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Effects of density and sowing pattern on weed suppression and grain yield in three varieties of maize under high weed pressure

C MARÍN* & J WEINER†

*Department of Biological and Environmental Sciences, Jorge Tadeo Lozano University, Bogotá, Colombia, and †Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

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Summary

We tested the hypothesis that improved weed suppression by maize can be achieved through increased crop density and spatial uniformity. Field experiments on three varieties of maize sown at three densities (5, 7 and 10.5 seeds m^{-2}) and in two spatial patterns (grid pattern and rows) under very high weed pressure from *Brachiaria brizantha* were performed in 2012 and 2013. We measured weed biomass 1 month after sowing and at harvest, and grain yield at harvest. Density, variety and sowing pattern all had strong and significant effects on both weed biomass and yield. On average, weed biomass was reduced (by 72% in the first year and 58% in the second year), and grain yield was increased (by 48% and 44%) at the highest density in the grid pattern compared with standard sowing practices (medium density, row pattern). There was a significant density × variety interaction, which is evidence for genetic differences in the response of the varieties to density in characteristics that influence weed suppression. The variety that suppressed weeds best at high density had the lowest variation in the angle of insertion of the oldest living leaf at harvest (leaf 6), supporting the hypothesis that reduced phenotypic plasticity may be advantageous for weed suppression under high density and spatial uniformity. Increased density and uniformity can contribute to weed management in maize in many cases, potentially reducing the need for herbicides or mechanical weed control.

Keywords: crop/weed competition, phenotypic plasticity, sowing density, spatial uniformity, *Brachiaria brizantha*, *Zea mays*.

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Introduction

Weeds are the largest source of yield losses worldwide, and there is a great need for new and environmentally friendly approaches to weed management as alternatives or supplements to chemical and mechanical weed control. Several studies have shown increased weed suppression with decreased crop row distance or increased crop density, and yield can also be higher in a more uniform pattern even without weed pressure. Narrower rows are reported to suppress weeds in organic wheat (Drews *et al.*, 2009) and rice (Chauhan & Johnson, 2011). In maize (*Zea mays* L.), where yield losses due to weeds can be very high, especially in developing countries (Kwiligwa *et al.*, 1994), there are several studies showing increased weed suppression

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Correspondence: J Weiner, Department of Plant and Environmental Sciences, University of Copenhagen, Rolighedsvej 21, DK-1958 Frederiksberg, Denmark. Tel: (+45) 3533 2822; Fax: (+45) 3533 2821; E-mail: jw@plen.ku.dk

resulting from narrower crop rows and/or increased density (Teasdale, 1995, 1998; Murphy *et al.*, 1996; Begna *et al.*, 2001; Saberali *et al.*, 2008; Mashingaidze *et al.*, 2009; Fanadzo *et al.*, 2010; McDonald *et al.*, 2010; Mohammadi *et al.*, 2012).

Several studies over the past decade have taken this approach further and shown that weed suppression by wheat can be greatly improved through a combination of increased crop density and spatial uniformity in wheat (Weiner *et al.*, 2001, 2010; Olsen *et al.*, 2005a,b, 2006, 2012; Olsen & Weiner, 2007; Kristensen *et al.*, 2008). Increased crop density increases the degree of 'size-asymmetric competition', to the advantage of the crop, which almost always has an initial size advantage in competition with annual weeds (Weiner *et al.*, 2001). A more uniform spatial pattern of the crop reduces competition among crop plants early in the growing season, increasing crop/weed competition, while the crop still has its initial size advantage. Increased crop density and uniformity results in increased collective shading of weeds by the crop, suppressing the weeds before they can 'catch up' with the crop's initial size advantage. Unlike other methods for weed management, such as herbicides or mechanical weed control, this approach has no negative environmental effects. Indeed, increased standing biomass in the field, which is one result of increased crop density, generally increases sustainability (Weiner *et al.*, 2010).

Spatial uniformity makes interactions among crop plants more co-operative. In a spatially uniform pattern, plants have approximately the same available area for resource uptake (Fisher & Miles, 1973; Mithen *et al.*, 1984; Regnier & Bakelana, 1995). In the standard row-sowing pattern, crop plants are very crowded in one dimension, and much farther apart in the other dimension. In a uniform pattern, individual crop plants are equally crowded in both dimensions. Intraspecific competition within the crop is delayed, while interspecific competition with weeds begins sooner. This allows the crop population to shade and suppress the weeds, increasing the effect of the crop's initial size advantage (Weiner *et al.*, 2010). Our hypothesis is that shading of weeds by the crop population is maximised under high density, high uniformity conditions. It has also been hypothesised that a reduction in some forms of phenotypic plasticity can increase the collective shading of weeds by the crop under high density/high uniformity conditions, as plasticity in response to competition represents an 'individual defensive' strategy, whereas weed suppression is a 'collective offensive' strategy (Weiner *et al.*, 2010).

Acciaresi and Chidichimo (2007) showed that a grid pattern in maize increased yield and weed suppression compared with the standard row-sowing pattern and that this effect varied among varieties. They concluded that increased spatial uniformity could play an important role in weed suppression, increasing yield in maize under weed pressure. Their study used a fixed density and did not explore the combined effect of uniformity and increased density. The potential for a weed control strategy based on both increased density and spatial uniformity has not yet been well investigated in maize.

There are several reasons to expect that this approach could be useful in maize. As maize is much larger than other cereals and density is inversely related to size, 'high density' for maize is much more manageable agronomically than 'high density' for wheat, which was 720 grains m^{-2} in some of the above-mentioned studies. Also, because of its large size, high spatial uniformity is common in maize production, but not in other cereals. Early in the growing season, maize is usually a good competitor against weeds (Page *et al.*, 2009), even against some perennial weeds, such as *Datura stramonium* L. (jimson weed) and *Cannabis sativa* L. (marijuana; Cavero *et al.*,1999; Karimmojeni *et al.*, 2010; Lehoczky *et al.*, 2005), as well as annuals such as *Chenopodium album* L. (lambs quarters), *Xanthium strumarium* L. (cocklebur) and *Abutilon theophrasti* Medik.(velvetleaf; Baghestani *et al.*, 2007; Karimmojeni *et al.*, 2010; Lindquist & Mortensen, 1999; McDonald & Riha, 1999). It has been hypothesised that this is because maize has a high leaf area index, allowing it to shade weeds effectively (Cavero *et al.*, 1999; McDonald & Riha, 1999; McDonald *et al.*, 2010). According to our current understanding of resource competition among plants, this effect should increase with density and spatial uniformity. Here, we take the first step in testing the potential for improved weed suppression by maize through a combination of increased crop density and spatial uniformity.

Materials and methods

The experiment was carried out on 16 January 2012 and repeated on 14 January 2013 on a farm in the El Tambo municipality, Cauca, Colombia (2°25′45.90″N, 76°43′34.68″W), at 1730 m altitude. Maize was previously grown on the site. Soil was a sandy clay inceptisol, with an organic matter content of 3%. Organic fertiliser (Gallinaza, Productora Avícola de Occidente S.A.S®, Carrera 2C No. 30-03, Cali, Colombia) was applied at a rate of 40 kg N ha^{-1} 8 days before and 1 month after sowing. The highest temperature for the sowing season in 2012 was 29.2°C, and the minimum was 13.4°C, with an average of 18.7°C and a mean monthly precipitation of 175 mm. For 2013 season, the highest, minimum and average temperatures were

30.1, 13.8 and 19.2°C, respectively; the mean monthly precipitation was 140 mm.

We used three sowing densities (5, 7 and 10.5 plants m^{-2}), two spatial patterns (grid and rows; Table 1), and three varieties of maize (*Novillero*, *Amarillo ICA V-305*, *Híbrido HR Oro-Amarillo*), all of which are cultivated in the region. *Novillero* is a traditional variety, while *Amarillo ICA V-305* and *Híbrido HR Oro-Amarillo* are recently developed (SemillasArroyave®, Calle 162 No. 18A-32, Bogotá, Colombia). *Híbrido HR Oro-Amarillo* matures more quickly than the other varieties. All seeds were sown with a seeding machine at 5 cm depth; conventionally mechanised, intensive tillage practices (including seedbed) were implemented. Factors were (i) three densities, (ii) three varieties and (iii) two spatial arrangements, giving a total of 18 treatments. Plots were 6 by 6 m with two replicates, giving a total of 36 plots (1296 m^2 sown). To obtain high weed pressure, *Brachiaria brizantha* Hochst. Ex A. Rich. (bread grass) seeds (Semicol S. A.®, Calle 34 No. 19-38, Bogotá, Colombia) were manually dropped into the soil at a rate of 0.7 g seeds m^{-2}, corresponding to a density of 30 seeds m^{-2}, immediately after the maize was sown. *Brachiaria brizantha* is an invasive and aggressive weed in this region, especially in grasslands. The critical period for weed control in maize is around 1 month after sowing (Page *et al.*, 2009), so we measured weed biomass on 15 February in 2012 and 13 February in 2013, and at harvest both years, which for *Novillero* and *Amarillo ICA V-305* was 180 days (14 July in 2012 and 13 July in 2013), and for *Híbrido HR Oro-Amarillo* was 120 days (15 May in 2012 and 14 May in 2013). All aboveground weed biomass within one half of each plot, a 6 × 3 m quadrat, was cut at the soil surface 1 month after sowing, and the other half at harvest, dried for 48 h at 70°C and weighed. Total grain yield was manually collected in the 6 × 3 m quadrats in which aboveground weed biomass was cut at harvest. To obtain information on phenotypic plasticity, we measured the angle of insertion of oldest living (i.e. green and not withered) leaf at harvest (leaf six for all plants) on 10 randomly selected plants within each quadrat. Leaf insertion angles were measured using photographs of the leaf bases taken perpendicularly to the plane formed by the leaf base and the stem. The photographs were analysed with the software ImageJ 1.45 (U.S. National Institutes of Health, http://rsbweb.nih.gov). Standard deviation of leaf insertion angle among replicates was analysed with ANOVA to investigate phenotypic variation among varieties and treatments. Data for both years were analysed with mixed linear models in SPSS 20.0, using the MIXED Test of Fixed Effects (SPSS, 2005), based on likelihood principles, in which blocks and years are treated as random effects.

Table 1 Distances of sowing in different densities and spatial patterns

Density (seeds m^{-2})	Spatial pattern	Inter-row distance (m)	Intrarow distance (m)
5	Rows	1.000	0.200
5	Grid	0.447	0.447
7	Rows	0.850	0.168
7	Grid	0.378	0.378
10.5	Rows	0.700	0.136
10.5	Grid	0.309	0.309

Results

Sixty-eight per cent of the total weed biomass harvested was *B. brizantha* (the sown weed), 11% *Brachiaria* sp., 9% *Pteridiumaquilinum* L. Kuhn (bracken fern), 7% *Dichondramicrantha* Urban. (kidney weed) and 5% other species.

There were strong and highly significant effects of all three independent variables: density, pattern and variety on weed biomass, both 1 month after sowing and at harvest in both years (Tables 2 and 3). Most interactions among these variables were also

Table 2 Mixed linear model for dry weed biomass in g m^{-2} 1 month after sowing in both years, using a Type III Test of Fixed Effects (SPSS, 2005)

	2012				2013			
Source	Num d.f.	Den d.f.	*F*	*P* > *F*	Num d.f.	Den d.f.	*F*	*P* > *F*
Density	2	18	45.917	<0.001	2	18	34.569	<0.001
Variety	2	18	12.733	<0.001	2	18	7.206	0.005
Pattern	1	18	280.725	<0.001	1	18	265.188	<0.001
Density*variety	4	18	14.756	<0.001	4	18	12.516	<0.001
Density*pattern	2	18	6.017	0.010	2	18	3.274	0.061
Variety*pattern	2	18	17.459	<0.001	2	18	19.020	<0.001
Density*variety*pattern	4	18	5.311	0.005	4	18	5.533	0.004

Table 3 Mixed linear model for dry weed biomass in g m^{-2}at harvest in both years, using a Type III Test of Fixed Effects (SPSS, 2005)

	2012				2013			
Source	Num d.f.	Den d.f.	*F*	*P* > *F*	Num d.f.	Den d.f.	*F*	*P* > *F*
Density	2	18	203.810	<0.001	2	18	126.552	<0.001
Variety	2	18	26.041	<0.001	2	18	13.501	<0.001
Pattern	1	18	436.936	<0.001	1	18	343.370	<0.001
Density*variety	4	18	5.319	0.005	4	18	2.984	0.047
Density*pattern	2	18	0.140	0.870	2	18	0.335	0.720
Variety*pattern	2	18	11.105	0.001	2	18	5.236	0.016
Density*variety*pattern	4	18	3.088	0.042	4	18	2.030	0.133

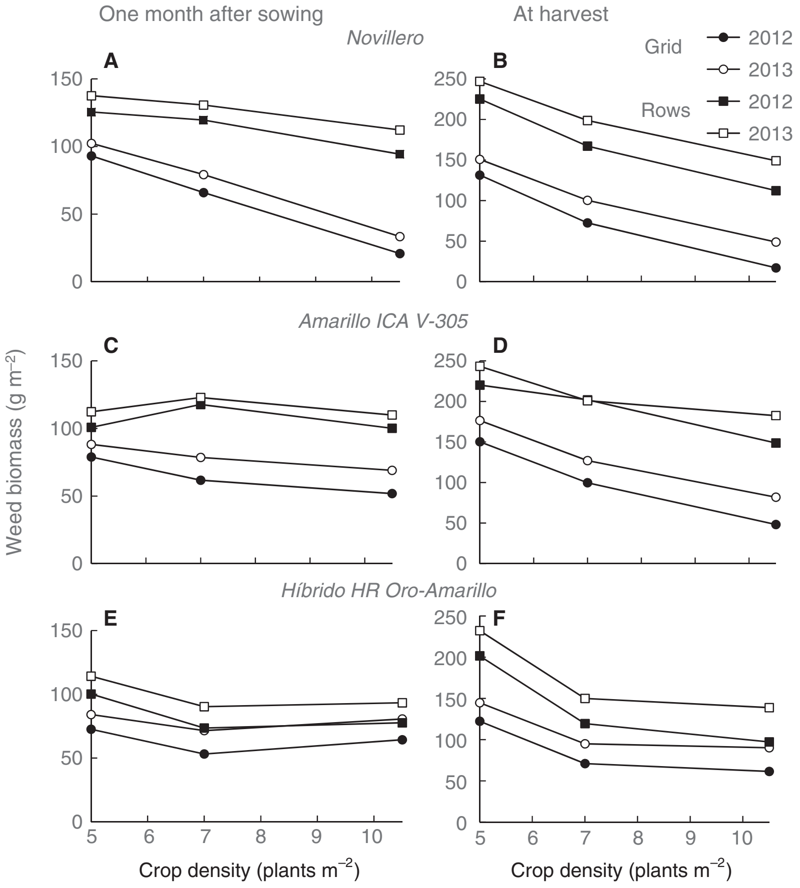

Fig. 1 Dry weight of weed biomass for three varieties of maize (A and B, *Novillero*; C and D, *Amarillo ICA V-305*; E and F, *Híbrido HR Oro-Amarillo*) sown at three densities (5, 7 and 10.5 seeds m^{-2}), at 1 month after sowing (A, C and E) and at harvest (B, D and F). Experiment was performed in two spatial patterns (circles: grid pattern; squares: rows), in 2012 (filled symbols) and 2013 (empty symbols).

significant, but the interaction effects were not as strong as the main effects. Comparing the grid pattern at the highest density to normal practice (row pattern, medium density), weed biomass at harvest was reduced by 89.8%, 76.1% and 48.6%, in *Novillero*, *Amarillo ICA V-305* and *Híbrido HR Oro-Amarillo* in 2012 and 75.4%, 59.2% and 39.9% in 2013 respectively. For one variety, *Híbrido HR Oro-Amarillo*, weed biomass increased slightly from medium to high density 1 month after sowing and decreased only slightly from medium to high density at harvest (Fig. 1).

The strength of density effects on grain yield also differed among the three varieties (Table 4). In both years, *Híbrido HR Oro-Amarillo* showed a decrease in grain yield from the medium to the high density, whereas grain yield always increased with density and spatial uniformity in all other cases (Fig. 2). Comparing the high density, spatially uniform pattern with our treatment closest to standard practice, the yield of *Novillero*, *Amarillo ICA V-305* and *Híbrido HR Oro-Amarillo* in 2012 was increased by 70.0%, 66.7% and 7.6%, in 2012 and 65.5%, 58.0% and 7.1% in 2013 respectively.

Table 4 Mixed linear model for the effects of density, variety and sowing pattern on grain yield (g m^{-2}) at harvest of maize grown under very high weed pressure in 2012 and 2013, using a Type III Test of Fixed Effects (SPSS, 2005)

Source	2012 Num d.f.	2012 Den d.f.	2012 *F*	2012 *P* > *F*	2013 Num d.f.	2013 Den d.f.	2013 *F*	2013 *P* > *F*
Density	2	18	56.334	<0.001	2	18	52.064	<0.001
Variety	2	18	16.397	<0.001	2	18	16.289	<0.001
Pattern	1	18	30.915	<0.001	1	18	25.173	<0.001
Density*variety	4	18	9.867	<0.001	4	18	9.318	<0.001
Density*pattern	2	18	0.701	0.509	2	18	0.597	0.561
Variety*pattern	2	18	0.684	0.517	2	18	0.685	0.517
Density*variety*pattern	4	18	0.131	0.969	4	18	0.168	0.952

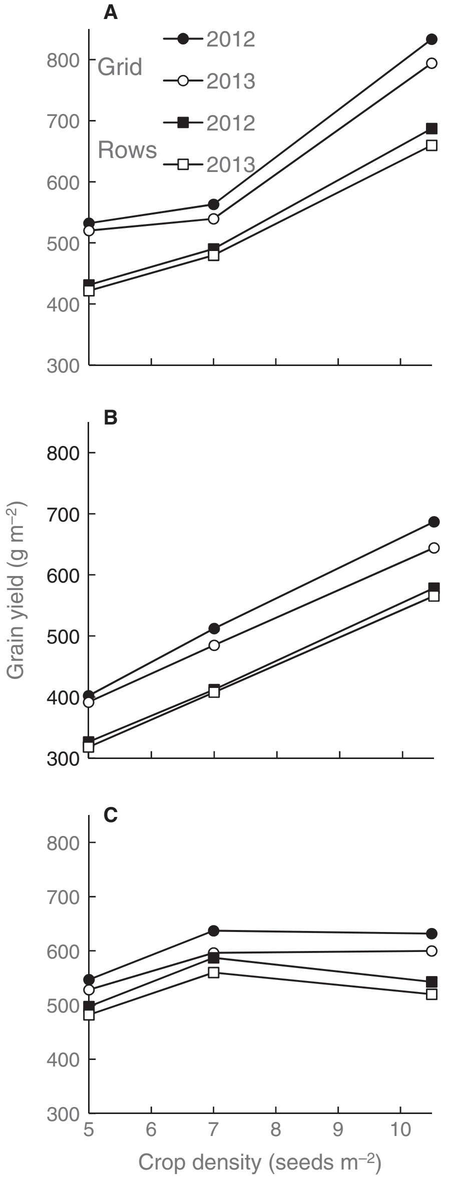

Fig. 2 Dry weight of grain yield for three varieties of maize (A, *Novillero*; B, *Amarillo ICA V-305*; C, *Híbrido HR Oro-Amarillo*) sown at three densities (5, 7 and 10.5 seeds m^{-2}). Experiment was performed in two spatial patterns (circles, grid pattern; squares, rows), in 2012 (filled symbols) and 2013 (empty symbols).

Under high weed pressure, as here, weed biomass was inversely related to grain yield, and this relationship was stronger in the grid pattern than the row pattern (Fig. 3).

Variation in leaf angle was lower in the grid than in the row pattern and decreased with density, except for *Híbrido HR Oro-Amarillo*, where at high density in the grid pattern, there was an increased variation in leaf angle in both years (Fig. 4). In an ANOVA, standard deviation of leaf angle was significantly affected by variety (d.f. = 2, $F = 14.6$, $P < 0.0001$), density (d.f. = 2, $F = 6.8$, $P = 0.006$) and sowing pattern (d.f. = 1, $F = 240.4$, $P < 0.0001$). *Novillero* showed the lowest average variation (average SD = 5.03), *Amarillo ICA V-305* was intermediate (6.36) and *Híbrido HR Oro-Amarillo* showed much higher variation (10.22).

Discussion

All the treatment variables had strong and significant effects on grain yield as well as weed biomass, but the only significant interaction for yield was density × variety (Table 4). *Híbrido HR Oro-Amarillo* benefitted much less from increased density and spatial uniformity than did the other two varieties. Our results agree with those of Acciaresi and Chidichimo (2007), who found that uniformity at a single density promoted weed suppression and increases grain yield in maize. They also found early weed suppression and more collective shading in a grid pattern.

The results were similar in both years, but there were some differences. Weed suppression and grain yield were lower in 2013 than 2012. One possible explanation for this is that 2013 was a drier year than 2012 (rainfall averaged175 mm per month in 2012 and 140 mm per month in 2013). Maize, like many crops, is highly dependent on water availability, and lower water availability often reduces the crop's competitive advantage over weeds (Olsen *et al.*, 2012). Another difference between the 2 years was that in 2013 variation in leaf insertion angles were slightly less pronounced

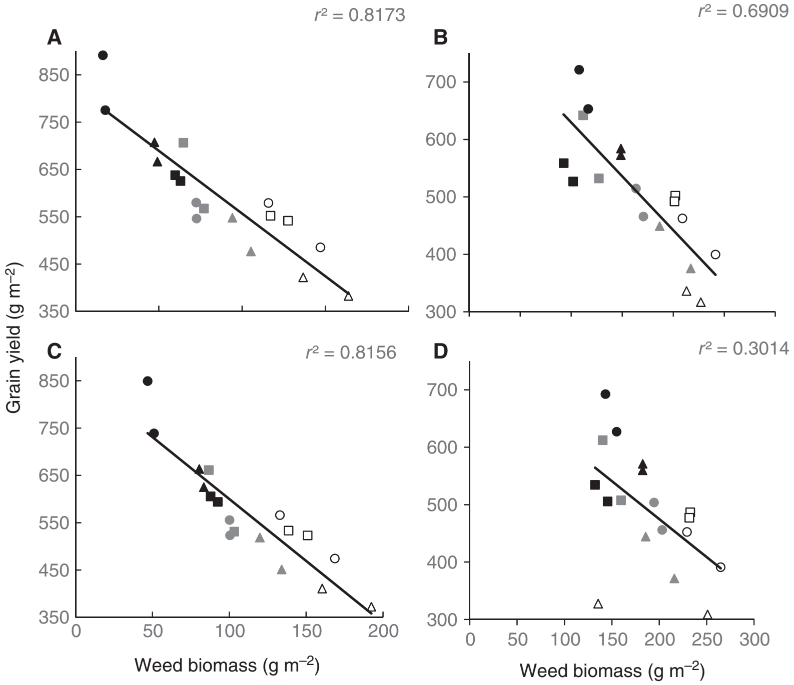

Fig. 3 Relationship between weed biomass at harvest and grain yield in a grid pattern, in 2012 (A) and 2013 (C), and in a row pattern in 2012 (B) and 2013 (D). The densities are 5 (white), 7 (grey) and 10.5 plants m^{-2} (black), and the varieties *Novillero* (circles), *Amarillo ICA V-305* (triangles) and *Híbrido HR Oro-Amarillo*, (squares).

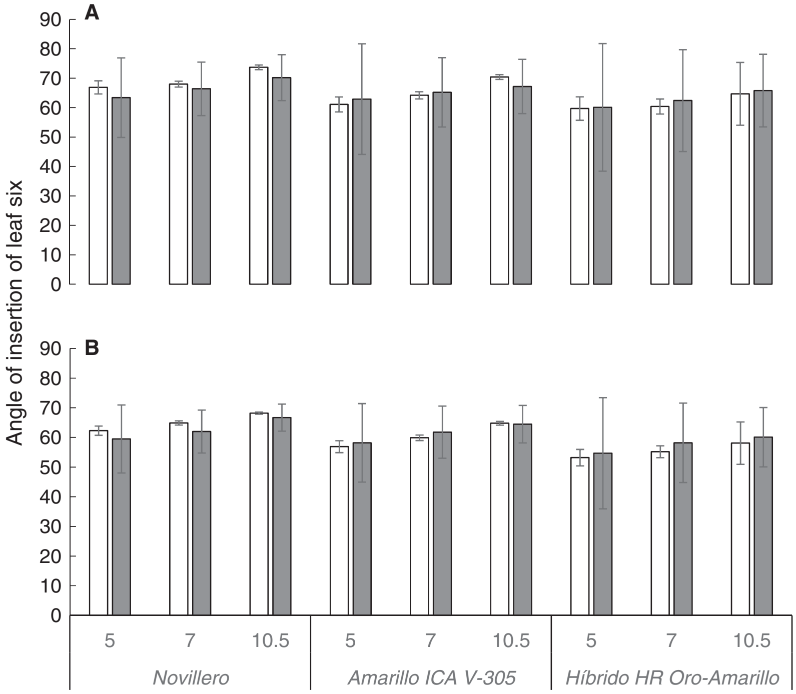

Fig. 4 Angle of insertion of the oldest living leaf (leaf 6) at harvest on 10 randomly selected plants in each plot for three varieties of maize (*Novillero*; *Amarillo ICA V-305*; *Híbrido HR Oro-Amarillo*) sown at three densities (5, 7 and 10.5 seeds m^{-2}) in 2012 (A) and 2013 (B). Error bars are standard deviations. White columns represent the grid pattern, and grey columns the row pattern.

than in 2012 (Fig. 4). These could also be due to the lower rainfall, which can reduce canopy cover.

Density and 'competitive ability'

The highly significant interaction between density and variety for both weed biomass and grain yield in both years means that the different varieties reacted differently to increased density, as observed in experiments on wheat (Weiner *et al.*, 2001, 2010). Weed scientists have tended to talk about a crop's competitive ability against weeds as a general, genetically determined attribute, but our results emphasise that competitive ability against weeds varies with crop density; the best competitor at low density was not the best at high density. Thus, the attributes that give the best competitive performance against weeds at low density do not necessarily do so at high density. Attributes determining competitive ability also depend on spatial arrangement, as variety × pattern and density × pattern interactions were also highly significant for weed suppression (Table 2). Researchers need to rethink the concept of crop competitive ability. Recent results, including those presented here, are evidence against the idea of general 'competitive ability' of a variety against weeds, independent of density. As weeds can only be effectively suppressed at high crop density, we need to investigate the characteristics increasing weed suppression at high density if we want to better utilise the potential for weed suppression by crops.

What attributes improve weed suppression at high density? One hypothesis is that reduced phenotypic plasticity can increase weed suppression under high density, spatially uniform conditions (Weiner *et al.*, 2010), because reduced plasticity increases the collective competitive effect of the crop population on the weeds, whereas plasticity is a defensive strategy by individuals to reduce the effect of competition on them. Variation in the angle of insertion was much greater in the row than the grid pattern, and this might contribute to increased weed suppression in the grid pattern (Fig. 4). The one exception to this was in the variety *Híbrido HR Oro-Amarillo*, which had by far the highest variation in leaf angle and the smallest decrease in this variation in the grid pattern at high density. Consistent with our hypothesis, this was the only case in which grain yield decreased at high density (Fig. 2) and weed biomass increased from medium to high density 1 month after sowing, decreasing only slightly at harvest (Fig. 1). Although this variety grew and developed faster, the high variation of the canopy structure may have reduced weed suppression, as hypothesised. At high density, the variety that suppressed weeds best and had the highest yield (*Novillero*) had lowest variation in the angle of insertion. These results were similar in both years, strengthening the evidence that reduced phenotypic plasticity can increase weed suppression and yield under high weed pressure.

Perspectives for weed management

Our results support the hypothesis that increased density and spatial uniformity can make a valuable and environmentally friendly contribution to weed management in maize, reducing the need for chemical or mechanical weed control. In combination with an appropriate crop rotation, it might even be possible to eliminate chemical and mechanical weed control under some conditions. Of course, further research is needed before we have a sufficient understanding of the environmental conditions under which such a strategy can be effective. Our results with maize in Colombia and previous results with wheat in Denmark show there is potential under a wide range of mesic conditions. Dry conditions seem to reduce the potential for weed suppression by cereal crops (Olsen *et al.*, 2012), and there may be additional yield losses under increased competition for water at higher densities.

This potential for the suppression of weeds by cereal crops can be greatly improved if we can identify the characteristics promoting weed suppression under high density – high uniformity conditions. Our results suggest the possibility of developing 'high density, weed suppressing' varieties of maize and other crops, because attributes that will prove advantageous under such conditions are not those natural selection or plant breeding to date have promoted. Weed suppression is a group activity, which can potentially be improved by breeding 'co-operative' varieties (Donald, 1968), whereas natural selection (and, until recently, plant breeding) increases individual performance, which can be at the expense of population performance (Weiner *et al.*, 2010).

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