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Rhyssomatus subtilis (Coleoptera: Curculionidae) Impact in Soybean Plant Stands¹

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Abstract The black soybean weevil, *Rhyssomatus subtilis* (Fiedler), is an important but infrequently studied insect pest of soybean, *Glycine max* (L.) Merrill, in South America. Severe crop damage occurs when the adult weevils attack soybean seedlings, resulting in reduced plant stands, and when weevils feed on and oviposit in seedpods. The objectives of this 3-yr study were to quantify early-season damage caused by *R. subtilis* to terminal buds and plant stands in soybean and evaluate insecticide seed treatments under field conditions relative to this damage. Terminal bud damage is the most consistent criteria for determining early-season damage by *R. subtilis* to soybean. The results indicated that *R. subtilis* can cause as much as 36% plant stand loss in soybean. Thiamethoxam and a mixture of ethiprole + fipronil provided significant levels of control of *R. subtilis* damage, with the higher doses of each product tending to provide longer-lasting protection.

Key Words *Glycine max*, black soybean weevil, crop damage, insecticides, seed treatment

The black soybean weevil, *Rhyssomatus subtilis* Fiedler (Coleoptera: Curculionidae), is an infrequently documented pest that affects soybean, *Glycine max* (L.) Merrill, production in Argentina. The insect recently expanded its range in the northwest of Argentina, a major soybean production area (Cazado et al. 2013). All life stages of *R. subtilis* occur in soybean. Overwintering adults emerge from early November to June in association with rainfall occurrence (L.E.C. unpubl. data). Newly emerged adult weevils feed on the terminal buds of both seedlings and well-developed plants. The damage caused to soybean during early vegetative stages can affect the development of the plant negatively, even causing plant death, forcing the reseeded of infested fields to maintain an adequate plant stand (Cazado et al. 2013, Socías et al. 2009). During the plant's R5 reproductive stage (Fehr et al. 1971), the female weevils deposit their eggs inside the pods. Subsequently, the larvae hatch and feed on the seed within the pods, developing through four larval instars, at which time the larvae drop to the ground to pupate (Cazado et al. 2014). Pod damage from these activities also promotes the development of phytopath-

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ogens that may impact soybean seed quality (Escobar et al. 2009). Yield losses attributed to injury by *R. subtilis* during soybean vegetative and reproductive stages have reportedly reached high levels in Argentina, but there is a lack of documentation of this impact on soybean production.

Another important pest of soybean in Brazil and Argentina is *Sternechus subsignatus* Boheman (Coleoptera: Curculionidae). Adults of this pest feed on stem tissues in early vegetative stages, causing the death of the plant (Hoffman-Campo et al. 1991, Socías et al. 2011). In both countries, fipronil and thiamethoxam used as seed treatments provide effective protection of damage caused by *S. subsignatus* populations up to 20 d after planting (Casmuz et al. 2009, Tonnet 2000). There is, however, little available information on insecticidal activity against *R. subtilis*, with the exception of a study by Zunino et al. (2012) that evaluated botanical oils against this pest.

Seed treatment technologies have advanced rapidly during the past few years, and various seed treatments have effectively controlled a range of pests in many crops (Bonham et al. 2009, Casmuz et al. 2009, Seidenglanz et al. 2010, Sidhu et al. 2014). In addition, this insecticide strategy is compatible with integrated pest management strategies because it requires much less active ingredient per unit area while providing control levels similar to those obtained with in-furrow drench insecticide applications (Nault et al. 2006). The objective of the present study was to document the impact of *R. subtilis* on soybean stand loss. Insecticide seed treatments, typically used to control *S. subsignatus*, were evaluated for controlling *R. subtilis* in early soybean developmental stages; therefore, persistence of control provided by seed treatments was also assessed. The hypotheses were that: (a) *R. subtilis* would cause significant stand loss in soybean, (b) thiamethoxam and a mixture of ethiprole + fipronil treatments would provide significant reductions in *R. subtilis* damage to soybean relative to an untreated check, and (c) higher rates of insecticide treatment would significantly reduce damage over the lower rate of the insecticide.

Materials and Methods

Field trials were conducted in summer of 2010 and 2011 in Rosario de la Frontera (S 25°39'57", W 64°56'58") (Salta province, Argentina) and in 2014 in Rapelli (S 26°25'21", W 64°33'1") (Santiago del Estero province, Argentina) using natural *R. subtilis* infestations. *Sternechus subsignatus* was not found at these sites during the time of these tests. Soybean seeds of cultivar 'A 8000 RG' were hand-planted in plots measuring four rows (52-cm spacing) × 5-m-long plots, using a randomized complete block design with three replications. Seed treatments were applied at least 2 weeks before planting each year and were prepared according to Casmuz et al. (2009). Briefly, 1 kg of commercial seed was treated with either 0.350 g (low rate) or 0.787 g (high rate) active ingredient (AI) of thiamethoxam (Cruiser® 35 FS or Cruiser® PLUS, Syngenta Agro, Argentina) as a liquid mixed with water in the planter seed hopper. A commercial mixture of ethiprole + fipronil (Regent® ET PACK, Bayer CropScience, Argentina) 0.437 g + 0.312 g (low rate) or 0.525 g + 0.375 g (high rate) AI per 1 kg seed were similarly applied. Planting date, insecticide

Table 1. Planting dates, localities, insecticide treatments, rates, and sampling dates of field studies conducted in 2009, 2010, and 2014, on *Rhyssomatus subtilis* control in 'A 8000 RG' soybean.

Planting Date	Locality	Insecticide Treatment		Sampling (DAP)*
		Active Ingredient	Rate (g AI/100 kg seed)	
7 Jan. 2010	Rosario de la Frontera	Thiamethoxam	35.0	12, 20, and 28
		Ethiprole + fipronil	43.7 + 31.2	
			52.5 + 37.5	
		Untreated control		
19 Jan. 2011	Rosario de la Frontera	Thiamethoxam	35.0	15, 20, 27, and 33
			78.7	
		Ethiprole + fipronil	43.7 + 31.2	
			52.5 + 37.5	
	Untreated control			
18 Feb. 2014	Rapelli	Thiamethoxam	35.0	15 and 21
			70.0	
		Ethiprole + fipronil	52.5 + 37.5	
		Untreated control		

* Days after planting (DAP).

treatments, doses, and sampling dates are listed in Table 1. All rows were treated, but only 30 plants of the two center rows of each plot were used for data collection.

Terminal bud damage is the most consistent criterion for determining early-season damage by *R. subtilis* to soybean (L.E.C. unpubl. data). The method for determining this damage was to inspect the shoot apex above the youngest trifoliolate leaf on 60 plants per plot on the dates listed in Table 1 and determine the percentage of plants with significant damage to the apical meristem and terminal leaf buds. The percentage of plants with terminal bud damage and the percentage of plant stand loss were analyzed using a one-way ANOVA, and means were separated using Fisher LSD tests ($P < 0.05$) with InfoStat (Di Rienzo et al. 2008). Where necessary, data were arcsine square root transformed before analysis; untransformed means are presented in the tables and figures.

Results

In 2010, there was low, but significant stand loss in the untreated plots compared with the thiamethoxam-treated plots (Table 2). On the first sampling date (12 d after

Table 2. Percentage of plant stand loss for *Rhyssomatus subtilis* in each of the three years of study.

Treatments	% Plant Stand Loss		
	2010	2011	2014
	28 DAP*	27 DAP	21 DAP
Thiamethoxam (low rate)	0.4 ± 0.4 a**	0.0 ± 0.0 a	1.7 ± 1.0 a
Thiamethoxam (high rate)	—	0.0 ± 0.0 a	2.2 ± 0.5 a
Ethiprole + fipronil (low rate)	2.1 ± 0.4 b	1.8 ± 0.8 ab	—
Ethiprole + fipronil (high rate)	2.6 ± 0.1 b	1.5 ± 0.7 ab	2.2 ± 1.5 a
Untreated control	2.6 ± 0.7 b	3.2 ± 1.9 b	35.6 ± 9.3 b

* Days after planting (DAP).

** Means ± SE within columns followed by the same letters indicate no significant difference (LSD test, $P < 0.05$).

planting [DAP]), there were only marginally significant differences among treatments in terms of the percentage of plants with terminal bud damage ($F = 3.1$; $df = 3, 11$; $P = 0.109$), likely due to the initial low infestation with *R. subtilis* (Fig. 1). At 12 DAP, all insecticide treatments resulted in <3% of plants with terminal bud damage caused by *R. subtilis* with no differences among treatments. On the second sampling date (20 DAP), significant differences were observed ($F = 18.4$; $df = 3, 11$; $P = 0.002$) among all insecticide treatments and the untreated control (Fig. 1). On the subsequent sampling date (28 DAP), there were no statistical differences ($F = 0.9$; $df = 3, 11$; $P = 0.508$) among treatments (Fig. 1). Thus, the seed treatment effect on bud damage appeared to have dissipated by the third sampling date. On this sampling date, there were significant differences among treatments for the percentage of plant stand loss ($F = 6.2$; $df = 3, 11$; $P = 0.018$) (Table 2), with the check exhibiting $2.6 \pm 0.7\%$ plant loss.

In 2011, there was slightly more *R. subtilis* pest population pressure (Table 2). The percentage of plants with terminal bud damage differed among insecticide treatments and the untreated control at 15 DAP ($F = 11.6$; $df = 4, 14$; $P = 0.002$), 20 DAP ($F = 6.9$; $df = 4, 14$; $P = 0.011$), 27 DAP ($F = 8.3$; $df = 4, 14$; $P = 0.006$), and 33 DAP ($F = 4.5$; $df = 4, 14$; $P = 0.035$). At 15 and 20 DAP, a high rate of thiamethoxam and ethiprole + fipronil tended to provide greater control of adults of *R. subtilis*, resulting in <10% of plants with terminal bud damage. The low rate of both insecticides was less effective in preventing damage; for example, the low rate of ethiprole + fipronil exhibited 23% bud damage (Fig. 2). At 27 DAP, all insecticide treatments differed from the untreated control, with exception of the low ethiprole + fipronil rate. Although significant differences were recorded between insecticide treatments and the untreated controls at 33 DAP, the percentage of plants with terminal bud damage in insecticide treatments was up to 47%, suggesting that the effects of the treatments were declining (Fig. 2). In this sampling date, there were marginal differences among treatments for the percentage of plant stand loss ($F =$

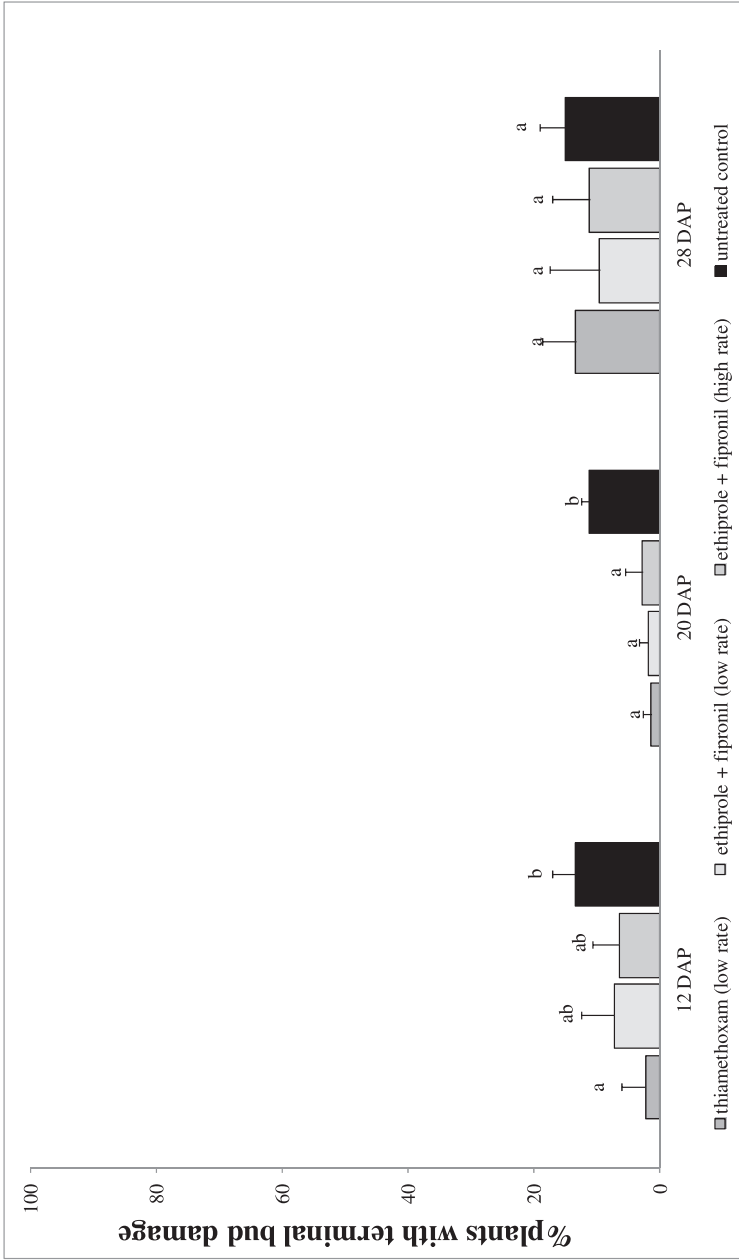


Fig. 1. Percentage of plants with terminal bud damage caused by *Rhyssomatus subtilis* at 12, 20, and 28 d after planting (DAP), year 2010. Means \pm SE within DAP accompanied by different letters indicate significant differences (LSD test, $P < 0.05$).

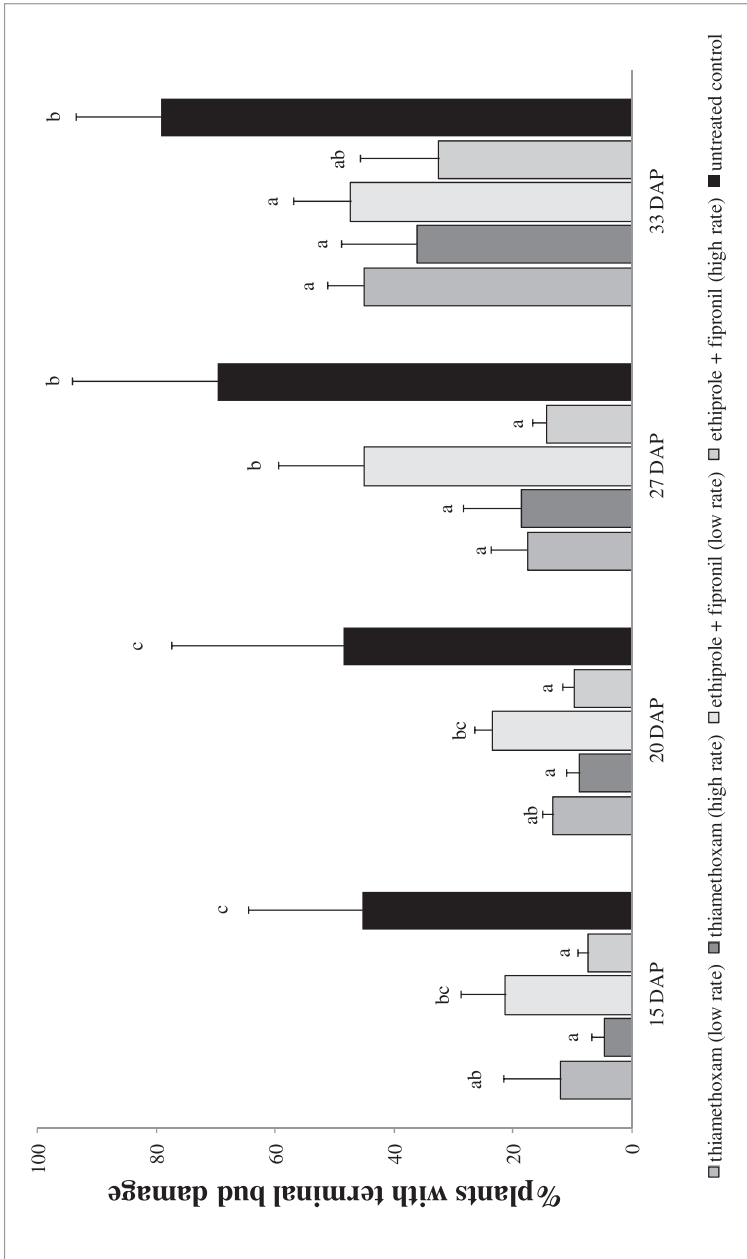


Fig. 2. Percentage of plants with terminal bud damage caused by *Rhyssomatus subtilis* at 15, 20, 27, and 33 d after sowing (DAS), year 2011. Means \pm SE within DAP accompanied by different letters indicate significant differences (LSD test, $P < 0.05$).

2.3; $df = 4, 14$; $P = 0.129$) with the check exhibiting $3.2 \pm 1.9\%$ plant stand loss (Table 2).

The *R. subtilis* pest population pressure was greatest in 2014 (Table 2). The effect of insecticide treatments on adult weevils and, subsequently terminal bud damage, proved to be significant on the first sampling date, 15 DAP ($F = 33.8$; $df = 3, 11$; $P < 0.001$), but not at 21 DAP ($F = 2.6$; $df = 3, 11$; $P = 0.145$). At 15 DAP, all insecticide treatments showed a significant reduction of *R. subtilis* damage, but less damage was observed with the higher rates of both insecticides ($<20\%$ damage). At 21 DAP, no significant difference in damage among treatments was observed, presumably due to the high infestation level of *R. subtilis* (Fig. 3). However, on this sampling date, all insecticide treatments differed from the untreated control in terms of stand loss ($F = 12.0$; $df = 3, 11$; $P = 0.006$), with the check exhibiting $35.6 \pm 9.3\%$ plant stand loss (Table 2).

Discussion

To our knowledge, this is the first report to quantify soybean plant stand loss caused by *R. subtilis*, which reached as high as 36%. During this 3-yr study, insecticide seed treatments were observed to provide effective protection of the crop from damage caused by *R. subtilis* adults during the early vegetative stages of soybean. Seed treatments should not only improve plant stand by reducing plant mortality, but could potentially reduce the amount of insecticide active ingredient per hectare required. For example, the difference in the amount of active ingredient of thiamethoxam per hectare for the highest seed treatment rate in this study (78 g AI/ha) and, for the maximum cumulative foliar rate that is labeled (141 g AI/ha), could reduce insecticide usage by 45%. Our results agreed with those obtained by various authors, whose reports demonstrate the advantages of using a seed treatment for controlling various curculionid species and other crop pests, for example, *S. subsignatus* in soybean (Casmuz et al. 2009, Tonnet 2000), *Listronotus oregonensis* LeConte (Coleoptera: Curculionidae) in carrot (*Daucus carota* L.) (Bonham et al. 2009), *Lissorhoptrus oryzophilus* Kuschel (Coleoptera: Curculionidae) (Lanka et al. 2014) and *Chironomus tepperi* Skuse (Diptera: Chironomidae) (Stevens et al. 1998) in rice (*Oryza sativa* L.), and *Delia antiqua* Meigen (Diptera: Anthomyiidae) in onions (*Allium cepa* L.) (Nault et al. 2006).

In this study, both thiamethoxam and a mixture of ethiprole + fipronil provided similar levels of damage reduction through the control of *R. subtilis* adults at the higher dose rates. A lower rate of ethiprole + fipronil did not always significantly reduce damage as compared with the untreated control, except when there was a low pest pressure. In our trials, higher doses of both insecticide seed treatments protected soybean for a period that extended up to 20 DAP, offering protection and possibly reducing the emergence of the first adult weevils. The control of *R. subtilis* adults until now has been based primarily on foliar applications. These results are similar to those reported by Tonnet (2000) and Casmuz et al. (2009) in Brazil and Argentina, who reported equal periods of protection from *S. subsignatus* in soybean field studies where thiamethoxam and fipronil had been used as an at-plant application, but with different rates from those used herein.

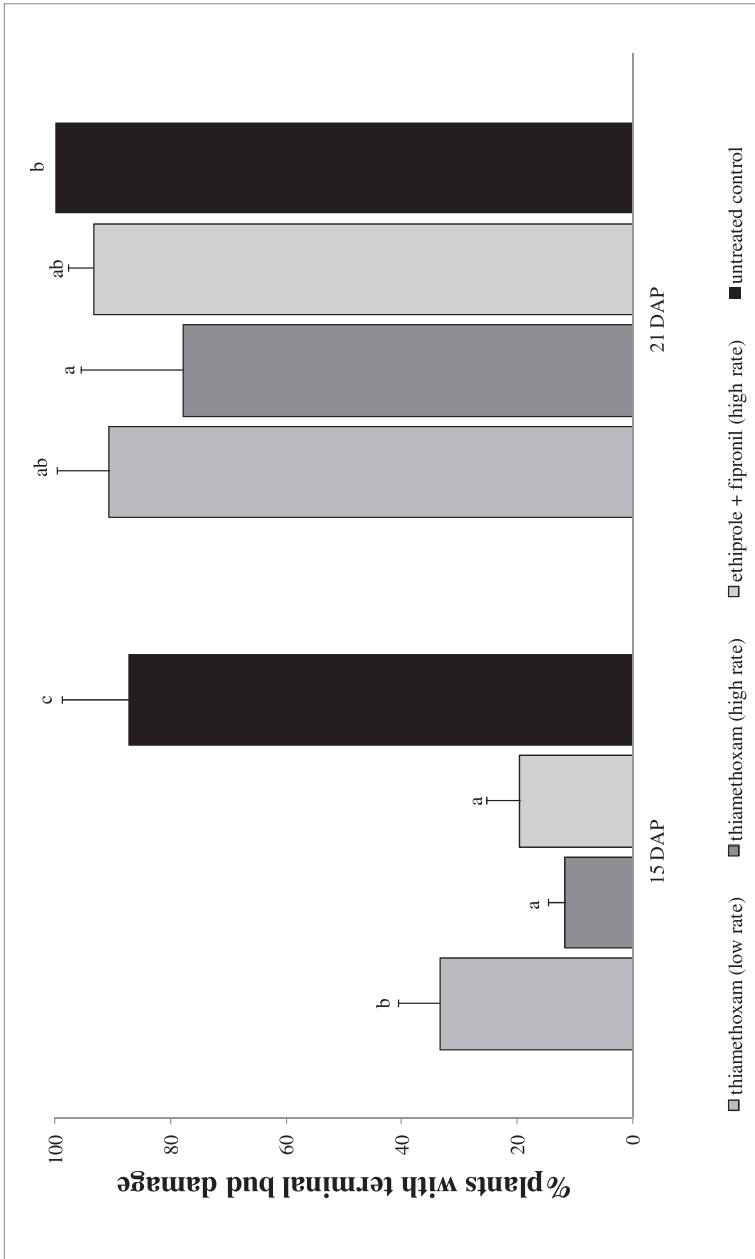


Fig. 3. Percentage of plants with terminal bud damage caused by *Rhyssomatus subtilis* at 15 and 21 d after planting (DAP), year 2014. Means \pm SE within DAP accompanied by different letters indicate significant differences (LSD test, $P < 0.05$).

Seed treatments are easily applied and economically justified, as they require smaller amounts of active ingredient. Moreover, effective seed treatments reduce the number of aerial insecticide applications leading to reductions in both expenses and environmental impact (Sidhu et al. 2014). The results of this study suggest that seed treatments such as thiamethoxam and a mixture of ethiprole + fipronil might be used to improve management of *R. subtilis* in soybean. The effective protection achieved with both insecticide treatments used in this study would allow for rotating insecticide modes of action which, in turn, should prolong insecticide efficacy by reducing insecticide resistance development.

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