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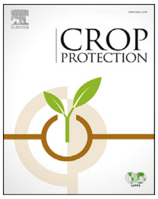

Cultural practices to reduce damage by borer insects in commercial cultivars of *Amaranthus*

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ABSTRACT

Stem borer insects are one of the most important pest groups of *Amaranthus* crops on a global scale. In this study, we evaluated the magnitude of borer herbivory in five cultivars of *Amaranthus* experimentally in La Pampa, Argentina, during two growing seasons. We tested the effect of several plant attributes of five cultivars of *Amaranthus* on the herbivory caused by three stem borer species. In turn, we evaluated the effect of cultivar and plant density (both as factors modifying the thickness of the stems) and the effect of cultivar and planting time (both as factors modifying the length of the life cycle of the plants) on the herbivory caused by the stem borers in two cultivars of *Amaranthus hypochondriacus*.

We report a wide variation in the susceptibility of the cultivars to stem borer herbivory and discuss the effects of the plant features investigated. Phenological and morphological features of the stems (especially the diameter) influenced the selectivity of host plants by the adult females. The management practices tested here, including plant density and sowing date manipulations, modified plant structure and consequently influenced the damage by stem borers. High density sown plants presented thinner stems and suffered reduced damage by borers than plants sown at low density, whereas delayed sown plants had thinner stems and were less attacked by borer insects than earlier sown plants. The implementation of these cultural practices seems to be a promising alternative for the management of borer species, to which *Amaranthus* is particularly vulnerable.

1. Introduction

Resistance to diseases and pests is one of the objectives in *Amaranthus* plant breeding, since the crop is affected by numerous insects (Bürki et al., 1997; Brenner et al., 2010). Among them, the stem borer guild is one of the prevalent pest groups, with about twenty-six different species reported worldwide (Louw et al., 1995; Niveyro and Salvo, 2014). The stem borer guild includes mainly weevils (Curculionidae: Coleoptera), cerambycid beetles (Cerambycidae: Coleoptera) (Louw et al., 1995; Niveyro and Salvo, 2014), but also flies (Diptera: Agromyzidae) (Torres-Saldaña et al., 2004) and moths (Lepidoptera: Crambidae) (Oliveirade et al., 2012). The occurrence of borer species varies widely across the world e.g. the weevil *Hypolixus truncatulus* (Fabricius) is reported in India and Mexico (Gupta and Rawat, 1954; Torres-Saldaña et al., 2004), *Conotrachelus cervinus* Hustache in both America and Europe (Niveyro and Salvo, 2014), while *Gasteroclisus* cf. *cuneiformis* (Fahraeus) has been reported only in Africa (Louw et al., 1995). Stem bored damage is caused when females oviposit into the stems and then juvenile stages bore into the main and secondary stems; but depending on the borer species, thick stalks and even roots can be affected (Niveyro and Salvo, 2014). As a consequence of borer feeding, plants are prone to breakage, with subsequent loss of seeds (Terry and Lee, 1990). Moreover, some studies mentioned that entry or exit holes caused by borers allowed the entrance of fungi and bacteria, contributing to further plant decline (Anno-Nyako et al., 1991). In spite of a great number of reports describing damage by different stem borer species on *Amaranthus* crops, no studies have yet been performed to determine action threshold levels. In turn, the habit of boring and feeding into stems limits the efficiency of chemical control (Wilson, 1989) underlying the need to search for pest management alternatives with low economic and environmental costs.

In the semi-arid region of La Pampa, the simultaneous

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occurrence of the three species: *Conotrachelus cervinus* Hustache, *C. histrio* Boheman (Coleptera: Curculionidae) and *Aerenea quadriplagiata* Boheman (Coleoptera: Cerambycidae) is reported in *Amaranthus*, coinciding with advanced phenological stages of the crop (Niveyro and Salvo, 2014). A previous study demonstrated that *A. quadriplagiata,* tends to oviposit in cultivars with particular stem diameters (Riquelme et al., 2013). Therefore, the choice of cultivars displaying features less preferred by stem borer females for oviposition could be a recommendable measure to reduce damage in *Amaranthus* crops, but further information about *Amaranthus* genotypes grown under field conditions is required. Agronomic practices such as altering sowing date and plant density are modifiers of the size and structure of plants in crops (Gimplinger et al., 2008). Both agronomic practices have been employed as pest management strategies in other crop species (Dosdall et al., 1999; Echezona, 2007) but scarcely studied in grain amaranth (Torres-Saldaña et al., 2004).

In this study, we assessed whether differences in morphological and phenological features of five commercial cultivars of *Amaranthus* affect the herbivory caused by stem borers. In addition, we studied the effect of cultivar and planting density (both as factors modifying the thickness of the stems) experimentally and the effect of cultivar and planting time (both as factors modifying the length of the life cycle of the plants) on the herbivory caused by these insects in two cultivars of *Amaranthus hypochondriacus*. We hypothesized that differences in stem thickness and life cycle length of the cultivars will affect the resource availability for borers and consequently, herbivory levels will decrease (Riquelme et al., 2013). Cultivars with thicker stems and longer cycles are expected to be more heavily attacked by borers and hence, they will harbor a greater number of larvae than plants with thinner stems or shorter cycles (Dubbert et al., 1998). Furthermore, we predicted that plants sown at higher densities and sown later will display lower levels of herbivory by borer insects given their thinner stems and shorter life cycles.

2. Material and methods

2.1. Experiment 1: susceptibility of *Amaranthus* cultivars to borer insects

In order to assess the effects of plant features on the susceptibility of *Amaranthus* cultivars on the herbivory by stem borer insects, a field experiment was conducted during the summer season of 2008/2009 at the Experimental Station of the Agronomy Faculty, in Santa Rosa, La Pampa, Argentina (36°37′S, 64°16′W). Five cultivars of *Amaranthus* belonging to three different species were chosen on the basis of their good agronomic performances (Covas, 1987; Niveyro et al., 2013). Plants of different cultivars have shown differences in morphological, phenological and chemical features (phenolic acids and betalains contents) (Niveyro et al., 2013; Niveyro, 2015). The studied cultivars were: one cultivar of *Amaranthus cruentus* (Don León), three cultivars of *A. hypochondriacus* (San Antonio, 280 FK-FH1 and Artasa 9122) and one of *A. mantegazzianus* (Don Juan), hereinafter named as "*Cruentus*", "*Hyp SA*"; "*Hyp 280*"; "*Hyp Artasa*" and "*Mantegazzianus*", respectively (Table 1).

The experiment was arranged in a Latin Square design of 5 × 5 plots ($n = 25$). Plots were sized 3.20 m × 2 m (6.4 m^2) and separated from each other by a distance of 1 m. Seeds were planted into five rows of 3.20 m length, separated by 0.50 m, using approximately 3.5 kg of seeds per hectare (Henderson et al., 2000). Weeding was done manually and no fertilizers, herbicides or pesticides were used during the trial.

Variables relating to plant morphology were recorded in 5 plants per cultivar and plot (total $n = 125$ plants), at maturity stage (R7) according to the methodology of Fomsgaard et al. (2010). The measured variables were: total length of the stem (from the ground to the neck of the panicle), stem diameter (40 cm above the ground) and length of panicle, in all cases expressed in cm. On seven dates during the growing season (9th, 16th, 22nd, 29th of January, 24th of February, 5th of March and 11th of April), the phenological stages of 5 plants randomly chosen from each plot were measured following the scale of Mujica and Quillahuamán (1989), and the number of days required to reach anthesis stage (R4) and to complete the ontogenetic cycle (R7) were quantified.

On two sampling dates: at the middle of the plant cycle (21st to 22nd of February 2009) and at end of the plant cycle (15th to 16th of April 2009), herbivory by *Conotrachelus cervinus*, *C. histrio* and *Aerenea quadriplagiata* was estimated in 10 plants randomly chosen from each experimental unit (total $n = 500$ plants). Stems and panicles were longitudinally sectioned and borer larvae and galleries were quantified. The variables measured were percentage of damaged stems and panicles (number of damaged organs/total of observed organs), number of galleries per stem, number of larvae per plant organ (stem and panicle) and damaged stem area (cm^2). To calculate the latter variable, stems damaged by borer insects was estimated visually in the field by assigning a percentage of stem area lost by herbivory on a 0–10 scale with the following categories: 0: 0%, 1: 1–5%, 2: 6–10%, 3: 11–20%, 4: 21–30%, 5: 31–40%, 6: 41–50%, 7: 51–65%, 8: 66–75%, 9: 76–95%, 10: 100%. The percentages were later converted to area values (cm^2) to allow comparisons among cultivars. For this conversion, the diameter and length of dissected stems were also measured and the formula of

Table 1
Morphological and phenological features and plant tissue pigmentation in five cultivars of *Amaranthus*.

Features		Cruentus	Hyp SA	Hyp 280	Hyp Artasa	Mantegazzianus
Origin		Argentina	Mexico	Hungary	Argentina	Argentina
Specie		*Amaranthus cruentus*	*Amaranthus hypochondriacus*	*Amaranthus hypochondriacus*	*Amaranthus hypochondriacus*	*Amaranthus mantegazzianus*
Growing cycle (days)		142	126	59	175	162
Height (cm)		166.8 ± 5.4 (n = 37)	152.1 ± 5.3 (n = 40)	107.6 ± 2.2 (n = 45)	121.46 ± 4.3 (n = 35)	195.1 ± 7.4 (n = 36)
Foliage		abundant	abundant	poor	abundant	abundant
Panicle	shape	amaranthiform	amaranthiform	amaranthiform	amaranthiform	globular
	size	medium	large	small	large	large
	color	yellowish green	purple	purple	purple	yellow/orange
Stem	thickness	thick	thick	thin	thick	thick
	branching	scarce	scarce	scarce	intermediate	abundant
	color	green	reddish green	reddish	reddish green	green
Grain yield (kg/ha)		1763 ± 446 (n = 13)	858 ± 125.4 (n = 12)	1215 ± 245 (n = 12)	1626 ± 281 (n = 11)	2045.4 ± 522 (n = 12)

Table 2
Mean values (±standard errors) of plant attribute measurements in the cultivars. Different letters between columns indicate significant differences according to Scott and Knott test ($P < 0.05$). LD: Low density, HD: High density; S1: Early seeding, S2: Late seeding. The bold in table means the highest and statistically significant values.

Exp.	Cultivar	Plant per m^2 (number)	Plant branch (number of branches/plant)	Stem length (cm)	Stem diameter (cm)	Panicle length (cm)	Plant height (cm)	Grain yield (kg/ha)
1	Cruentus	23.75 ± 2.9 a	20.8 ± 0.8 b	95 ± 4.7 c	2.1 ± 0.1 c	**60.6 ± 2.2 b**	155.3 ± 12.4 c	1158.2 ± 297.6 a
	Hyp SA	21.25 ± 2.5 a	**41.9 ± 3.0 c**	90.1 ± 5.4 c	1.8 ± 0.1 b	**60.7 ± 3.9 b**	150.8 ± 12.7 c	1088.1 ± 132.9 a
	Hyp 280	**32.28 ± 1.8 b**	10.7 ± 0.4 a	49 ± 1.9 a	0.9 ± 0.1 a	51.9 ± 2.3 a	101.0 ± 8.4 a	1098.1 ± 174.4 a
	Hyp Artasa	**29.91 ± 0.7 b**	19.04 ± 0.9 b	65.7 ± 0.6 b	1.5 ± 0.1 b	50.2 ± 3.3 a	115.9 ± 7.2 b	1450.5 ± 278 a
	Mantegazzianus	19.06 ± 1.3 a	**44.8 ± 4.1c**	**105.4 ± 5.4 d**	**2.8 ± 0.2 d**	**100.3 ± 4.7 b**	**205.7 ± 14.5 d**	1505.9 ± 258.6 a
2	Hyp 280-LD	28.2 ± 2.5 b	_	66.3 ± 0.5 a	1.3 ± 0.07 b	**55.0 ± 0.9 b**	120.4 ± 3.8 a	546.6 ± 19.2 a
	Hyp 280-HD	**38 ± 3.1 c**	_	65.8 ± 4.8 a	0.9 ± 0.07 a	46.4 ± 1.1 a	111.5 ± 9.1 a	791.1 ± 63.9 a
	Hyp Artasa-LD	17.5 ± 0.4 a	_	**83.9 ± 1.9 b**	**2.2 ± 0.02 c**	**60.1 ± 1.3 b**	**144.1 ± 3.4 b**	**1550.3 ± 303.2 b**
	Hyp Artasa-HD	30 ± 1.3 b	_	**80.1 ± 1.7 b**	1.4 ± 0.08 b	49.6 ± 1.2 a	128.9 ± 6.1 a	**2201.1 ± 363.1 b**
3	Hyp 280-S1	25.36 ± 5 a	_	62.2 ± 1.5 a	1.3 ± 0.02 b	**48.2 ± 0.1 b**	**110.4 ± 1.4 b**	670.9 ± 46.1 a
	Hyp 280-S2	23.9 ± 4.9 a	_	54.7 ± 6.3 a	0.9 ± 0.04 a	34.2 ± 0.5 a	88.9 ± 3.3 a	621.3 ± 104.5 a
	Hyp Artasa-S1	20.7 ± 9.6 a	_	79.3 ± 7 a	**1.8 ± 0.02 c**	**49.3 ± 4.2 b**	**128.6 ± 11.2 b**	1950.4 ± 799.3 a
	Hyp Artasa-S2	32.8 ± 1.8 a	_	68.3 ± 1.9 a	1.2 ± 0.01 b	27.4 ± 1.3 a	95.7 ± 3.1 a	875.9 ± 297.0 a

cylinder area (2. π. r. h) was applied. Borer larvae were not discriminated by species or family in the calculation of the number of larvae per plant organ.

2.2. Experiments 2 and 3: effect of cultivar, plant density and sowing date on incidence of borer insects

During the summer season of 2010/2011, two experiments were conducted to assess the effect of cultivar and cultural practices (sowing density and planting date) on the incidence of borer insects, following a two-factor randomized block design with 3 replicates per treatment. In both experiments, each of the 12 experimental units consisted of five rows of 3.20 m length, spaced 0.50 m apart.

In the first trial (further “experiment 2”), two cultivars were chosen on the basis of their variation in stem thickness: *Hyp 280* (thin stems) and *Hyp Artasa* (thick stems) (Niveyro, 2015), whereas levels of the plant density treatment were 3 kg of seed per hectare (low density) and 6 kg of seed per hectare (high density), in both cases the sowing was carried out on 30 November 2010. In experiment 3, the same two cultivars were used taking into account differences in cycle duration: *Hyp 280* (short cycle) and *Hyp Artasa* (long cycle) (Niveyro, 2015). The sowing date differed by 18 days (30th November 2010 for “early planting” level and 18th December 2010 for “late planting” level), in both cases plant density was 3.5 kg ha of seed per hectare. To compare the conditions under which the plants were grown, mean monthly temperature and photoperiod were taken in the two summer seasons. Soil temperature (taken at 1 cm depth) was taken in the second season and it was calculated as an average of 5 measurements (one per day) taken two days before the sowing date and two days after planting. Since there is no information about thermal requirements (base temperature and growing degree days) of *Amaranthus* plants in the study region, differences of growth cycle among cultivars were estimated as the number of days needed to reach the anthesis (R4) and maturity (R7) stages.

In experiments 2 and 3, variables related to plant morphology were recorded in 5 plants per cultivar, following the methodology described for experiment 1. In turn, 240 plants per trial (total n = 480) were dissected to estimate herbivory, following the same methodology for experiment 1, but adding other herbivory indicators such as the number of holes per plant organ, the number of larvae per plant organ discriminated by insect family (Curculionidae or Cerambycidae) and panicle damage. The latter variable was estimated visually in the field by assigning the percentage of area lost by following the herbivory scales mentioned above. The insect identification was carried out using reference material and corroborated by specialists Dr. Charles O'Brien (University of Arizona) and Ing. Ana María de Haro (Universidad Nacional del Centro de la Provincia de Buenos Aires).

In order to avoid bias due to the size of stems (experiment 2) and growing time in plants (experiment 3), the number of larvae per organ was measured as the mean the number of larvae per m^2 in experiment 2, and the mean number of larvae per day in experiment 3. When necessary, data were arcsine transformed to meet the assumptions of normality for statistical tests. The sampling dates were: 20th to 22nd of February 2011 and 21st to 24th of April 2011 for experiment 2 and 26th of February to 1st of March 2011 and 22nd to 26th of April 2011 for experiment 3.

At the end of the growth season, after plants of experiments 1, 2 and 3 reached physiological maturity (R7), all panicles were cut, kept at room temperature until dried and then threshed by hand. The amount of grain produced was standardized to 14% moisture content and it was expressed in kg per hectare of crop.

2.3. Data analysis

The data obtained from plants and insects were analyzed by ANOVA according to each experiment design and Scott and Knott as

Table 3
ANOVA results of experiment testing the effects of cultivar on plant attributes, herbivory and grain yield.

Variable	Experiment 1			
	Trial		Cultivar	
	F	P	F	P
Plant attributes				
Plant per m^2	9.57	**0.0002**	19.26	**<0.0001**
Stem diameter (cm)	6.86	**0.001**	18.69	**<0.0001**
Stem length (cm)	23.71	**<0.0001**	63.77	**<0.0001**
Panicle length (cm)	12.17	**0.0001**	34.58	**<0.0001**
Phenology (days to R7)	18.21	**<0.0001**	18.21	**<0.0001**
Phenology (days to R4)	25.22	**<0.001**	25.22	**<0.001**
Herbivory				
Number of damaged stems (%)	8.33	**0.0004**	9.65	**0.0010**
Number of damaged panicles (%)	1.96	0.1293	2.81	0.0738
Galleries in stem	5.66	**0.002**	11.92	**0.0004**
Damaged stem area (cm^2)	4.62	**0.006**	8.10	**0.002**
Total larvae in plant	2.53	0.06	4.10	**0.02**
Larvae in stem	3.34	**0.02**	3.34	**0.02**
Larvae in panicle	0.74	0.69	2.53	0.06
Grain Yield (kg/ha)	2.08	0.109	1.17	0.37

The bold in table means the highest and statistically significant values.

test *posteriori*. In all cases, an alpha of 0.05 was considered. Multiple regression analysis was used to analyze the relationship between the three herbivory estimators (dependent variables) with the stem features: diameter and length (predictor/independent variables).

3. Results

3.1. Experiment 1: susceptibility of five cultivars

Morphological and phenological features of plants differed significantly among *Amaranthus* cultivars. *Hyp 280* presented the smallest sized plants and the shortest cycle, while *Mantegazzianus* presented the highest values in most of the morphological attributes and the longest ontogenetic cycle (Tables 2 and 3, Fig. 1). *Hyp 280* and *Hyp SA* reached the stage of anthesis in the shortest time (44–56 days), whereas *Mantegazzianus* needed a significantly longer time (68–77 days) (Table 3, Fig. 1).

During the performed experiments, adults and larvae of the three stem borer species were collected from *Amaranthus* plants: *Conotrachelus histrio, C. cervinus* (both Coleoptera: Curculionidae) and *Aerenea quadriplagiata* (Coleoptera: Cerambycidae). Analysis of the percentages of damaged organs indicated *Hyp Artasa* had the highest percentage of drilled stems (83%), while *Cruentus* and *Mantegazzianus* showed the lowest percentages (45 and 57%, respectively) (Table 3 and Fig. 2 a). No significant differences in the percentage of damaged panicles and total larval density (number of larvae observed in stems and panicles) among cultivars were observed (Table 3). However, when the analysis was partitioned by drilled organ, *Hyp Artasa* stems had the largest damaged area (Fig. 2 b) and the highest number of larvae (Fig. 2 c) compared to the other cultivars (Table 3). The damaged stems did not show any symptoms of fungal diseases. Plants supported other species of phytophagous insect (Niveyro and Salvo, 2014).

3.2. Experiment 2: cultivar and plant density

The number of plants per m^2 varied among treatments with different sowing densities. At the high density, the cultivar *Hyp 280* presented the highest value of plants per m^2 while at the low density *Hyp Artasa* treatment had the lowest plant per m^2 (Table 2). Plants grown at high density showed thinner stems and shorter panicles than plants seeded at lower density, with a significant interaction between factors observed only for stem diameter (Tables 2 and 4). In turn, the stem length significantly varied between cultivars, regardless of the effect of plant density (Table 4).

From the total number of dissected plants (total $n = 240$), damage by borer insects was observed in 97.5% of stems and 90.8% of panicles. Numbers of galleries and holes in stems (Table 4, Fig. 3a and b) and damaged stem area (Table 4, Fig. 3 c) were affected by plant density and *Amaranthus* cultivar, with no significant interaction between the factors (Table 4). In turn, the number of holes in the panicles was affected by plant density and cultivar, with a significant interaction between factors (Table 4). *Hyp Artasa* at low plant density had the highest number of galleries per stem and the highest values of damaged area (Fig. 3b and c). The susceptibility of *Hyp Artasa* sown at low density was also evident in the significantly higher number of Curculionid larvae observed in stems and panicles (Table 5).

The multiple regression analysis indicated a significant relationship between the percentage of affected stems and the attributes of the stem (model: $R^2 = 0.49$, $R^2_{\text{adjusted}} = 0.38$, $N = 12$, $F_{(2,9)} = 4.31$, $P = 0.04$; $P_{\text{diameter}} = 0.04$, $P_{\text{length}} = 0.01$). The stem diameter was positively related to the number of larvae/m^2 (model: $R^2 = 0.74$, $R^2_{\text{adjusted}} = 0.78$, $N = 12$, $F_{(2,9)} = 12.96$, $P = 0.002$; $P_{\text{diameter}} = 0.01$) and to the damage area (model: $R^2 = 0.91$, $R^2_{\text{adjusted}} = 0.89$, $N = 12$, $F_{(2,9)} = 4.76$, $P < 0.0001$; $P_{\text{diameter}} = 0.0004$) (Table 6).

Grain yield was affected by the cultivar (Table 4), being *Hyp Artasa* the most productive, with similar values at both levels of plant density (Table 2).

3.3. Experiment 3: cultivar and sowing date

The average temperature of the soil for the first planting date was 18.44 ± 0.15° C (n = 5), while for the second date was 23.44 ± 0.27° C (n = 5). Mean monthly values of temperature and photoperiod are shown in Table 7.

The number of days to reach (stage VE following Mujica and Quillahuamán (1989)) the anthesis stage (R4) differed between sowing treatments (Table 8). In both cultivars, plants sown on the second date reached the stage of maturity faster in comparison to

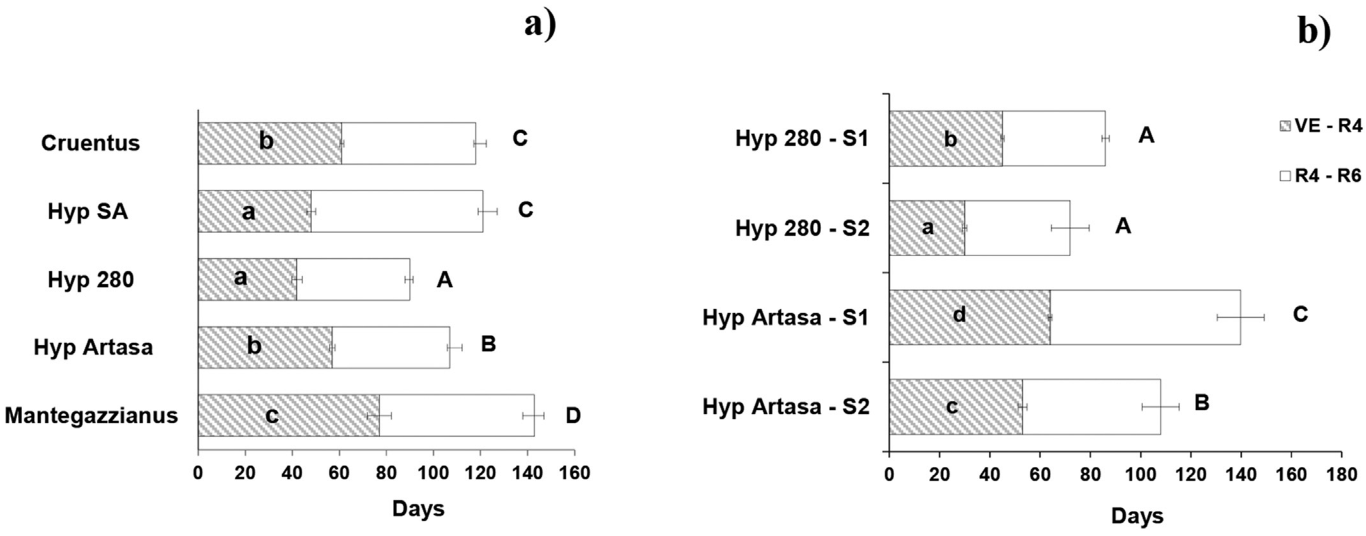

Fig. 1. Mean days required by plants to reach the phenological state of anthesis (R4) and to complete the ontogenetic cycle (R6) in: **a)** five cultivars of *Amaranthus* and **b)** two cultivars of *Amaranthus hypochondriacus* sown in different dates, S1 = Early sown, S2 = Late sown. The horizontal lines indicate the standard errors. Different lower case letters indicate significant differences in time required from emergence to R4 and capital letters indicate significant differences in time required for the entire cycle, in both cases according to Scott and Knott test.

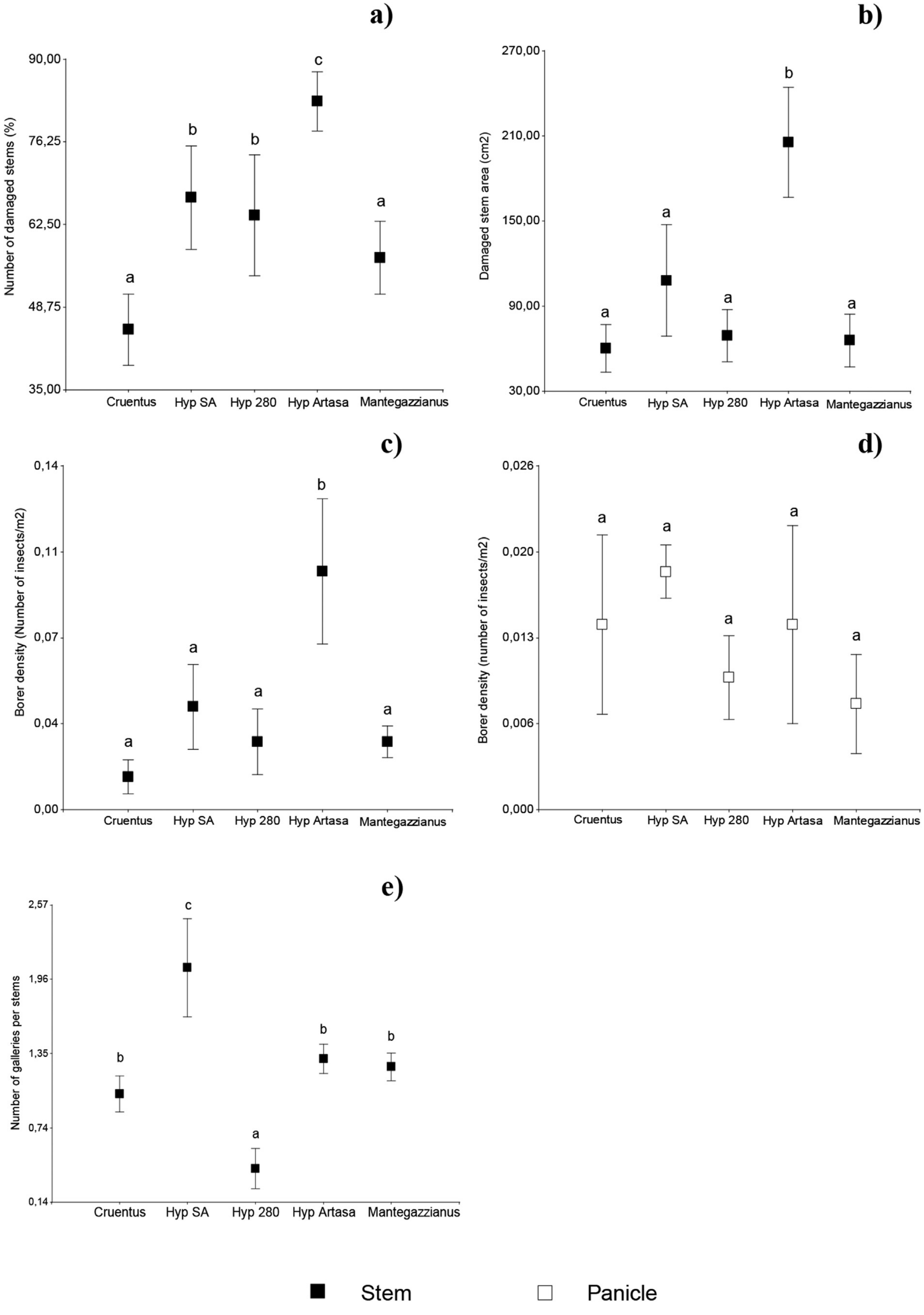

Fig. 2. Variables estimating herbivory by borer insects in five cultivars of *Amaranthus* **a)** Number of damaged stems **b)** Damaged stem area **c)** Larval density in stems **d)** Larval density in panicles **e)** Number of galleries per stem. Vertical lines indicate standard errors. Different letters indicate significant differences according to Scott and Knott test.

Table 4
ANOVA results of experiment testing the effects of cultivar, plant density and their interaction on plant attributes, herbivory and grain yield.

Variable	Experiment 2							
	Trial		Cultivar		Density		C D interaction	
	F	P	F	P	F	P	F	P
Plant attributes								
Plant per m^2	13.34	**0.003**	25.28	**0.002**	36.10	**0.001**	0.55	0.48
Stem diameter (cm)	34.21	**0.0002**	93.72	**0.0001**	69.35	**0.0002**	7.71	**0.032**
Stem length (cm)	8.92	**0.009**	39.95	**0.0007**	0.72	0.429	8.42	0.531
Panicle length (cm)	17.85	**0.001**	13.53	**0.010**	71.84	**0.0001**	0.76	0.416
Herbivory								
Number of damaged stems (%)	1.00	0.48	0.00	0.99	3.00	0.134	0.00	0.99
Number of damaged panicles (%)	3.60	0.07	0.00	0.99	11.44	**0.014**	0.14	0.720
Holes in stems	13.12	**0.003**	39.11	**0.0008**	20.12	**0.0042**	4.4^{-03}	0.949
Holes in panicle	34.28	**0.0002**	47.87	**0.005**	109.12	**<0.0001**	10.60	**0.017**
Galleries in stem	6.8	**0.01**	14.96	**0.008**	15.92	**0.007**	1.66	0.244
Damaged stem area (cm^2)	32.27	**0.0003**	116.54	**<0.0001**	36.84	**0.0009**	0.73	0.424
Damaged panicle (%)	1.94	0.221	0.23	0.65	5.63	0.055	0.36	0.557
Total larvae in plant	5.61	**0.029**	17.25	**0.006**	9.2	**0.023**	1.09	0.336
Larvae in stem	2.77	0.120	11.11	**0.015**	2.64	0.155	0.17	0.698
Larvae in panicle	10.33	**0.006**	22.57	**0.003**	23.70	**0.002**	2.89	0.139
Curculionidae in stem	4.51	**0.047**	9.64	**0.021**	8.29	**0.028**	4.36	0.081
Curculionidae in panicle	10.75	**0.005**	16.11	**0.007**	30.95	**0.001**	6.07	0.048
Cerambycidae in stem	1.50	0.316	3.65	0.104	0.61	0.465	3.13	0.127
Cerambycidae in panicle	1.70	0.267	4.44	**0.079**	0.62	0.459	1.11	0.332
Grain Yield (kg/ha)	10.9	**0.007**	38.17	**0.0008**	5.25	0.06	1.08	0.33

The bold in table means the highest and statistically significant values.

those sown earlier (Table 8). However, the time from emergence to maturity was significantly higher only in plants of *Hyp Artasa* (Fig. 1). Stem lengths and diameters were affected by both cultivar and sowing date, with a significant interaction between factors for stem diameter (Table 8). Panicle length was affected only by sowing date, without significant interaction with cultivar (Table 8).

From the total number of analyzed plants (total n = 240), 94.2% had galleries in stems and 73.8% in panicles. The percentage of affected stems and panicles varied according to sowing date and no interactions with cultivar were observed (Table 8). Earlier sowing tended to increase the borer herbivory in both cultivars (Table 8, Fig. 4a and b and d); whereas damaged area in stems was greater in earlier sown plants, but differences were statistically significant only in *Hyp Artasa*.

The total number of larvae observed in plants (stems and panicles) differed between cultivars and sowing date treatments, with a significant interaction among factors (Table 8). *Hyp Artasa* had a higher density of larvae per day in earlier sown plants than in the later sown, whereas no significant differences due to density were observed in *Hyp 280* (Fig. 4 c). In both cultivars, a higher density of Curculionid larvae was recorded in panicles of plants of the first sowing date, without differences for the Cerambycidae family (Table 8). Symptoms of fungal diseases in the damaged stem tissues were not observed in plants of experiments 2 and 3.

The multiple regression analysis indicated a significant relationship between the percentage of affected stems and length of the stems (model: $R^2 = 0.83$, $R^2_{adjusted} = 0.78$, $N = 12$, $F_{(2,9)} = 17.12$, $P = 0.002$, $P_{length} = 0.04$) (Table 6). No relationship between stem features and others herbivory estimators (larval density: $R^2 = 0.37$, $R^2_{adjusted} = 0.21$, $N = 12$, $F_{(2,10)} = 2.34$, $P = 0.15$ damaged stem area: $R^2 = 0.24$, $R^2 = 0.05$, $N = 12$, $F_{(2,10)} = 1.24$, $P = 0.33$) were found (Table 6). Regarding grain yields, no differences related to planting dates were found (Table 8).

4. Discussion

4.1. Susceptibility of five cultivars to borer insects

Morphological and phenological variations were observed among cultivars. The plant growth rate was the highest in *Hyp 280*, the lowest in *Mantegazzianus* and intermediate in *Cruentus*, *Hyp SA* and *Hyp Artasa*. These differences are probably related to architectural features of the plants, since *Mantegazzianus* presented the largest plants, *Hyp 280* the smallest ones and plants of the remaining cultivars had intermediate sizes. The greatest taxonomic relatedness of the cultivars *Hyp SA*, *Hyp 280* and *Hyp Artasa* (all belonging to *A. hypochondriacus* species) was not linked to similar values in morphological or phenological features, which is in concordance with the wide range of genetic variation reported in *A. hypochondriacus* (Kietlinski et al., 2013; Sogbohossou and Achigan-Dako, 2014; Raut et al., 2014). However, similarities in morphological characters of plants between pairs of *A. hypochondriacus* cultivars were observed, such as the stem diameter in *Hyp SA* and *Hyp Artasa*, and panicle lengths, which were similar in *Hyp 280* and *Hyp Artasa*.

Plants with large dimensions and architectural complexity generally exhibit greater abundance and richness of phytophagous insects (Price et al., 1980; Rudgers and Whitney, 2006). In this study, the damage by borer insects appeared not to be strictly related with the resources offered by the plants, since the cultivar with the largest size (plant height and stem diameter), the most complex architecture (number on branches, leaf density) and the longest exposure time at field (*Mantegazzianus*) harbored a number of larvae per plant and damage similar to the variety with the smallest size, the simplest architecture and the shortest cycle (*Hyp*

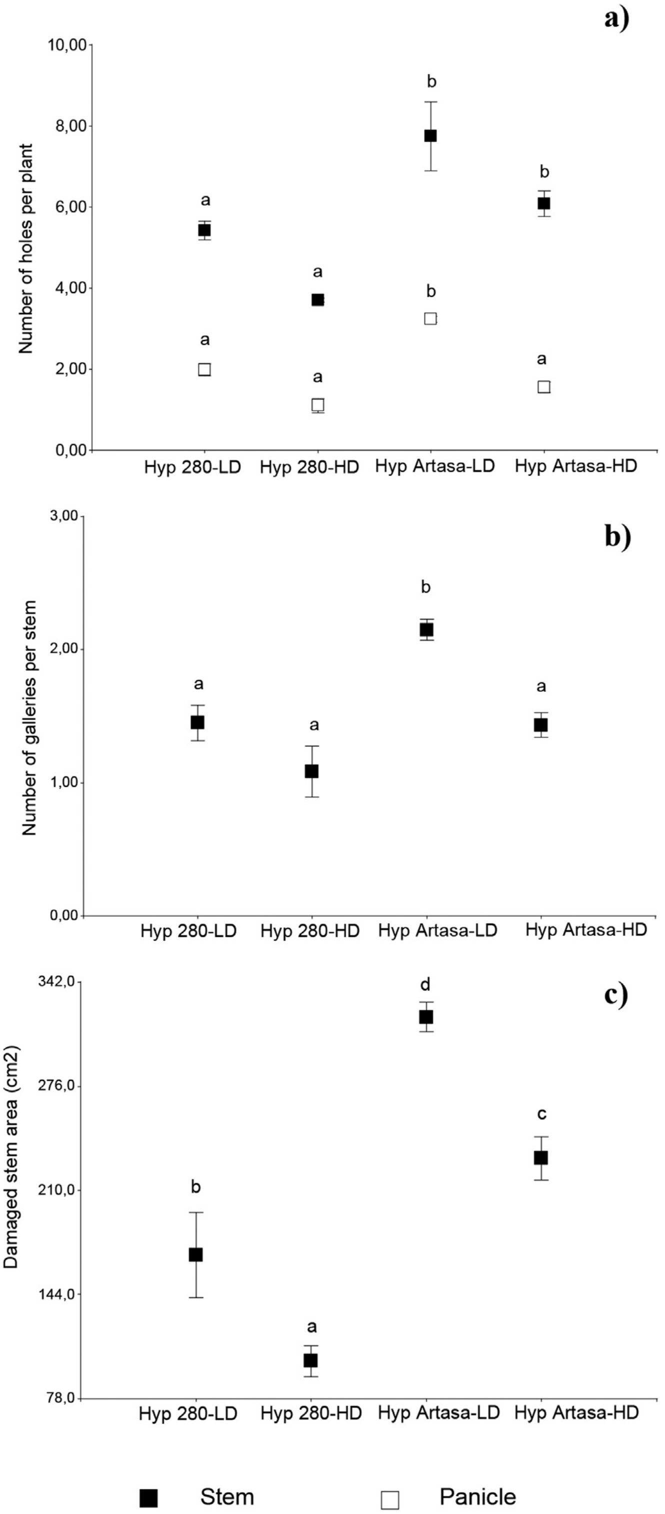

Fig. 3. Variables estimating herbivory by borer insects in two cultivars of *Amaranthus hypochondriacus* with two levels of planting density. **a)** Number of holes on stems and panicles, **b)** Number of galleries on stems and **c)** Damaged stem area. LD: Low density; HD: high density. Vertical lines indicate standard errors. Different letters indicate significant differences according to Scott and Knott test.

280). Instead, *Hyp Artasa*, a cultivar with plants of intermediate sizes and exposure time in the field, was the most affected cultivar by stem borer guild. The lower preference for *Mantegazzianus* displayed by borer insects could be linked to the higher energy expenditure by female adults to reach oviposition sites in these very branched plants. On the other hand, vegetation complexity can generate diversification of ecological niches, and harbor predators and parasites insects with impact on phytophagous insects (Alonso and Herrera, 1996; Mulatu et al., 2004).

In addition to morphological characteristics, other plant features such as stem hardness, nutritional quality or plant defense could be contributing to the observed pattern in herbivory. Differences in phenological cycle suggest a different growth rate in the five cultivars. It has been proposed that the growth rate in plant species may determine differences in the levels of defenses against herbivores (Coley et al., 1985). Because the production of chemical defenses implies costs to be maintained by plants, the presence of immobile structures and compounds such as lignins and tannins that have a high initial cost but a low rate of renewal (non-mobile compounds) (Walker, 1975), would confer greater adaptive advantages to slow growing plants.

In this study, *Mantegazzianus*, the cultivar with slower growth rate, presented low levels of stem damage, which was also observed in *Hyp 280*. However, the difference in growth rate of these cultivars could compromise different forms of defense. Although lignins and tannins were not quantified in the cultivars, they might have influenced the lower herbivory observed in *Mantegazzianus*. On the other hand, in plants with rapid growth, other defensive compounds with low costs and high rates of renewal are expected, such as alkaloids, cyanogenic glycosides and phenolics (Coley et al., 1985). Previous studies indicated *Hyp 280* showed high concentration in several phenolic compounds (e.g. caffeic acid, ferrulic acid and salicylic acid; Niveyro et al., 2013).

Furthermore, in this study, we observed that plants with green pigmentation (*Cruentus* and *Mantegazzianus*) were less preferred for oviposition than reddish ones. The red pigmentation in *Amaranthus* plant tissues is due to the presence of betalains, a nitrogen-containing alkaloid (Cai et al., 2001; Strack et al., 2003). Betalains accumulation are genotype-dependent in *A. hypochondriacus* (Casique-Arroyo et al., 2015), and the biosynthetic genes and enzymes of these pigments are induced in response to insect folivory (Casique-Arroyo et al., 2015). Based on our results, we do not rule out both compounds, betalains and phenolic compounds as possible factors influencing herbivory differences on these cultivars (Barbehenn and Kochmanski, 2013; Richards et al., 2016). Chemical and physical plant traits in *Amaranthus* that may function as anti-herbivore defense need further experimental and comparative studies aimed to improve the understanding of the mechanisms underlying these effects.

4.2. Effect of cultivar, plant density and sowing date on borer insect incidence

In both cultivars, plants grown at higher density reached a lower height, smaller stem diameter and length of the panicles, according to previous reports (Law-Ogbomo and Ajayi, 2009). Differences in the number of raised plants and their size (Table 3) suggested a greater intraspecific competition in plants of *Hyp Artasa* than *Hyp 280*. Competition in *Amaranthus* plants begins early, from about the fifth week after emergence, and it has been mentioned that plants at low density tend to expand and fill the available space (Mnzava and Reuben, 1982). The effect of space limitation observed for *Hyp Artasa* agreed with the expected, considering that plants of this cultivar were bigger, and thus required a bigger space for development than plants of *Hyp 280*.

Plant density affected plant morphology and, at the same time, influenced the susceptibility to borer insects. Although the percentage of damaged stems did not differ between plant density treatments, we have recorded a greater reduction of the damaged

Table 5
Average larvae density (number of larvae per m^2 ± standard error) observed in two cultivars of *Amaranthus hypochondriacus* planted in different densities. Different letters between columns indicate significant differences according to Scott and Knott test ($P < 0.05$). LD = low density; HD = high density.

Variable	Cultivar – Density			
	Hyp 280 - LD	Hyp 280 - HD	Hyp Artasa - LD	Hyp Artasa - HD
Total larvae	0.29 ± 0.04 a	0.16 ± 0.03 a	**0.63 ± 0.09 b**	0.37 ± 0.06 a
Larvae in stems	0.16 ± 0.03 a	0.11 ± 0.02 a	0.32 ± 0.05 a	0.23 ± 0.03 a
Curculionidae	0.08 ± 0.003 a	0.06 ± 0.01 a	**0.24 ± 0.04 b**	0.09 ± 0.03 a
Cerambycidae	0.08 ± 0.03 a	0.05 ± 0.01 a	0.08 ± 0.01 a	0.14 ± 0.02 a
Larvae in panicles	0.14 ± 0.01 a	0.05 ± 0.01 a	**0.32 ± 0.04 b**	0.13 ± 0.04 a
Curculionidae	0.09 ± 0.01 a	0.01 ± 0.01 a	**0.24 ± 0.03 b**	0.05 ± 0.03 a
Cerambycidae	0.04 ± 0.01a	0.03 ± 0.02 a	0.05 ± 0.01 a	0.07 ± 0.01 a

The bold in table means the highest and statistically significant values.

Table 6
Results of multiple regression analysis of herbivory and characteristics of the stem (diameter and length). (df = degrees of freedom; β = standardized coefficient; SE = standard error).

Exp.	Dependent variable	Predictor variable	df	β	SE	F	P-value
2	Number of damaged stems (%)	Stem length	1.12	−0.48	0.18	8.01	**0.01**
		Stem diameter	1.12	9.84	3.48	6.72	**0.02**
	Number of larvae/m^2	Stem length	1.12	3^{-03}	0.01	0.32	0.58
		Stem diameter	1.12	0.30	0.10	9.43	**0.01**
	Damaged stem area (cm^2)	Stem length	1.12	1.84	1.36	1.83	0.20
		Stem diameter	1.12	140.84	25.72	30.00	**0.0004**
3	Number of damaged stems (%)	Stem length	1.12	−0.47	0.20	−2.42	**0.04**
		Stem diameter	1.12	3.38	7.50	0.45	0.66
	Number of larvae/m^2	Stem length	1.12	1.1E-03	5.2E-04	2.05	0.07
		Stem diameter	1.12	−0.03	0.02	−1.63	0.14
	Damaged stem area (cm^2)	Stem length	1.12	6.18	6.10	1.01	0.34
		Stem diameter	1.12	−113.29	234.57	−0.48	0.642

The bold in table means the highest and statistically significant values.

stem area and larvae density in plants sown at high density (thin stems) than in plants sown at low density (thick stems). Regarding the plant density, the results seem to be in opposition to the expected from the “resource concentration hypothesis” (Root, 1973), which predict a positive relationship between herbivore density and plant density. However, the thicker stems of plants at lower densities may be a stronger factor driving the higher density of insects in these plants. In this regard, our results showed that density of plants, as a modifying factor of stem thickness, had a greater effect on Curculionidae than in the Cerambycidae species. Previous works on several Cerambycidae species have indicated they are highly selective of the stem diameter for oviposition (Paulino-Neto et al., 2005). Particularly, females of *A. quadriplagiata* tend to oviposit more frequently in plants with stem diameters between 5 mm and 7 mm (Riquelme et al., 2013). The fact that stems of plants here studied were thinner than those preferred by *A. quadriplagiata*, could explain at least in part, the little response of this insect to plant density. The relatively low abundance of *A. quadriplagiata* regarding *Conotrachelus* spp. could be related to its geographic distribution, prominent in the northeast of the country where it is reported as the only borer species affecting *Amaranthus* crops (Dr. Riquelme, personal communication). On the other hand, our data suggest that *Conotrachelus* females could also be selecting the stems according to their diameter in *Hyp Artasa*, the cultivar in which differences in stem diameter due to density changes were bigger (X = 0.79 cm). Previous assessments of the effect of plant density on borer species *H. truncatulus* and *Amauromyza abnormalis*, in cultivars of *A. hypochondriacus* failed to find effects of plant density on larval density and herbivory damage (Torres-Saldaña et al., 2004). Differences in studied cultivars, characteristics of the plants and particular behaviors of the involved insect borer species could explain the discordances between that study and ours.

Regarding the third experiment, our data indicated that later sown plants of both cultivars completed their cycles in a shorter time than plants of early sowing date treatment, reducing the length of all plant organs measured (with the exception of the stem length), differences being even more evident in *Hyp Artasa*. This was expected in *Amaranthus* plants given the C4 photosynthetic metabolism that achieve high growth rate under high temperature (Grubben, 1980). Moreover, the transition from vegetative to reproductive stages in *Amaranthus* was accelerated when the length of the days begins to decrease (Sawhney et al., 1980), photoperiod sensitivity being a variable character in *Amaranthus* species (Kauffman and Weber, 1990). The critical value of flowering in *A. hypochondriacus* was reported with 16 h of light (Amhed, 2005). In the study area (36°37′S, 64°16′W), shorter days conditions (13–15 h of light per day) accelerated the flowering in both cultivars, but *Hyp 280* was the most sensitive cultivar to photoperiod, starting the reproductive stage about a month before *Hyp Artasa*.

Significantly less severe damage by borers (holes in the stems, galleries and the average damage area in stems and panicles) was observed in plants with delayed sowing date. This lower level of herbivory could be a consequence of the shorter exposure time of plants in the field, creating asynchrony between peak population of insects and crop cycle, but it may also be a consequence of insect females selecting the longer and thicker stems of early sown plants for oviposition. In turn, lower herbivory was registered in later

Table 7
Mean temperature values (±standard error) and photoperiod during seasons 2008–2009 and 2010–2011.

Variable	2008–2009						2010–2011					
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Mean monthly temperature (°C)	22.91 ± 3.56 (n = 30)	22.50 ± 3.06 (n = 31)	24.42 ± 3.02 (n = 31)	23.90 ± 3.12 (n = 28)	22.44 ± 2.69 (n = 31)	17.97 ± 3.83 (n = 30)	19.0 ± 0.45 (n = 30)	23.1 ± 0.66 (n = 31)	22.5 ± 0.55 (n = 31)	21.6 ± 0.53 (n = 28)	19.6 ± 0.50 (n = 31)	15.9 ± 0.51 (n = 30)
Photoperiod (Daylighthours) (a)	14.53–15.43	15.45–15.63	15.62–14.89	14.85–13.82	13.78–12.59	12.55–11.54	14.53–15.43	15.45–15.63	15.62–14.89	14.85–13.82	13.78–12.59	12.55–11.54

[a] Number of daylight hours, minimum and maximum.

sown plants (thinner stems), which was most noticeable in *Hyp 280*, where at similar density of larvae within stems, a significantly smaller area was consumed. Furthermore, we cannot discard the possibility that physiological changes due to phenological differences among treatments can modify plant chemistry and defensive compounds effectiveness (Johnson and Agrawal, 2005; Lamarre et al., 2014) throughout plant development (Boege and Marquis, 2005).

4.3. Herbivory and cultural practices incidence on grain yield in *Amaranthus*

Values of grain yield per hectare obtained here were within the previously reported value range for the *Amaranthus* crop (Gimplinger et al., 2008). Similar yields among treatments with different levels of herbivory in experiments 1 and 3 suggested plants were not affected enough to reduce seed production or that they were able to compensate for the resources lost by insect consumption. According to previous works, *Amaranthus* plants are tolerant to defoliation and compensation strategies are used to prevent the reduction of yield (Vargas-Ortiz et al., 2013). The underlying mechanism of this tolerance is given by the rapid uptake of carbon stocks stored during the pre-flowering stages in leaves, stems and roots, mostly in the form of starch and it is conditioned by the amount of damage, the environment and the particular species (Castrillón-Arbeláez et al., 2012). Further studies should be made to evaluate *Amaranthus* damage thresholds. Even though similar yields were observed in the treatments, we cannot affirm that plants were able to compensate the damage. In this regard, the three species of stem borers occurred in advanced stages of crop development (flowering and filling of grain) and this could have allowed the plant to store carbon stocks before the insect damage was produced (Castrillón-Arbeláez et al., 2012). In turn, it is probable that these particular species of borers consume parts of the stems that do not compromise the conduction of nutrients during grain filling. However, this does not disregard the damage by these species given the fact that they can produce the total loss of seed due to plant break.

On the other hand, in this study we observed that grain yield did not differ significantly between treatments of density, as it has been previously reported by Henderson et al. (2000) and Guillen-Portal et al. (1999) where the density ranged from 7.7 to 27.2 plant/m^2 and 4–200 plant/m^2, respectively. However, higher yields have been reported with low densities in trials ranging from 8, 7, 35 and 70 plants per m^2 (Gimplinger et al., 2008). By contrast, higher yield at densities of 22 plants per m^2 than in 6 plants per m^2 have been indicated (Apaza-Gutierrez et al., 2002). Considering the competitive nature of *Amaranthus* plants (Gimplinger et al., 2008), a very dense crop should reduce grain yield. However, a proper distribution of seeds in the rows and an adequate distance between them would favor crop performance, even at high densities. On the other hand, sowing in very low density in order to avoid intraspecific competition could decrease the production of grain as a consequence of the smaller number of plants in the field. In this study, on the basis of the density levels and the cultivars tested, the damage by stem borer decreased in plants sown at high density (30–38 plants/m^2) and therefore, it may be a recommendable practice for both cultivars, particularly for the most susceptible, *Hyp Artasa*. Further experiments should be made to elucidate the optimal plant density of *Amaranthus* crop. As our results showed, this information is closely related to several morphological features of plants (i.e. branching, height, growth rate, etc.), and it must be simultaneously considered to increase crop yields.

In conclusion, our results constitute a preliminary contribution to *Amaranthus* damage thresholds, which are not currently

Table 8
ANOVA results of experiment testing the effects of cultivar, sowing date and their interaction on plant attributes, herbivory and grain yield.

Variable	Experiment 3							
	Trial		Cultivar		Sowing date		C S interaction	
	F	P	F	P	F	P	F	P
Plant attributes								
Plant per m^2	0.51	0.76	0.11	0.754	0.67	0.445	1.05	0.345
Stem diameter (cm)	115.7	<**0.0001**	215.33	<**0.0001**	328.31	<**0.0001**	32.97	**0.001**
Stem length (cm)	5.56	**0.029**	17.25	**0.006**	6.18	**0.047**	0.22	0.656
Panicle length (cm)	70.81	**0.002**	1.80	0.228	70.81	**0.0002**	3.40	0.114
Phenology (days to R7)	18.71	<**0.0001**	44.49	<**0.0001**	11.57	**0.0036**	1.75	0.204
Phenology (days to R4)	122.49	<**0.0001**	288.8	<**0.0001**	111.33	<**0.0001**	2.34	0.145
Herbivory								
Number of damaged stems (%)	3.23	0.09	0.27	0.624	13.07	**0.011**	0.27	0.624
Number of damaged panicles (%)	18.20	**0.001**	1.81	0.226	88.93	**0.0001**	0.04	0.853
Holes in stems	5.36	**0.03**	4.41	0.080	18.66	**0.0050**	2.11	0.196
Holes in panicle	13.87	**0.003**	3.58	0.097	58.28	**0.0003**	6.85	**0.039**
Galleries in stem	7.22	**0.01**	0.71	0.432	30.36	**0.001**	3.15	0.126
Damaged stem area (cm^2)	15.11	**0.002**	9.04	**0.023**	62.08	**0.0002**	2.56	0.160
Damaged panicle (%)	13.59	**0.003**	3.67	0.104	62.93	**0.0002**	0.67	0.443
Total larvae in plant	8.60	**0.01**	16.33	**0.006**	8.33	**0.027**	16.33	**0.006**
Larvae in stem	3.80	0.06	8.33	**0.027**	0.33	0.584	8.33	**0.027**
Larvae in panicle	3.14	0.09	1.5^{-03}	0.970	12.53	**0.012**	2.24	0.185
+Curculionidae in stem	2.25	0.17	6.58	**0.042**	2.40	0.172	1.86	0.221
Curculionidae in panicle	7.30	**0.01**	0.55	0.487	30.22	**0.001**	4.94	**0.067**
Cerambycidae in stem	0.71	0.63	0.27	0.620	2.45	0.168	0.27	0.620
Cerambycidae in panicle	1.30	0.37	0.20	0.669	5.88	0.051	0.15	0.710
Grain Yield (kg/ha)	1.05	0.46	2.49	0.16	1.34	0.29	1.12	0.33

The bold in table means the highest and statistically significant values.

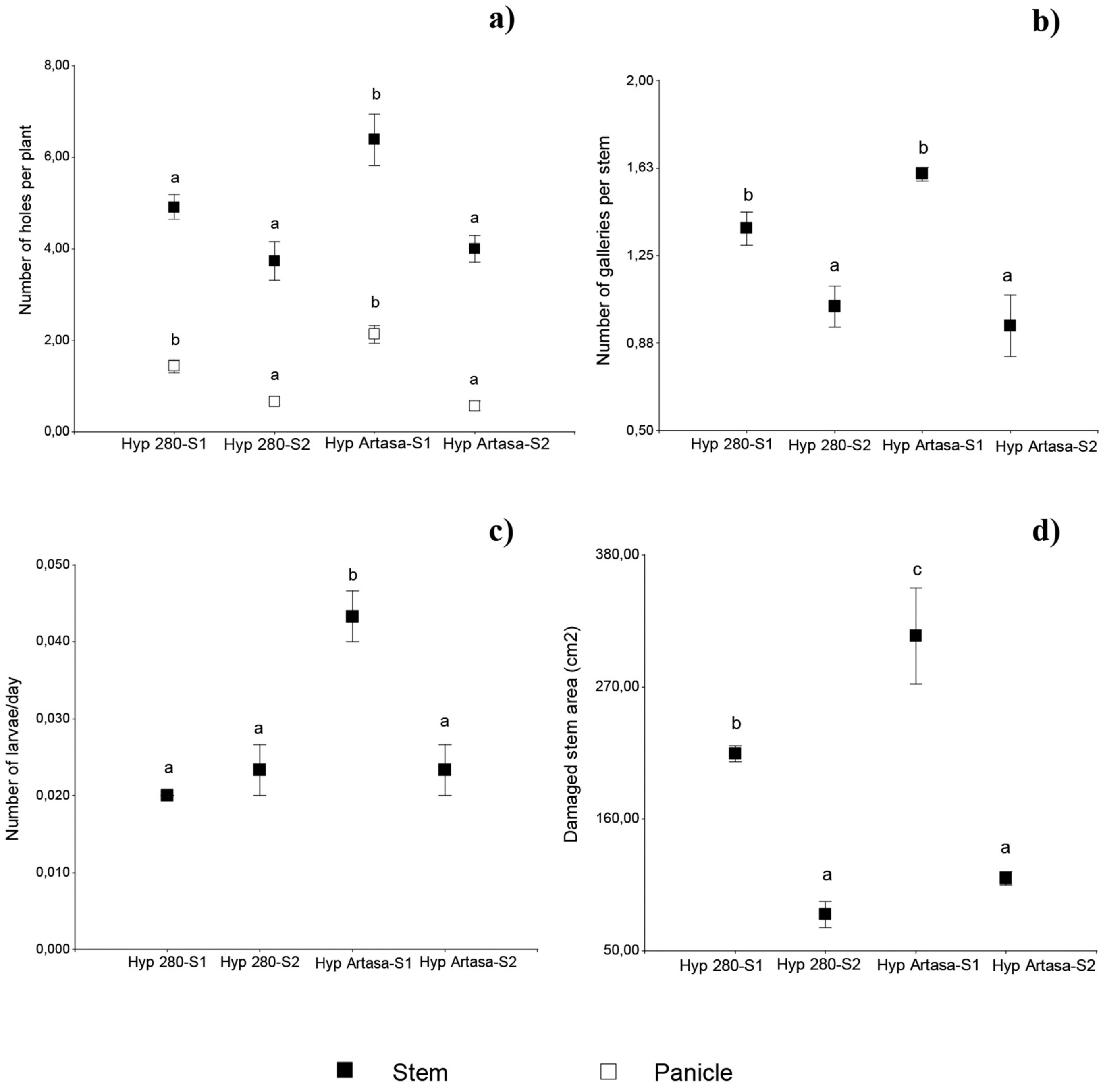

Fig. 4. Variables estimating herbivory by borer insects in two cultivars of *Amaranthus hypochondriacus* in two different sowing dates. **a)** Number of holes on stems and panicles, **b)** Number of galleries on stems, **c)** Larval density in stem and **d)** Damaged stem area. S1 = Early sown; S2 = Late sown. Vertical lines indicate standard errors. Different letters indicate significant differences according to Scott and Knott test.

developed for this crop. We provide information about morphological and phenological features and plant tissue pigmentation of *Amaranthus* cultivars had influenced on the susceptibility of plant to borer insects under field conditions. In turn, we reported two agronomic practices: increasing plant density and delaying sowing dates (both practices causing thinner stems) which were effective to minimize the herbivory by stem borers in cultivars of *A. hypochondriacus* with a large impact on the most susceptible cultivar. Finally, these results indicate that crop management in *Amaranthus* either by artificial selection of attributes in the cultivar (e.g. physical or chemical) or by modifying agriculture practices, may reduce the vulnerability of plants to associated stem borer insects.

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