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

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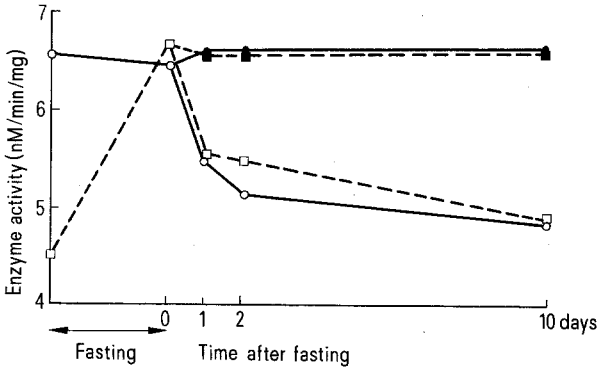
Summary. The effects of some biological parameters on β-N-acetylglucosaminidase activity have been investigated in *S. oryzae*. There is no significant influence of sex and developmental time on the enzyme activity level, which appears in contrast to be greatly influenced by food (wheat or sorghum). 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 $p = 0.05$).

Table 2. Effect of developmental time on β-N-acetylglucosaminidase activities. Summary of variance analysis

Strains	Reared on	Developmental time	Enzyme activity[b]	F
Symbiotic (Sfr)	Sorghum	31.66±0.16(1402)	4.28 (140)	0.0526[c]
	Wheat	30.77±0.15(360)	5.73 (30)	0.0002[d]
Aposymbiotic (SS/Sfr)	Wheat	43.46±0.29(858)	9.94 (50)	0.0066[e]

[a]Expressed in days (mean±SE, sample size in brackets); [b]expressed in nmoles p-nitrophenol×mg^{-1} (fresh wt)×min^{-1} (mean sample size in brackets), [c]calculated from 14 values of developmental time (27-41.5 days); [d]calculated from 3 values of developmental time (28, 31, 34.5 days); [e]calculated from 5 values of developmental time (34, 38, 42, 47, 51 days).

Table 3. Effect of food. Comparison of β-N-acetylglucosaminidase activities in strains reared on wheat and sorghum

Strains		Enzyme activity[a] On wheat	On sorghum	Relative activity (%) wheat/sorghum
Symbiotic	Sfr	5.74±0.27 (20)	4.29±0.34 (20)	134[b]
	RR	6.48±0.28 (30)	5.95±0.39 (20)	109[b]
	LL	4.27±0.17 (20)	3.11±0.20 (20)	137[b]
Aposymbiotic	SS/Sfr	10.18±0.44 (20)	8.71±0.60 (20)	117[b]
	SS/RR	5.42±0.33 (20)	3.06±0.12 (20)	177[b]
	SS/LL	4.31±0.15 (30)	3.28±0.17 (20)	131[b]

[a]Expressed in nmoles p-nitrophenol×mg^{-1} (fresh wt)×min^{-1} (mean±SE, sample size in brackets); [b]significant ($p=0.05$).

variations) to which β-N-acetylglucosaminidase remains insensitive.

Influence of food. The 6 strains of weevil exhibit a lower activity when fed on sorghum than on wheat (table 3).

The influence of fasting has also been studied (figure). Thus, experiments were carried on a strain Sfr continuously reared either on wheat (denoted *ω*) or sorghum (denoted *sor*). After activity measurement, both types of insects (*ω* and *sor*) were submitted to fasting for 3 days. Enzyme activity was measured after this period, and then half of the insects of each type were put on either wheat or sorghum; so *ω* was fed either on wheat (*ω* → *ω*) or sorghum (*ω* → *sor*). (*sor* → *sor*) and (*sor* → *ω*) were obtained according to a similar procedure. Enzyme activity was assayed 1, 2 and 10 days after the end of fasting in the 4 conditions. The figure shows that before fasting, β-N-acetylglucosaminidase activity is lower for *sor* insects than for *ω* insects ($p=0.01$). Just before the end of fasting, the activity level of *ω* decreased a little, but not significantly, whereas there was a 48% increase in *sor*, so that 2 activities did not differ significantly. Insects which were fed with wheat after fasting (*ω* → *ω* and *sor* → *ω*) recovered very quickly (as early as the 1st day) the characteristic activity level of Sfr *ω*, whereas insects which were put on sorghum (*ω* → *sor* and *sor* → *sor*) exhibited a diminished activity even on the 1st day (inhibition exceeds 15%). Then, activity decreased continuously, but at a slower rate. The inhibition reached 25% on the 10th day and differed significantly from that observed on the 1st day ($p=0.05$). From these results it can be inferred that sorghum inhibits β-N-acetylglucosaminidase activity. This inhibition, lost during fasting, is recovered shortly after feeding; this result suggests that the inhibitory effect acts on the gut.

To investigate whether the inhibition by sorghum acts competitively, K_m values were determined in Sfr strains reared on wheat and sorghum, before and after dialysis of the homogenate: K_m values are as follows: 0.21 and 0.22 mM on wheat respectively and 0.44 and 0.29 mM on sorghum. The correlation coefficients lie between 0.98 and 0.99. After dialysis, the K_m in insects reared on sorghum decreased: dialysable inhibitors are therefore present in homogenates and could come from components of sorghum with a larger molecular size. In spite of the decrease, the K_m value still differs significantly from that of insects reared on wheat: different kinds of inhibitors probably act in sorghum: dialysable and non-dialysable. A study of these has not yet been initiated. However, it can be noted that sorghum contains a large amount of glucosamine (7.8% proteins)[9], and when it is added to ground wheat, enzyme activity is inhibited, but less than with ground sorghum alone, even if the amount of glucosamine is multiplied by 10[10]. It seems obvious therefore that the presence of glucosamine cannot explain all the inhibitory effect of sorghum.

Studies on the influence of sorghum on metabolism show that tannins especially are responsible for the delayed growth of chickens[11]; they also depress the digestive use of nitrogen in the growing rat[12]. In insects, tannins damage the midgut and caecum epithelia in the migratory locust[13]; toxic effects have also been described in *Drosophila*[14]; in the boll weevil, they are thought to suppress gut bacteria[15].

The present study provides additional information on the deleterious influence of sorghum on metabolism, without defining which components inhibit β-N-acetylglucosaminidase in the midgut. But that action in symbiotic strains could be responsible for a slower growth on sorghum compared to that on wheat. It can be suggested that this enzyme plays a role in digestion because more than a third of its activity is concentrated in the midgut of the weevil (unpublished data). On the contrary, sorghum seems to enhance the development of aposymbiotic insects[3]. A more detailed study of the precise mode of action of wheat and sorghum appears to be necessary for explaining all the observed facts.

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