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Genetical analysis of the ability of the rice weevil *Sitophilus oryzae* (Coleoptera, Curculionidae) to breed on split peas

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Sitophilus weevils are mainly pests of cereals, but some populations of *S. oryzae* are able to feed and breed on split peas. Genetical analyses and resistance tests were individually conducted by crossing individuals of a pea-resistant strain from China with those of a pea-susceptible strain from the laboratory (SFr). Adults from parental populations and progenies from F_1 and F_2 generations (two reciprocal crosses), as well as from backcrosses, were obtained on wheat and thereafter tested for their ability to survive on yellow split peas for 14 days. The expression of pea-resistance in *S. oryzae* is under simple Mendelian control implicating only one recessive and no sex-linked gene. Resistant populations able to develop on split peas are homozygous for the recessive gene, but some wild susceptible populations can contain heterozygous individuals at a very low frequency. The possible role of intracellular symbionts in pea detoxification was tested by comparing aposymbiotic and normally symbiotic populations. Symbionts were not directly required but might play a role in supplying disposable energy for the metabolism implicated in the detoxification of allelochemicals.

Keywords: detoxification, food plant preference, Insecta, legumes, Mendelian character, rice weevil, *Sitophilus oryzae*.

Introduction

Weevils from the genus *Sitophilus* (Coleoptera, Curculionidae) can be considered as major pests of stored cereals all over the world. Although the three main *Sitophilus* species are mostly specific to cereals, some strains of *S. oryzae* (L.), the rice weevil, are able to feed and breed on split peas; the two other species, *S. zeamais* Motsch., the maize weevil, and *S. granarius*, the grain weevil, have never been observed to survive on these legumes. Cotton (1920) noticed that *Sitophilus* adults could be found feeding on various fruits and that larvae could develop on chickpeas (*Cicer arietinum*). Vayssière (1943) described severe damage by *S. oryzae* to split peas and lentils. These breeding abilities on legume seeds were confirmed by Coombs *et al.* (1977) and Holloway (1986). More recent studies have confirmed that the alimentary spectrum of these cereal weevils contains species belonging to the families Fabaceae, Caesalpiniaceae, Chenopodiaceae, Clusiaceae and Fagaceae (Delobel & Tran, 1993). Weevils could even show higher efficiencies on chestnuts and acorns than on wheat (Delobel & Grenier, 1993).

Genetical analyses of the ability to feed and breed on split peas, using crosses between pea-breeding and non-pea-breeding strains, led to inconsistent conclusions. Thind & Muggleton (1981) showed that this character was controlled by only a single autosomal gene in a strain from Trinidad, but other factors (such as a sex-linked lethal gene) might act in addition to this autosomal gene in another strain from Africa. The results obtained by Holloway & Smith (1985) were not consistent with a simple Mendelian mode of inheritance, but might implicate a maternal factor, such as a symbiont. Therefore, the genetic background of the pea-breeding ability remained to be clarified in these cereal weevils, as well as the possible intervention of their intracellular symbionts located in larval and ovarian bacteriomes (Nardon, 1971; Nardon & Wicker, 1981).

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Materials and methods

Culture of Sitophilus *strains*

In the laboratory, weevils were maintained for several years on wheat at 27.5°C and 70 per cent relative humidity (r.h.) in plastic latticed boxes kept in a climatic cabinet, according to Laviolette & Nardon (1963). After screening for pea-breeding ability among numerous strains available in the laboratory (54 strains coming from all over the world), four *S. oryzae* strains were selected which had some individuals able to feed and achieve their complete development on peas (Delobel & Grenier 1993). These strains were thereafter kept on split peas of commercial origin (Vivien Paille), to maintain the selection pressure. In our experiments on pea resistance, we used the pea-resistant strain from China which had the highest ratio of resistant individuals at the beginning of the selection (12.5 per cent; 36 pea-feeding adults out of 289). This strain was maintained for 24 generations on peas before the experiment. The non-pea-breeding population came from a reference strain (SFr) studied in our laboratory for more than 25 years.

Genetical experiments and tests of pea resistance in S. oryzae

In *S. oryzae*, reciprocal crosses between the pea-resistant strain (China) and the pea-susceptible strain (SFr) were conducted on wheat, in order to obtain both pea-resistant and pea-susceptible progenies. Each mating was individually effected by isolating a virgin pair in a plastic tube, and allowing the female to lay eggs on wheat kernels for 2 weeks. Genetical studies were conducted on pairs: (i) in each parental cross (control); (ii) in the two reciprocal F_1 hybrids (one between a Chinese female and an SFr male: the CS cross; and the other between an SFr female and a Chinese male: the SC cross); (iii) in F_2 hybrids coming from the progeny of F_1 hybrids but maintained in separate tubes; and (iv) in backcrosses between F_1 hybrids and each parent. About 1 month after egg laying, the progeny of each pair emerged from the wheat kernels. Tests of resistance were effected by maintaining adults (about 15 day-old) on split peas for 14 days. This time was sufficient to observe the ability of weevils to survive on this legume. In previous experiments, Delobel & Grenier (1993) confirmed that when adults were able to feed for 14 days on split peas, the complete development from egg to adult was always observed on these seeds.

Pea varieties and dose-response mortality of S. oryzae

Pea strains, both commercial and noncommercial, were provided by the Station d'Amélioration des Plantes (INRA, Versailles, France) as untreated seeds. As many as 31 different varieties of split peas were tested for the dose-response mortality of *S. oryzae* adults. When necessary, seeds were powdered in a blender and bolted through a 0.2 mm screen. Yellow split-pea and wheat flours were mixed together in various proportions (0, 2.5, 5, 10, 20, 40, 60 and 80 per cent pea by weight) and presented to insects as reconstituted pellets by adding water, according to Holloway's method (1986). Pellets were allowed to dry overnight before experiments. For each dose, 30 adults from the sensitive strain SFr were put on pellets and dead insects were observed each day, until 95 per cent mortality in the most concentrated sample occurred, and the LC_{50} (concentration required to kill 50 per cent of individuals) was calculated for each pea variety. The appearance of the seed was also observed. It could be smooth (*l* = 'lisse') or wrinkled (*r* or *rb* = 'ridé'), *r* and *rb* seeds differing in their starch composition.

Aposymbiotic populations

In order to test the possible intervention of intracellular symbionts in the mechanism of detoxification, an aposymbiotic population was obtained from the normally symbiotic Chinese strain by heat treatment (35°C for 3 weeks; Nardon, 1973). The dose-response curve to pea supplementation in the food was studied both in aposymbiotic and in normally symbiotic populations of the resistant strain (China), and the non-pea-breeding strain (SFr) was used as control.

Statistical tests

Classical χ^2-tests for comparison of distributions and the *t*-test for comparison of proportions were used to analyse results. The dose-response curves of mortality of the weevil on different varieties of pea or in the presence or absence of symbionts were obtained using the program TOXICOLOGIE (Febvay & Rahbé, 1991). This program used the probit transformation of a sigmoid curve of mortality and calculated the equation of the regression curve as well as the LC_{50} for each pea variety.

Results

Genetical analysis of the ability to breed on whole split peas in the rice weevil

Adults were tested on whole yellow split peas commercialized by Vivien Paille (Valenciennes, France). Results are given in Table 1. In parental populations, as expected, the progeny derived from pea-breeding Chinese weevils were pea-resistant (R) and the progeny from non-pea-breeding weevils were pea-susceptible (S).

The total F_1 hybrid progeny from 65 pairs was mainly pea-susceptible (only 3.05 per cent resistant). If we consider reciprocal hybrids: 1.50 per cent of offspring of CS crosses (China female × SFr male) were resistant and 4.89 per cent of offspring of SC crosses (SFr female × China male) were resistant. By studying individual pairs, it was possible to separate two kinds of hybrid progeny in each reciprocal cross. In CS crosses, 30 pairs out of 31 gave only S individuals, but one pair furnished a mixed progeny with about half R and half S adults (56 per cent resistant). In SC crosses, 30 pairs out of 34 gave only S adults, but three pairs furnished a mixed progeny (51.5 per cent resistant) and one pair gave a weaker resistance (9 per cent resistant) (this last pair is not analysed in Table 1). In our case, heterozygosity χ^2 could not be calculated because it was impossible to distinguish heterozygous individuals from homozygous ones, in the sensitive population.

In the F_2 generation, the total progeny issuing from F_1 hybrids was 19.61 per cent resistant with 17.57 per cent resistant in CS crosses and 21.51 per cent resistant in SC crosses, respectively. However, by considering F_2 progeny according to the previous classification of F_1 generations, we could observe that in F_2 progeny coming from 100 per cent susceptible F_1 hybrids, we obtained 18.18 per cent resistant insects (17.39 per cent resistant in CS and 18.97 per cent resistant in SC). By contrast, if we consider the F_2 progeny coming from mixed F_1 hybrids we obtained 45.42 per cent resistant insects (32 per cent resistant in CS and 48.05 per cent resistant in SC).

From the analysis of F_1 hybrids, it appeared that the pea-resistant character was not expressed in the F_1 generation and might be recessive. Moreover, the two reciprocal crosses gave nearly similar progenies, therefore this character did not seem to be sex-linked. If we suppose that the resistant character was controlled by a single recessive gene, the existence of a mixed F_1 progeny in four pairs (52.33 per cent resistant) could be explained by the occurrence of heterozygous adults in the pea-susceptible parental population (SFr). If so, this F_1 progeny should contain half homozygous resistant insects and half susceptible heterozygous ones. This hypothesis was confirmed because no significant difference occurred when comparing observed proportions to the theoretical values $\frac{1}{2}$:$\frac{1}{2}$ ($\chi_1^2 = 0.42$).

In the F_2 generation, progenies from both recipro-

Table 1 Inheritance of pea resistance in *Sitophilus oryzae* fed on whole split peas

Generation	Type of mating (female × male)	No. of pairs	Observed R:S progeny	Observed R %	Expected R % †	χ^2-test
Parents	China × China	14	861:0	100	100	—
	SFr × SFr	15	0:1449	0	0	—
F_1 CS	China × SFr (total)	31	29:1901	1.50	0	—
	From 100% S progeny	30	1:1879	0.05	0	—
	From 50% R: 50% S progeny	1	28:22	56	50	0.72 NS
F_1 SC	SFr × China (total)	34	79:1535	4.89	0	—
	From 100% S progeny	30	0:1404	0	0	—
	From 50% R: 50% S progeny	3	73:70	51.05	50	0.06 NS
F_2 $(CS)^2$	CS × CS (total)	31	726:3406	17.57	25	121.65
	From 100% S F_1 progeny	30	710:3372	17.39	25	125.96
	From 50% R: 50% S F_1 progeny	1	16:34	32	56.25	6.48
F_2 $(SC)^2$	SC × SC (total)	34	949:3461	21.51	25	28.58
	From 100% S F_1 progeny	30	762:3255	18.97	25	77.91
	From 50% R: 50% S F_1 progeny	3	123:133	48.05	56.25	0.39 NS

C, China; S, SFr. Reciprocal crosses were effected between resistant (China) and susceptible (SFr) strains and studied in F_1 and F_2 generations.

†Expected R% from the hypothesis of recessive autosomal inheritance.

NS, observed and expected proportions were not signficantly different at the 5% level ($\chi_1^2 = 3.84$).

cal crosses using 100 per cent susceptible F_1 hybrids were not significantly different from each other ($\chi_1^2 = 3.38$). However, all χ^2 tests were inconsistent with the hypothesis of a recessive gene with segregation of characters in the F_2 generation, according to theoretical values $\frac{1}{4}$ R:$\frac{3}{4}$s the observed numbers of resistant weevils were all lower than expected, because of the very high toxicity of the pea variety used in this experiment.

Nevertheless, in the F_2 generation, the results obtained from mixed F_1 hybrids were significantly different from those obtained from 100 per cent susceptible F_1 progeny ($\chi_1^2 = 202.62$) and gave proportions closer to 9/16 R : 7/16 S, as expected in such crosses ($\chi_1^2 = 14.57$); however, these results also were affected by an increase in pea toxicity.

Measurements of LC_{50} *of different pea varieties on* S. oryzae *survival*

The excess of susceptible insects observed in the first genetical analysis was apparently correlated with an increase in the toxicity of the new pea seeds (Vivien Paille) used in this experiment. Therefore, we tested as many as 31 pea varieties for their toxicity on the rice weevil.

First we calculated the dose-response mortality curve and LC_{50} for each variety by using 30 adults of the sensitive strain in 0–80 per cent pea pellets. For 4 days exposure on all varieties, most of the weevils died at higher concentrations and for 6 days exposure most of the weevils were killed, even at lower pea doses. Therefore, we calculated the LC_{50} for each pea variety for 4 days exposure and classified peas according to their decreasing toxicity (Table 2). For example, the Colmo variety was the most toxic with an LC_{50} of about 7 per cent, whereas the 829 variety was the least toxic with an LC_{50} of about 200 per cent (i. e. about only 50 per cent weevils died at higher pea concentrations in the pellets). The pea variety used in the previous genetical analyses was classified in the group of intermediate toxicity ($LC_{50} = 60.93$ per cent), which did not explain the excess in toxicity with respect to Mendelian proportions.

Secondly, we tested the mortality of the resistant weevil strain from China by exposing 30 adults for 14 days on whole split peas from these 31 varieties. Surprisingly, there was no correlation between LC_{50} on pellets for the sensitive strain and mortality percentages for a 14 days exposure of the resistant strain. The pea variety currently used in the laboratory (because available in large quantity and with perenniality) was the most toxic for weevils and some varieties with a high LC_{50} (low toxicity) could lead to 50 per cent mortality for the resistant strain. The lethal effect of pea seemed to be correlated with the phenotype of the grain (smooth or wrinkled) which was itself correlated with a difference in starch quality. Therefore, a high mortality was observed on *r* or *rb* seeds (increased hardness) rather than on *l* smooth seeds which were softer, independently of the pea allelochemicals composition.

In conclusion, in order to obtain a better genetical analysis by avoiding an artificial increase in mortality caused by the hardness of the seed, we chose to use pellets with a pea concentration which was lethal for the susceptible strain but not for the resistant strain, in place of whole seeds. Standard curves of mortality in both populations on mixed pellets showed that 60 per cent pea pellets was a concentration which was sufficient to kill susceptible weevils without affecting the survival of resistant ones.

Genetical analysis for weevil ability to survive on 60 per cent pea–40 per cent wheat pellets

Comparable crosses to those in the first experiment were conducted on 60 per cent pea pellets, as defined in the previous section. Results are shown in Tables 3a and 3b. As expected, when Chinese weevils were intercrossed the progeny was entirely pea-resistant (R) ($n = 468$), and when SFr weevils were intercrossed the progeny was completely pea-susceptible (S) ($n = 280$). The total F_1 hybrid progeny from 60 pairs was nearly completely pea-susceptible (only 0.004 per cent resistant; $n = 2284$) and no difference appeared between reciprocal hybrid CS and SC crosses, as in the case of a recessive gene which is not sex-linked.

In the F_2 generation, the total progeny of F_1 hybrids was 25.32 per cent resistant with, respectively, 25.97 per cent resistant in CS crosses and 24.11 per cent resistant in SC crosses. These results did not differ significantly from the segregation of characters of a recessive gene in the F_2 generation. Observed values for both crosses and total progeny were tested with the expected values $\frac{3}{4}$:$\frac{1}{4}$ and χ^2-values were in agreement with this hypothesis.

In order to perform the genetical analysis we also studied reciprocal back-crosses (BC) between each hybrid (CS and SC) and each parent (resistant or susceptible). Results are shown in Table 3b. When a hybrid (female or male) was mated with the susceptible parent, all its progeny was susceptible, whereas when it was mated with the resistant parent, its progeny was half resistant : half susceptible. In this

Table 2 Comparison of the toxicity of the different varieties of pea for *Sitophilus oryzae* adults

Pea variety	LC_{50} (4 d) test of SFr on 0–80% pea pellets	% mortality (14 d) test of China on whole split pea	Pea variety: Winter (W) Spring (Sp)	Seed aspect: gene 'ridé' (*r*, *rb*) or 'lisse' (*l*)
Colmo	6.96	6.67	Sp	*l*
Bingo	7.15	3.33	—	*l*
Countess	8.02	0	—	*x*
Stampede	10.17	23.33	—	*x*
Diabolo	11.07	0	Sp	*l*
Solara	11.17	0	Sp	*l*
Figaro	11.59	3.33	—	*l*
Micro	14.44	46.67	—	*r*
Finale	14.50	3.33	Sp	*l*
776	15.27	3.33	—	*l*
Amac	15.89	0	W	*l*
777	16.00	0	—	*l*
Mini	18.44	13.33	—	*r*
Amino	23.29	6.67	Sp	*l*
Aldot	26.11	0	—	*l*
Terese	26.71	0	Sp	*l*
Progretta	27.10	0	Sp	*x*
Alfi	27.89	13.33	—	*r*
Madria	28.72	0	Sp	*l*
667	28.98	40.00	—	*rb*
Alaska Sweet	29.14	0	Sp	*rb*
Frilene	29.32	6.67	W	*l*
Frogel	38.96	13.33	W	*r*
Frisson	40.29	0	W	*l*
Lab. pea strain	*60.93*	*76.67*	—	*x*
Cennia	61.65	16.67	—	*rb*
Frigloo	67.20	3.33	W	*l*
206	81.67	0	W	*l*
Rafale	83.85	3.33	W	*l*
831	141.68*	50.00	—	*rb*
829	198.26*	26.67	—	*rb*

The susceptible weevil strain (SFr) was tested on pellets and toxicity expressed as 50 per cent lethal concentration at day 4. The resistant weevil strain (China) was tested on whole split peas and toxicity expressed as adult mortality percentage for a 14 day exposure. Some characteristics of pea varieties and seed appearance are also reported. *x* = unknown.
*Extrapolated values.

latter case, χ^2-test values did not differ significantly from the ratio $\frac{1}{2}$:$\frac{1}{2}$ for both total reciprocal progenies, as expected for the hypothesis of a recessive and autosomal inheritance of the resistant character.

This new genetical analysis on F_1, F_2 and BC was fully in agreement with the hypothesis of Mendelian heredity of resistance to pea in *Sitophilus oryzae* caused by only one recessive autosomal gene.

Moreover, in order to know if one sex was more affected than the other by pea resistance, we observed the sex ratio in both resistant and susceptible progenies in F_2 generations and resistant backcrosses (Table 4). For each mating, the ratio of females was determined. This ratio was similar between susceptible and resistant progenies (*t*-tests showed no significant differences). In each progeny, females and males were identically affected by toxicity. However, differences in female ratios between two crosses appeared, but will not be discussed in this paper.

Possible effects of symbionts

In a previous assay we observed that when aposymbiotic adults (produced by heat-treatment from the pea-resistant strain) were put on whole yellow split peas for 14 days, the mortality ratio was 53.33 per cent ($n = 90$). Subsequently, the dose-response mortality curve to pea supplementation in food was studied, both in aposymbiotic and in normally

Table 3a Analysis of the inheritance of the ability to feed on pea pellets (60% pea) in *Sitophilus oryzae* studied in parental, F_1 and F_2 generations

Generation	Type of mating (female × male)	No. of pairs	Observed R:S values	Observed R %	Expected R % †	χ^2-test
Parents	China × China	10	468:0	100	100	—
	SFr × SFr	10	0:280	0	0	—
F_1 CS	China × SFr	30	6:1642	0.004	0	—
SC	SFr × China	30	3:633	0.005	0	—
	Total CS + SC	60	9:2275	0.004	0	—
F_2 $(CS)^2$	CS × CS	30	1086:3095	25.97	25	2.12 NS
$(SC)^2$	SC × SC	30	528:1662	24.11	25	0.93 NS
	Total $(CS)^2 + (SC)^2$	60	1613:4758	25.32	25	0.34 NS

Abbreviations and symbols have the same meaning as in Table 1.

Table 3b Analysis of the inheritance of the ability to feed on pea pellets (60% pea) in reciprocal back-crosses in *Sitophilus oryzae*

	Type of mating (female × male)	No. of pairs	Observed R:S values	Observed R %	Expected R % †	χ^2-test
Backcross CS	CS × SFr	27	6:2081	0.003	0	—
	CS × China	26	1063:963	52.47	50	4.94
	SFr × CS	18	0:922	0	0	—
	China × CS	13	400:404	49.75	50	0.02 NS
	Total resistant progeny CS	39	1463:1367	51.70	50	3.26 NS
Backcross SC	SC × SFr	16	0:1377	0	0	—
	SC × China	17	269:251	51.73	50	0.62 NS
	SFr × SC	19	5:1499	0.003	0	—
	China × SC	20	533:608	46.71	50	4.93
	Total resistant progeny SC	37	802:859	48.28	50	1.96 NS
Both BC	*Total resistant progeny BC*	*76*	*2265:2226*	*50.43*	*50*	*0.34* NS

BC, back-cross. On the last line, progenies of both back-crosses (CS and SC) showing resistant individuals were pooled (76 pairs). Other abbreviations and the symbols have the same meaning as in Table 1.

symbiotic populations. In each population, 30 individuals for each pea concentration were tested. For a 6-day exposure, as expected, the symbiotic sensitive population exhibited a high level of mortality at pea concentrations as low as 10 per cent (Fig. 1), whereas the symbiotic resistant population from China was not affected. Aposymbiotic Chinese weevils did not die below 60 per cent pea concentration but they became sensitive for higher concentrations. Their LC_{50} was high, 97.54 vs. 11.54 for the sensitive population (LC_{50} of the symbiotic China strain was close to infinite).

Discussion and conclusion

Ability to breed on split peas

The evolution of the ability to feed and breed on peas seems to have occurred recently in cereal weevils (Coombs *et al.*, 1977; Pemberton & de Rodriguez, 1981). The great barriers to feeding on legumes which *S. oryzae* needs to overcome are the toxicants present in most Leguminosae which contain a wide range of phytotoxins with an insecticidal function (Harbone, 1982). Adaptation processes of herbivorous insects to deleterious plant allelochemicals include various strategies such as sequestration, target site insensitivity, occurrence of surfactants and/or an alkaline pH in the gut, differential absorption by the peritrophic membrane, induction of detoxifying enzymes, and excretion (Slansky, 1992).

Nonlegume feeding adult *S. oryzae* die on yellow split peas because of intake of toxins, rather than through the effects of antifeedants (Holloway, 1986). This author also found that rice weevils fed on peas had enhanced adult survival and reproduction after

Table 4 Comparison of distributions of females and males in F_2 and back-cross progeny of *Sitophilus oryzae*

	Type of matings (female × male)	S or R character	No. of progeny	Female %	*t*-test %
F_2 $(CS)^2$	CS × CS	Susceptible	3092	61.48	0.680 NS
		Resistant	1086	60.31	
$(SC)^2$	SC × SC	Susceptible	1662	49.16	0.261 NS
		Resistant	528	49.81	
Back-cross	CS × China	Susceptible	959	47.13	0.759 NS
		Resistant	1061	48.82	
	China × CS	Susceptible	401	53.12	0.094 NS
		Resistant	406	53.45	

Susceptible and resistant progeny were considered separately. Symbols have the same meaning as in Table 1. Observed and expected proportions were not significantly different at the 5% level.

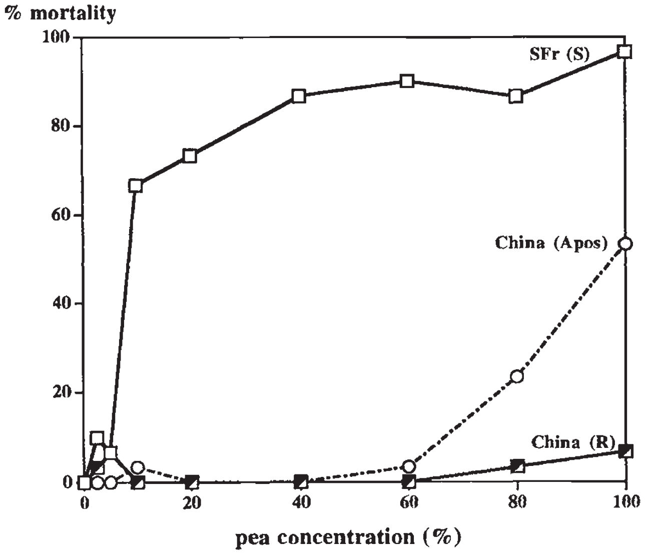

Fig. 1 Dose-response mortality curve at 6 days for *Sitophilus oryzae* feeding on pea/wheat pellets, studied in the susceptible strain (SFr) and in the resistant strain (China). The mortality of the aposymbiotic resistant strain (Apos) obtained from the China strain is represented by the interrupted line.

removal from the legume. This may be indirect evidence for an allelochemical-induced beneficial enzyme system(s) in this species. By studying enzyme activities, Baker & Woo (1992) showed that despite a small decrease in β-glucosidase activity in the gut of pea-breeding weevils when transferred to wheat, there was no obvious relationship between β-glucosidase activity in adults and their adaptation to feeding and breeding on legumes.

Holloway & Mackness (1988) described mechanisms of detoxification of legume secondary compounds in legume-feeding *S. oryzae*. They studied pea-resistant and susceptible weevil strains (only breeding on wheat) on six legume seeds: adzuki bean (*Vigna angularis*), split pea (*Pisum sativum*), brown lentil (*Lens esculenta*), cowpea (*Phaseolus vulgaris*), green gram (*Vigna radiata*) and soybean (*Glycine max*). Wheat-breeding populations survived very poorly on legumes, whereas the legume-breeding populations survived better. Soybean was clearly more toxic than the five other legumes.

Genetical analysis of the pea-breeding character

From the genetical analysis conducted in our study, it can be concluded that only one autosomal recessive gene is involved in the expression of pea resistance in *S. oryzae*. Resistant populations able to develop on peas are homozygous for the recessive gene. Selection of weevils on pea seeds has led to a population (China) which is completely resistant to peas. Even if this population is bred thereafter on wheat from egg to adult, weevils remained fully resistant to peas.

It can be noted that in the first experiment, the progeny of some crosses between Chinese resistant and SFr susceptible strains was half resistant and half susceptible, showing that the susceptible SFr population may contain some individuals (5 out of 65 = 7.69 per cent) which were heterozygous for the resistant gene. In order to estimate the frequency of this gene in the SFr strain we maintained a large number of adults (about 4000) on peas and we found only five insects alive after 2 weeks. The proportion of homozygous resistant weevils in SFr was then 0.13 per cent leading to an estimate of the resistance frequency, p_{res}, of 0.0354 in this population. The theoretical proportion of heterozygous weevils could be calculated (6.83 per cent) and this value was very similar to that observed in the experiment (7.69 per cent).

The demonstration of the possible occurrence of heterozygous insects in susceptible populations allows us to discuss previous conclusions dealing with genetical transmission of the ability of *S. oryzae* to feed on peas. Holloway & Smith (1985) compared survival of *S. oryzae* adults after 14 days on yellow split peas, in two resistant populations (Trinidad and Tanzania) and a susceptible one (India). In F_1 hybrids among these three strains, they noticed important deviations from expected Mendelian monogenic frequencies. Especially, reciprocal hybrids obtained with the susceptible Indian strain showed a significant level of resistance. In fact, an analysis of their data, considering that none of the population was homozygous for the studied character, showed that in the Trinidad strain, depending of the data used, about 1 per cent of weevils were homozygous for pea susceptibility, and up to 6 per cent were homozygous in the Tanzania strain. In the reverse situation, the Indian population contained 4 per cent of resistant homozygous insects, which implies that 33 per cent were heterozygous. These calculated values allow the interpretation of all the results of crossing experiments between the three strains, especially the unexpected high ratio of surviving insects observed in the cross containing the Indian strain, and also the high ratio of susceptible insects in the crosses between Tanzania and Trinidad lines (14.2 per cent). Therefore, the results of Holloway & Smith (1985) are not contradictory to those of Thind & Muggleton (1981), and like ours point to a resistance borne by a single recessive autosomal gene.

Toxicity levels in peas

The analysis of mortality of the sensitive strain at day 4 on ground peas, showed a wide range of variation in the content of the toxic substances (LC_{50} between 7 and 200). Winter peas appeared to be less toxic than spring peas.

On whole seeds, the mortality of the pea-feeding population was nil or nearly nil for 77 per cent of the pea strains. Most of the pea strains which appeared to be resistant to weevil attack were of the wrinkled phenotype (*r* or *rb*). In peas, the *r* and *rb* loci are known to encode genes affecting the starch biosynthetic pathway by modifying the crystalline structure of starch granules and their contents in amylopectin (Bogracheva *et al.*, 1995; Lloyd *et al.*, 1996). The presence of a special starch can modify the physical resistance of the seeds, either by enhancing hardness or lowering the water content in the same conditions. The action of other secondary compounds could not be excluded, although these compounds should also have acted on the sensitive strain, even after grinding.

Relationships between detoxification and symbiosis

In the rice weevil, the aposymbiotic population obtained from the pea-resistant strain by heat treatment was resistant for pea concentrations up to 60 per cent. From this result, it may be concluded that resistance to pea is not linked to symbiosis. However, at higher concentrations, mortality appears, which reduces the ability to feed and breed on peas.

The effect of symbionts may be either direct caused by their own action in detoxification (as shown by Dowd (1989, 1992) in *Lasioderma serricorne* where symbiotic yeasts produced enzymes able to detoxify xenobiotics) or more probably indirect caused by their modifying the energy metabolism implicated in detoxification of allelochemicals. Indeed, it may be hypothesized that the loss of symbionts leading to modifications in the energy metabolism diminishing the oxidation capacities in the weevil (Heddi *et al.*, 1993), could weaken the detoxification mechanisms in the aposymbiotic strains. This detoxification could be reduced not only in the case of the toxicity studied but also in the case of other allelochemicals present in peas.

In conclusion, the expression of pea resistance in *S. oryzae* is under the control of a single Mendelian autosomal recessive gene. Resistant populations able to develop on pea are homozygous for the recessive gene, but some wild susceptible populations can contain some heterozygous individuals. Symbionts are not directly required but might play a role in supplying disposable energy for the metabolism implicated in the detoxification of allelochemicals.

This study may be an illustration of how a minor genetical change can modify the pest status of an insect through an enlargement of its food adaptation. Thus *S. oryzae*, which is mainly a pest of cereals, may also be able to feed and breed on Legumes.

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