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Herbicide tolerance and seed survival of grain amaranth (Amaranthus sp.)

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Abstract

Amaranth is receiving increasing attention as an alternative crop to small grain cereals. From a weed control point of view cultivation of amaranth poses two problems. Firstly, amaranth grows slowly after emergence and hence is very susceptible to competition by weeds and secondly, seed losses at harvest are significant due to an uneven maturing and volunteer amaranth plants could potentially become a weed problem in following crops. Nonetheless, no studies are available on the tolerance of amaranth to herbicides or the survival of seeds in the soil. In this study we examined 1) the tolerance of amaranth to a range of herbicides in a series of outdoor pot experiments and in one field experiment and 2) the survival of amaranth seeds buried at 4 depths (2.5, 5, 10 and 25 cm) in 3 countries. The results showed that amaranth is very susceptible to broadleaved weed herbicides. Of the broadleaved herbicides examined only clomazone, clopyralid, phenmedipham and triflusulfuron were tolerated by amaranth. Applying clomazone early post-emergent instead of pre-emergent provided full crop tolerance even at the highest doses ($P<0.05$). The post-emergence herbicides phenmedipham and triflusulfuron caused less crop damage applied at the 4-6 leaf stage compared to the 2-4 leaf stage while clopyralid was selective at both growth stages. The seed survival studies revealed differences between the countries with higher viability in Spain (up to 18%) than in Argentina and Denmark (up to 6%). Our results showed that chemical weed control in amaranth is possible and despite a significant loss of seeds at harvest problems with volunteer amaranth plants in the following crop should not be a major issue due to a high susceptibility of amaranth to herbicides and the short longevity of amaranth seeds.

Keywords: chemical weed control, clomazone, clopyralid, phenmedipham, triflusulfuron, seed longevity, seed survival.

Introduction

Grain amaranth is an annual pseudocereal crop originating from Central and South America where it was the main grain crops of the Aztecs (Kaufman and Weber, 1990). Amaranth has been characterized as a plastic plant that can adapt to wide range of environmental conditions and in recent years there has been an increasing interest in cultivating grain amaranth also in temperate regions of the world (Kaufmann, 1992; Bavec and Mlakar, 2002). Several species of cultivated amaranth exist but the most studied species are *Amaranthus cruentus*, *Amaranthus hypochodriacus* and *Amaranthus caudatus*. The three species are related and can hybridize but the F_1 plants have low pollen fertility and set fewer seeds than their parents (Gupta and Gudu, 1991). Amaranth seeds are high in protein and the amino acid lysine and the starch and oil contained in the seeds have properties suitable for industrial purposes (Kaufman and Weber, 1990). Another interesting feature of amaranth grain is that they contain no gluten hence amaranth is an alternative to wheat to people with celiac disease. It is estimated that ca. 1 out of 100 persons is affected by celiac disease and this section of the population represents a potentially large market for amaranth production. Focus of the research on grain amaranth cultivation in recent years have been on evaluation of cultivars (Gelinas and Seguin, 2008a), germination (Bavec and Mlakar, 2002), seeding date (Henderson et al., 1998; Gelinas and Seguin, 2008b), seeding rate and row distance (Henderson et al., 2000; Gimplinger et al., 2007; 2008; Gelinas and Seguin, 2008b) and fertilization (Pospisil et al., 2006; Gelina and Seguin, 2008b). Amaranth grows slowly in the first weeks after germination and hence is very susceptible to competition by weeds. Although it is evident that effective weed control is important to ensure maximum yields only one study has been published on weed control in grain amaranth. Ojo (1997) studied the effect of weeding frequency in transplanted grain amaranth under tropical conditions and found that three weedings at 2, 5 and 8 weeks after transplanting was required to ensure maximum yield. No herbicides are registered for use in grain amaranth in Europe, USA or South America and weed control is presently only possible by interrow cultivation requiring that amaranth is sown at a row distance that allows the use of interrow cultivators. Another aspect of amaranth cultivation where no information is available is on the survival of amaranth seeds in the soil. Amaranth is a small seeded crop that matures non-uniformly and seed losses at harvests are unavoidable and can be significant (Kaufman and Weber, 1990) and volunteer amaranth plants could be a potential weed problem in succeeding crops. No information is available on the survival of seeds of cultivated amaranth in soil. Omami et al. (1999) reported that the loss of viability seeds of *A. retroflexus*, a weedy amaranth species, buried at various soil depths decreased with depth but at 5 and 10 cm depth a significant part of the seeds survived more than a year. The present study was initiated to provide information on the susceptibility of grain amaranth to a wide range of herbicides and is the first

Table 1. Details of the outdoor pot experiments and field experiment reported in the paper.

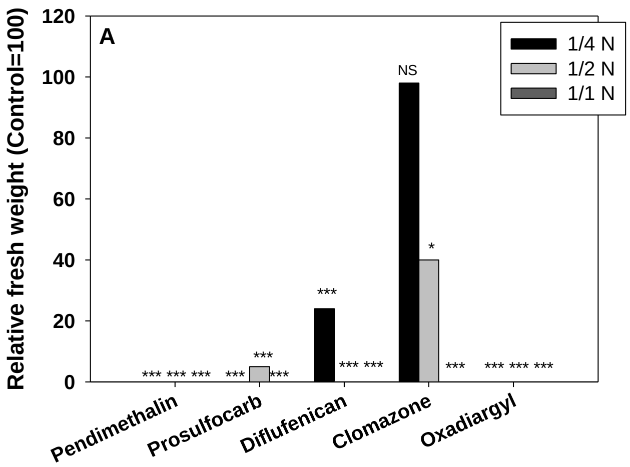
Exp.	Type	Amaranth species	Growth stages	Sowing depth (cm)	Herbicides	Maximum dose (g/ha)	Volume rate (L/ha)
1	Outdoor pot experiment	*A. cruentus* cv. Don Armando	Pre-emergence 0-2 leaf stage	0.5	Pendimethalin Prosulfocarb Diflufenican Clomazone Oxadiargyl	2400 3200 125 360 400	154.4
2	Outdoor pot experiment	*A. cruentus* cv. Don Armando	Pre-emergence 2 leaf stage	0.5 1.5	Clomazone	480	146.1
3	Outdoor pot experiment	*A. cruentus* cv. Don Armando	4-6 leaf stage	0.5	Bentazone Phenmedipham Fluroxypyr Clopyralid Fenoxprop-P Fluazifop-P Iodosulfuron Bifenox	720 240 144 80 55.2 125 2.5 480	154.4
4	Outdoor pot experiment	*A. cruentus* cv. Don Armando & Candil *A. caudatus* cv. CAC 48A *A. mantegazzianus* cv. Don Juan	2-4 leaf stage 4-6 leaf stage	0.5	Bentazone Phenmedipham Triflusulfuron Clopyralid	1440 320 10 200	146.1
5	Field experiment	*A. cruentus* cv. Candil	Pre-emergence 6-8 leaf stage	0.5	Clomazone Prosulfocarb Bentazone Phenmedipham Clopyralid Triflusulfuron	240 1600 1440 320 200 10	200

study of its kind. Furthermore, we studied the survival of cultivated amaranth seeds in Argentina, Denmark and Spain.

Results

Herbicide tolerance

Pendimethalin, prosulfocarb, diflufenican and oxadiargyl applied pre-emergence killed grain amaranth, irrespectively of the dose applied (Fig 1A). Clomazone showed selectivity but only at the lowest dose (90 g /ha). Applied post-emergence at the 0-2 leaf stage all five herbicides were significantly more selective but only clomazone did not reduce amaranth fresh weight significantly even at the highest dose of 360 g /ha (Fig 1B). Post-emergence application of bleaching herbicides like clomazone and diflufenican caused chlorosis on the leaves formed at the time of application but new leaves showed no symptoms and growth of the new leaves was only slightly affected by the herbicides. A subsequent experiment examined the influence of sowing depth on the tolerance of amaranth to pre-emergence application of clomazone at doses up to 480 g/ha. Increasing sowing depth enhanced crop tolerance but the improvement was insignificant compared to the effect of delaying application of clomazone until the 0-2 leaf stage of the crop (Fig 2) confirming the results of the previous experiment with clomazone. As expected, amaranth tolerated applications of the post-emergence graminicides fluazifop-p-butyl and fenoxaprop-p-ethyl although biomass was reduced at the highest fenoxaprop-p-ethyl dose (Fig 3). Fluroxypyr, iodosulfuron and bifenox significantly reduced fresh weight of amaranth irrespectively of the dose whereas clopyralid was tolerated well by the crop at all doses. Bentazone and phenmedipham were intermediate showing some degree of selectivity. Iodosulfuron is a sulfonylurea herbicide and experiments with other sulfonylurea herbicides revealed a very low tolerance to this group of herbicides except for triflusulfuron, a sulfonylurea herbicide developed for use in sugar beet (data not shown). Triflusulfuron was therefore included in the subsequent experiments. The influence of growth stage on tolerance of amaranth to post-emergence herbicides was examined in a trial with two cultivars of *A. cruentus* (cv. Don Armando and cv. Candil) and one cultivar of each of the species *A. caudatus* (cv. CAC48A) and *A. mantegazzianus* (cv. Don Juan). While the response to bentazone was not significantly affected by growth stage tolerance to phenmedipham and particularly triflusulfuron increased with growth stage (Fig 4A-D). In contrast clopyralid had no effect on biomass at any of the growth stages. Significant differences were observed between amaranth species and cultivars but differences were not consistent and no conclusions as to the susceptibility of the accessions could be drawn. The field experiment confirmed that clomazone was tolerated better by amaranth than prosulfocarb, however, in contrast to the outdoor pot experiments no significant differences were observed between the pre- and post-emergence applications of clomazone (Figs 5). Similar to the outdoor pot experiments bentazone significantly reduced fresh weight accumulation whereas only minor and non-significant fresh weight reductions were observed following application of phenmedipham, clopyralid and triflusulfuron. In some instances fresh weight was even significantly increased following application of clopyralid and triflusulfuron.



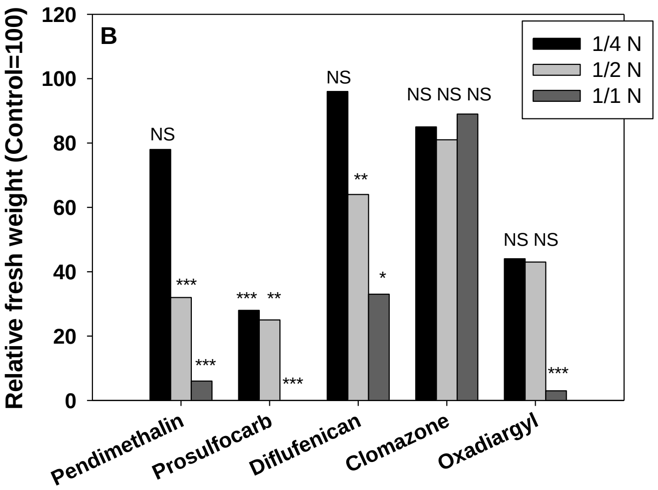


Fig 1. Relative fresh weight of *A. cruentus* (cv. Don Armando) following application 5 residual herbicides applied (A) pre-emergence and (B) post-emergence (0-2 leaf stage) at 25% (1/4 N), 50% (1/2N) and 100% (1/1 N) of the maximum dose (Experiment 1 in Table 1). NS: not significantly different from the control, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

Seed survival

In Argentina and Denmark very few seeds survived one year burial irrespectively of the depth of burial (Figs 6A-D). Seed survival in Spain was higher than at the two other locations and this difference was confirmed in the second experiment where up to 40% of the seeds survived burial at 25 cm for 5 months.

Discussion

Of the wide variety of herbicides included in the present studies the majority caused unacceptable high levels of damage to amaranth. Of the pre-emergence treatments tested only clomazone was tolerated by amaranth (Figs 1A-B and 2). Clomazone is a pro-herbicide that has to be converted to 5-keto clomazone in the plant to be active. 5-keto clomazone inhibits the isoprenoid biosynthesis pathway leading to blockage of the carotenoid biosynthesis in susceptible plants (Ferhatoglu and Barrett, 2006). Clomazone is generally recommended applied shortly after sowing to avoid damage to the crop but experiences in other crops such as cabbage have shown that application of clomazone early post-emergence may increase crop tolerance (Norsworthy and Smith, 2007). Although the field experiment revealed no differences between the pre- and post-emergence applications of clomazone the outdoor pot experiments, comprising higher doses than the field experiment, clearly indicated that selectivity is improved by post-emergence applications. Another parameter determining crop tolerance is sowing depth. Increasing sowing depth improved tolerance of clomazone applied pre-emergent. Being a small-seeded crop amaranth should be sown in the upper soil layer to ensure high and uniform germination thus improving tolerance to residual herbicides by sowing the seeds deeper into the soil may hamper field germination and require higher seeding rates. Previous research on clomazone effects against weedy amaranth species showed a poor effect on *A. hybridus* whereas *A. lividus* and *A. retroflexus* were more susceptible (Wallinder et al., 1986). Vencill et al. (1990) found that the difference in susceptibility could be related to differential root absorption. In the present experiments two cultivars of *A. cruentus* were included but future registration trials should include other cultivated amaranth species to examine if differences in susceptibility also exist between cultivated amaranth species. Several post-emergence broadleaved weed herbicides caused severe damage to amaranth and only clopyralid was selective irrespective of dose while phenmedipham and bentazone was selective at the lower doses (Fig 3). This order of selectivity was confirmed in an experiment with 4 species/cultivars of amaranth and two application times (Figs 4A-D). Triflusulfuron was included in the latter experiment and revealed a higher level of selectivity than of phenmedipham and bentazone. In the field trial the selectivity of triflusulfuron was comparable to that of clopyralid while phenmedipham was intermediate between clopyralid and bentazone, the latter resulting in an unacceptable level of crop damage (Fig 5). The excellent selectivity of clopyralid was expected as it is primarily active on plant species belonging to the Asteracea and Fabacea families (Thompson and Cobb, 1987). Several studies have shown that phenmedipham applied at doses up to 1700 g/ha, in contrast to the chemically very closely related herbicide desmedipham, do not provide effective control of weedy amaranth species. (e.g. Schweizer and Weatherspoon, 1971; Schweizer, 1974). Hendrick et al. (1974) found that extensive metabolisation of phenmedipham within the first 5 h after herbicide application explained the differential response to the two herbicides. Our results suggest that cultivated amaranth can also metabolise phenmedipham. Triflusulfuron is a relatively new herbicide developed for sugar and fodder beet (Peeples et al., 1991) but also used in chicory (Wilson et al., 2004). Triflusulfuron is claimed to be effective against *A. blitoides* and *A. retroflexus* (Peeples et al., 1991) and a good effect against *A. retroflexus* has been demonstrated in several studies (Wilson, 1994; Wilson et al., 2004). Selectivity of triflusulfuron in sugar beet was attributed to very rapid metabolism of and differences in the rate of metabolism also explained the differences in susceptibility between weed species (Wittenbach et al., 1994). In view of the susceptibility of *A. retroflexus* and *A. blitoides* to triflusulfuron it was unexpected that triflusulfuron provided good selectivity in all 4 species/cultivars of amaranth. Understanding the underlying mechanisms explaining the tolerance of cultivated

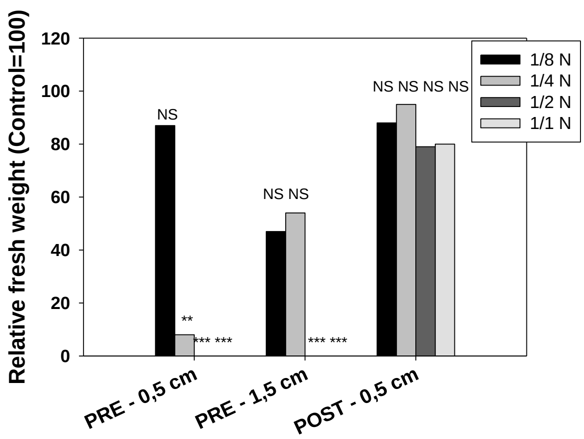


Fig 2. Relative fresh weight of *A. cruentus* (cv. Don Armando) following application of clomazone applied pre-emergence and post-emergence (2-leaf stage) to plants sown at 0.5 and 1.5 cm depth. Clomazone was applied at 12.5% (1/8 N), 25% (1/4 N), 50% (1/2 N) and 100% (1/1 N) of the maximum dose (Experiment 2 in Table 1). NS: not significantly different from the control, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

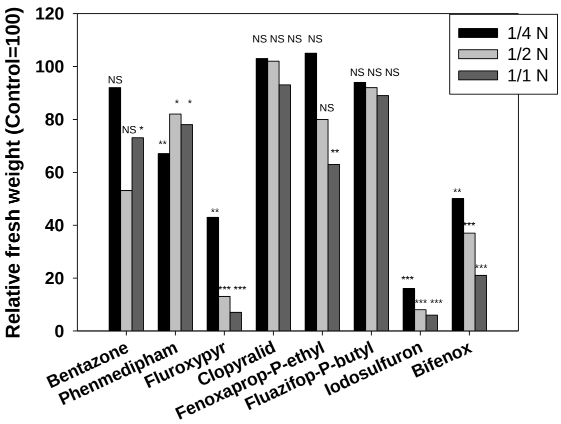


Fig 3. Relative fresh weight of *A. cruentus* (cv. Don Armando) following application of 8 post-emergence herbicides at the 4-6 leaf stage at 25% (1/4 N), 50% (1/2 N) and 100% (1/1 N) of the maximum dose (Experiment 3 in Table 1). NS: not significantly different from the control, *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$.

amaranth to triflusulfuron should be the topic of future studies. Like other broadleaved crops amaranth was tolerant to selective graminicides belonging to the group of aryloxyphenoxypropionate herbicides although some damage was observed with fenoxaprop-p-ethyl (Fig 3). No cyclohexandione herbicides were included in the present study but compounds belonging to this herbicide group can also be assumed to be selective, i.e. several herbicide options are available for the control of annual and perennial grass weeds in grain amaranth. Overall a very good correlation was found between the results of the outdoor pot experiments and field experiment. For post-emergence applications responses in the outdoor pot experiments and the field experiment were very similar. Damages by clomazone applied pre-emergence were more pronounced in the outdoor pot experiments than in the field experiment. This may be due to the standard practice of watering the pots on the top shortly after application of residual herbicides to simulate worst case conditions with rain occurring immediately after herbicide application. Our results suggest that the outdoor pot experiments provide a good proxy to the more expensive and time-consuming field experiments for evaluating crop tolerance to herbicides.

Seed survival

The seed burial experiments revealed that survival of amaranth seeds after 12 months (in Denmark 10 months) in the soil was generally very low compared to the germinability of the fresh seeds which was >80% for all accessions. Maximum survival rates were observed in Spain where 19% of seeds of *A. cruentus* survived burial at 10 cm depth. Survival of the two other amaranth species was lower, irrespectively of depth of burial. In the Argentinean trial the maximum survival rate was 8% and in Denmark it was 5%. In Argentina and Denmark *A. hypochondriacus* tended to survive better than the other two species but differences were not significant. Average soil temperatures during the burial period were somewhat higher in Spain (13.8^{o}C) and Argentina (14.9^{o}C) than in Denmark (9.6^{o}C) but the most significant difference between the three regions was the average monthly rainfall being notably higher in Argentina (36.3 mm) and Denmark (40.3 mm) than in Spain (10.1 mm). A plausible explanation of the lower survival rate in Argentina and Denmark is that the seeds either germinated or were destroyed by fungi under the more humid soil conditions in these two locations. In the second experiment in Spain the seeds were exhumed after 5 months. Survival was lower than in the experiment of the previous year which ran for one year suggesting that the majority of seeds are destroyed or germinate within the first 5 to 6 months. Omami et al. (1999) placed seeds of *A. retroflexus* at the soil surface and at 2.5, 5 and 10 cm depth and exhumed the seeds after 1, 3, 6, 9 and 12 months. They found cyclic changes in germinability but the changes were identical at all depths suggesting that external climatic factors caused seasonal dormancy. Seed viability declined exponentially, most rapidly for seeds at the soil surface, whereas the germinability of seeds kept at 10 cm depths only declined by 10%. Buhler and Hartzler (2001) recovered 12% of the *A. rudis* seeds buried at 0-5 cm depth 4 years after burial, while Steckel et al. (2007) only found 0.004%, averaged over tillage treatments, of the original seed bank of *A. rudis* 4 years after having allowed a dense population of *A. rudis* to set and shed seeds. Jha et al. (2010) also found that burial for 3 to 6 months induced dormancy in seeds of *A. palmeri*.

Materials and methods

Outdoor pot experiments

Selectivity of herbicides in grain amaranth was studied in outdoor pot experiments conducted from 2007 to 2009 and in a field experiment carried out in 2009. For outdoor pot experiments seeds of cultivated amaranth (*A. cruentus* cv. Don Armando and cv. Candil, *A. hypochodriacus* cv. San Antonio, *A. caudatus* cv. CAC 48 A and *A. mantegazzianus* cv. Don Juan) were grown either in 1 L pots in soil collected in the experimental field of the Dept. of Agroecology (sandy-loam soil) for experiments with pre-emergence herbicide applications or in 2 L pots in a potting mix consisting of field soil, sand and peat (2:1:1 w/w) for experiment with post-emergence applications. In one experiment with pre-emergence treatments amaranth seeds were sown at two depths (0.5 and 1.5 cm) to assess the influence of seeding depth on herbicide selectivity. Other

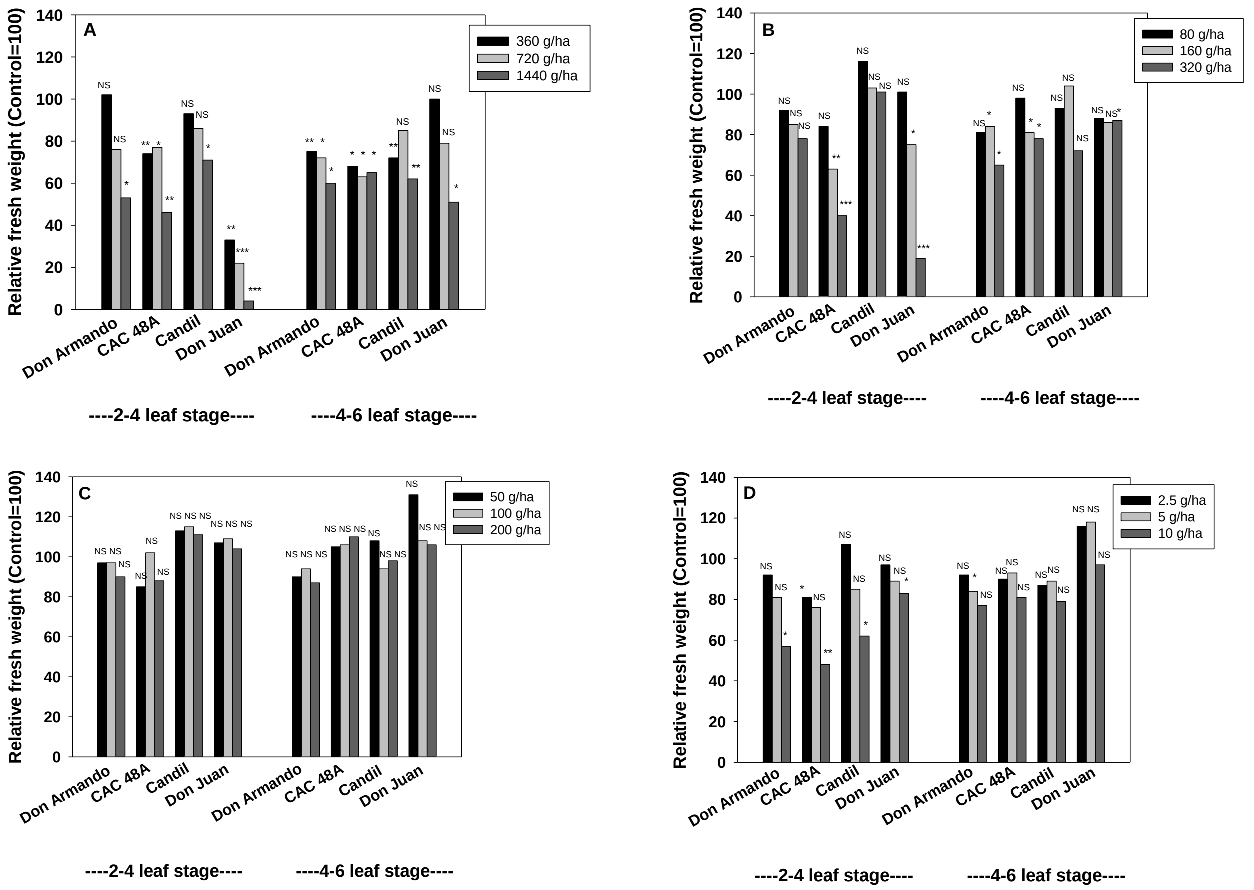


Fig 4. Relative fresh weight reduction of *A. cruentus* (cv. Don Armando and Candil), *A. caudatus* (cv. CAC48A) and *A. mantegazzianus* (cv. Don Juan) following application the post-emergence herbicides (A) bentazone, (B) phenmedipham, (C) clopyralid and (D) triflurusulfuron at two growth stages (Experiment 4 in Table 1). NS: not significantly different from the control, *: P<0.05, **: P<0.01, ***: P<0.001.

experiments examined the influence of growth stage on the tolerance to post-emergence herbicide applications. Pots were placed on outdoor tables and sub-irrigated automatically up to 5 times per day. Prior to application of foliar applied herbicides the number of plants per pot was reduced to a pre-set number. Herbicides were applied in a laboratory pot sprayer fitted with a spray boom with two Hardi ISO LD 110-02 nozzles operated at 3 bars and a speed of 5.6 km/h. Volume rate varied from 150 to 155 L/ha between experiments. Details on herbicides and doses used in the outdoor pot experiments are shown in Table 1. Following application of pre-emergence treatments the pots were irrigated on the soil surface with 50 mL deionised water equivalent to 20 mm rain to simulate a worst case scenario. Plants were harvested 3-4 weeks after treatment depending on the time of year the experiment was conducted. At harvest fresh and dry weights were recorded and results are expressed as percent fresh weight compared to the corresponding control plants. Relative fresh weight reduction was taken as measure of herbicide tolerance. All pot experiments were complete randomized designs with three replicates per treatment.

Field experiment

Seeds of *A. cruentus* cv. Candil were sown on 29 June 2009 in a field at the Dept. of Agroecology in Flakkebjerg. Soil type was sandy-loam containing 15% clay, 15% silt, 67.5% sand and 2.5% organic matter. Pre-emergence herbicides were applied on 30 June 2009 while post-emergence herbicides were applied on 15 and 22 July 2009 (Table 1). The herbicides were applied with a self-propelled experimental plot sprayer equipped with Hardi ISO LD 4110-02 nozzles delivering a volume rate of 200 L/ha. Fresh weight and number of surviving plants were recorded on 20 August. Fresh weight results are expressed as percent fresh weight compared to the untreated control. The experiment was not taken to yield. A randomized complete block design with four replications was used.

Seed burial experiments

Seeds of *A. cruentus* cv. Don Guiem, *A. hypochondriacus* cv. Nutrisol and *A. mantegazzianus* (cv. Don Juan) were collected immediately after harvest. Samples consisting of 100 or 400 seeds were prepared. The samples consisting of 100 seeds were used to determine the germinability of the harvested seeds. The seeds were germinated on filter paper at 20^0 C and a light regime of 16 h day and 8 h night. The 400 seed samples were each mixed with 0.2 L sterilised field soil and placed in fabric mesh bags and buried at 2.5, 10 and 25 cm depth. Each bag was divided into 3 parts, one for each of the 3 amaranth species. Each treatment had 4 replicates. The bags were placed in a vegetation free area and the experiments were initiated within 1 to 2 weeks after harvesting the seeds. The experiment was conducted in

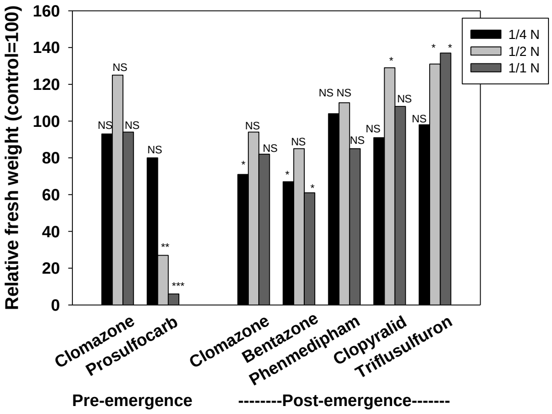


Fig 5. Relative fresh weight reduction of *A. cruentus* (cv. Candil) following application of the pre- and post-emergence herbicides. Post-emergence herbicides were applied at the 6-8 leaf stage (Experiment 5 in Table 1). NS: not significantly different from the control, *: P<0.05, **: P<0.01, ***: P<0.001.

Lleida in Spain (41° 37′ 0″ N, 0° 38′ 0″ E), in Santa Rosa in Argentina (36° 37′ 0″ S, 64° 17′ 0″ W) and in Flakkebjerg in Denmark (55 19' 00" N, 11 24' 00" E). The soil type at all locations was a sandy loam or a loam soil. The seeds used in Spain and Denmark originated from the same demonstration field in Spain while the seeds used in Argentina were collected in an Argentinean demonstration trial In Spain and Argentina the experiment was initiated in November 2007 and May 2008, respectively. It was the intention also to start the Danish experiment in November 2007 but due to very wet conditions in the autumn of 2007 the experiment was not initiated until January 2008. Due to this delay the bags were exhumed 10 months after burial instead of the intended one year. An additional experiment was conducted in Spain in November 2008 but in that experiment the bags were exhumes after 5 months. Average monthly soil temperatures and precipitation were recorded. Germination tests were done by spreading the soil samples out on a 1 cm thick layer on filter paper. Soil moisture was controlled by sub-irrigating regularly or by placing a wick between the filter paper and a water reservoir. The germination tests were carried out at ca. 20^{0}C at a light regime of 16 h day and 8 h night and continued until germination ceased. Then soil samples were dried out for a week and rewetted with water. The germination test was then repeated using the same method as in the first test. Seed survival was expressed as percent germinated seeds of the 400 seeds that each sample contained.

Statistical analyses

Fresh and dry weight data and seed survival data were analysed by an analysis of variance. In the herbicide tolerance experiments means of all treatments were compared to the corresponding untreated control using a Student t-test. In the seed survival experiments means were compared by a Duncan multiple range test (P<0.05). Due to variance heteroscedacity data of some of the experiments were square root transformed. Non-transformed means are reported but the interpretation is based on the transformed data.

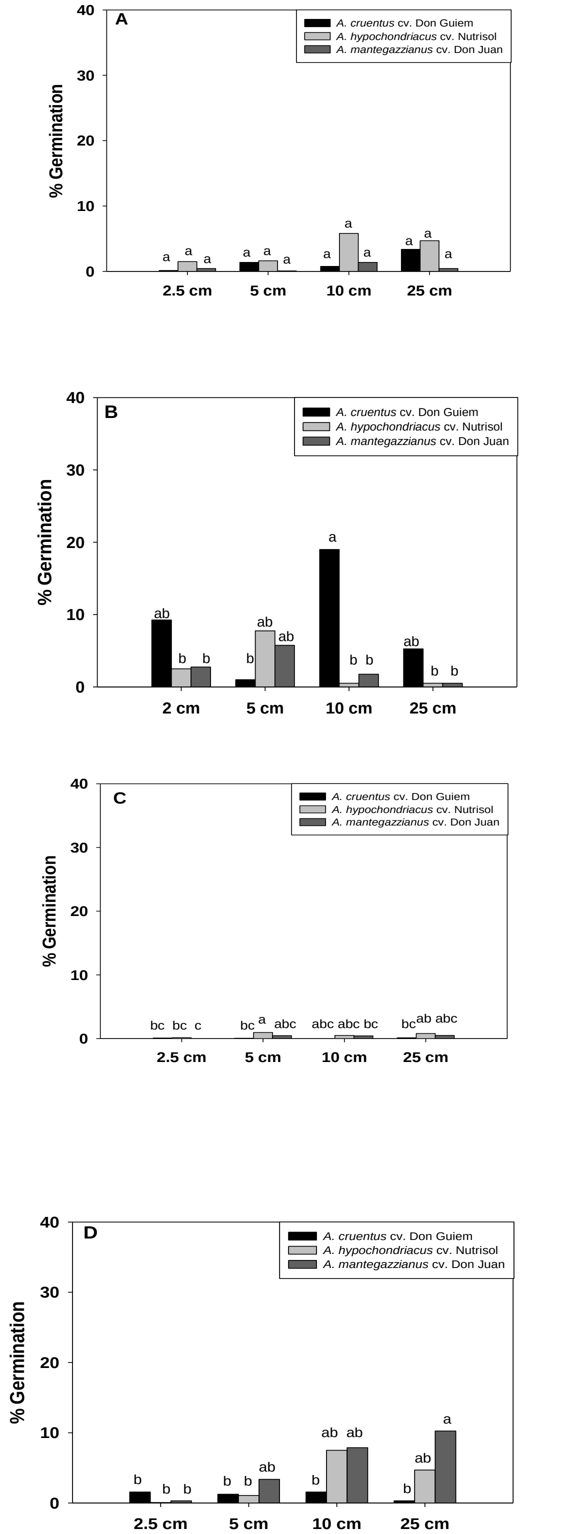


Fig 6. Seed survival of *A. cruentus* (cv. Don Guiem), *A. hypochondriacus* (cv. Nutrisol) and *A. mantegazzianus* (cv. Don Juan) when buried for 12 months in (A) Argentina and (B) Spain, 10 months in (C) Denmark and 6 months in (D) Spain at 2.5, 5, 10 and 25 cm depth. Means followed by different letters are statistical significant (P<0.05).

Conclusions

Two hypotheses exist regarding the origin of *A. caudatus*, *A. cruentus* and *A. hypochondriacus*. One assumes that they originate from three different wild relatives while the other suggests that they originate from one progenitor (Gupta and Gudu, 1991). Such a close relationship to wild relatives of which some are important weeds may explain the generally very similar response of cultivated and wild amaranth species to the tested herbicides. Except for triflusulfuron all the herbicides showing a potential to be registered for use in cultivated amaranth had previously been reported to be ineffective against weedy amaranth species. Future search for selective herbicides in cultivated amaranth should therefore focus on herbicides that do not control weedy amaranth species very well. Survival of seeds of cultivated amaranth was significantly shorter than that reported for weedy amaranth species hence cultivated amaranth will not pose a rotational problem although seed loss can be abundant. It should be stressed that in the present experiment no attempt was made to check whether lack of germination was caused by secondary dormancy or mortality. Being a domesticated species it was assumed that secondary dormancy was low in cultivated amaranth.

Acknowledgement

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