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Sulfur fertilization increases defense metabolites and nitrogen but decreases plant resistance against a host-specific insect

N.A. Santos[1], N.C. Teixeira[1], J.O.S. Valim[1], E.F.A. Almeida[2], M.G.A. Oliveira[3,4] and W.G. Campos[1,4]*

[1]Department of Biosystems Engineering, Federal University of São João del Rei, São João del Rei, MG, 36301-160, Brazil: [2]Institute of Agricultural Science, Federal University of Minas Gerais, Montes Claros, MG, 39525-000, Brazil: [3]Department of Biochemistry and Molecular Biology, Federal University of Viçosa, Viçosa, MG 36570-900, Brazil: [4]National Institute of Science and Technology in Plant-Pest Interactions, Brazil

Abstract

We tested the sulfur-modulated plant resistance hypothesis using potted cabbage (*Brassica oleracea* var. *capitata*) plants that were grown without and with increasing levels of sulfur fertilization. Changes in plant chemical traits were assessed and developmental performance of *Plutella xylostella*, a highly host-specific leaf-chewing insect, was followed. Leaf sulfur concentration gradually increased with growing addition of sulfur in soil; however, there was a generalized saturation response curve, with a plateau phase, for improvements in total leaf nitrogen, defense glucosinolates and insect performance. *Plutella xylostella* performed better in sulfur-fertilized cabbage probably because of the higher level of nitrogen, despite of the higher content of glucosinolates, which are toxic for many non-specialized insects. Despite the importance of sulfur in plant nutrition and production, especially for *Brassica* crops, our results showed that sulfur fertilization could decrease plant resistance against insects with high feeding specialization.

Keywords: plant defenses, glucosinolates, plant quality, plant nutrition, herbivory, specialist herbivores, *Plutella xylsotella*, *Brassica oleracea*

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Introduction

Many plants of the family Brassicaceae are grown as annual crops worldwide. Such plants in general use multiple defense mechanisms against herbivorous insects and glucosinolates are thought to be their first line of defense (Ahuja *et al*., 2010; Bohinc *et al*., 2012). There are approximately 120 described glucosinolates and they are degraded by myrosinase, upon plant damage, to a variety of hydrolysis products, which are responsible for virtually all of the biological activities of this compound class (Rask *et al*., 2000; Wittstock *et al*., 2003; Holst & Williamson, 2004; Halkier & Gershenzon, 2006). Glucosinolates hydrolyzed into toxic compounds negatively affect a wide variety of generalist or polyphagous herbivores; however, insects that specialize in feeding on brassicaceous plants have evolved specific adaptations to detoxify glucosinolates or inhibit the formation of toxic hydrolytic products (Kos *et al*., 2012).

Among the main cosmopolitan pest insects that use brassicaceous plants as food sources are the diamondback moth *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Zalucki *et al*.,

*Author for correspondence: Tel: +55 (32) 3379-5566
Fax: +55 (32) 3379-5565
E-mail: wgcampos@ufsj.edu.br

2012), a highly host-specific herbivore (Sarfraz *et al.*, 2006; Ahuja *et al.*, 2010). Varieties of *Brassica oleracea*, such as the cabbage (var. *capitata*), are its preferred hosts, where it feed preferably on the new foliage with higher levels of glucosinolates and nitrogen (Campos *et al.*, 2016; Moreira *et al.*, 2016). The *P. xylostella* larvae overcome the toxic barrier of the glucosinolate–myrosinase system and use some metabolites as feeding stimulant. Moths even rely on some glucosinolates for host location and oviposition stimulation (Ratzka *et al.*, 2002; Sarfraz *et al.*, 2006; Hopkins *et al.*, 2009; Winde & Wittstock, 2011).

The glucosinolates are sulfur-rich compounds and each molecule contains two to three sulfur atoms (Halkier & Gershenzon, 2006; Falk *et al.*, 2007). In addition, sulfur is found in methionine and cysteine amino acids, which are incorporated into most eukaryotic proteins (Droux, 2004; Brosnan & Brosnan, 2006). Variations in amino acids and protein contents in plants are vitally important and strongly affect many life-history traits of herbivorous insects (Mattson, 1980; Scriber & Slansky, 1981; Leather, 1990), as foliar nitrogen affects behavior and performance of *P. xylostella* in *Brassica* (Staley *et al.*, 2009). The total sulfur content in plant tissues varies in decreasing quantities and demands among Brassicaceae and Liliaceae (high-sulfur demand), Leguminosae (medium), and Gramineae (low) (Jordan & Ensminger, 1958; Scherer, 2001).

Sulfur interacts with nitrogen in such a way that lack of one reduces the uptake and assimilation of the other (Hesse *et al.*, 2004). In some cases, sulfur availability in soil benefits the metabolism of sulfur-containing amino acids and proteins (Spencer *et al.*, 1990; Naito *et al.*, 1995; Zhao *et al.*, 1999*a*), the nitrogen uptake and fixation (Zhao *et al.*, 1999*b*; Abdallah *et al.*, 2010), and the activity of nitrate reductase (Friedrich & Schrader, 1978; Hesse *et al.*, 2004). Therefore, sulfur fertilization is expected to increase the concentration of nitrogen in plant tissues (Andrew, 1977; Ahmad *et al.*, 2007; Järvan *et al.*, 2008; Rehman *et al.*, 2013). In addition, glucosinolate biosynthesis is based on sulfur directly or on the sulfur-containing amino acids cysteine and methionine (Hesse *et al.*, 2004), and sulfur assimilation is a prerequisite for synthesizing glucosinolates (Aarabi *et al.*, 2016). Genes coding for sulfur assimilation pathway, sulfate transporters, and enzymes involved in the biosynthesis of sulfur-containing amino acids and glucosinolates are all regulated under sulfur conditions (Hawkesford & De Kok, 2006; Falk *et al.*, 2007; Aarabi *et al.*, 2016). Therefore, sulfur fertilization has usually led to increases in glucosinolates levels in *Brassica* and other plants (De Pascale *et al.*, 2007; Falk *et al.*, 2007; Badenes-Perez *et al.*, 2010).

If soil amendment with sulfur increases plant nitrogen and glucosinolates, then sulfur is expected to change the quality of host plants as food for herbivorous insects. Sulfur fertilization may modulate plant resistance against herbivores. Our specific hypothesis is that sulfur fertilization decreases resistance of *Brassica* plants against highly host-specific herbivores. For this, we assumed that: (1) sulfur-fertilized *Brassica* will have higher leaf contents of total nitrogen and glucosinolates, and (2) the specialist *P. xylostella* will perform better on sulfur-fertilized host plants because of higher levels of nitrogen, despite of higher concentration of glucosinolates.

Material and methods

Soil conditions and sulfur treatments

Cabbages (*B. oleracea* var. *capitata*) were grown in 10 L plastic pots in a greenhouse (9 m × 25 m) covered with transparent waterproof plastic and laterally coated with anti-aphid netting. The pots were filled with subsoil lacking organic matter and humus content. First, soil fertility was analyzed, and improvements in nutrients were carried out at the beginning and during plant growth. We used a standard fertilization recommended by Malavolta (1980) for growing potted plants. Macronutrients (N, P, K, and Mg) were added via NPK fertilizer 4 : 14 : 8, urea, and potassium chloride; micronutrients (Zn, B, Cu, Mn, and Mo) by means of zinc sulfate, boric acid, copper sulfate, manganese chloride, and ammonium molybdate. The soil pH was stabilized in 6.8 using dolomitic limestone. Physical soil analysis indicated a sandy texture.

After the initial soil amendment with macro and micronutrients, sulfur concentration was 7.03 mg dm^{-3}, which was the experimental control condition (C) for sulfur in soil. The amounts of sulfur delivery required to achieve different treatment conditions in soil were 25 mg dm^{-3} (T1), 75 mg dm^{-3} (T2), and 100 mg dm^{-3} (T3), since an ideal sulfur fertilization for potted cabbage is 50 mg dm^{-3}. We used the Sulfurgran 90®, with 90% sulfur purity. The fertilizer was added on soil at 250, 750, and 1000 mg per 10 L pot, respectively, for T1, T2, and T3 treatments.

Seedlings of cabbage were initially grown in polystyrene seedling trays for approximately 30 days, when they acquired four leaves and were ±7 cm high. Then, they were transplanted into 3 L plastic pots, where they were maintained under standardized organic substrate for another 20 days. After that, the seedlings were transplanted again into the 10 L pots with the four sulfur conditions. They received 300 ml of water per day. The pots were placed on greenhouse benches at 1 m high. The sulfur treatments (C, T1, T2, and T3) were arranged on the greenhouse benches in a uniform distribution. Each row consisted of four pots (plants), one for each treatment, which were changed in position along the rows. Thus, each plant was an experimental unit. The plants in each treatment were allowed to grow and to adapt for 45 days.

Measurements of plant responses

Leaf sulfur

To determine total leaf sulfur, five plants per sulfur treatment ($n = 20$) were grown as described above. All the leaves from the five plants in each treatment were collected. The leaves were wrapped in paper bags and immediately taken to the laboratory. The samples were blotted dry and subsequently fully dried in a forced-air-circulation oven at 55°C until constant weight. The dry material was then ground in a Wiley mill equipped with a 1 mm mesh screen and sealed in plastic pots for later analyses. The analyses were requested and paid to the Soil and Plant Tissue Laboratory Services, at the Federal Institute of Education, Science and Technology, Southeast of Minas Gerais, Barbacena, Brazil. Leaf tissue samples were submitted to nitric-perchloric digestion, following Tabatabai & Bremner (1970). Leaf concentrations of total sulfur were determined by turbidimetry (Tedesco *et al.*, 1995).

Leaf nitrogen

To determine total leaf nitrogen, ten plants per sulfur treatment ($n = 40$) were grown as described above. All the leaves from the ten plants in each treatment were collected. The leaves were wrapped in paper bags and immediately taken to the laboratory. The samples were blotted dry and

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subsequently pre-dried in a forced-air-circulation oven at 55°C until constant weight. The pre-dried material was then ground in a Wiley mill equipped with a 1 mm mesh screen and sealed in plastic pots for later chemical analyses. First, triplicate samples of 0.5 g of pre-dried matter were again placed in the oven at 105°C for 16 h to obtain the dry matter. The chemical analyses were made using the pre-dried matter; however, data were converted to dry matter. The analyses were performed three times for each experimental unit (plant), from which we used the average values.

We used the standard method 990.03 (Kjeldahl method) for total nitrogen determination, following AOAC (2000). A sample of 0.25 g of pre-dried matter was placed in a Kjeldahl tube, to which 3 g of a mixture of chemical catalysts (Na_2SO_4 and $CuSO_2$) and 10 ml of H_2SO_4 95% were added. The tubes were placed in a Gerhardt® digestion block, Kjeldatherm, located inside a fume hood, whose temperature was gradually increased to 400°C, where they remained for 4 h. The tubes with the digested samples were coupled to a Kjeldahl nitrogen distiller. The ammonia that was released during the distillation was collected in an Erlenmeyer flask containing 20 ml of boric acid solution 4%. The material was titrated with molar HCl 0.1. The volume of HCl that was used in the titration was recorded and used to calculate the nitrogen concentration of the sample. The total nitrogen (%) was calculated as: $(V \times N \times 0.014 \times 100)/W$; where V = volume of HCl, N = normality HCl, 0.014 = milliequivalent gram of nitrogen, and W = sample weight.

Leaf glucosinolates

To determine total glucosinolates, leaves were harvested from another ten plants per treatment ($n = 40$), which were grown as described above. They were frozen and ground to a powder in liquid nitrogen and maintained at −20°C (Rosa & Heaney, 1996) for further analysis. The determination of the secondary metabolites was based on Jezek *et al.* (1999) and Gallaher *et al.* (2012), with adaptations. Alternatively to chromatographic methods, we used a procedure based on alkaline hydrolysis of glucosinolate to yield 1-thioglucose. Ferricyanide oxidizes 1-thioglucose, and the loss of the chromogenic ferricyanide can be assessed spectrophotometrically and used to determine the total glucosinolates content (Jezek *et al.*, 1999).

A leaf sample of 0.5 g received 7.5 ml of boiling acetate buffer (0.2 M, pH 4.2), and it was kept in a water bath at 100°C for 15 min. After that, 1.5 ml of barium and lead acetate was added, and the mixture was vortexed. An amount of 0.4 g of polyvinylpolypyrrolidone was added and the mixture was incubated at room temperature for 15 min. After incubation, 1.5 ml of sodium sulfate (Na_2SO_4) was added. The mixture was homogenized by vortexing and centrifuged (3500*g*) for 10 min at room temperature. Thereafter, 0.9 ml of the extract was mixed with 0.9 ml of sodium hydroxide (NaOH 2M) and incubated for 30 min at room temperature. Then, 0.138 ml of concentrated (37%) hydrochloric acid (HCl) was added, and the mixture was again centrifuged (3500*g*) for 10 min. For the spectrophotometric assay, 0.9 ml of the supernatant was mixed with 0.9 ml of potassium ferricyanide (2 mM), which was previously prepared in a phosphate buffer (0.2 M, pH 7). The mixture was homogenized by vortexing and centrifuged at 3500*g* for 3 min, and the absorbance of the supernatant was measured at 420 nm against a blank control. The control solution was prepared in the same manner as the test solution, but without plant material.

The absorbance was converted to a concentration using a calibration curve, which beforehand was constructed using sinigrin (Sigma-Aldrich®) 5 mg ml^{-1} as standard, with dilutions of 0.025, 0.05, 0.075, 0.1, and 0.15 ml of solution (Gallaher *et al.*, 2012). First, 5 mg of sinigrin was diluted in 1 ml of distilled water. Then, 0.5 ml of NaOH 2M was added to 0.5 ml of the solution, and the mixture was incubated for 30 min at room temperature. Soon after, 0.077 ml of concentrated (37%) HCl was added, so that the final concentration of sinigrin monohydrate was 2.3364 mg ml^{-1}. From this solution, volumes from 0.025 to 0.15 ml were separated, to which a phosphate buffer was added until the solution reached a volume of 0.5 ml. Finally, 0.5 ml of potassium ferricyanide was added, and absorbance at 420 nm was immediately read against a phosphate buffer, pH 7.0. The total glucosinolates concentration was converted into lmol g^{-1} of fresh leaf weight.

Measurements of insect responses

The insects were obtained from a breeding stock that was kept for ≈20 generations in a climate-controlled room (23°C, 12 h light), where they were fed leaves of collard (*B. oleraceae* var. *acephala*) and cabbage before the trial period. The *P. xylostella* breeding was installed with larvae and pupae collected from *Brassica* fields in São João del Rei (MG), Brazil. The test began with groups (cohorts or experimental units) of 50–60 eggs, which were separated for rearing with plants of each sulfur treatment ($n = 40$ cohorts). Because each plant was individually insufficient for feeding an entire cohort, we had to use two labeled plants to rear each cohort. Thus, 20 plants of each treatment were grown as described above.

First, to stimulate oviposition, macerated pieces of cabbage leaves were inserted in a paper towel envelope. The envelope was placed in a cage with approximately 250 pairs of moths that had emerged the previous day. The envelope was offered for oviposition during the late afternoon and removed early the following morning, and it was then incubated at a constant temperature (25 ± 0.5°C), relative humidity (65 ± 5%), and photoperiod (12 D/12 L). Pieces of paper towel with 50–60 eggs were individually placed in petri dishes. After 2 days, each egg cohort was placed on a cabbage plant. The plants were wrapped in a bag made of white organza fabric to prevent the escape of insects. Upon emergence, the very small first instar larvae enter the leaf mesophyll, where they mine until the second instar. The number of first instar endophytic larvae was estimated by deducting the number of non-viable eggs that remained in the piece of paper towel. As soon as the larvae reached the third to fourth instar, we transferred them to a new plant with a fine paintbrush.

The percentages of preimaginal survival (or mortality) were estimated by comparing the number of first instar larvae with the number of adults that emerged from pupae. The duration of the larval phase was expressed as the number of days between eggs hatching and pupation. From the total number of pupae that developed in each cohort, ten couples were sexed using a stereomicroscope after removing the silk cocoon with fine tweezers. We placed each couple in a separate acrylic box of 10 × 10 × 5 cm. After the emergence of moths, we added a piece (6 cm^2) of a fourth expanded leaf of cabbage to the box for oviposition. We did not supply the moths with honey solution or water. The eggs were counted using a stereomicroscope, and the leaf pieces were changed daily until all moths

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had died. The eggs laid on the walls of the box were also counted and removed daily with tweezers. Fecundity was expressed as the average number of eggs laid per female using the average of the ten couples for each cohort.

The most commonly used measure of fitness or performance is the intrinsic rate of increase (*r*) (Mylius & Diekmann, 1995). The growth rate *r* was calculated using the equation $r = LnRo/Gt$, where $Ro = (FP/100)/2$, being F = fecundity and P = % of preimaginal survival, halved at the end because the sexual ratio of *P. xylostella* is 50% female. Thus, *Ro* indicates the estimated number of adult females generated by each female. *Gt* is the generation time, obtained by the sum of the preimaginal period (days from egg to adult) and half the adult longevity (Howe, 1953). Therefore, *r* indicates the *Ln* of the number of adult females generated by each female per day.

Statistical analyses

Analyses were performed using GraphPad Prism 5.0 software, Prism Graph Inc., San Diego, CA, USA, all with a significance level of 0.05. Data normality was verified using the Kolmogorov–Smirnov test, and homogeneity of variances was assessed by the Bartlett's test. Plant and insect responses were subjected to one-way analysis of variance followed by the Tukey's *post hoc* test. Correlations of intrinsic rate of increase with leaf nitrogen and leaf glucosinolates were verified with the Pearson's correlation coefficient.

Results

Sulfur, glucosinolates, and nitrogen concentrations in leaves of cabbage changed with the amount of sulfur added in soil (respectively, $F_{3,19} = 45$, $P < 0.0001$; $F_{3,39} = 11$, $P < 0.0001$; and $F_{3,19} = 51$, $P < 0.0001$). The addition of 25, 75, and 100 mg dm^{-3} of fertilizer to the natural condition of 7.03 mg dm^{-3} of sulfur in soil, respectively, increased leaf sulfur in 48, 69, and 83%. Thus, total leaf sulfur concentration gradually increased with the addition of sulfur in soil (fig. 1a); however, total leaf glucosinolates and nitrogen reached maximum concentrations at the lowest dose of sulfur added in soil of 25 mg dm^{-3}. The higher doses of sulfur in the soil did not continue to increase leaf glucosinolates and nitrogen (fig. 1b, c).

There was no difference in fecundity of moths that developed from larvae fed with plants under different conditions of sulfur fertilization ($F_{3,39} = 1$, $P = 0.39$). On the other hand, plant sulfur fertilization affected both the percentage of eggs that developed until adult stage (preimaginal survival) ($F_{3,39} = 8$, $P < 0.001$) and the time spent for the preimaginal development ($F_{3,39} = 11$, $P < 0.0001$). Because of this and despite a high fecundity variation within treatments, the intrinsic rate of increase (*r*) was significantly affected by the amount of sulfur in soil ($F_{3,39} = 12$, $P < 0.0001$).

The preimaginal development was slower when larvae fed plants that did not receive sulfur fertilization, but increasing doses of the fertilizer did not proportionately accelerate their development (fig. 2a). Similarly, the preimaginal survival and the rate *r* were lower in control condition, but increasing doses of sulfur did not proportionately improve the insect performance (fig. 2b, c). There was a generalized pattern of improvement in leaf nitrogen (fig. 1b), leaf glucosinolates (fig. 1c), and herbivore performance (fig. 2a–c) with sulfur content in soil, and the intrinsic rate of increase was positively correlated with leaf nitrogen ($r = 0.67$, $P < 0.0001$) and leaf glucosinolates ($r = 0.58$, $P < 0.0001$).

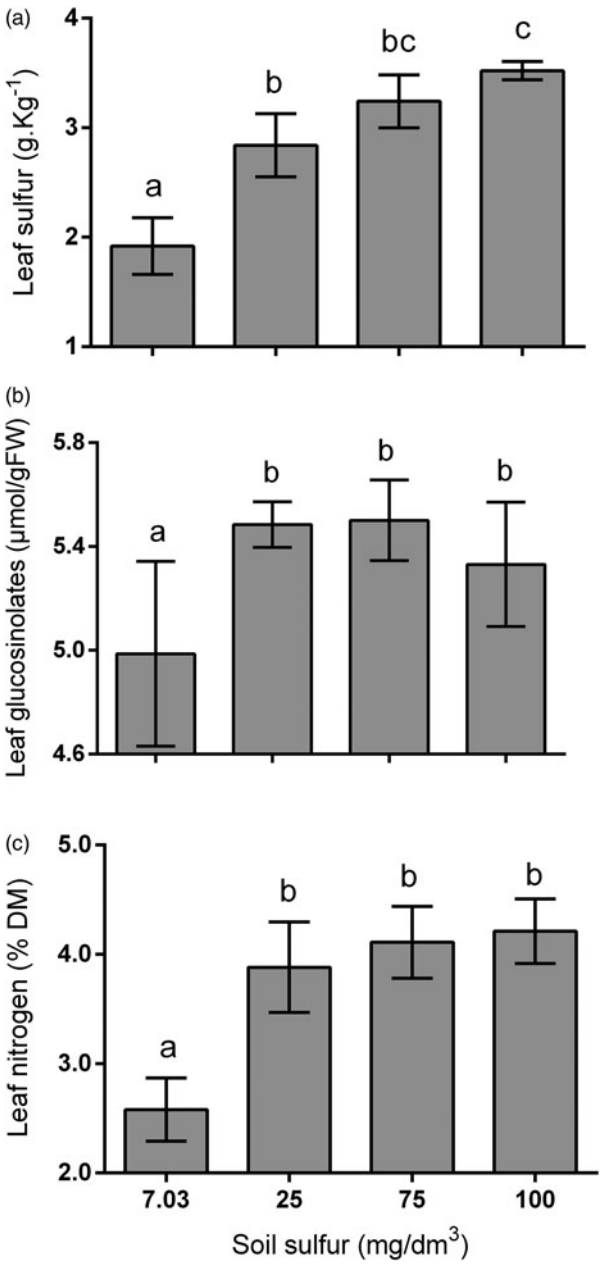

Fig. 1. Concentrations of total sulfur (a), glucosinolates (b) and nitrogen (c) in leaves of cabbages (*Brassica oleraceae var. capitata*) that were grown under different sulfur contents in soil. *Bar* and *line* indicate average ± SD. *Different letters* between columns indicate significant differences ($P < 0.05$) by the Tukey's *post hoc* test.

Discussion

Despite the importance of sulfur in plant nutrition and production, especially for *Brassica* crops (Tabatabai, 1984; Scherer, 2001; Grant *et al.*, 2012), our study showed that sulfur

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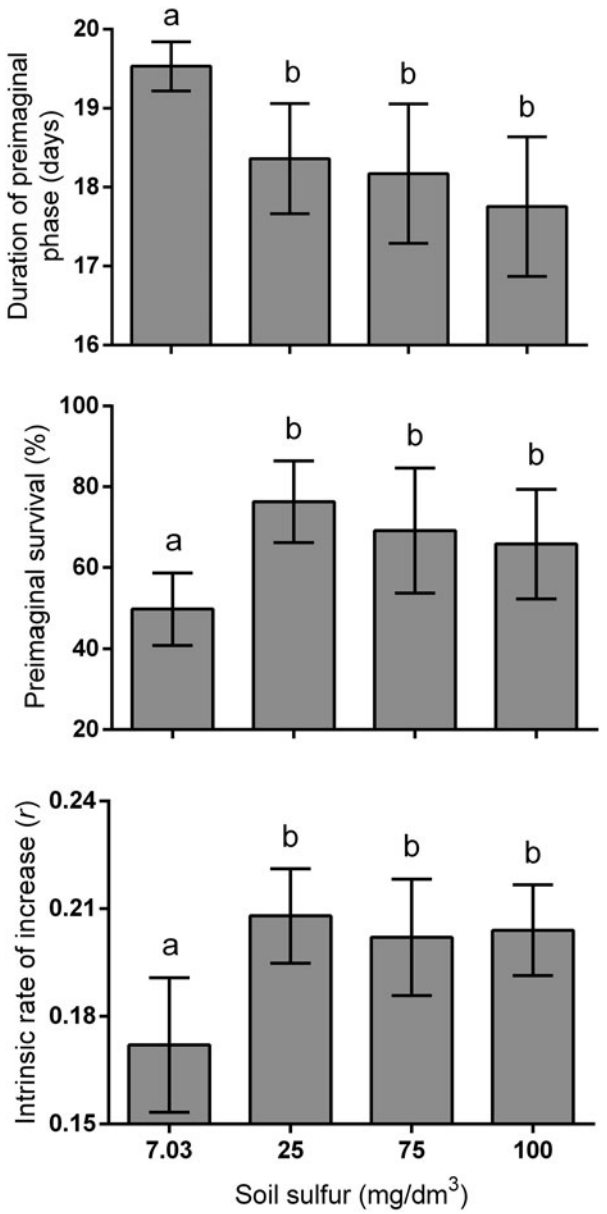

Fig. 2. Developmental time (a) and survival (b) of the immature phase and intrinsic rate of increase (c) of *Plutella xylostella* reared with cabbages (*Brassica oleraceae var. capitata)* that were grown under different sulfur contents in soil. *Bar* and *line* indicate average ± SD. *Different letters* between columns indicate significant differences ($P < 0.05$) by the Tukey's *post hoc* test.

fertilization decreases resistance of some plants against host-specific herbivores, particularly crops with high-sulfur demands, such as members of Brassicaceae. We showed that the specialist *P. xylostella* performed better on sulfur-fertilized cabbages probably because of higher levels of nitrogen, despite of higher concentration of toxic secondary metabolites.

Sulfur uptake efficiency varies among genotypes of *Brassica* (Ahmad *et al*., 2005), but it also depends on the availability of the oxidized sulfate (SO_4^{2-}) in soil (Dick *et al*., 2008). Large accumulations of sulfate in plant tissues are commonplace and ought to provide a buffer of available sulfur when external supply fluctuates (Parmar *et al*., 2007). Brassicaceous plants are known to accumulate and remove from soil large amounts of sulfur (Spencer, 1975). Excess sulfate accumulates in leaf vacuoles of adequately fertilized *Brassica* and other plants, and is remobilized if supply is interrupted (Hawkesford, 2000; Parmar *et al*., 2007). Thus, cabbage in our study was clearly sensitive to the sulfur fertilization, because stored sulfate gradually increased in the leaves with increasing sulfur addition in soil.

Limited sulfur supply can cause a general decrease in plant biomass and a reduction in the levels of proteins, amino acids, chlorophyll, and total RNA (Falk *et al*., 2007). Our results showed, and literature has related (Andrew, 1977; Zhao *et al*., 1999b; Ahmad *et al*., 2007; Järvan *et al*., 2008; Rehman *et al*., 2013), that soil sulfur fertilization enhances plant nitrogen or protein content, although increasing levels of sulfur in soil do not improve nitrogen concentration beyond a maximum value. Thus, considering protein content in isolation, sulfur fertilization is expected to improve plant nutritional quality for humans (Malhi *et al*., 2007; Järvan *et al*., 2008) and herbivores in general, while overfertilization is unnecessary and ineffective.

In addition to nitrogen compounds of the primary metabolism, sulfur fertilization affects plant secondary metabolites. Studies have shown that total glucosinolates or specific glucosinolates are dropped in conditions of limited supply of sulfur in soil, while sulfur fertilization increases their concentration in many species of *Brassica* (Mailer, 1989; Kim *et al*., 2002; Kopsell *et al*., 2003; Marazzi *et al*., 2004; Aires *et al*., 2006; Ahmad *et al*., 2007; Falk *et al*., 2007; Martínez-Ballesta *et al*., 2013). In fact, the fertilization with the lowest amount of 25 mg dm^{-3} of sulfur in soil increased leaf concentration of glucosinolates; however, concentration did not improve beyond a maximum value when sulfur was used in higher levels in soil.

Earlier works revised by Rausch & Wachter (2005) have indicated that formation of sulfur-containing defense compounds saturates at a higher sulfur supply than plant growth does. The response of plant growth to sulfate as a sulfur source shows a typical saturation curve, the plateau phase varying according to the plant species and growth conditions. In our study, the amount of soil sulfur in the control condition was below that required for cabbage nutrition; however, the lowest sulfur dose used in soil fertilization was sufficient to reach the plateau saturation of total glucosinolates in leaves, as well as total nitrogen.

The concentration ranges of total leaf nitrogen and glucosinolates in our study (2.6–4.2%DM and 4.9–5.5 μmmol g^{-1} FW, respectively) were consistent with results of other studies that used the same analytical methods. Similar values were found in varieties of *B. oleraceae* under changing conditions of plant and leaf age in the field (≈4.9–5.9 μmmol g^{-1} FW) (Campos *et al*., 2016) or drought stress in the greenhouse (≈3.0–6.5% DM and 5.3–5.7 μmmol g^{-1} FW) (Valim *et al*., 2016). Thus, the plateau saturations of plant glucosinolates and nitrogen due to sulfur fertilization were similar to the maximum values that were found in other variable conditions of plant growth. In addition, the plateau saturations due to sulfur fertilization are probably influenced by plant genotype, age and phenology, water stress, and other soil nutrients, especially nitrogen.

The *P. xylostella* performance was positively correlated with leaf nitrogen and glucosinolates. It is widely known that *P. xylostella* overcomes the toxic barrier of the glucosinolates and can benefit itself by using these metabolites (Ratzka *et al*., 2002; Wittstock *et al*., 2003; Sarfraz *et al*., 2006; Hopkins et al., 2009; Winde & Wittstock, 2011; Bohinc et al., 2012).

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Insect herbivores that are well adapted to exploit their hosts are able to access individual plants or plant parts of higher nutritional quality (Feeny, 1976; Rhoades & Cates, 1976). Higher content of foliar nitrogen greatly benefits *P. xylostella* in *Brassica* (Staley et al., 2009; Soufbaf et al., 2012; Campos et al., 2016; Moreira et al., 2016). Thus, *P. xylostella* performed better on more nutritious host plants, with higher content of nitrogen compounds, despite or because of higher content of glucosinolates. While well-adapted herbivores overcome toxic defenses of their host plants, non-adapted species may be strongly inhibited (Li et al., 2000; Kliebenstein et al., 2002; Arany et al., 2008; Gutbrodt et al., 2011). Thus, the sulfur-modulated plant resistance hypothesis (SMPRH) may be relative, depending on the degree of insect feeding specialization, so that sulfur-induced glucosinolates would be toxic against non-adapted insect herbivores. The effect of plant sulfur fertilization on polyphagous or generalist *Brassica* herbivores has not been investigated, but it has been shown that sulfur-modulated defenses can increase plant resistance against phytopathogenic fungi (Dubuis et al., 2005; Falk et al., 2007). The generalization of the SMPRH to insect herbivores with different degrees of food specialization requires more evidence.

For insect herbivores that feed exclusively on members of Brassicaceae, sulfur fertilization may actually decrease resistance, as we found in our study. Although leaf glucosinolates and nitrogen were not measured, Marazzi & Stadler (2004) concluded that an optimal supply of sulfur to *Brassica napus* plays an important role in acceptance of the host plant by *P. xylostella*, and can benefit insect performance, both at the adult and larval stage. Marazzi *et al.* (2004) found a relation between sulfur fertilization, increased total glucosinolates and higher oviposition preference by *P. xylostella*. Badenes-Perez *et al.* (2010) suggested that sulfur fertilization could increase the effectiveness of *Barbarea vulgaris* as a trap crop for *P. xylostella*. The effect of plant sulfur fertilization on *P. xylostella* oviposition preference was associated with a quantitative glucosinolate increase. Yusuf & Collins (1998) showed that *B. oleracea* var. *gemmifera* fertilized with sulfur were more infested by the aphid *Brevicoryne brassicae*, a host specialist, than plants receiving lower levels of sulfur, despite increased glucosinolate content. Thus, sulfur fertilization perhaps decreases host plant resistance to different insect-feeding guilds. However, insect guilds usually differ in their responses to changes in plant chemistry and structure (Larsson, 1989; Stam *et al.*, 2014), and a generalized prediction of sulfur-decreased plant resistance among leaf-chewing and sap-sucking guilds of host-specific insects also requires more evidence.

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