

## Mechanical weed control strategies for grain amaranth (*Amaranthus cruentus* L.)

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**Abstract.** Currently, no herbicide is registered for grain amaranth in Europe, the United States and South America. Hence, weed control must be addressed with alternative methods. Field trials were conducted in 2018 and 2019 in Central Italy by comparing some mechanical weed control treatments in grain amaranth (*Amaranthus cruentus* L.). In 2018, the five treatments were: untreated control (T1<sub>18</sub>), cutter hoeing (T2<sub>18</sub>), flat share cuts and one central duck foot tine (T3<sub>18</sub>), flat share cuts and two central duck foot tines (T4<sub>18</sub>), and three duck foot tines (T5<sub>18</sub>). In 2019, the five treatments were: untreated control (T1<sub>19</sub>), three duck foot tines (T2<sub>19</sub>), flex tine harrowing (T3<sub>19</sub>), flex tine harrowing plus finger weeding with red fingers (T4<sub>19</sub>), and finger weeding with red fingers (T5<sub>20</sub>). In 2018, amaranth was a successful competitor against weeds from 40 days after emergence (10 true leaf stage, corresponding to BBCH code 15). The competitive ability was showed by excellent seed yields averaging 1.2 t ha<sup>-1</sup>, for all treatments. This feature was also confirmed to some degree in 2019. However, seed yield in 2019 was more strongly influenced by treatment as well as by the lower emergence of plants. All the mechanical methods employed can be effectively used for weed control in grain amaranth. Treatments with the flex tine harrower and finger weeder negatively affected the plant density at harvest, necessitating further optimization. However, combined mechanical strategies proved the most effective, especially in controlling dicot weeds. There is a need to optimize strategies, with mechanical equipment, to anticipate and improve the ground cover of amaranth. These strategies include selecting optimal plant density and the correct distancing between the rows for easier mechanical control.

**Key words:** pseudocereal, grain amaranth competition, weed control.

### INTRODUCTION

The rediscovery and the improvement of various pseudocereals (including exotic types) for European environments has led to the valorization of species that have remained neglected for a long time. Buckwheat (*Fagopyrum esculentum* Moench.) and quinoa (*Chenopodium quinoa* Willd.) have contributed to the establishment of new markets in both food and non-food sectors. Another species that has managed to occupy a good market segment is amaranth (*Amaranthus* spp.), native to Mexico and Central America which, together with corn, beans and various pumpkin species, represented one of the main foods of the Maya and/or Aztecs (Sauer, 1950; Turchi, 1987).

The rediscovery of this plant as a precious food resource dates back to the 1970s with studies published by Downton (1973), highlighting the remarkable nutritional properties of the most widespread species: *Amaranthus cruentus* L., *A. hypochondriacus* L., *A. caudatus* L. and *A. edulis* Speg. Regarding the former two species, research has developed to such an extent that important markets both within and outside the areas of origin have been established (Tucker, 1986; Granados & Lòpez, 1990).

The main characteristic of this species is related to the nutritional content of the seeds, that are rich in protein (15–18%), and with averages of 5.2 and 0.37 g<sup>-1</sup> 100 g<sup>-1</sup> dry matter in lysine and calcium, respectively (Saunders & Becker, 1984; Petr et al., 2003). Moreover, amaranth is characterized by the absence of gluten and is therefore, suitable for celiac nutrition (Ballabio et al., 2011). These characteristics provides this species with high market potential, especially where up until now, it has been confined almost exclusively to the health sector (Hackman & Mayers, 2003). In addition to being the basis of a large number of food preparations, amaranth is also used for the formulation of bars, snacks, muesli, puffed seeds, extruded materials and other products such as cooked vegetable (Mulandana et al., 2009).

Equally interesting, is the use of amaranth in the non-food sector, although this aspect has not been well investigated. The cosmetic and pharmacological sectors benefit mostly from the high content of squalene, a triterpene with an average content of 4.2% (ranging from trace levels to 7.3%) in amaranth seed oil, which is the most plentiful plant source of squalene (Han-Ping & Corke, 2003). The oil content of the seeds is on average 6.0%, from which both tocopherol and squalene are used in the cosmetic industry, especially in the skin and hair care sectors and, more generally, in hypoallergenic formulations.

Although the potential of this pseudocereal has been established even beyond the areas of origin (Carlsson, 1980; Gimlinger et al., 2007), amaranth has not received much attention in Italy, despite promising agronomic trials conducted over multiple years. Based on good yields of *Amaranthus cruentus* L. and *A. hypochondriacus* L. (Alba et al., 1997; Lovelli et al., 2005; Rivelli et al., 2008; Ercoli et al., 1987; Massantini et al., 1987; El Gendy et al., 2018), the possibility of introducing this species was highlighted despite the fact that studies reporting agronomic techniques are as yet still limited (Ercoli et al., 1987; Casini & La Rocca, 2014; Pulvento et al., 2015; Casini & Biancofiore, 2020a; Casini & Biancofiore, 2020b; Gresta et al., 2020; Pulvento et al., 2021).

Amaranth is a C4 plant that is characterized by a very slow initial growth for the first 3–5 weeks after emergence, a period during which it is very susceptible to weed competition (Sooby et al., 1998; Bavec & Mlakar, 2002; Kudsk et al., 2012; Brust et al., 2014). However, the critical period may vary considerably according to both the cultivation environment and the variety used (Nurse et al., 2016). The variability in the duration of the reproductive cycle of amaranth, and the potential seed production, can be attributed to various causes. The presence of weeds is considered the most important. The extent to which weed competition reduces amaranth yield varies considerably, and is dependent on both the density and the prevalent species of weed (Chaudhari et al., 2019). An uncontrolled infestation of amaranth also leads to a decrease in the quality of the grain and an increase in production costs.

Amaranth is very susceptible to broadleaf weed herbicides. Previous studies have shown that colazone, clopyralid, phenmdipham and triflusulfuron are tolerated by amaranth (Kudsk et al., 2012). However, problems with the use of herbicides were reported, such as loss of seeds at harvest and the presence of volunteer amaranth plants in the following

cop (Kudsk et al., 2012). Although the application of 50 g ha<sup>-1</sup> oxyfluorfen 40 days after sowing of amaranth has been demonstrated to be useful in the control of weeds (Chaudari et al., 2019), currently no herbicide is registered for this species in Europe, the United States and South America. Hence, weed control must be addressed with integrated management and cultural practices which have been found to be effective in controlling weeds (Ojo, 1997). These methods should include the selection of seeding density and distances between the rows, as well as the use of different types of inter-row cultivators. Peiretti & Gesumaria (1998) observed that single rows with plants spaced at 0.30 and 0.45 m are the most appropriate in terms of the rate of inter-row coverage, which offers advantages for weed control by competition. However, amaranth yield is only slightly affected with an increase in density from 47 to 100 plants m<sup>-2</sup>. Similar results were reported previously (Casini & La Rocca, 2014; Casini & Biancofiore, 2020b). It was shown that the use of double rows permitted taking advantage of a better ground cover than single rows. Moreover, together with the possibility of mechanical intervention for weed control, the double rows also provided a higher yield.

Under many environmental conditions, the use of inter-row cultivation is the only weed control method available to organic farmers (Gélinas & Sequin, 2008; Nurse et al., 2016). However, mechanical methods may only be performed within a limited time period as the development of the crop can hinder the passage of equipment causing damage to the crop. These methods require that amaranth is sown at row distances based on the available equipment in the farm, to permit an easy inter-row cultivation.

After the first weeks of emergence, starting from the 10 true leaf stage, ground cover is almost complete, thereby permitting the crop to compete with weeds for light, water and nutrients (Casini & La Rocca, 2014).

The need for effective weed control is driven, not only by the negative effects on seed yield, but by the presence of wild relatives, such as *Amaranthus retroflexus* L. (redroot pigweed) and the survival of grain amaranth seeds in the soil. Contamination with black seeds of the wild relatives in grain amaranth makes seed cleaning and processing difficult (Ojo, 1997). Although the hybridization between cropped and weedy *Amaranthus* spp., has not yet been ascertained (Brenner et al., 2000, 2013; Nurse et al., 2016), and has thus far only been detected between *A. hypochondriacus* L. and *A. hybridus* L. (Kauffman & Weber, 1990), the possibility of hybridization may represent a serious problem for crops intended for the multiplication of certified seed. Regarding the survival of amaranth seeds in the soil and their potential to be a weed problem for subsequent crops, it must be taken into consideration that amaranth seeds are very small (1,000 seed weight 0.5–0.8 g) and that their ripening is uneven. Furthermore, the loss of seed at the time of harvest can be significant (Kauffmann & Weber, 1990). The few studies that have been conducted relating to the survival of the seed of cultivated amaranth in the soil, report that it is significantly shorter than weed relatives and that it highly unlikely constitute a rotational problem, although seed loss can be abundant (Omani et al., 1999; Kudsk et al., 2012).

Reducing weed competition for the first 4–6 weeks after sowing, results in increased biomass, seed proteins and yield (Ojo, 1997). Although it is not feasible to use residual herbicides, this scenario does not present a problem in grain amaranth cultivation as cultural and mechanical methods can serve as excellent alternatives to guarantee a qualitatively and economically sustainable production (Russell, 1977; Coolman & Hoyt, 1993; Morse, 1993).

Given that there is little research available on weed control in grain amaranth, the present research was initiated to evaluate the effectiveness of some mechanical methods in field experiments.

## MATERIALS AND METHODS

Two field experiments were carried out in 2018 and 2019 in Tuscany, Central Italy at ‘Tenuta di Cesa’ agricultural research station (43° 18’ north; 11° 47’ east, 246 m asl) on a neutral, loamy-sandy soil. The physical and chemical characteristics of the soil (depth of 20 cm) were as follows: sand 36.2%, loam 37.9%, clay 25.9%, total N 0.121% and P (Olsen) 13 ppm. Exchangeable Ca, Mg and K, were 4180, 641 and 142 ppm, respectively. Meteorological data was recorded using SIAP automatic equipment, controlled and validated by the Regional Hydrological and Geological Sector.

Initially, the research envisaged identical treatments over both years. However, the prolonged rainy period that occurred during the first year, did not permit mechanical weeding at the appropriate times with both flex-tine harrowing and the finger weeder. Therefore, the treatments were different over the two-year trial and are detailed in Table 1.

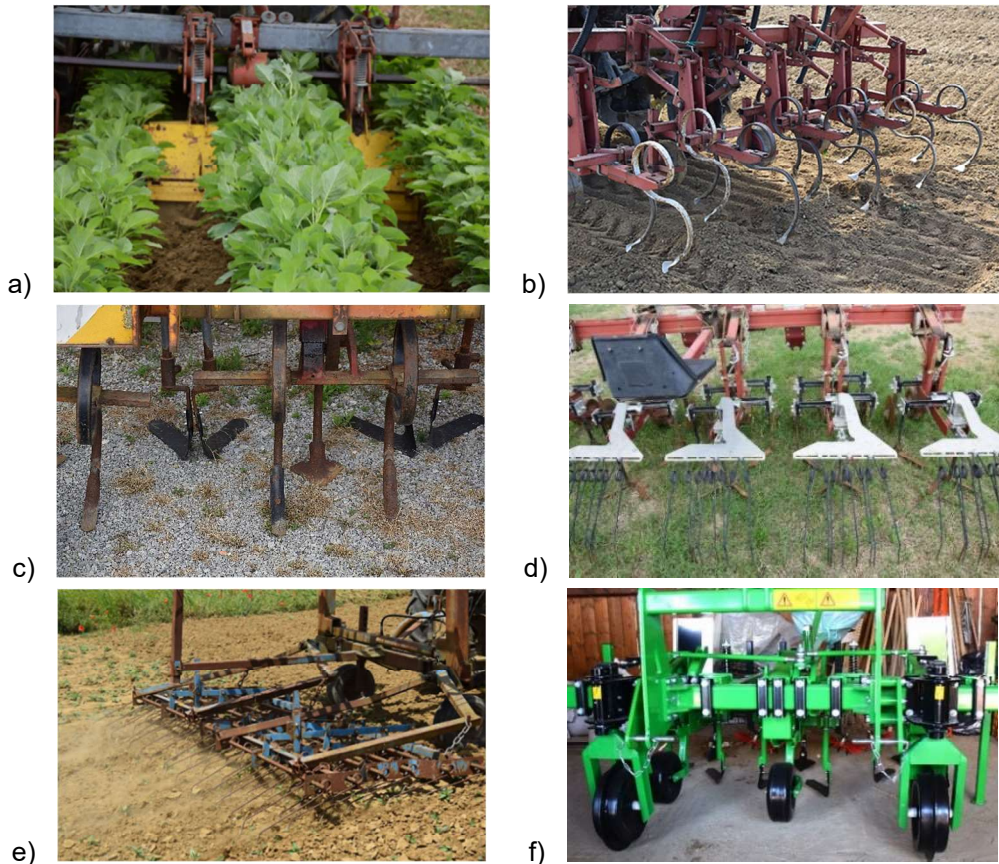
**Table 1.** Details of mechanical weed control treatments and application dates according to amaranth BBCH codes (Martínez-Núñez et al., 2019)

Treatment Code	Experiment 1 – 2018			Treatment Code	Experiment 2 – 2019		
	Treatment detail	Date	BBCH growth stage*		Treatment detail	Date	BBCH growth stage*
T1 <sub>18</sub>	Untreated Control	-	-	T1 <sub>19</sub>	Untreated Control	-	-
T2 <sub>18</sub>	Cutter Hoeing	June 5 (37 DAE)	15	T2 <sub>19</sub>	Three duck foot tines	June 18 (34 DAE)	13
T3 <sub>18</sub>	Flat share cuts and one central duck foot tine	June 5 (37 DAE)	15	T3 <sub>19</sub>	Flex tine harrowing	June 6 (22 DAE)	12
T4 <sub>18</sub>	Flat share cuts and one central duck foot tines with second operator	June 5 (37 DAE)	15	T4 <sub>19</sub>	Flex tine harrowing + Finger weeding with red fingers	June 6 (22 DAE) June 18 (34 DAE)	12 13
T5 <sub>18</sub>	Three duck foot tines	June 5 (37 DAE)	15	T5 <sub>19</sub>	Finger weeding with red fingers	June 18 (34 DAE)	13

\*BBCH codes corresponding to the following amaranth growth stages: 12 – (3–4 true leaves); 13 – (6–7 true leaves); 15 – (8–10 true leaves). Weed growth stage at time of weeding: 2018: from 4 to 8 true leaves; 2019: from 2 to 4 true leaves. Meteorological data occurred at the time of the treatments: 2018: clear sky, air temperature 20.0 °C, wind speed 2.4 m s<sup>-1</sup>. 2019 T3 and T4: clear sky, air temperature 21.2 °C, wind speed 2.3 m s<sup>-1</sup>; T2 and T5: clear sky, air temperature 24.5 °C, wind speed 1.7 m s<sup>-1</sup>.

The cutter hoeing treatment (T2<sub>18</sub>) was performed with 0.5 m wide units equipped with 15 cm cutters at a cultivation depth of 7–10 cm and driving speed of 2.5 km h<sup>-1</sup>. T3<sub>18</sub> was carried out with two 20 cm flat share cuts and one 15 cm duck foot tine at a cultivation depth of 5–7 cm and driving speed of 3.0 km h<sup>-1</sup>. Equipment used for T4<sub>18</sub> was equipped with 20 cm flat cuts and 15 cm duck foot tines at a cultivation depth of 5–7 cm and driving speed of 3.0 km h<sup>-1</sup>. T5<sub>18</sub> and T2<sub>19</sub> treatments were performed

with 20 cm duck foot tines at a cultivation depth of 5–7 cm and driving speed of 3.5 km h<sup>-1</sup>. T3<sub>19</sub> and T4<sub>19</sub> were equipped with 0.7 cm diameter flexible tines inclined by -35° at a cultivation depth of 2–3 cm and a driving speed of 6.5 km h<sup>-1</sup>. Finger weeding for T4<sub>19</sub> and T5<sub>19</sub> was carried out at a cultivation depth of 3–4 cm and a driving speed of 3.5 km h<sup>-1</sup>. Details of the equipment are reported in Fig. 1 while agronomic techniques are reported in Table 2.



**Figure 1.** Equipment utilized in the experiments: a) T2<sub>18</sub> – ‘Breviglieri’ equipment, M21-2 model; b) T5<sub>18</sub> and T2<sub>19</sub> – ‘Gaspardo’ equipment, HL-6R model; c) T4<sub>18</sub> – ‘Spapperi’ equipment without crop guard between horizontal knives; d) T3<sub>18</sub> – ‘Badalini’ equipment. Due to the amaranth growth stage, flex tine harrows were not used; e) T3<sub>19</sub> and T5<sub>19</sub> – Equipment without brand name (handcrafted design); f) T4<sub>19</sub> and T5<sub>19</sub> – ‘Sfoggia’ equipment, Kress model.

The experiments were carried out under rainfed conditions according to a Randomized Complete Block (RCB) design with four replicates. In order to carry out treatments with open field equipment, plots were 30 m long consisting of 8 rows spaced 0.6 m apart (surface area of 144 m<sup>2</sup>). Within each plot, a test area (the 4 central rows for a length of 5 m) was permanently allocated for all phenological, morphological and productive data, as well as weed community analysis. Seeding rate was 0.120 kg plot<sup>-1</sup> corresponding to an expected plant density of 60 m<sup>-2</sup>. UNIFI6161, a new line of *Amaranthus cruentus* L., obtained by the University of Florence, was used.

**Table 2.** Agronomic techniques during the field trials

	2018	2019
Preceding crop	Protein pea	Sunflower
Soil preparation	0.3 m plowing, August 2017 Disc harrowing, October 12, 2017 Rotary harrowing, April 20, 2018	0.3 m plowing, August 2018 Disc harrowing, January 11, 2019 Rotary harrowing, May 7, 2019
Pre-sowing fertilization	N 55.2 kg ha <sup>-1</sup> (Urea) P 78.0 kg ha <sup>-1</sup> (Triple superphosphate + Mineral superphosphate)	N 55.2 kg ha <sup>-1</sup> (Urea) P 78.0 kg ha <sup>-1</sup> (Triple superphosphate + Mineral superphosphate)
Insecticide treatment	Deltametrine 250 g 100 <sup>-1</sup> L water May 29	Deltametrine 250 g 100 <sup>-1</sup> L water May 25
Date of sowing	April 20	May 8
Date of emergence	April 29	May 15
Date of harvesting	September 13	September 19

The effectiveness of the treatment was evaluated by counting weeds and visually estimating the ground cover of the latter both immediately before the treatment and before the harvest of the crop, using the Braun-Blanquet cover-abundance scale (Maabel, 1979). A quadrat (0.5×0.5 m) was randomly positioned in each sampling area and three replicate analyses were performed. An average of the three samples was utilized for the statistical analysis. Both the average weed counts performed in all test areas and the percentage composition of the dominant species were considered as part of the weed community typical of the experimental area. Immediately following the pre-harvest counting, the weeds were removed manually and the total fresh and dry weight estimated. With regard to the main weed species, the effectiveness of the treatments was calculated and expressed as a percentage reduction in the number of plants compared to those present in the T1 control.

Visual ground cover of the crop as well as the dates of the different phenological stages were recorded. These stages, according to the specific BBCH codes (Martínez-Núñez et al., 2019) were as follows: 12 (four true leaves), 13 and 15 (six and ten true leaves, respectively), 50 (full panicle appearance), 65 (full flowering), 75 and 85 (milky and waxy maturity, respectively) and 89 (maturation), respectively. For the maturation stage, seed consistency was taken into consideration together with complete filling (non-translucent endosperm). Morphological crop traits were evaluated by considering the average of ten plants, randomly harvested in each sample plot.

The harvest was performed by a combine harvester. Seed humidity was recorded on a 100 g sample. After drying the seeds to a standard humidity of 12% (airflow at 35 °C for 48 h), yield calculations were then performed. Treatments were considered as a factor with fixed effects in the ANOVA model. Data on the percentage composition of weed flora and visual ground cover were subjected to the angular transformation as follows:

$$Y = \arcsine \sqrt{\frac{p}{100}}$$

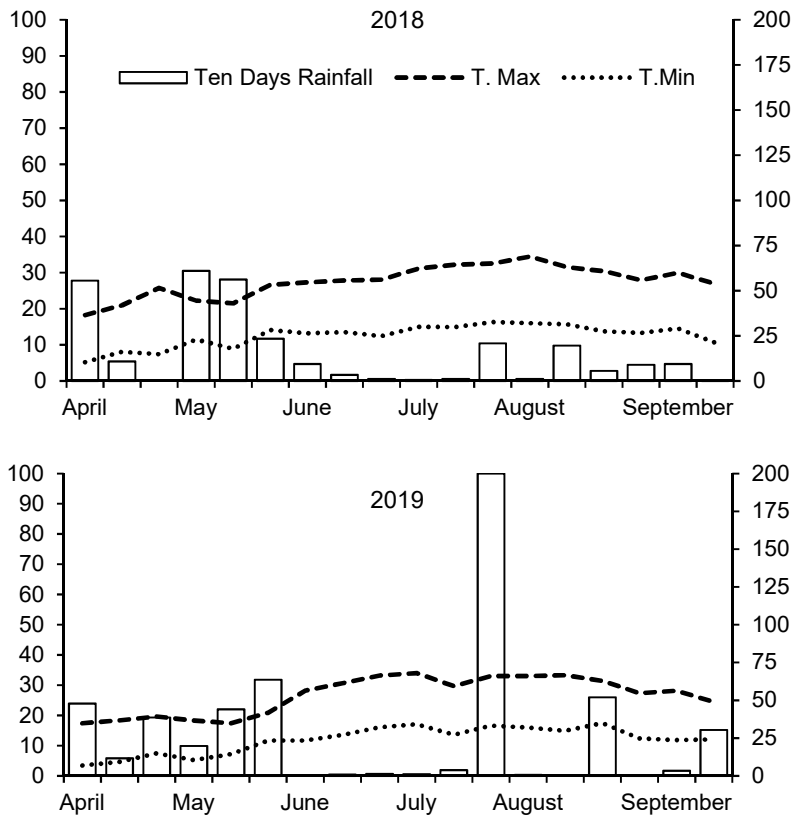
Differences between means were tested utilizing the Tukey test at  $P \leq 0.05$ ,  $P \leq 0.01$  or  $P \leq 0.001$ . COSTAT 6.45 software was used for the statistical analysis.

## RESULTS AND DISCUSSION

### Meteorological data

Fig. 2 shows the climatic trends during the field experiments. The average minimum and maximum temperatures recorded in 2018 were 12.5 and 27.5 °C, respectively, conforming to the ten-year average of the geographical area. The intense and persistent rains over March-April 2018 (110 mm) led to a slight delay in the sowing date, relative to that predicted to be most suitable for the area. Even over the May-June period, the rains were of an unusual frequency and intensity (75 mm), resulting in a change in some of treatments planned due to the excessive height of the amaranth plants.

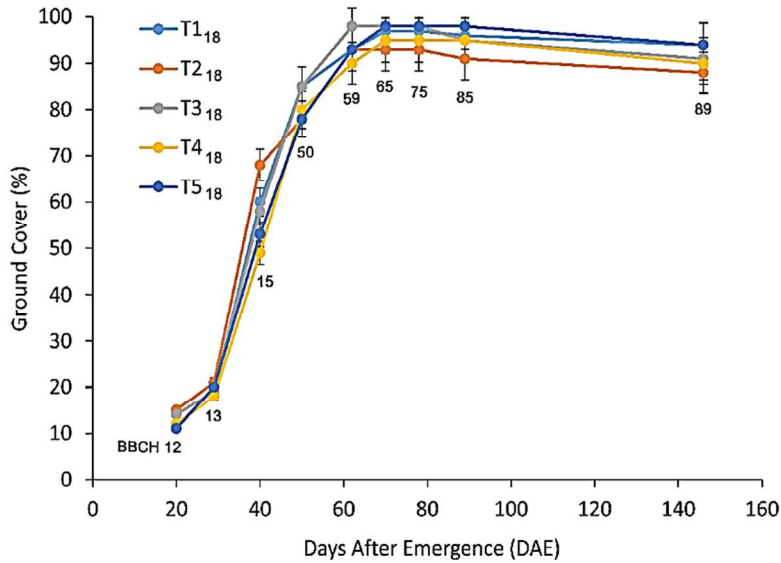
The average minimum and maximum temperatures recorded in 2019 were 11.8 and 26.6 °C, respectively. Rainfall, that occurred in both the second and third ten day periods of April led to a delay in the sowing, that was then postponed to May. During the month of May, when the crop was at the 12–13 phenological stage, temperatures ranged from 6.5 and 18.0 °C (below average monthly levels) with a rainfall of 127 mm. These environmental conditions led to a delay in plant development and physiological parameters characterizing the species. The last 10 days of July were characterized by both heavy and abundant rainfall, attaining a level of 233 mm of the total of 552 mm recorded in April-September period.



**Figure 2.** Temperature and rainfall recorded during the field experiments.

### Experiment 1 – 2018

For experiment 1 in 2018, the time of the phenological growth stages of amaranth, together with the ground cover trend, are shown in Fig. 3. The maturation of the crop in the different treatments was completed 145 days after emergence (DAE). Growth in the early growth stages, between the emergence and stage 13, was particularly slow (about 30 days), a characteristic that distinguishes this species.



**Figure 3. Experiment 1.** Amaranth ground cover in relation to the treatments and phenological growth stages according to BBCH codes. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at  $P \leq 0.05$ . T1<sub>18</sub>: Untreated Control; T2<sub>18</sub>: Cutter Hoeing; T3<sub>18</sub>: Flat share cuts and one central duck foot tine; T4<sub>18</sub>: Flat share cuts and one central duck foot tines with second operator; T5<sub>18</sub>: Three duck foot tines.

The beginning of the reproductive phase, coinciding with stage 59, occurred at 63 DAE. Full flowering (stage 65) in this species is strongly scaled, with an acropetal trend on the panicle. The time interval between stages 85 and 89 was 55 DAE, which was particularly long. Fig. 3 also evidences the rapid growth of amaranth from the stage 13.

Significant differences in amaranth ground cover were observed starting from stage 15, corresponding to one week after the treatments (37 DAE). In this phase, the highest ground cover of 76.3% was found in T2<sub>18</sub>. This was significantly different from the remaining treatments, particularly in T4<sub>18</sub>, where the coverage was 49.1%.

Stage 15, together with stage 50, highlighted significant differences between some treatments. The rapid development of the foliage in T2<sub>18</sub> may have been favored by improved soil aeration and consequent reduction in water loss due to capillary rise. Thereafter, in the subsequent phenological growth stages, starting from 65 onwards, significant differences were only reported between T1<sub>18</sub> and T2<sub>18</sub>.

Table 3 showed that the weed community in the area of the experimental trial was mostly composed of dicots (94.5%), with the remaining percentage composed of monocots. Of the dicots, 51.7% consisted of *Portulaca oleracea* L., followed by



*Solanum nigrum* L. (36.2%), *Convolvulus arvensis* L. (5.4%) and *Chenopodium album* L. (0.4%), respectively. Regarding the monocots, 58.0% was represented by *Echinochloa crus-galli* (L.) P. Beauv.

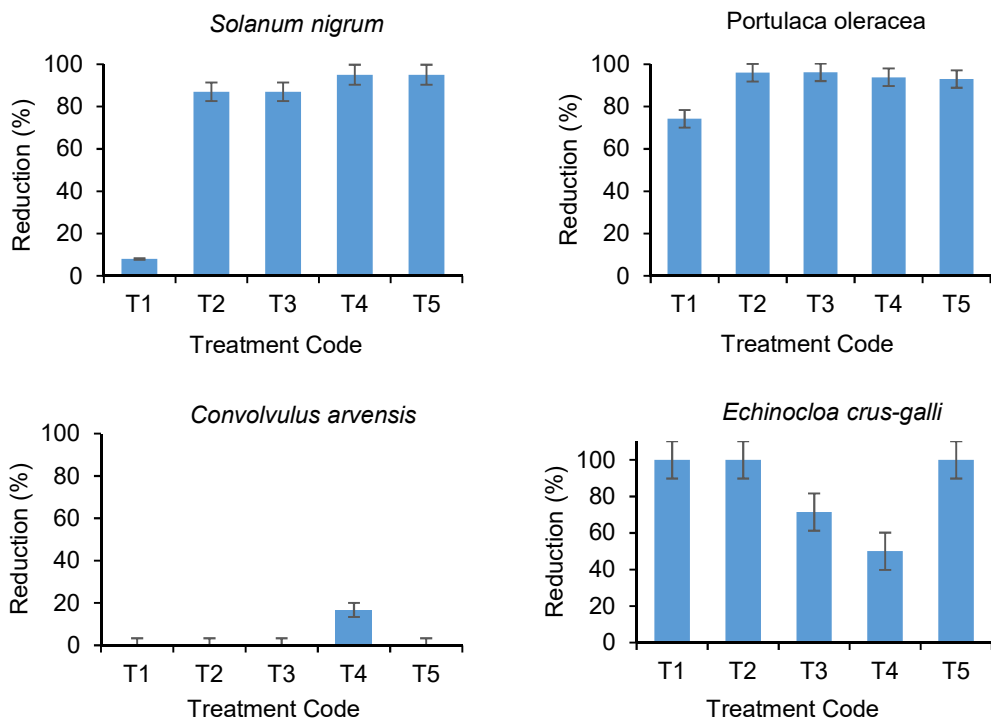
Hence, the weed community appeared simplified, with only two dicot species representing 87.9% of the weed community, typical for all the conventional agriculture farms within the area. Moreover, the seasonal trend may also have influenced the predominance of *P. oleracea*.

**Table 3. Experiment 1.** Mean floristic composition detected before treatments as a percentage of abundance compared to the total weed number

Species/ Abundance	<i>Portulaca oleracea</i>	<i>Solanum nigrum</i>	<i>Convolvulus arvensis</i>	<i>Chenopodium album</i>	Other dicots	<i>Echinochloa crus-galli</i>	Other monocots	Total dicots	Total monocots
Abundance, %	51.7	36.2	5.4	0.4	0.8	5.0	0.5	94.5	5.5
Other minor species	<i>Helianthus pauciflorus</i> Nutt., <i>Fallopia convolvulus</i> (L.) Holub, <i>Abutilon theophrasti</i> Medik., <i>Amaranthus retroflexus</i> L., <i>Cirsium arvense</i> (L.) Scop., <i>Fumaria officinalis</i> L., <i>Heliotropium europaeum</i> L., <i>Senecio vulgaris</i> L., <i>Taraxacum officinalis</i> Web., <i>Brassica nigra</i> L., <i>Phytolacca americana</i> L., <i>Mercurialis annua</i> L., <i>Sinapis arvensis</i> L., <i>Cynodon dactylon</i> (L.) Pers., <i>Avena</i> spp., <i>Lolium</i> spp., <i>Digitaria sanguinalis</i> (L.) Scop., <i>Setaria viridis</i> (L.) Beauv.								

The effectiveness of the treatments in reducing the number of predominant weeds is shown in Fig. 4. A drastic reduction of dicots, exceeding 85%, was observed with all mechanical treatments, with the exception of *C. arvensis*. Specifically, amaranth did not appear to compete effectively against *S. nigrum* (T<sub>18</sub>). There was a 8.3% reduction of the latter species compared to the remaining weeds under all treatments, of which T<sub>4</sub><sub>18</sub> and T<sub>5</sub><sub>18</sub> were especially effective (reduction exceeding 95%), and significantly better compared to T<sub>2</sub><sub>18</sub> and T<sub>3</sub><sub>18</sub>.

*P. oleracea*, by far the most widespread weed in all plots, was significantly reduced by all treatments. However, the untreated amaranth T<sub>18</sub> also showed an excellent competitive ability in reducing this weed by 75.8%. In contrast with the results reported for *P. oleracea*, for *C. arvensis* all treatments were practically ineffective, probably attributable to the prostrate posture and long climbing branches of the weed, and to the presence of rhizomes continuously forming new shoots. With regard to *E. crus-galli*, control was particularly effective with the T<sub>2</sub><sub>18</sub> and T<sub>5</sub><sub>18</sub> treatments (98.0% of reduction), while significantly smaller differences were observed with T<sub>3</sub><sub>18</sub> (68.5%) and T<sub>4</sub><sub>18</sub> (50.1%), respectively. The excellent effectiveness of T<sub>2</sub><sub>18</sub> was attributable to the type of action performed by this equipment (rotating and cutting parts at a shallow depth) which acts on weeds that were characterized by a bundled and still superficial root system at the time of treatment. However, once again (Fig. 4), the good competitive ability of untreated amaranth (T<sub>18</sub>) resulted in a 98.1% reduction of *E. crus-galli*. This effect can be ascribed to two main factors. The first resides in the root system of amaranth, characterized by rapid growth, and the deep penetration of taproot by the stages 13 and 15. The second resides in the rapid epigeal growth, starting from the same phenological growth stages, resulting in a rapid and almost complete ground cover, and in so doing, exercising an excellent competitive advantage for water, nutrients and light.



**Figure 4. Experiment 1.** Percentage reduction in the number of weeds compared to control belonging to the main species. If the bars do not overlap, the difference between averages is significant at  $P \leq 0.05$ . T1<sub>18</sub>: Untreated Control; T2<sub>18</sub>: Cutter Hoeing; T3<sub>18</sub>: Flat share cuts and one central duck foot tine; T4<sub>18</sub>: Flat share cuts and one central duck foot tines with second operator; T5<sub>18</sub>: Three duck foot tines.

The data reported in Table 4 showed significant differences for all weed parameters. The ground cover data allowed the identification of two groups. The first was from T1<sub>18</sub> to T3<sub>18</sub>, with an average of 52.4%, which was significantly higher than that observed in the second group comprised of T4<sub>18</sub> and T5<sub>18</sub> (8.2%). This was also confirmed in part by the number of weeds per m<sup>2</sup> and by the total dry weight of the weeds per m<sup>2</sup>. For the latter, T1<sub>18</sub> was significantly higher with 150.5 g m<sup>-2</sup>, while the lowest dry weight was recorded in T2<sub>18</sub> (38.7 g m<sup>-2</sup>). For amaranth, there were no significant differences in plant height and plant density per m<sup>2</sup>, with respective averages of 155.9 cm and 57.8 m<sup>-2</sup> plants. From the present experiment, it was not possible to identify the critical weed-free period, defined by Nurse et al. (2016) and Knezevic et al. (2002). Given the rapid increase in ground cover of amaranth and associated increased growth competitiveness, the weeds were shown to spread rapidly in the first growth phases of the crop, corroborating previous work (Sooby et al., 1998; Bavec & Mlakar, 2002; Kudsk et al., 2012; Brust et al., 2014). However, despite an excess of 51.0 weeds per m<sup>2</sup> observed in T1<sub>18</sub> (untreated control), the weeds did not affect negatively the morphological characteristics of the crop. Therefore, the present results indicate the excellent competitive ability of the crop, also confirmed by grain production. All seed yields exceeded 1.3 t ha<sup>-1</sup>, with the exception of those found in T5<sub>18</sub>. The only significant reduction compared to T1<sub>18</sub> was observed in T5<sub>18</sub> (1.19 t ha<sup>-1</sup>), and was likely attributed

to the greater depth of the moving parts of the equipment used that could have damaged the more superficial roots of the plants since the mechanical intervention was performed at a fairly advanced stage of growth (37 DAE). Furthermore, no significant differences were observed in the humidity of the seeds at harvest (12.7% on average).

**Table 4. Experiment 1.** Plant height, density, seed humidity and yield of the amaranth crop recorded at harvest, as well as total weed ground cover, density and dry weight

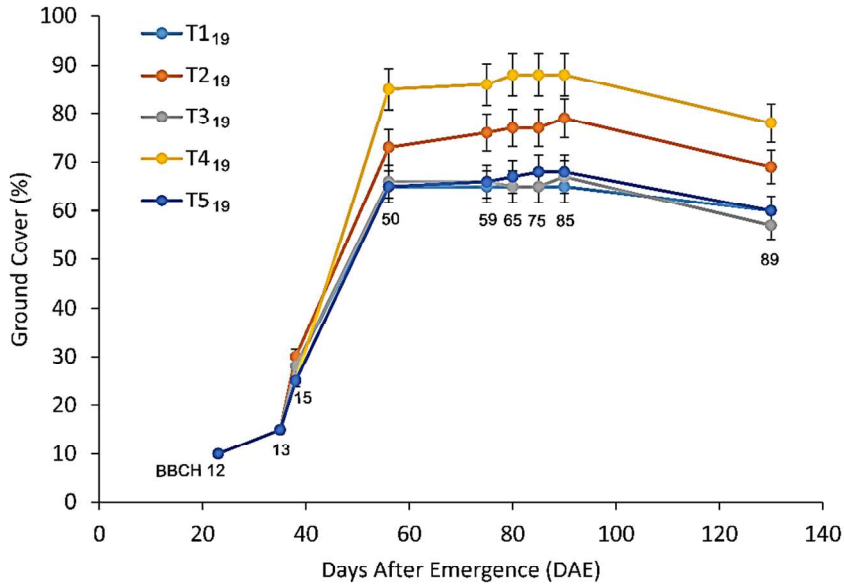
Treatment Code	Weeds			Grain amaranth			
	Ground cover, %	Total weed density, n m <sup>-2</sup>	Dry weight, g m <sup>-2</sup>	Plant height, cm	Plant density, n m <sup>-2</sup>	Seed humidity, %	Seed yield, t ha <sup>-1</sup>
T1 <sub>18</sub>	62.9 <sup>a</sup>	51.0 <sup>a</sup>	150.5 <sup>a</sup>	145.2	58.8	12.7	1.40 <sup>a</sup>
T2 <sub>18</sub>	50.0 <sup>a</sup>	8.1 <sup>b</sup>	38.7 <sup>d</sup>	158.1	63.3	12.4	1.36 <sup>ab</sup>
T3 <sub>18</sub>	44.3 <sup>a</sup>	12.3 <sup>b</sup>	63.7 <sup>c</sup>	160.0	62.2	12.6	1.34 <sup>ab</sup>
T4 <sub>18</sub>	8.2 <sup>b</sup>	14.0 <sup>b</sup>	84.5 <sup>b</sup>	166.3	54.9	12.7	1.38 <sup>ab</sup>
T5 <sub>18</sub>	8.3 <sup>b</sup>	6.4 <sup>b</sup>	51.6 <sup>cd</sup>	150.3	63.4	13.0	1.19 <sup>b</sup>
<i>Significance</i>	**	*	**	<i>ns</i>	<i>ns</i>	<i>ns</i>	*

*ns*: not significant; \*: significant at  $P \leq 0.01$ ; \*\*: significant at  $P \leq 0.05$ . Means within rows followed by the same letter(s) are not different at 5% level as per Tukey's test; T1<sub>18</sub>: Untreated Control; T2<sub>18</sub>: Cutter Hoeing; T3<sub>18</sub>: Flat share cuts and one central duck foot tine; T4<sub>18</sub>: Flat share cuts and two central duck foot tines with second operator; T5<sub>18</sub>: Three duck foot tines.

### Experiment 2 – 2019

For experiment 2 in 2019, the general trend of the phenological growth stages of amaranth (Fig. 5) in Experiment 2, confirmed that observed in Experiment 1. There was a rapid growth starting from stage 15, approximately to 40 DAE. Moreover, an extended period of 40 days was similarly required for the seed filling (stage 85). Fig. 5, showing crop ground cover in relation to the different treatments, showed significantly different effects starting from stage 50. In this growth stage, the ground cover showed significant differences between T2<sub>19</sub> (72.5%) and T4<sub>19</sub> (87.5%), as well as between T2<sub>19</sub> and T4<sub>19</sub> and the remaining treatments. Interestingly, in T4<sub>19</sub> there were 13.8 fewer plants per m<sup>-2</sup> than in T2<sub>19</sub>. The lower plant density in T4<sub>19</sub> probably led to less intraspecific competition and, therefore, a greater leaf area production and, consequently, ground cover. This dynamic is important and assumes a role of primary importance in providing a competitive advantage of amaranth against weeds, which in turn may have a positive effect on seed yield (Nurse et al., 2016). Considering the aforementioned plasticity of the amaranth sown at different densities, it is important to underline that when selecting both the plant density and the distance between the rows various aspects need to be taken into consideration. These include the type of equipment available for weeding and the behavior of the amaranth in a specific geographical area. Moreover, seeding rate flexibility depends on both the spectrum and density of weeds in the field. If thermophilic broad-leaf weeds predominate, then sowing amaranth seeds in narrow or double rows could accelerate ground cover, thereby providing a competitive advantage. As was shown previously, the rate of ground coverage is doubled using the aforementioned technique compared to that recorded with single rows (Casini & Biancofiore, 2020b). Good ground coverage by the crop also influences ground temperature and the red/far-red light ratio, which are lower below the plant canopy, resulting in a lower germination of weed seeds (Teasdale & Daughy, 1993; Batlla et al., 2000). Furthermore,

for *A. cruentus* and *A. hypochondriacus*, the release of allelopathic substances able to improve the competitive effect has also been demonstrated (Connick et al., 1989; Allemann & Denner, 2006; Tejada-Sartorius et al., 2011).



**Figure 5. Experiment 2.** Amaranth ground cover in relation to the treatments and phenological growth stages according to BBCH codes. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at  $P \leq 0.05$ . T1<sub>19</sub>: Untreated Control; T2<sub>19</sub>: Three duck foot tines; T3<sub>19</sub>: Flex tine harrowing; T4<sub>19</sub>: Flex tine harrowing and finger weeding with red fingers; T5<sub>19</sub>: Finger weeding with red fingers.

Additionally, in the present experiment (Table 5) the dicot weeds were undoubtedly the predominant species (95.1%), mainly represented by *S. nigrum* and *P. oleracea*.

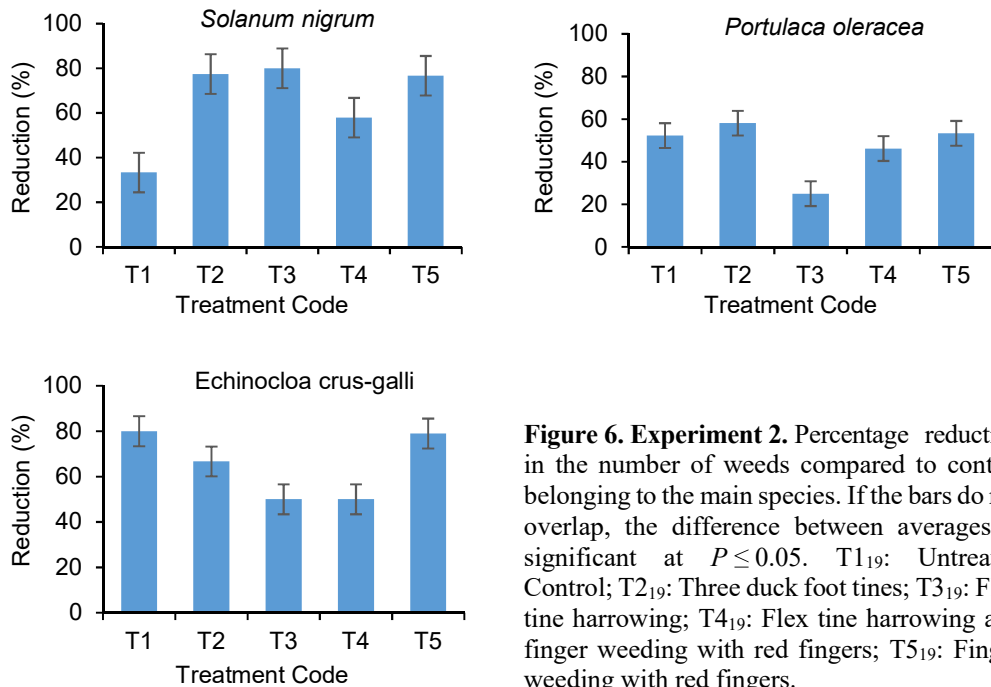
**Table 5. Experiment 2.** Mean floristic composition detected before treatments as a percentage of abundance compared to the total weed number

Species/ Abundance	Portulaca oleracea	Solanum nigrum	<i>Convolvulus arvensis</i>	<i>Amaranthus retroflexus</i>	Other dicots	Echinochloa crus-galli	Other monocots	Total dicots	Total monocots
Abundance, %	48.8	43.8	0.8	1.0	0.7	4.3	0.6	95.1	4.9
Other minor species	<i>Abutilon theophrasti</i> Medik., <i>Amaranthus retroflexus</i> L., <i>Brassica nigra</i> L., <i>Fallopia convolvulus</i> (L.) Holub., <i>Fumaria officinalis</i> L., <i>Helianthus pauciflorus</i> Nutt., <i>Heliotropium europaeum</i> L., <i>Mercurialis annua</i> L., <i>Sinapis arvensis</i> L., <i>Taraxacum officinalis</i> Web., <i>Avena</i> spp., <i>Cynodon dactylon</i> (L.) Pers., <i>Digitaria sanguinalis</i> (L.) Scop., <i>Setaria viridis</i> (L.) Beauv.								

By analyzing the effectiveness of the treatments against the most common weeds (Fig. 6), excellent control was shown against *S. nigrum*. The best results were obtained

in T3<sub>19</sub> (79.2%) and T2<sub>19</sub> (78.1%), followed by T5<sub>19</sub> (76.0%). In the T4<sub>19</sub>, a significantly lower result of 56.6% was recorded.

Additionally, with regard to *P. oleracea*, an average reduction of 53.2% compared to the control was recorded. In T3<sub>19</sub>, the reduction was equal to 23.5%. Given the postponement of the mechanical treatments, *P. oleracea* with its prostrate habitus and deep taproot was more difficult to eradicate under these conditions.



**Figure 6. Experiment 2.** Percentage reduction in the number of weeds compared to control belonging to the main species. If the bars do not overlap, the difference between averages is significant at  $P \leq 0.05$ . T1<sub>19</sub>: Untreated Control; T2<sub>19</sub>: Three duck foot tines; T3<sub>19</sub>: Flex tine harrowing; T4<sub>19</sub>: Flex tine harrowing and finger weeding with red fingers; T5<sub>19</sub>: Finger weeding with red fingers.

In T1<sub>19</sub> amaranth demonstrated an excellent competitive effect against the most widespread monocot, *E. crus-galli*. This was attributable to the rapid and good ground coverage of the crop, starting from stage 15, and also from the limited leaf area of the weed. The most effective treatment in reducing this weed occurred in T2<sub>19</sub> (65.3%) and in T5<sub>19</sub> (79.8%).

Data on the ground cover of weeds and their density at the time of harvest (Table 6), confirm the good competitive ability of amaranth. A weed density of 55.8 m<sup>-2</sup> was found in T1, not significantly different from that observed in T3<sub>19</sub> and T5<sub>19</sub>. This was also confirmed by the dry weight of the weeds which was 75.2 g m<sup>-2</sup>. For this parameter, the results also highlighted how the combined T4<sub>19</sub> treatment was effective not only in reducing weed number, but above all in reducing weed development, significantly lowering dry weight to 39.3 g m<sup>-2</sup>.

Table 6 also shows how some of the mechanical treatments negatively affected crop density. Compared to the average of 20.3 m<sup>-2</sup> plants in T1<sub>19</sub> and T2<sub>19</sub>, plant number was significantly reduced to 7.9 with the flex tine harrowing associated with the finger weeder (T4<sub>19</sub>). The negative effect of flex tine harrowing (machinery developed for cereal crops rather than dicots) was also due to the choice of the phenological phase in which to perform the treatments. This equipment should ideally be utilized as soon as

the deep taproot has formed in order to protect the plant from uprooting. However, given the unfavorable climatic conditions, treatments were performed coinciding with stage 13, evidently causing substantial damage to the hypogeal part of the crop.

**Table 6. Experiment 2.** Plant height, density, seed humidity and yield of the amaranth crop recorded at harvest, as well as total weed ground cover, density and dry weight

Treatment Code	Weeds		Grain amaranth				
	Ground cover, %	Total weed density, n m <sup>-2</sup>	Dry weight, g m <sup>-2</sup>	Plant height, cm	Plant density, n m <sup>-2</sup>	Seed humidity, %	Seed yield, t ha <sup>-1</sup>
T1 <sub>19</sub>	67.7 <sup>a</sup>	55.8 <sup>a</sup>	75.2 <sup>b</sup>	118.1	18.9 <sup>ab</sup>	22.4	0.75 <sup>c</sup>
T2 <sub>19</sub>	51.6 <sup>c</sup>	25.9 <sup>b</sup>	49.5 <sup>c</sup>	122.3	21.7 <sup>a</sup>	24.8	0.99 <sup>b</sup>
T3 <sub>19</sub>	72.1 <sup>a</sup>	47.2 <sup>a</sup>	99.0 <sup>a</sup>	119.5	15.7 <sup>ab</sup>	23.1	0.94 <sup>b</sup>
T4 <sub>19</sub>	51.9 <sup>c</sup>	26.9 <sup>b</sup>	39.3 <sup>c</sup>	133.2	7.9 <sup>c</sup>	25.2	1.12 <sup>a</sup>
T5 <sub>19</sub>	59.7 <sup>b</sup>	37.0 <sup>ab</sup>	48.3 <sup>c</sup>	124.5	15.5 <sup>ab</sup>	23.0	0.98 <sup>b</sup>
<i>Significance</i>	**	**	**	<i>ns</i>	*	<i>ns</i>	**

*ns*: not significant; \*: significant at  $p \leq 0.01$ ; \*\*: significant at  $p \leq 0.05$ . Means within rows followed by the same letter(s) are not different at 5% level as per Tukey's test T1<sub>19</sub>: Untreated Control; T2<sub>19</sub>: Three duck foot tines; T3<sub>19</sub>: Flex tine harrowing; T4<sub>19</sub>: Flex tine harrowing and finger weeding with red fingers; T5<sub>19</sub>: Finger weeding with red fingers.

Despite the lower plant density at harvest compared to that expected at sowing, the treatments using flex tine harrowing (T3<sub>19</sub>) and the finger weeder (T4<sub>19</sub>), as well as the combined equipment treatment (T5<sub>19</sub>), did not result in significantly different yields. Seed production was on average of 1.0 t ha<sup>-1</sup>. The lower plant density invariably stimulated side-branching with the development of secondary panicles to compensate for the lower seed production of the main panicle. This aspect resulted in a gradual maturation which was delayed over time, ensuing a higher seed humidity of 25.2% at harvest in T4, which was higher (although not significantly) than that in T1<sub>19</sub> and T5<sub>19</sub>. Nonetheless, with the humidity levels in the seeds under open-field cultivation, drying is essential to attain the threshold level of 11–12% for safe storage.

Overall, the best yield of 1.12 t ha<sup>-1</sup> was obtained with the combined T4<sub>19</sub> treatment, which was significantly higher than the lowest yield of 0.75 t ha<sup>-1</sup> in the untreated control T1<sub>19</sub>.

## CONCLUSIONS

Based on the seasonal trend recorded in Experiment 1, which resulted in a delay in both the sowing time and that of the mechanical treatments, it was not possible to completely assess the efficacy of the different treatments. Nonetheless, the predominant dicot weeds were effectively controlled under all treatments, contrary to that reported with interventions against monocots. Despite the fact that the various mechanical treatments were carried out over a period of time varying between 22 and 37 DAE, for the purposes of the competitive effects of the weeds on the crop, this interval selected is compatible with previous studies in another agroclimatic environment (Nurse et al., 2016). After 30 DAE, the problem shifts from the possible competitive effects to the difficulty of late mechanical intervention which is linked to the excessive development of both the crop and weeds.

Amaranth, at least in these experiments, proved to be an extremely competitive species against weeds starting from about 40 DAE at stage 15 corresponding to the 10 true leaf stage. Similar results were also obtained by Jena et al. (2009) and Shukla et al. (2014) in *Amaranthus hypochondriacus*, where the untreated control was compared with both hand weeding and the use of a herbicide in post-emergence.

The competitive ability of amaranth was also confirmed by the seed yield. Not only was the seed yield not significantly different between the treatments, but was also shown to be of a good level, exceeding  $1.2 \text{ t ha}^{-1}$  on average. This characteristic was also confirmed to some degree in the second year of experimentation, even if strongly influenced by treatments and a lower plant density compared to the previous year. A plant density of less than  $30 \text{ plants m}^{-2}$  was considered optimal according to Sooby et al. (1998) and Casini & La Rocca (2014). In addition, seed yield in the second year was also more strongly influenced by mechanical treatment compared to the first year.

The results of the single treatment repeated in both years (three duck foot tines; T5<sub>18</sub> and T2<sub>19</sub>) confirmed the effectiveness of this equipment, even though it performed less well in controlling weeds, equivalent to 25%, in the second year.

Treatments with the flex tine harrower and finger weeder (T3<sub>19</sub>, T4<sub>19</sub> and T5<sub>19</sub>) negatively influenced the plant density at harvest, a clear sign necessitating adjustments in these interventions to achieve optimal results. However, the combined treatment (T4<sub>19</sub>) was shown to be the best in weed control, specifically the dicot weeds. Furthermore, this treatment facilitated the rapid and extensive ground cover of the crop, providing a competitive advantage against weeds.

The present study suggests that all the mechanical methods for inter-row cultivation can be effectively used to control weeds in grain amaranth. However, there is a need to optimize strategies to anticipate and improve the ground cover of amaranth.

Single row width as a parameter does not significantly influence crop production on the experimental site. The choice of distance must be implemented according to the both type of machinery available and the soil type. Loose soils, for example, will easily permit treatment closer to the row using horizontal knives or rotating finger weeders. Instead for clay-rich soils, machinery with adequate crop guard systems would need to be used.

Optimal plant density is a priority, as well as effectively choosing the correct distance between the rows according to easier mechanical control (Endres 1986; Jamriška 1998; Chaudhari et al. 2009; Olofintoye et al., 2011; Singh et al., 2017). According to Casini & Biancofiore (2020b), the use of double rows (18 + 60 cm) permitted taking advantage of a better ground cover than single rows, together with the possibility of mechanical treatments for weed control. Future research on the mechanical control of weeds in grain amaranth in Central Italy, should focus on the type of equipment, the false seedbed technique and the possible integration of thermal methods.

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