germination was a result of direct action of the barbiturates on the pollen grains independent of any external nutrient supply. However, the nutritional attributes of Oxoid Agar No.3 as a solid medium or aqueous extracts thereof described here indicate instead that the adverse actions of barbiturates on the germination and growth behaviour of this species of pollen described previously[2-4] was that of interference with the

Impatiens holstii pollen germination and tube growth after 1 h on agar extract and other media

Germination medium*	Germination (%)	Mean pollen tube length (mm ± SE)
a	12	(−)**
b	63	0.97 ± 0.09
c	55	0.81 ± 0.05
d	94	1.92 ± 0.28
e	18	(−)**
f	26	(−)**
g	10	(−)**
h	91	1.83 ± 0.23

*The figures listed in the table are based on the 5 agar batches of 100 pollen grains per germination medium. **The germination percentages were too low to yield meaningful measurements of pollen tube lengths.

uptake and/or utilization of the germination promoting nutrient material(s) supplied by the agar. Consequently, the nutritive aspects of Oxoid Agar No.3 in relation to *Impatiens holstii* pollen germination and tube growth may be a suitable experimental system for investigating the manner by which barbiturates interfere with the nutritional requirements for the germination and growth processes of this species of haploid organism.

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2 H.A. Kordan and P.M. Mumford, Ann. Bot. *43*, 997 (1978).
3 H.A. Kordan and P.M. Mumford, Eur. J. Cell Biol. *19*, 299 (1979).
4 H.A. Kordan and P.M. Mumford, J. biol. Educ. *13*, 102 (1979).
5 The Oxoid Manual, 3rd edn, p.50. Oxoid Ltd, Hampshire 1976.
6 J.L. Brewbaker and B.H. Kwack, in: Pollen Physiology and Fertilization, p.143. Ed. H.F. Linskens. North-Holland, Amsterdam 1964.
7 W.G. Rosen, A. Rev. Pl. Physiol. *19*, 435 (1969).
8 R.G. Stanley and H.F. Linskens, in: Pollen: Biology, Biochemistry, Management. Springer-Verlag, Berlin 1974.

Influence of sex, developmental time and food on β-N-acetylglucosaminidase activity in the rice weevil *Sitophilus oryzae* L.

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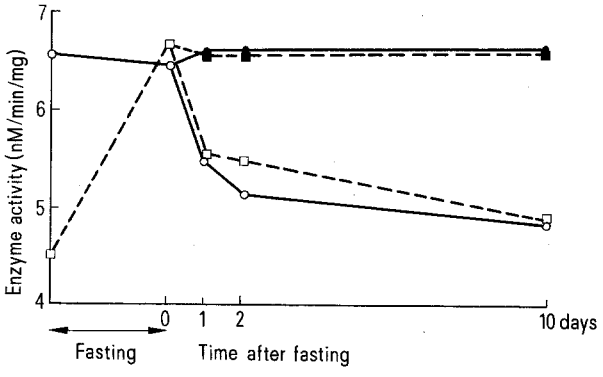
Summary. The effects of some biological parameters on β-N-acetylglucosaminidase activity have been investigated in *S. oryzae*. There is no significant influence of sex and developmental time on the enzyme activity level, which appears in contrast to be greatly influenced by food (wheat or sorghum). Sorghum contains competitive inhibitors which are almost completely removed after dialysis. Fasting relieves this inhibition very quickly, suggesting that inhibitors act directly at the gut level.

The rice weevil *Sitophilus oryzae* contains bacteria-like symbiotes in gut and ovaries[1,2], the number of which is constant in a given strain but varies among different strains[3]. Genetic control of symbiote density by the host has been demonstrated[4]. The physiological mechanism involved in the control is unknown; but since a degeneration process (myelin figures) of symbiotes in bacteriocytes has been observed[2], a bacteriolytic system could be inferred. Because β-N-acetylglucosaminidase is known to exhibit bacteriolytic properties[5] and is present in ovaries, it may be involved in that control. In order to investigate the role of this enzyme, we thought it necessary to test the influence of some factors on its activity level.

Materials and methods. Insects were reared on wheat or sorghum at 27.5 °C and 75% relative humidity. Experiments were carried out with 3 symbiotic strains named Sfr, RR and LL, and with aposymbiotic strains, which were obtained from Sfr, RR and LL and called SS/Sfr, SS/RR and SS/LL, respectively[3]. RR and LL were selected from Sfr to obtain a quick a slow developmental time respectively. Enzyme activity was assayed in adult homogenates by spectrophotometric measurement of p-nitrophenol released from p-nitrophenyl N-acetyl-β-D-glucosaminide, as previously reported[6]. For evaluation of K_m[7], activity was assayed at 4 substrate concentrations (5–10 measures for each concentration); homogenates were dialyzed overnight against 0.05 M citrate buffer pH 4.7.



Influence of food. Enzyme activity of insects ω (○——○) and *sor* (□– –□) before and after fasting (3 days); then half of the insects ω and *sor* were put on either wheat (solid symbols) or sorghum (open symbols).

Results and discussion. Influence of sex. There was no significant change with sex (table 1). In symbiotic strains, only female adults retain great number of symbiotic bacteria throughout their life (these symbiotes are almost all located in the ovaries). Since males and females have the same activity level, symbiotes do not appear to exert a direct influence on it. Since the activity of symbiotic and aposymbiotic insects appears to differ markedly, we can conclude that symbiotes may exert an indirect influence which probably involves an action on regulatory genes. This point has been discussed in a previous paper[8].

Influence of developmental time. For a given strain, developmental time has no influence on enzyme activity (table 2). That point seems important, since strains characterized by different developmental times also contain different enzyme activity levels[6]. These differences are probably due to genetic factors[8]. But for each strain, activity levels do not depend on developmental time which itself is dependent on environmental factors (i.e. temperature

Table 1. Effect of sex on β-N-acetylglucosaminidase activities in symbiotic and aposymbiotic strains (reared on wheat)

Strains	Sex	Enzyme activity[a]	t
Symbiotic (Sfr)	Male	5.84 ± 0.34 (20)	0.53[b]
	Female	5.96 ± 0.36 (20)	
Aposymbiotic (SS/Sfr)	Male	9.83 ± 0.47 (20)	0.33[b]
	Female	9.73 ± 0.45 (20)	

[a] Expressed in nmoles p-nitrophenol × mg^{-1} (fresh wt) × min^{-1} (mean ± SE, sample size in brackets); [b] Not significant (at $p = 0.05$).