Fertilization Effects of Phosphorus and Sulfur on Chemical Composition of Seeds of *Pisum sativum* L. and Relative Infestation by *Bruchus pisorum* L.

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The effect of different rates of phosphorus (P) and sulfur (S) application to soil on chemical composition and level of seed infestation by *Bruchus pisorum* was studied under field conditions using three pea (*Pisum sativum* L.) varieties. The cultivar Ballet (BA) had greater concentration of total phenols, phytic acid, and protein ($P < 0.05$) than cultivars Renata (RE) and Solara (SO). Tannin content in RE was less than that in SO and BA ($P < 0.05$). A close correlation ($r^2 = 0.928$, 0.987, 0.984) was observed between different P application rates and phytic acid levels in RE, SO, and BA, respectively. Otherwise, only SO showed a linear correlation between S fertilization and total protein content ($r^2 = 0.867$). Sulfur application rate was correlated with albumin fraction ($r^2 = 0.987$, 0.979, 0.750), Cys ($r^2 = 0.951$, 0.884, 0.764), and Met ($r^2 = 0.995$, 0.893, 0.964) contents of RE, SO, and BA cultivars, respectively. The proportion of grains infested by *B. pisorum* was greater in BA than in SO and RE ($P < 0.05$). Infestation levels in all cultivars were unaffected by fertilization rates ($P > 0.05$). However, a linear correlation between both protein and phytic acid content and *B. pisorum* infestation was observed ($r^2 = 0.735$ and 0.732, respectively). The results suggest that greater phytate and protein contents reduce the risk of *Bruchus* infestation in pea seeds.

Keywords: *Tannins; phytates; nutritional quality; Pisum sativum; insect infestation; Bruchus pisorum; fertilization*

INTRODUCTION

Legume crops, particularly pea (*Pisum sativum*), faba bean (*Vicia faba*), and kidney bean (*Phaseolus vulgaris*), are important sources of protein, carbohydrates, and minerals for both animal and human nutrition throughout the world (Bressani and Elías, 1988).

Genotypic variation and cultivation methods are two major factors influencing levels of chemical constituents in pea seeds. Because pea has the ability to fix nitrogen (N), the application of N-containing fertilizers has been considered unimportant, and more attention has been paid to P or S enrichment (Shukla *et al.*, 1993; Straw *et al.,* 1994).

Many seeds contain substances that are referred to as antinutritional factors (ANFs). The main ANFs in legume seeds are tannins, phytates, saponins, lectins, and protease inhibitors. These factors disturb metabolic processes and reduce use of nutrients by animals (Liener, 1986; Gupta, 1987; Nyman and Bjorck, 1989; Marzo *et al.,* 1991). The presence of various ANFs, in most species, is controlled by the expression of various genes and their interaction with specific culturing methods (Annis and O'Keeffe, 1991; Richardson, 1991).

Pea (*Pisum sativum* L.) is commonly used in animal and human nutrition, and the presence of ANFs limits the amount that can be used. However, these ANFs protect seeds against insect attack and diseases (Riemer and Whittaker, 1989; Fornal and Ciepielewska, 1995).

One of the most damaging parasites of *P. sativum* seed is *Bruchus pisorum* L. (Coleoptera: Bruchidae). Different species of *Bruchus* that infest different legumes are distributed worldwide and present problems in some countries (Srivastava *et al.,* 1988; Clement, 1992; Modgil and Mehta, 1993a,b). In the spring, the insects emerge from hibernation. They feed on pollen-flowering peas to complete their sexual maturation, and eight or nine days after feeding, egg-laying begins. Female *Bruchus* glue their eggs on the surfaces of pods that have reached their maximal size. The newly hatched larva penetrates through the pod tissues, enters immature pea grains, and feeds on cotyledon content with little damage to the seed coat (Balachowsky, 1962; Makasheva, 1983). At the time of larval penetration, the concentration of tannins and phenols in the seed coat is minimal (Sudesh *et al.,* 1995). Larvae eat up to 30−40% of the cotyledons, and, during their development, cause a substantial reduction in the levels of available carbohydrates, proteins, phosphorus, vitamins, and minerals (with a relative increase in the proportion of ANFs) in the pea seeds. Seeds damaged by *Bruchus* are unfit for use either as food or for sowing purposes (Modgil and Mehta, 1993a, 1994).

This work describes, with field trials, the effect that various rates of P and/or S soil enrichment had on the chemical composition of pea seeds and its role in infestation by *B. pisorum*.

MATERIALS AND METHODS

Field Trials. Three pea cultivars (*P. sativum* L. cv. Renata, Solara, and Ballet) were selected to test their responses to soil enrichment with P and S and to infection of *B. pisorum* (Coleoptera: Bruchidae). Field trials were carried out during the 1992 growing season in Navarra, Spain, 42° 40′ N, 2° 2′ W. During the growing period, average rainfall was 61 mm month^{-1}, and the mean temperature was 11.5 °C. Experiments were conducted on a sandy clay loam soil [47% sand, 32% clay, and 21% silt (USDA classification); Soil Survey Staff, 1975] with 70, 30, and 331 ppm of available soil P (Bray and Kurtz, 1945), S, and K, respectively, and 0.13% N. The pH of the soil was 7.42 measured in 0.1 N KCl (1:2.5).

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Table 1. Effect of P and S Fertilization on Nutritional Characteristics of *P. sativum* L. Cv. Renata, Solara, and Ballet

cultivar	fertilization[a]	phytic acid, mg g^{-1}	total phenols, mg g^{-1}	tannins, mequiv kg^{-1}	protein, %
Renata	P_0S_0	8.56 ± 0.01[b]	0.37 ± 0.07	188 ± 10	18.9 ± 0.3
	P_0S_{30}	8.53 ± 0.03	0.47 ± 0.08	173 ± 3	19.9 ± 0.1
	P_0S_{60}	8.51 ± 0.03	0.56 ± 0.10	175 ± 5	18.9 ± 0.2
	$P_{40}S_0$	8.76 ± 0.01	0.49 ± 0.08	179 ± 1	19.5 ± 0.1
	$P_{40}S_{30}$	8.59 ± 0.03	0.48 ± 0.0 8	179 ± 5	18.4 ± 0.3
	$P_{40}S_{60}$	8.58 ± 0.03	0.50 ± 0.09	175 ± 7	19.6 ± 0.2
	$P_{80}S_0$	8.83 ± 1.01	0.60 ± 0.11	174 ± 5	19.6 ± 0.2
	$P_{80}S_{30}$	8.65 ± 0.01	0.51 ± 0.04	176 ± 5	19.6 ± 0.1
	$P_{80}S_{60}$	8.64 ± 0.04	0.45 ± 0.04	173 ± 5	19.0 ± 0.2
Solara	P_0S_0	8.75 ± 0.01	0.40 ± 0.04	244 ± 6	17.7 ± 0.2
	P_0S_{30}	8.68 ± 0.01	0.47 ± 0.03	243 ± 3	18.9 ± 0.1
	P_0S_{60}	8.70 ± 0.07	0.49 ± 0.02	240 ± 5	19.1 ± 0.1
	$P_{40}S_0$	8.83 ± 0.03	0.54 ± 0.09	238 ± 7	18.9 ± 0.2
	$P_{40}S_{30}$	8.78 ± 0.03	0.44 ± 0.03	236 ± 14	19.4 ± 0.1
	$P_{40}S_{60}$	8.80 ± 0.05	0.52 ± 0.05	244 ± 10	18.6 ± 0.1
	$P_{80}S_0$	8.95 ± 0.03	0.46 ± 0.04	238 ± 10	19.0 ± 0.2
	$P_{80}S_{30}$	8.86 ± 0.02	0.50 ± 0.06	243 ± 7	19.1 ± 0.1
	$P_{80}S_{60}$	8.90 ± 0.01	0.33 ± 0.01	243 ± 15	19.8 ± 0.1
Ballet	P_0S_0	8.92 ± 0.01	0.55 ± 0.05	257 ± 7	20.8 ± 0.1
	P_0S_{30}	9.08 ± 0.03	0.53 ± 0.03	249 ± 5	21.0 ± 0.4
	P_0S_{60}	9.12 ± 0.06	0.60 ± 0.05	228 ± 21	20.7 ± 0.3
	$P_{40}S_0$	9.58 ± 0.03	0.53 ± 0.04	233 ± 19	21.2 ± 0.1
	$P_{40}S_{30}$	9.59 ± 0.02	0.50 ± 0.03	236 ± 18	21.1 ± 0.2
	$P_{40}S_{60}$	9.65 ± 0.04	0.54 ± 0.07	240 ± 8	22.3 ± 0.4
	$P_{80}S_0$	10.00 ± 0.04	0.50 ± 0.04	250 ± 10	19.0 ± 0.2
	$P_{80}S_{30}$	10.16 ± 0.14	0.530.05	234 ± 13	22.2 ± 0.2
	$P_{80}S_{60}$	10.12 ± 0.10	0.53 ± 0.02	230 ± 16	20.5 ± 0.2
cultivar	CD ($P < 0.05$)	0.05	0.05	10	0.2
fertilization	CD ($P < 0.05$)	0.08	NS	NS	0.3
cv × fertilization	CD ($P < 0.05$)	0.14	NS	NS	0.5

[a] Subscripts of P and S express elemental rate in kg ha^{-1}. [b] Entries are means ± SE of either phytic acid, total phenols, tannin (n = 10 determinations), or total protein content (n = 5 determinations) of raw pea flour. CD denotes critical difference. Differences of two means between fertilization/cultivar exceeding this level are significant.

The soil was prepared by moldboard plowing and disking in the early autumn followed by rototilling just before planting. Nitrogen was applied to all plots as NH_4NO_3 at the rate of 40 kg ha^{-1}. Eighty-one 9 m^2 plots (4.5 × 2) were prepared with 0.95 m borders. The experimental design was a randomized complete block with three replicates per variety. Peas were sown using a rate of 110 seeds m^{-2} planted at a depth of 2−2.5 cm. Plants were fertilized two weeks after emergence at rates of P (0, 40, 80 kg ha^{-1}) and/or S (0, 30, 60 kg ha^{-1}). Phosphorus and S were applied as $CaHPO_4 \cdot 2H_2O$ and $CaSO_4 \cdot 2H_2O$, respectively.

Determination of Grain Infestation Levels. Seeds of each of the three pea cultivars with different P and/or S enrichments were harvested and distributed in two groups. The first was stored at −20 °C before freeze-drying, milling, sieving through a 0.4 mm sieve, and chemical analyses. The second group of seeds was stored in a chamber at 4 ± 2 °C and 40 ± 5% relative humidity for a maximum of 30 days to evaluate the levels of infestation by *B. pisorum*. After this period, the samples were placed in 125 mL glass vessels (100 grains per vessel) in three replicates per variety and soil enrichment treatment. The vessels were covered with muslin cloth held in place with elastic bands and placed in the climatized chamber at a temperature of 22 ± 2 °C and a humidity of 40 ± 5% for the 20 days required for the development of *Bruchus* adults. On the 21st day the vessel temperature was increased to 60 °C for 5 min to kill the parasites. Both insects and parasitized seeds of each set were counted.

Protein, Amino Acids, Phytic Acid, Total Phenols, and Tannin Assays. Moisture as well as N content was determined according to the AOAC method (AOAC, 1990). Crude protein was calculated using the 6.25 conversion factor for Kjeldahl N. Different protein components were isolated from pea flour as follows. The extraction buffer was chosen according to the guidelines of Murray and Vairhinos (1982). Albumins and globulins were separated using the procedure of Schroeder (1982) as modified by Gueguen and Barbot (1988). Amino acids were analyzed according to the method of Llames and Fontaine (1994) using a Waters HPLC system with the Pico-Tag method. This procedure is based on the precolumn derivatization reaction with a phenyl isothiocyanate reagent solution (Bidlingmeyer *et al.*, 1987). Phytic acid was determined after HCl extraction, anion-exchange chromatographic purification, and spectrophotometric measurement of diluted extracts with the modified Wade reagent (Frühbeck *et al.*, 1995). Total polyphenols were extracted with water at 70 °C and estimated spectrophotometrically after addition of Folin−Denis reagent (Salunkhe *et al.*, 1990). Tannins were determined with acidified vanillin and estimated as milliequivalents of catechin (Broadhurst and Jones, 1978).

Statistical Analysis. One-way ANOVA, followed by Fisher's least significant difference (lsd), was used to determine the differences among treatments (Miller and Miller, 1993).

RESULTS AND DISCUSSION

Cultivar by Fertilization Effects on Crude Protein, Total Phenol, Tannin, and Phytic Acid Contents. The protein content in seed samples of each pea cultivar was affected by P and S fertilization (Table 1). Protein content expressed as percent on dry matter varied from 18.4 to 19.9, from 17.7 to 19.8, and from 19.0 to 22.3 in Renata, Solara, and Ballet seeds, respectively. Ballet had greater protein content than the Renata and Solara ($P < 0.05$). Only Solara showed a linear correlation between total protein content and S fertilization ($y = 17.86 + 0.02x$; $r^2 = 0.867$, $P < 0.05$). Total phenol and tannin levels of each of the three cultivars were unaffected by fertilization treatments ($P < 0.05$). Ballet had greater content of total phenols than Renata and Solara ($P < 0.05$). Tannin content in Renata was less than that in Solara and Ballet ($P < 0.05$). There were no differences in the number of seeds

Table 2. Effect of P and S Fertilization on Protein Fractions and Amino Acid Profile of *P. sativum* L. Cv. Renata, Solara, and Ballet

cultivar	fertilization[a]	protein fractions[b]		amino acid (62.5 mg g^{-1} of N)																
		globulins	albumins	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Trp	Val	Ala	Asp	Cys	Glu	Gly	Pro	Ser	Tyr
Renata	P_0S_0	56.9*	20.7	8.45	2.29	3.57	7.91	8.08	0.99	4.68	3.68	0.92	5.10	4.48	4.32	1.04	12.7	4.72	3.91	4.67	3.80
	P_0S_{30}	58.1	21.0	8.64	2.36	3.69	7.98	8.15	1.03	4.74	3.94	0.98	5.20	4.59	4.35	1.11	12.8	4.89	4.15	4.98	4.03
	P_0S_{60}	56.0	21.2	8.47	2.30	3.61	7.92	8.09	1.08	4.69	3.72	0.92	5.14	4.49	4.32	1.27	12.7	4.77	3.94	4.71	3.82
	$P_{40}S_0$	56.5	22.0	8.51	2.32	3.63	7.96	8.09	0.92	4.72	3.81	0.94	5.17	4.51	4.33	1.03	12.7	4.82	4.01	4.74	3.93
	$P_{40}S_{30}$	56.7	21.3	8.42	2.26	3.54	7.91	8.07	1.05	4.68	3.66	0.92	5.09	4.46	4.31	1.16	12.6	4.72	3.83	4.56	3.79
	$P_{40}S_{60}$	57.9	22.1	8.59	2.34	3.67	7.98	8.13	1.07	4.73	3.89	0.97	5.19	4.57	4.35	1.22	12.8	4.86	4.11	4.91	4.00
	$P_{80}S_0$	57.9	21.4	8.56	2.33	3.65	7.97	8.12	1.05	4.72	3.86	0.96	5.19	4.56	4.34	1.14	12.8	4.85	4.07	4.87	3.97
	$P_{80}S_{30}$	57.8	21.5	8.52	2.33	3.64	7.97	8.11	1.06	4.72	3.82	0.95	5.18	4.53	4.33	1.19	12.8	4.85	4.03	4.83	3.95
	$P_{80}S_{60}$	57.1	22.2	8.49	2.30	3.62	7.94	8.09	1.07	4.69	3.77	0.93	5.17	4.49	4.33	1.20	12.7	4.79	3.94	4.72	3.85
Solara	P_0S_0	55.5	19.7	8.36	2.18	2.94	6.87	6.90	0.91	4.71	3.43	0.92	4.54	3.90	4.26	0.90	12.3	4.42	3.94	4.71	3.56
	P_0S_{30}	55.8	20.0	8.37	2.19	2.99	6.90	7.03	0.93	4.75	3.43	0.92	4.52	3.91	4.31	0.93	12.4	4.43	3.97	4.72	3.59
	P_0S_{60}	57.1	20.5	8.43	2.23	3.13	7.06	6.98	1.01	4.82	3.59	0.96	4.69	4.05	4.38	1.06	12.6	4.54	4.08	4.82	3.69
	$P_{40}S_0$	55.6	19.6	8.38	2.21	3.01	6.92	6.92	0.87	4.76	3.47	0.93	4.57	3.95	4.33	0.80	12.4	4.45	3.99	4.78	3.60
	$P_{40}S_{30}$	57.1	19.6	8.48	2.24	3.18	7.15	7.03	0.94	4.86	3.65	0.98	4.74	4.11	4.46	0.95	12.9	4.59	4.17	4.86	3.79
	$P_{40}S_{60}$	55.8	20.2	8.27	2.15	2.89	6.83	6.89	1.07	4.71	3.41	0.92	4.52	3.83	4.25	1.11	12.1	4.35	3.89	4.66	3.53
	$P_{80}S_0$	56.4	19.3	8.38	2.22	3.04	6.94	6.94	0.99	4.77	3.51	0.94	4.62	3.98	4.35	0.98	12.5	4.48	4.02	4.79	3.61
	$P_{80}S_{30}$	57.0	19.3	8.41	2.23	3.11	6.95	6.97	0.99	4.77	3.53	0.95	4.66	4.03	4.37	1.03	12.5	4.52	4.07	4.82	3.68
	$P_{80}S_{60}$	57.4	19.2	8.45	2.24	3.14	7.07	7.03	1.05	4.83	3.63	0.97	4.72	4.06	4.39	1.07	12.8	4.56	4.15	4.85	3.71
Ballet	P_0S_0	57.4	18.6	8.80	2.65	3.01	8.33	8.18	0.89	5.23	4.09	1.00	5.40	4.93	4.77	1.37	12.7	4.77	4.93	4.81	3.95
	P_0S_{30}	57.7	19.2	8.85	2.67	3.04	8.33	8.19	0.93	5.23	4.12	1.03	5.47	4.95	4.81	1.39	12.7	4.80	4.94	4.82	4.01
	P_0S_{60}	57.1	19.2	8.64	2.63	2.99	8.32	8.16	0.95	5.22	4.11	0.94	5.40	4.83	4.72	1.41	12.7	4.73	4.89	4.79	3.93
	$P_{40}S_0$	58.3	18.0	8.89	2.69	3.13	8.35	8.21	1.02	5.30	4.13	1.11	5.51	5.02	4.85	1.41	12.8	4.82	5.00	4.89	4.06
	$P_{40}S_{30}$	58.0	18.2	8.89	2.69	3.11	8.34	8.20	1.03	5.26	4.12	1.06	5.49	4.98	4.82	1.43	12.8	4.81	4.98	4.86	4.03
	$P_{40}S_{60}$	58.6	18.5	8.92	2.77	3.18	8.38	8.23	1.15	5.34	4.14	1.22	5.59	5.19	4.88	1.50	12.9	4.89	5.03	4.97	4.15
	$P_{80}S_0$	57.0	18.9	8.61	2.61	2.89	8.28	8.09	0.83	5.17	4.08	0.87	5.35	4.76	4.70	1.33	12.7	4.65	4.74	4.66	3.88
	$P_{80}S_{30}$	58.5	19.6	8.91	2.72	3.14	8.36	8.23	0.87	5.32	4.13	1.15	5.56	5.15	4.86	1.36	12.8	4.86	5.01	4.92	4.07
	$P_{80}S_{60}$	57.0	20.8	8.63	2.58	2.94	8.29	8.16	1.07	5.19	4.09	0.94	5.37	4.78	4.72	1.46	12.7	4.72	4.87	4.92	4.07

[a] Subscripts of P and S express elemental rate in kg ha^{-1}. [b]Expressed as a percentage of the protein fraction. *Entries are means of three determinations.

per pod among the cultivars and the treatments (data not shown). In all three varieties differences were observed in phytic acid levels depending on the enrichment treatments ($P < 0.05$). Close positive linear correlation between phytic acid content and P fertilization for each cultivar was observed ($y = 858.17 + 0.34x$, $r^2 = 0.928$, $P < 0.05$; $y = 874.33 + 0.25x$, $r^2 = 0.987$, $P < 0.05$; $y = 896.00 + 1.35x$, $r^2 = 0.984$, $P < 0.05$ for Renata, Solara, and Ballet, respectively). Phytic acid levels in Ballet were greater than those found in Renata and Solara ($P < 0.05$). Our results are in agreement with results obtained by other authors that fertilization with different rates of P and S directly affects the chemical composition of legume seeds (Omar *et al.*, 1990; Singh and Singh, 1992)

Cultivar by Fertilization Effects on Protein Fractions and Amino Acid Profile. Globulin content expressed as percent of protein fraction varied from 56.0 to 58.1, from 55.5 to 57.4, and from 57.0 to 58.6 in Renata, Solara, and Ballet seeds, respectively. Albumin fraction varied from 20.7 to 22.2 in Renata, from 19.2 to 20.5 in Solara, and from 18.0 to 20.8 in Ballet seeds (Table 2).

In all three pea cultivars, the albumin fraction increased with greater rates of S application ($y = 20.72 + 0.01x$, $r^2 = 0.987$, $P < 0.05$; $y = 19.66 + 0.01x$, $r^2 = 0.979$, $P < 0.05$; $y = 10.70 + 0.01x$, $r^2 = 0.750$, $P < 0.05$ for Renata, Solara, and Ballet, respectively). The same relation was observed between S fertilization and sulfur amino acid levels such as cysteine ($y = 1.025 + 0.038x$, $r^2 = 0.951$, $P < 0.05$; $y = 0.883 + 0.003x$, $r^2 = 0.884$, $P < 0.05$; $y = 0.536 + 0.017x$, $r^2 = 0.764$, $P < 0.05$ for Renata, Solar, and Ballet, respectively) and methionine ($y = 0.988 + 0.002x$, $r^2 = 0.995$, $P < 0.05$; $y = 0.900 + 0.002x$, $r^2 = 0.893$, $P < 0.05$; $y = 0.893 + 0.001x$, $r^2 = 0.964$, $P < 0.05$, for Renata, Solara, and Ballet, respectively).

Amino acid profile showed that the level of each amino acid increased as the seed N content increased. This relationship means that any variation, whether or not

Table 3. Effects of P and S Fertilization on *B. pisorum* Infestation Levels in *P. sativum* L. Cv. Renata, Solara, and Ballet

cultivar	fertilization[a]	infestation, %
Renata	P_0S_0	14.7 ± 1.1[b]
	P_0S_{30}	13.3 ± 1.4
	P_0S_{60}	13.0 ± 1.1
	$P_{40}S_0$	12.3 ± 0.6
	$P_{40}S_{30}$	15.3 ± 1.4
	$P_{40}S_{60}$	13.3 ± 1.1
	$P_{80}S_0$	13.7 ± 0.3
	$P_{80}S_{30}$	14.0 ± 1.3
	$P_{80}S_{60}$	15.7 ± 1.3
Solara	P_0S_0	13.8 ± 0.8
	P_0S_{30}	12.2 ± 0.8
	P_0S_{60}	13.3 ± 1.0
	$P_{40}S_0$	14.2 ± 0.9
	$P_{40}S_{30}$	14.0 ± 0.8
	$P_{40}S_{60}$	14.3 ± 1.1
	$P_{80}S_0$	14.0 ± 1.1
	$P_{80}S_{30}$	15.0 ± 0.7
	$P_{80}S_{60}$	13.2 ± 1.2
Ballet	P_0S_0	25.0 ± 1.8
	P_0S_{30}	25.7 ± 2.2
	P_0S_{60}	23.0 ± 2.3
	$P_{40}S_0$	24.7 ± 1.5
	$P_{40}S_{30}$	22.3 ± 0.9
	$P_{40}S_{60}$	22.4 ± 0.9
	$P_{80}S_0$	23.7 ± 1.1
	$P_{80}S_{30}$	23.0 ± 1.6
	$P_{80}S_{60}$	22.3 ± 0.9
cultivar	CD ($P < 0.05$)	1.0
fertilization	CD ($P < 0.05$)	NS
cultivar × fertilization	CD ($P < 0.05$)	NS

[a] Different subscripts of P and S express elemental rate in kg ha^{-1}. [b] Entries are means ± SE of infested seeds ($n = 3$ determinations). CD denotes critical difference. Differences of two means between fertilization/cultivar exceeding this level are significant.

genetic in origin, induces the same changes in amino acids.

Insect Infestation Levels. Infestation levels varied from 12.3 to 15.7, from 12.2 to 15.0, and from 22.3 to 25.7 insects per 100 grains, in Renata, Solara, and Ballet, respectively (Table 3). Ballet showed greater infestation levels than Solara and Renata ($P < 0.05$).

Neither of the cultivars showed differences in the infestation level caused by fertilization rates. A linear correlation was observed between *B. pisorum* infestation and protein ($y = 16.76 + 0.17x$, $r^2 = 0.735$, $P < 0.001$) or phytic acid content ($y = 763.78 + 7.99x$, $r^2 = 0.732$, $P < 0.001$). On the other hand, no correlation was observed between infestation levels and the antinutritional factors tannin and total phenols.

The life cycle of *Bruchus* encompasses a number of stages that might explain our results. At first, the adult seeks the flower on which it feeds and lays its eggs on well-developed pods. The *Bruchus* larva attacks the seed before the hull is totally synthesized and while it has lesser levels of the protective tannins and polyphenols (Jaglan *et al*., 1987). During its development, the larva feeds only on the cotyledon's constituents (carbohydrates, protein, and phosphorus) without using any of the hull as a food source.

In conclusion, our results suggest that in the conditions of this experiment, fertilization does not permit the development of a plant's absolute defensive mechanisms against *Bruchus* infestation, but it does induce changes in seed composition related with a better defense of pea seeds against this parasite.

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