



EFFECT OF SOPONINS AND ALKALOIDS ON STINK BUGS CONTROL AND YIELD OF COMMON BEAN VARIETIES (*Phaseolus vulgaris* L.)

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ABSTRACT

Stink bugs cause damage to a wide variety of agricultural crops worldwide, resulting in significant yield losses. The study aimed to evaluate the level of damage caused by stink bugs to common beans and to determine whether the presence of saponins and alkaloids in the legumes of various common bean varieties contributes to reducing the damage and yield losses caused by the stink bug complex in this crop. During the three planting seasons evaluated, five species of stink bugs were identified in common bean, with *Nezara viridula* being the most prevalent (41.07% of the total), followed by *Chinavia rolstoni* and *Chinavia marginatum*, with 21.84 and 18.16%, respectively. White-seeded varieties exhibited the highest susceptibility to stink bug incidence, with BAT-482 being significantly affected. The phytochemical screening revealed the presence of saponins and alkaloids in all bean varieties with higher levels in black-seeded varieties, reaching 30 and 161 mg/mL, respectively. The BAT-482 (white seed coat) variety exhibited the highest yield losses, ranging from 0.31 to 0.45 t/ha, with significant differences compared to the other common bean varieties, except for Velasco Largo (red seed coat). Black-seeded varieties recorded lower losses. These findings highlight the impact of stink bugs on common bean crops and the role of bean variety in susceptibility and chemical composition, which ultimately influence yield losses.

Keywords: fabaceae, hemiptera, pentatomidae, plant injury, secondary metabolites

INTRODUCTION

Stink bugs (Hemiptera: Pentatomidae) comprise an insect pest complex that affects agricultural crops worldwide. Among the primary species, *Nezara viridula* (L.) and *Piezodorus guildinii* (West), have been identified as infecting soybean (*Glycine max* Merr) and common bean (*Phaseolus vulgaris* L.) (Ramos et al., 2017; Chen et al., 2023). The stylet of stink bugs penetrates tissues of pods to varying depths, releasing saliva containing digestive enzymes. These enzymes promote digestion and tissue degradation,

while they also induce darkening. Feeding on legumes and immature seeds can lead to pod and seed malformation, as well as premature shedding. This may result in the production of wrinkled or empty seeds, along with delayed maturation and diminished seed vigor (Zerbino and Panizzi, 2019). Additionally, *N. viridula* has been implicated in the transmission of the yeast *Eremothecium coryli* (Peglion) Kurtzman (Esquivel and Medrano, 2019).

Plants contain a group of secondary metabolites that are natural biodegradable products, which are less toxic to humans and animals and categorized

based on their chemical structure into terpenes, phenolics, nitrogen-containing compounds, and sulfur-containing compounds (Zaynab et al., 2018). Terpenes are built from a basic 5-carbon isoprenoid unit and act as toxins that deter herbivores. The shikimic acid pathway produces compounds that form phenolics, which enhance the defensive capability of plants. Compounds containing nitrogen and sulfur are primarily derived from amino acids (Rosenthal and Berenbaum, 1992). These secondary metabolites protect plants against climatic factors, diseases, and herbivory caused by insect pests (Lyubenova et al., 2023). Saponins and alkaloids are among the most studied metabolites involved in plant-insect protection. Saponins are effective against insect pests by deterring their growth and reproduction. They disrupt insect digestive systems, alter gut microflora, and form complexes with digestive enzymes, hindering digestion. Additionally, saponins permeabilize membranes, leading to gut cell damage. They also bind with cholesterol, causing cellular toxicity and hindering insect molting (Singh and Kaur, 2018).

Fabaceae plants have the capacity to synthesize nitrogen-containing secondary metabolites, particularly alkaloids, which tend to accumulate within the seeds of legumes (Wink, 2013). These alkaloids exhibit toxicity towards insects, exerting adverse effects on their nervous system, DNA replication, protein synthesis, and enzyme activity (Divekar et al., 2022).

Most research on the incidence of stink bugs has been conducted in soybean cultivation, hence studies focusing on this aspect in common beans are very scarce. Furthermore, the role of secondary metabolites contained in common bean legumes as mechanisms for their protection has not been previously studied in Cuba. Therefore, this study aims to evaluate the damage level caused by the stink bugs to common beans, and to determine whether the saponins and alkaloids contained in the legumes of various common bean varieties contribute to reducing the damage and yield losses caused by the stink bug complex in this crop.

MATERIALS AND METHODS

Experimental setup

Six varieties of beans with different seed coat colors were planted in experimental plots (5 x 1.85 m and 0.45 m spacing between rows), resulting in 59, 65, and 50 seeds of black, red and white beans per plot. The black seed coat common bean varieties were Cubacuerdo 25-9 (B), ICA Pijao (B); the red seed coat varieties: Cubacuerdo 25-9 (R), Velasco Largo (R); and the white seed

coat varieties: BAT-482 (W) and Cubacuerdo 25-9 (W) (B, R and W stands for black, red and white seed coat, respectively). The fertilization method employed was organic, utilizing the same compost in all common bean plots. The compost consisted of 50% banana plant residues, 25% cattle manure, and 25% sugarcane bagasse. Additionally, the minimal tillage soil management method was employed, utilizing animal traction. No chemical pesticides were applied, and the plants received weekly irrigation. The plots were distributed in randomized blocks and replicated twice per sampling season.

Stink bugs sampling

Stink bug sampling was conducted on a weekly basis in the Sandino orchard of the Remedios municipality, Villa Clara, Cuba (22°57'56"N, 79°28'34"W), following the methodology proposed by Ramos et al. (2017). Five points were randomly marked in each plot, and five bean plants were quantified at each point, resulting in a total of 25 plants sampled per plot. Sampling began when the plants were in the BBCH (Biologische Bundesanstalt Chemical) 24 growth stage (fourth side shoots visible) until BBCH 97 (Senescence) (Meier, 2021). This procedure was carried out during three common bean planting seasons (first: September-December 2019; second: November 2019-January 2020, and third: February-April 2020).

Secondary metabolite determination

To determine one of the possible causes of the preference of stink bugs for common bean varieties of different seed coat colors, a phytochemical screening was carried out on healthy legumes taken during the grain formation period (growth stage BBCH 75). Once the metabolites present in the legumes were identified, those reported in the literature as possessing toxic effects against insects were quantified.

The phytochemical screening was conducted according to Public Health standard number 9 (MINSAP, 1994) at the Physiology and Biochemistry Laboratory of the Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas (UCLV). The legumes from the studied varieties were selected as standards, because they are the most commonly cultivated varieties of each seed coat color by common bean producers in Cuba. Legumes with forming grains were placed in the oven at 50°C until reaching constant weight, and then crushed to obtain a final particle size of 5 mm. Subsequently, 2 g of material from each variety was added to 50 mL of separate solvents: diethyl ether, 70% ethanol, and sterile distilled

water. The ethanolic and aqueous extracts were submitted to ultrasound for 5 min at a frequency range of 10 to 12 GHz with pulsations every 2 sec. The ethereal extracts were left to macerate for 24 h (Fernández et al., 2010).

Quantification of saponins

The quantification of saponins was carried out using the methodology of Harbome (1973). This technique is characteristic for determining total saponins, as the reagent used to develop the color is highly specific for this metabolite. The same common bean varieties mentioned earlier for the phytochemical screening were used.

For obtaining the extracts used as standards in the calibration curve, 2 g of dry and crushed plant material from the common bean varieties were first taken and degreased twice consecutively with 20 mL of chloroform for 24 h. The supernatant was filtered and discarded.

To the solid residues, 20 mL of 80% ethanol was separately added, refluxed for 1 h, and filtered. This process was repeated twice to obtain a greater amount of dissolved saponins. The ethanolic extract obtained from both refluxes of each common bean variety was combined individually according with each common bean variety and dried in a rotary evaporator at 40 °C and 30 rpm. Before and after evaporation, the flasks were weighed to finally determine the total dissolved solid in the samples (TDS). Different volumes of ethanol were added to each flask and the concentration was determined.

To provide coloration to the extracted saponins, a mixture of 99% acetic anhydride and concentrated sulfuric acid in a ratio of 1:5 was used. The solution was allowed to stand for 30 min for stabilization. Once the color reagent was prepared, 3.5 mL was added to each 1 mL sample. These solutions were then taken to a spectrophotometer, and a spectral scan was performed to determine the maximum absorption peak. Using this value, the absorbance of the extracts was measured. The obtained data allowed for the creation of the calibration curves, and the equation of the lines was derived.

For quantification, a sample of 2 g of dry and crushed plant material was taken and refluxed for 1 h with 20 mL of 80% ethanol following the same procedure described earlier for obtaining the extracts used as standards in the calibration curves. Subsequently, the color reagent was added to the sample (3.5:1), and absorbance was measured at the wavelength determined in the spectral scan. These data were substituted into the equation of the lines obtained in the calibration curves to determine the amount of total saponins in the common bean varieties.

Quantification of alkaloids

For the quantification of alkaloids, the methodology of García (2004) was employed. In this regard, 1 g of plant material from the six varieties mentioned for the phytochemical screening was taken, and 20 mL of distilled water was added to each. The mixtures were allowed to macerate for 24 h and then filtered. This procedure was repeated twice to extract the maximum amount of the metabolite and to fully utilize the plant material in the extraction. Subsequently, the aqueous extracts from each variety were filtered and combined.

To a 1 mL aliquot of extract, 2 mL of Dragendorff reagent was added and then centrifuged at a speed of 9000 rpm for 15 min in 10 x 1.5 cm plastic test tubes with caps. The supernatant was discarded, and the tubes were placed in an oven at 70 °C for 24 h to remove all aqueous residue. Before and after this process, the tubes were weighed to obtain gravimetrically the total alkaloids in 1 g of plant material.

Varietal preference determination and stink bug damage assessment

The total number of stink bugs (without species distinction) was quantified in each variety from BBCH 24 to BBCH 97 growth stages. Additionally, 10 plants per variety in each plot were sampled after harvest, and the percentage and level of grain damage were determined, the latter according to Jensen and Newson (1972). Two replications were carried out in each sampling season. Yield and losses caused by stink bugs to common bean crops were also determined for each planting season. To obtain these data, the number of legumes per plant, number of grains per legume, number of grains affected per legume, and the weight of 100 healthy grains of each variety were quantified. Yield and losses were determined according to Owens et al. (2013).

Statistical analysis

The obtained results were statistically analyzed using STATGRAPHICS PLUS version 5.1. For varietal preference (including mean of stink bugs and affected grains per variety), a non-parametric Kruskal-Wallis analysis of variance was conducted. For mean comparison, the Multiple Range Test contained in the STATISTIX version 1.0 program was used. For comparisons of secondary metabolites and yield losses, a one-way analysis of variance was performed followed by the Tukey's Honestly Significant Difference (HSD) test for post hoc analysis. All tests were conducted at a significance level of 0.05%.

RESULTS AND DISCUSSION

During the bean plantings carried out in different seasons, five species of stink bugs were detected. *Nezara viridula* was the predominant species with a total of 423 insects, representing 41.07% of the total. *Chinavia rolstoni* (Rolst.) and *Chinavia marginatum* (Pal de Beauv.) followed in order with 21.84 and 18.16%, respectively. Stink bugs with lower incidence were *P. guildinii* (13.40%) and *Euschistus bifibulus* (Pal de Beauv.) (5.53%).

The stink bugs exhibited a higher ($F = 42.31$; $df = 5$; $P = 0.0001$) level of attraction towards white-seeded varieties BAT-482 (W) and Cubacueto 25-9 (W), as well as the red-seeded variety Velasco Largo (R), compared to the black-seeded varieties ICA Pijao (B) and Cubacueto 25-9 (B). Similar trends were observed during the second and third planting seasons. No significant differences were found in the abundance of stink bugs between the varieties Cubacueto 25-9 (B) and Cubacueto 25-9 (R) across all planting seasons. Furthermore, varieties with the same seed coat color did not exhibit significant differences in terms of stink bug attraction (Fig. 1).

The preference of stink bugs for legumes has been documented by Smaniotto and Panizzi (2015), who also reported the incidence of *N. viridula* on *P. vulgaris* and *Desmodium tortuosum* (SW.) DC. These findings agree with the results obtained herein, further confirming the preference of *N. viridula* for common bean, even as it remains the primary stink bug affecting this agricultural crop.

Although stink bugs are known to exhibit a marked preference for soybean, they are phytophagous insects with a wide range of host plants, including species from the Fabaceae family, such as common beans. This is consistent with Smaniotto and Panizzi (2015), who reported the presence of *N. viridula* and *P. guildinii* in 21 and 23 species of the Fabaceae family, respectively. The species *C. rolstoni* and *C. marginatum* have also been reported to cause damage to common bean in Cuba (Ramos et al., 2017). However, Panizzi et al. (2021) found that they prefer other crop such as soybean, cotton (*Gossypium hirsutum* L.) and sunflower (*Helianthus annuus* L.). In addition, *Piezodorus guildinii* is also one of the most destructive stink bugs in legumes, such as soybean (Parys and Portilla, 2020), but it was

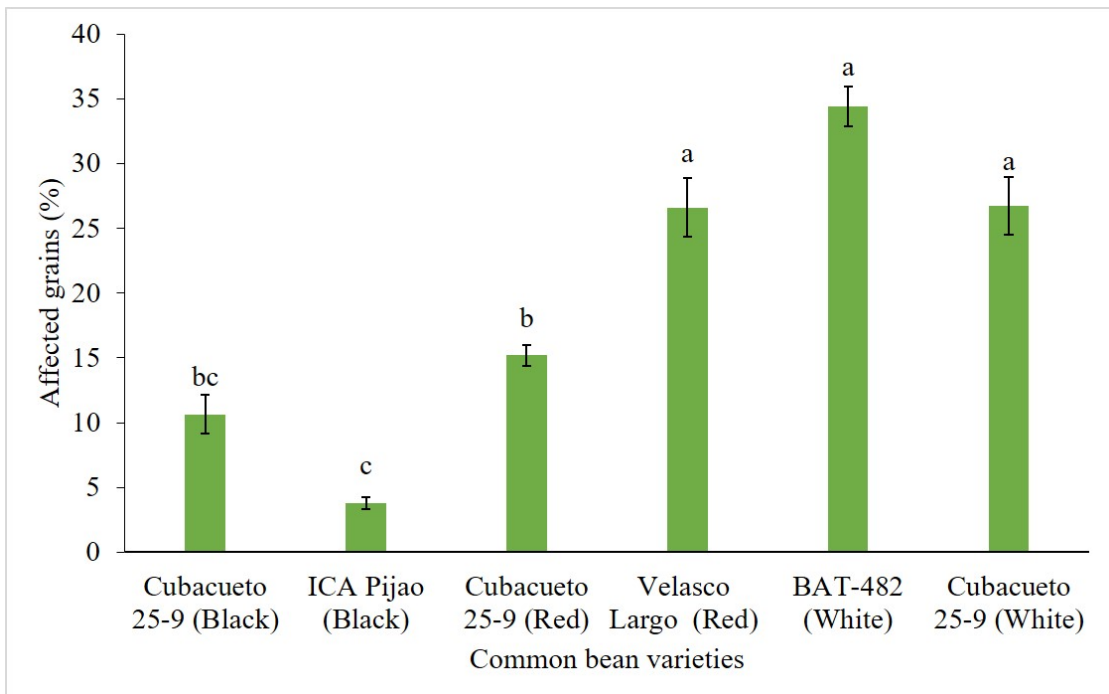


Fig. 1. Mean of stink bugs (\pm SE) associated with varieties of different seed coat colors in three planting seasons. Bars with different letters in the same planting season denote significant differences according to Multiple Range Test ($P < 0.05$).

poorly detected in common beans in the present study.

The damage caused by the stink bug complex to common bean varieties consisted of punctures on the legumes and subsequent formation of fermentation spots around each puncture (Fig. 2a). Grain wrinkling due to the suction of grain content during their formation (Fig. 2b) and pod malformation (Fig. 2c).

Throughout the three planting seasons, white-seeded varieties were the most susceptible ($F = 6.28$; $df = 5$; $P = 0.0006$) to stink bug incidence, particularly BAT-482 (W) with a 34.4% infestation. Red-seeded varieties followed in order, although they did not show marked differences compared to white-seeded varieties. Velasco Largo (R) was the most preferred variety ($F = 6.19$; $df = 5$; $P = 0.0241$) by these stink bugs, exhibiting similar infestation levels to those observed in Cubacueto

25-9 (W), with 26.6% and 26.73% grain infestation, respectively. Black-seeded varieties showed the least ($F = 11.74$; $df = 5$; $P = 0.0001$) susceptibility, with ICA Pijao being particularly notable at 3.79% damage (Fig. 3).

In addition to the percentage of affected common bean grains, the severity level recorded for each of the varieties under study demonstrates: a severe level for the most affected (white-seeded) varieties; a mild level for the black-seeded varieties; and moderate level for the red-seeded varieties (except for Velasco Largo).

The stink bugs feeding behavior results in economic losses for legume crop as (i) it affects fruiting structures, and leads to reduced photosynthesis and tissue degradation; (ii) it induces darkening, fruit and seed abortion, delayed maturation, and flat pod disorder; and (iii) it creates entry points for plant pathogens



Fig. 2. Direct damages caused by the stink bug complex on white-seeded beans, BAT-482 (W) variety. The figure depicts a severe level of infestation (Jensen and Newson scale, 1972); a: punctures surrounded by ferment spots; b: the arrows indicate grain wrinkling due to the suction of grain content during their formation; c: deformed legumes.

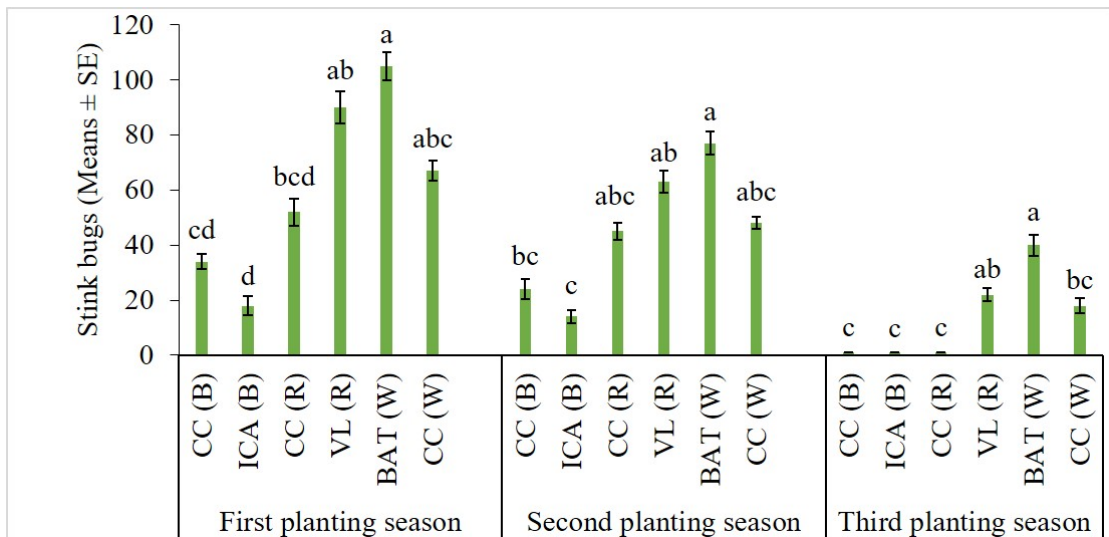


Fig. 3. Percentage of common bean grains affected by stink bugs among different seed coat color varieties. Bars with different letters denote significant differences according to Tukey's HSD test ($P < 0.05$).

Legend: CC (B): Cubacueto 25-9 (seed coat black); ICA (B): ICA Pijao (seed coat black); CC (R): Cubacueto 25-9 (seed coat red); VL (R): Velasco Largo (seed coat red); BAT (W): BAT-482 (seed coat white); CC (W): Cubacueto 25-9 (seed coat white).

(Esquivel and Medrano, 2019; Zerbino and Panizzi, 2019). Several of these damages were detected in common bean legumes in the present study, resulting in yield losses in the crop.

The phytochemical screening of the different varieties of common beans showed the presence of saponins in each of them when aqueous and ethanolic extracts were used, but not with the ethereal solvent. In the case of alkaloids, this metabolite tested positive in all varieties of beans studied but only with the aqueous extract (Table 1).

Throughout the three planting seasons of common beans, the quantified content of saponins and alkaloids was higher in black-seeded compared to white-seeded varieties. Red-seeded varieties exhibited an intermediate content of these metabolites. The ICA Pijao (B) variety (black seed coat) showed a higher ($F = 12.29$; $df = 5$; $P = 0.0001$) content of saponins and alkaloids compared to the rest of the evaluated varieties, coinciding with the lowest percentage of affected grains. Conversely, the BAT-482 (W) (white seed coat) variety presented lower ($F = 14.55$; $df = 5$; $P = 0.0001$) content of these metabolites, resulting in the highest indices of grain damage being quantified. However, no differences were observed between this variety and Velasco Largo (R) or Cubacueto 25-9 (W) (Fig. 4 a, b).

The resistance of black seed coat varieties

of common bean to stink bug infestation could be attributed to the high presence of saponins and alkaloids in the legumes. However, more studies need to be conducted to demonstrate this assumption. Saponin content has been detected in soybean, common bean, and adzuki bean (*Vigna angularis* (Wild.) Ohwi and Ohashi) (Fenwick and Oakenfull, 1981; Herrera et al., 2023; Moussa et al., 2024), while alkaloids have been detected in fava beans (*Vicia faba* L.) and species from *Lupinus* (Fabaceae) (Karolkowski et al., 2023; Madelou et al., 2023). However, studies on these compounds have traditionally focused on medical purposes. In contrast, the present study provides novel insights into how the natural presence of these compounds in common beans can act as an insecticide, reducing stink bug levels in the crop.

Many plant species, particularly legumes, protect themselves with saponins, while it has been observed that levels of oleanolic acid-derived saponins, such as hederagenin-derived compounds are correlated to the deterrence of *Trichopulsia ni* (Hübner) in the legume *Medicago truncatula* (Gaertn) (Chen et al., 2024). Furthermore, 14 triterpenoid saponins from *Clematis obscura* (Maxim) were discovered, which hold promise as botanical-based insecticide candidates against *Acyrtosiphon pisum* (Harris) and *Plutella xylostella* (L.) (Hao et al., 2024). This finding revealed that all saponins

Table 1. Phytochemical screening of legumes from six different varieties of common beans with varying seed coat colors.

Extracts	Secondary metabolites	CC 25-9 (B)	ICA Pijao (B)	CC 25-9 (R)	Velasco Largo (R)	BAT-482 (W)	CC25-9 (W)
Aqueous extract	Flavonoids	-	-	-	-	-	-
	Tannins and phenols	-	-	-	-	-	-
	Alkaloids	+++	+++	++	++	+	+
	Reducing carbohydrates	-	-	-	-	-	-
	Mucilages	-	-	-	-	-	-
Ethanollic extract	Saponins	++	+++	+	++	+	+
	Triterpenes and steroids	-	-	-	-	-	-
	Tannins and phenols	-	-	-	-	-	-
	Ninhydrins	-	-	+	+	+	+
	Saponins	+	+	+	+	+	+
	Flavonoids	-	-	-	-	-	-
	Alkaloids	-	-	-	-	-	-
	Coumarins	+	+	+	+	+	+
	Reducing carbohydrates	-	-	-	-	-	-
	Cardiotonic glycosides	-	-	-	-	-	-
	Quinones	-	-	-	-	-	-
	Fatty acids	+	+	+	+	+	+
Ether extract	Alkaloids	-	-	-	-	-	-
	Coumarins	+	++	++	+	++	+
	Fatty acids	+	+	+	+	+	+
	Triterpenes and steroids	-	-	-	-	-	-
	Flavonoids	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	

Legend: (-): absence of the secondary metabolite; (+): presence of the secondary metabolite; CC: Cubacueto; (B): black seed coat; (R): red seed coat; (W): white seed coat.

exhibited antifeedant activities against *A. pisum*, while compound 10 demonstrated significant insecticidal effects against third-instar larvae of *P. xylostella* after 72 h (Hao et al., 2024).

According to Meechuen et al., (2023), alkaloids are secondary metabolites with various roles in plants, including defense mechanisms. The authors identified a group of these compounds in *Piper retrofractum* (Miq.) C.DC. (Javanese long pepper) fruits, which offer promising alternatives to chemical insecticides due to their natural origin and insecticidal properties. Bioassays demonstrated that iridoid alkaloids exhibit good to excellent activities against *Plutella xylostella* (L.), *Tetranychus urticae* (C.L. Koch) and a specie of bean aphid. Compound 3n primarily demonstrated good insecticidal activity against the diamondback moth, with an LC₅₀ (35.6 µg/mL) comparable to that of the commercial insecticide rotenone (Xia et al., 2020).

Due to the chemical similarity of alkaloids with molecules involved in the transmission of

signals in the nervous system, the toxic effect of these compounds lies in their ability to block neuroreceptors involved in the transduction of neuronal signals and ion channels in vertebrates and insects (Wink, 2013).

BAT-482 (W) variety exhibited the highest yield losses ($F = 36.98$; $df = 5$; $P = 0.0001$) among the three planting seasons, ranging from 0.31 to 0.45 t ha⁻¹, with significant differences compared to the other common bean varieties, except for Velasco Largo (R). Black-seeded varieties experienced lower losses ($F = 30.08$; $df = 5$; $P = 0.0001$), even when compared with red-seeded varieties (Table 2).

CONCLUSIONS

The stink bug *N. viridula* had the highest incidence on common bean varieties. This species integrates with other stink bugs, forming a complex that causes damage to the legumes and grains, resulting in yield losses. Regarding

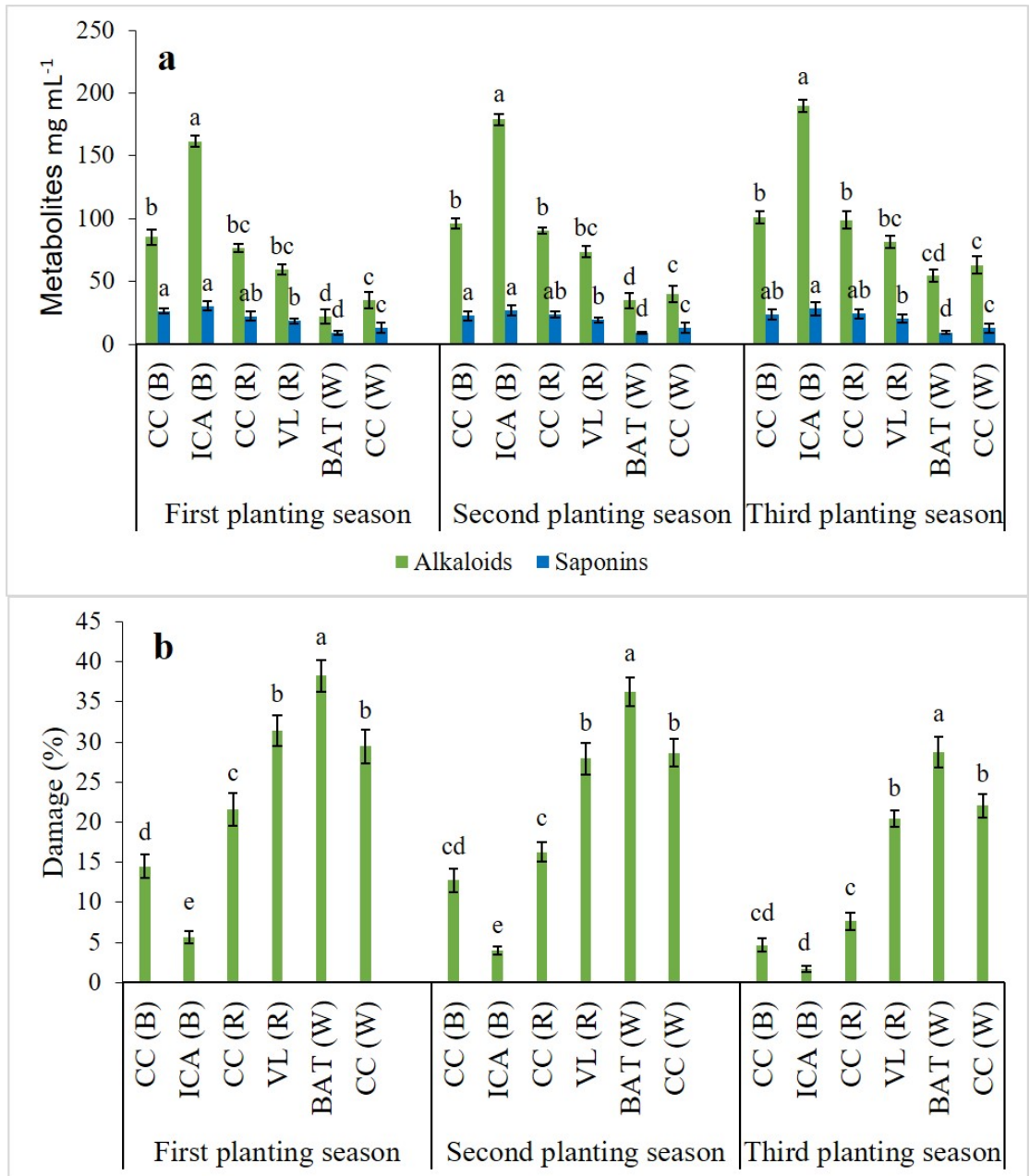


Fig. 4. Secondary metabolite content in common beans (\pm SE) and its relationship with damage caused by the stink bug complex; a: Content of saponins and alkaloids present in common bean varieties with different seed coat colors during three planting seasons; b: Damage caused by the stink bug complex to bean varieties of different seed coat colors during three planting seasons. Bars with different letters within the same planting season denote significant differences according to Tukey's HSD test ($P < 0.05$).

Legend: CC (B): Cubacuerdo 25-9 (seed coat black); ICA (B): ICA Pijao (seed coat black); CC (R): Cubacuerdo 25-9 (seed coat red); VL (R): Velasco Largo (seed coat red); BAT (W): BAT-482 (seed coat white); CC (W): Cubacuerdo 25-9 (seed coat white).

Table 2. Damage (\pm SE) caused by the stink bug complex on the yield of common bean during three planting seasons.

Varieties	Seed coat color	First Season		Second Season		Third Season	
		Yield (t/ha)	*Losses (t/ha) \pm SE	Yield (t/ha)	*Losses (t/ha) \pm SE	Yield (t/ha)	*Losses (t/ha) \pm SE
Cubacueto 25-9	Black	1.54	0.14 b \pm 0.005 c	1.91	0.13 \pm 0.06 c	2.0	0.07 \pm 0.05 c
ICA Pijao		1.54	0.11 \pm 0.007 c	1.73	0.08 \pm 0.04 c	1.87	0.04 \pm 0.03 c
Cubacueto 25-9	Red	1.62	0.28 \pm 0.005 b	1.75	0.20 \pm 0.07 b	1.91	0.15 \pm 0.07b
Velasco Largo		1.86	0.40 \pm 0.004 a	2.17	0.34 \pm 0.08a	2.25	0.27 \pm 0.09a
BAT-482	White	1.39	0.45 \pm 0.008 a	1.73	0.38 \pm 0.08 a	1.97	0.31 \pm 0.07a
Cubacueto 25-9		1.49	0.31 \pm 0.007 b	1.72	0.25 \pm 0.07 b	1.86	0.20 \pm 0.06 b

*Means with different letters in the same column denote significant differences according to Tukey's HSD test ($P < 0.05$).

varieties, black-seeded beans contained a higher concentration of saponins and alkaloids, and they recorded the lowest level of damage by stink bugs. Conversely, white seed coats contained lower amounts of these secondary metabolites, and they were the most affected by these pest insects. These findings demonstrate that the levels of saponins and alkaloids contained in common bean varieties with different seed coat colors can mitigate crop damage and yield losses caused by stink bugs. However, other factors such as the number of hairs and trichomes on the leaves and stems may have an impact in this regard. The obtained results provide bean producers valuable information to manage bean varieties, while they contribute to the understanding of resistance and susceptibility factors of common bean plants to the incidence of stink bugs.

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Author contributions

The authors declare active participation in the bibliographic review by Yordany Ramos and Ray Espinosa; in the development of the methodology: Yordany Ramos and Ray Espinosa; in the discussion of the results: Yordany Ramos; in review and approval of the final version of the article: Yordany Ramos and Ray Espinosa.

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