

Forage and grain yield of common buckwheat in Mediterranean conditions: response to sowing time and irrigation

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11	Running title: buckwheat for forage and grain
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1	Abstract. With the view to extending the cultivation of common buckwheat to Mediterranean
2	environments, we investigated the responses of two varieties to three sowing times, early
3	spring, late spring and late summer, in rainfed and irrigated conditions. Plants were harvested
4	at two ripening stages for forage production and at maturity for grain yield. The eultural crop
5	cycle lasted 82-88 days independent of sowing time, while the thermal time was
6	approximately 1000 °Cd in early spring and late summer sowings, and 1200 °Cd when sown
7	in late spring. Forage yield increased up to 75% between ripening stages. Early spring was the
8	best sowing time for forage (4 t ha ⁻¹ DW) and grain yield (2 t ha ⁻¹ DW) in rainfed conditions.
9	Late spring sowings give the highest forage yield when irrigated (6 t ha ⁻¹ DW), but were not
10	suitable for producing grain, for the adverse effect of high summer temperatures on seed set
11	and seed filling. Late summer sowings produced acceptable grain yield (1.5 t ha ⁻¹ DW),
12	whereas short days and low temperatures limited forage production. Thus, in Mediterranean
13	environments, buckwheat could be profitably introduced as a minor summer crop, sown in
14	early spring for grain production and in late spring for forage production.
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- 16 Additional keywords: alternative crops, dry matter production, grain yield, sowing time.
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1 Introduction

2 Common buckwheat (*Fagopyrum esculentum* Moench.), hereafter referred to as buckwheat, is 3 a dicotyledonous annual herb of the family *Polygonaceae* that was cultivated from ancient 4 times as pseudocereal crop. The stem is erect with a variable branching, and bears one leaf per 5 node. Inflorescences develop in the leaf axils and at the end of both the main stem and 6 branches. Each plant produces a lot of white to pink flowers, but only a few develop into 7 dark-hulled triangular achenes, containing one starch-filled seed (Marshall 1980; Halbrecq *et* 8 *al.* 2005).

9 The species originates from the north-west corner of the Yunnan province of China (25-10 30°N), which is a vast plateau in the Himalayan foothills, where it grows from 500 to 2,500 m 11 above sea level (Campbell 1997). Human consumption of buckwheat fruits dates back to 12 prehistory and, in the first millennium BC, its cultivation diffused from China to Russia and 13 Ukraine. Buckwheat became established in the rest of Europe in the Middle Ages, both as a 14 summer crop in rotation with rye, or on very poor soils, or as a pioneer species on new 15 farmland. The introduction of maize and potato from the new world and the diffusion of 16 higher yielding cereals, such as wheat and barley, caused a rapid decline in buckwheat cultivation, so that in the 19-20th centuries its cropping was associated with poverty and 17 18 hunger (Körber-Grohne 1987; Ahmed et al. 2014).

In recent years there has been renewed interest in buckwheat cultivation, driven by the rising demand for its products. Buckwheat fruit is generally milled to obtain gluten-free flour, which can be consumed by people affected by celiac disease (Alvarez-Jubete *et al.* 2010; Kaur *et al.* 2015). This flour possesses higher protein content and a better biological value compared to wheat and rice, due to the higher proportion of the amino acids lysine and arginine (Ratan and Kothiyal 2011; Zhang *et al.* 2012). The entire fruit is used to produce beer and to feed poultry and pigs (Körber-Grohne 1987).

1 Buckwheat leaves and young sprouts are consumed fresh as vegetables, or dried to 2 prepare tea, and the entire buckwheat plant contains a variety of compounds that can be used 3 to produce nutraceutical preparations and functional foods (Li and Zhang 2001; Baumgertel et al. 2010). It is also rich in rutin, a flavonoid employed in the prevention and treatment of 4 5 chronic cardiovascular diseases. Its potential as green and conserved forage and as a source of 6 nectar for honey bees has also been investigated (Omidbaigi and De Mastro 2004; 7 Amelchanka et al. 2010; Kälber et al. 2012; Mariotti et al. 2015). However, along with its 8 beneficial nutrients and phytochemicals, buckwheat also contains fagopyrin, which is a photo-9 sensitive substance, as well as compounds that can cause allergic reactions (Stojilkovski et al. 10 2013; Ahmed *et al.* 2014).

11 Buckwheat grows best in cool and humid conditions, and the optimal temperature range 12 for flowering and fruit maturation is 17-19 °C (Marshall 1980). Ecotypes differ in their 13 sensitivity to photoperiod (Angus et al. 1982; Cawoy et al. 2009), however buckwheat is 14 generally considered a non-specific short day crop (Hao et al. 1995). The crop cycle is quite 15 short, lasting 9-12 weeks, and it needs approximately 1,200 GDD, with a base temperature of 5 °C, to reach fruit maturity (Edwardson 1995; Ahmed et al. 2014). Buckwheat is thus 16 17 generally grown in cool temperate and even sub-arctic regions, as a minor summer crop sown 18 in May-June, or later, after the harvest of wheat and barley. However, in subtropical regions it 19 can also be grown as a second crop sown in late summer or autumn (Angus et al. 1982; 20 Amelchanka et al. 2010).

The leading buckwheat producers are China, Russia, Ukraine, France, and the USA (FAOSTAT 2015). The yield is highly variable, with a maximum grain production close to 3 t ha⁻¹ in France. The short crop cycle and the non-specific response to day length could facilitate the introduction of buckwheat cultivation into new geographical areas, with greatest potential in multiple cropping systems (Angus *et al.* 1982). In Italy, buckwheat was traditionally cultivated as a summer crop for flour production in restricted Alpine and

1 Apennine areas. Its introduction in Mediterranean environments, as the main or second crop 2 with a sowing time between early spring and late summer, would enable marginal lands to be 3 exploited and increase farm biodiversity (Tallarico et al. 2008). However, shifts from 4 conventional sowing times could affect both forage and grain production, since buckwheat is 5 sensitive to low temperatures at establishment and to high temperatures and water stress at flowering and grain set (Slawinska and Obendorf 2001; Taylor and Obendorf 2001; Ahmed et 6 7 al. 2014). Plants would also be exposed to a variety of day lengths, which could influence 8 growth patterns and seed set (Michiyama et al. 2005). In Mediterranean climates, high 9 temperatures and limited water availability could negatively affect buckwheat crops in 10 summer, but also in late spring and early autumn in warm and dry years. To the best of our 11 knowledge, no data are available on either buckwheat cultivation in plain areas with a typical 12 Mediterranean climate or on its response to irrigation.

In order to assess the best sowing time for forage and grain production in a typical Mediterranean environment, we cultivated buckwheat in a plain area of central Italy. The responses of two varieties to three sowings, performed in early and late spring and in late summer, were investigated in rainfed and irrigated conditions. Irrigation was applied to evaluate whether an additional water supply could increase yield and ameliorate the adverse effect of high temperatures. Since the stage of highest biomass accumulation is not well defined in buckwheat, forage harvest was performed at two stages of phasic development.

20

21 Materials and methods

22 Experimental site

The experiment was carried out in 2012 and 2013 at the Department of Agriculture, Food and Environment of the University of Pisa, Italy, which is located at a distance of approximately 4 km from the sea (43°40′N, 10°19′E) and is 1 m above sea level. The climate of the area is hot1 summer Mediterranean, with mean annual maximum and minimum daily air temperatures of

2 20.2 °C and 9.5 °C, respectively, and a mean rainfall of 971 mm per year.

The main physical and chemical properties of the soil were 51.1% sand (2 mm - 0.05 mm), 38.6% silt (0.05 mm - 0.002 mm), 10.3% clay ($\emptyset < 0.002$ mm), 8.2 pH, 22.6 g kg⁻¹ organic matter (Walkley and Black method), 14.2 g kg⁻¹ total CaCO₃ (Scheibler method), 0.91 g kg⁻¹ total nitrogen (Kjeldhal method), 10.2 mg kg⁻¹ available P (Olsen method), and 162.4 mg kg⁻¹ available K (ammonium acetate test method). Field capacity and permanent wilting point were determined with the pressure chamber method at 33 and 1500 kPa soil water tension, respectively, and were 23.1% and 10.3%.

10

11 Treatments and experimental design

12 In each year, treatments involved two buckwheat (Fyagopyrum esculentum Moench) 13 varieties, three sowing times, and two irrigation levels. We also compared two harvest stages 14 for forage production. The commercial varieties Bamby and Lileja were chosen, because of 15 their high and reasonably stabile grain yield and their wide cultivation throughout Europe 16 (Brunori et al. 2006; Kälber et al. 2012). Sowing times were early spring (ESp), late spring 17 (LSp) and late summer (LSu). Early spring, i.e. around mid April, was chosen as the earliest 18 period that escapes spring frost in central Italy. Late spring, i.e. end of May, is the 19 conventional sowing time for buckwheat in temperate climates (Edwardson 1995; Kalinova 20 and Dadakova 2013), and late summer, i.e. beginning of September, is the earliest sowing 21 period escaping summer drought. The sowing dates for the two years are reported in Table 1. 22 Irrigation treatments were rainfed and 100% replacement of the estimated evapotranspiration.

In order to estimate the optimal stage for forage yield, forage harvests were performed at peak-full flowering, when plant growth is presumed to stop (Cawoy *et al.* 2009), and at the beginning of fruit ripening, just prior to the onset of senescence. Following the growth scale of Arduini *et al.* (2016), these stages were identified with the appearance of 1-2 green achenes

at the base of the first inflorescence formed on the plant (stage 70 - First Green Achenes) and
 with the ripening of these achenes (stage 85 - First Brown Achenes). For grain yield, plants
 were harvested at maturity, when all achenes were dark brown or aborted (stage 88, Arduini
 scale).

5 In both years, the experiment was arranged in a split-split-plot design with three 6 replicates. Sowing date was the main plot factor, irrigation treatment was the sub-plot factor, 7 and variety was the sub-sub-plot factor. Sub-sub-plot dimensions were 5 by 9 m, each 8 separated by 4 m. The three harvests were performed within sub-sub-plots on randomly 9 chosen sample areas of 1 x 1 m.

10

11 Crop management

In both years, the preceding crop was rapeseed. Soil preparation consisted in medium depth ploughing (30 cm), carried out in October 2011 and 2012. Final seed bed preparation was carried out just prior to sowing by harrowing twice, with a disc harrow, and with a rotating harrow. Buckwheat was sown with 15-cm row spacing and with a density of 200 viable seeds per m². Nitrogen, phosphorous and potassium fertilisers were applied at rates of 40, 44 and 83 kg ha⁻¹, respectively as urea, triple mineral phosphate and potassium sulphate. Nitrogen was applied just before seeding, while P and K were applied before tillage.

19 The soil profile was close to field capacity at planting and, after crop emergence, 20 irrigation lines were permanently installed above ground in inter-rows. Starting from the stage 21 of first true leaf unfolded (stage 11, Arduini et al. 2016), water was distributed daily by drip irrigation (1 dripper per metre) and the flow application rate was 4 L h⁻¹ m⁻¹ of tubing. The 22 23 amount of water given daily was designed so that rainfall plus irrigation replaced the soil 24 moisture lost through evapotranspiration. The potential evapotranspiration (E_{θ}) of the 25 previous day was estimated from Class A pan evaporation. Actual evapotranspiration was 26 calculated as $E = kc \ge kc$, where kc is the crop coefficient. Because kc values of buckwheat are not known, we used those reported for wheat (Doorembos and Pruit 1977). Accordingly, *kc* values increased from 0.3 at 10 days after emergence to 1.15 at the first brown achenes stage, and declined to 0.25 at maturity. As a whole, 183, 240 and 55 mm irrigation were supplied, respectively, to ESp, LSp and LSu in 2012, and 193, 232 and 50 mm in 2013. No pest infestation was detected during the cultivation period, and weed cover was very low up to the end of flowering.

7

8 Measurements

9 For the entire period of the research, the minimum and maximum daily temperatures and 10 rainfall were obtained from a weather station located at about 100 m from the experimental 11 site. Cumulated rainfall from April to November was 671 in 2012 and 468 in 2013, which was 12 higher and lower, respectively, than the preceding ten-year average (573 mm), by 13 approximately 17% (Fig. 1). Over the same period, the mean temperature was 18.7 °C in both 14 years, which was slightly higher than that of the previous 10 years (17.9 °C), primarily due to 15 the higher temperature in the autumn. Day length increased from 12:46 h to 15:27 h between 16 1 April and 21 June, and then decreased to 9:12 h on 30 November (NOAA 2016).

17 Thermal time for buckwheat was calculated as the sum of heat units measured in growing 18 degree-days (GDD, °Cd), as GDD = ((Tmax + Tmin)/2) - Tb. In the formula, Tmax and Tmin 19 are the daily maximum and minimum air temperatures, and Tb is the base temperature below 20 which no significant crop development occurs. If Tmin < Tb then Tmin = Tb was also 21 incorporated into the equation. An upper threshold temperature (Tut), above which crop 22 development is negatively affected, was also incorporated, i.e. if Tmax > Tut then Tmax = 23 Tut (McMaster and Wilhelm 1997). Base temperature and Tut were set respectively at 5 °C 24 and 25 °C following Edwardson (1995).

At each harvest, plants were manually cut at ground level, counted and measured to determine the height. There were approximately 130 plants m^{-2} , without significant

differences among treatments (data were not reported). At forage harvests, plants were separated into leaves, stems and inflorescences, including developing achenes. At maturity, plants were separated into achenes and straw, which consisted of stems, leaves and inflorescence axes. The mean achene weight and harvest index (HI) were also determined. All plant parts were oven dried at 65 °C to constant weight for dry weight determination.

6

7 Statistical analysis

8 The results were subjected to analysis of variance (ANOVA) separately for forage and grain 9 production. For the forage, the main effects of year, sowing date, irrigation, variety, harvest 10 stage, and their interactions were tested. For the grain, we tested the main effects of year, 11 sowing date, irrigation, variety, and their interactions. The combined analysis over years was 12 conducted after verifying the homogeneity of error variances by the chi-square test. The 13 CoStat statistical package (version 6.4, CoHort Software, CA, USA) was used, and, in all 14 analyses, the year and imposed treatments were considered as fixed effects. Significantly 15 different means were separated at the 0.05 probability level by the least significant difference 16 test (Steel et al. 1997).

17

18 **Results**

19 *Climate conditions*

The year mean effect and all interactions of year with other treatments were not significant for any of the measured or calculated parameters, probably because between-year differences in temperature and rainfall were very low. Only LSu plants received 39% more rainfall in 2012 than in 2013, but close to the end of the crop cycle (Fig. 1). Accordingly, all data are presented as averaged over years.

25 Climate conditions experienced by buckwheat plants differed markedly in response to 26 sowing time. Cumulated rainfall over the entire growth cycle was 367, 153 and 35 mm for LSu, ESp and LSp plants, respectively (Table 1). Mean temperature ranged from 12 to 24 °C
in ESp, from 15 to 25 °C in LSp and from 6 to 23 °C in LSu, which corresponded to a mean
temperature calculated over the entire <u>eultural crop</u> cycles of 18, 22 and 17 °C, respectively
(Fig. 1). Average day length was 14:42 h in ESp, 15:06 h in LSp and 11:00 h in LSu.

5

6 *Phasic development*

The duration of growth phases was affected by sowing time but not by irrigation or variety. Calculated in days, the time to reach the green achenes stage was approximately 38 days in LSp and LSu, and 50 days in ESp, whereas a further 13-15 days were needed to reach the brown achenes stage in all sowings (Table 1). The period from the first brown achenes stage to crop maturity increased with the delay in sowing, which was 23, 28 and 36 days in ESp, LSp and LSu, respectively. The length of the entire growth cycle thus did not vary greatly in response to sowing date, which was between 82 and 88 days.

Calculated in thermal units, the time to reach the first green achenes stage was approximately 530 °Cd in all sowings (Table 1). Thereafter, ESp and LSu plants needed approximately further 170 °Cd to reach the first brown achenes stage and an additional 330 °Cd for achene maturity, while LSp plants required 35% and 45% more thermal units, respectively. As a result, the thermal time cumulated by buckwheat from sowing to maturity was slightly higher than 1000 °Cd in ESp and LSu, and close to 1200 °Cd in LSp.

20

21 Forage production

At both harvests, the dry biomass of buckwheat forage decreased with the delay in sowing from early spring to late summer in rainfed conditions (Fig. 2). However, at the green achenes stage, the dry biomass decreased progressively by 33%, whereas, at the brown achenes stage, the decrease was approximately 41% in LSp and LSu compared to ESp. Up to the stage of first green achenes, irrigation did not affect forage production in ESp and LSu, while

irrigation increased forage production by 91% in LSp. After the first forage harvest, the effect
 of irrigation was still not significant in ESp, but was much more pronounced in LSp (+180%)
 and LSu (+28%).

Forage dry weight always increased from the first green to the first brown achene stages, however increments differed greatly according to sowing time and irrigation (Fig. 2). The lowest increments were recorded in rainfed plants of LSp and LSu, (approximately 50 g m⁻²), and the highest increments were in irrigated plants of LSp (approximately 300 g m⁻²). As a result, maximum forage yield was obtained in LSp with the aid of irrigation (5.9 t ha⁻¹), and in ESp in rainfed conditions (3.8 t ha⁻¹).

Patterns of plant height matched those of forage yield, indicating that changes in forage production were essentially due to changes in plant size (Fig. 2). Maximum height was 111 cm in LSp irrigated plants, while it was only 72 and 60 cm in plants of ESp and LSu.

13 At both forage harvests, leaf and stem dry weight changed in response to treatments, but 14 the response to irrigation was more pronounced in stems than in leaves. In fact, at the second 15 harvest, water supply increased leaf biomass by 20% in LSu and by 150% in LSp, while water 16 supply increased the biomass of stems by 35% and 233%, respectively (Table 2). The 17 response of inflorescence biomass did not match that of leaves and stems. At the first green 18 achene stage, it did not differ significantly among sowing dates in rainfed conditions and was 19 increased by irrigation only in LSp. At the first brown achene stage, in rainfed conditions, 20 inflorescence biomass still did not differ significantly between ESp and LSu, but was much 21 lower in LSp. Irrigation increased dry weight of inflorescences in all sowings but increments 22 were much higher in LSp, so that inflorescence biomass decreased in the order LSp > LSu >23 ESp.

Plant parts changed with different patterns between forage harvests. Leaf biomass did not increase except in LSp irrigated plants, whereas stem biomass always increased in ESp, only when irrigated in LSp, and never in LSu (Table 2). Due to achene development, inflorescence

biomass increased markedly between harvests in all treatments, but increments were more pronounced in irrigated plants. The different growth patterns of leaves, stems and inflorescences and their different responses to treatments affected partitioning in forage. The most striking difference was in the proportion of inflorescences at the first brown achene stage, which was approximately 28% in ESp and LSp and 50% in LSu, irrespective of irrigation treatments (data not shown).

Varieties responded similarly to treatments, however Bamby was approximately 8 cm taller and produced 7% more forage than Lileja, averaged over years, sowing times, irrigation treatment and stage of forage harvest. The higher forage yield was due to the higher stem biomass, since leaf biomass was the same and inflorescence biomass was also higher in Lileja at the second harvest (Table 3). This slightly affected partitioning within forage, with a higher proportion of stems in Bamby than in Lileja (56% vs 53%) and a higher proportion of inflorescences in Lileja than in Bamby (24% vs 22%), averaged over harvests.

14

15 *Grain yield*

Grain yield differed markedly in response to sowing time, and the highest values of 224 g m⁻² 16 17 were achieved with ESp, irrespective of irrigation treatments (Fig. 3). In LSp, grain yield was very low (24 g m⁻²) in rainfed conditions, and increased to only 91 g m⁻² with the aid of 18 irrigation. In LSu, grain yield was approximately 150 g m⁻², with a slight positive effect due 19 20 to irrigation. The number of achenes per plant decreased with the delay in sowing from 63 to 21 approximately 43, but in non-irrigated plants, it fell dramatically in LSp (Fig. 3). Finally, the 22 dry weight of straw, showed similar patterns to grain yield in ESp and LSu, and was 23 approximately 67% higher in the former than in the latter. In LSp, straw biomass was between 24 the other two sowings in rainfed conditions, and approximately 158% higher when irrigated 25 (Fig. 3).

Mean achene weight and harvest index changed in response to sowing time and variety, but were not affected by irrigation. Mean achene weight was 13% higher and harvest index was 7% higher in LSu than in ESp, and both parameters were very low in LSp (Table 4). The mean achene weight was by 9% higher in Lileja than in Bamby, which, however, did not affect grain yield, which was 1.5 t ha⁻¹ in both varieties. In contrast, similar to forage harvests, straw biomass was higher in Bamby (295 vs 261 g m⁻²) and, consequently, the harvest index was lower in this variety.

8

9 **Discussion**

In our research, buckwheat vielded close to 4 t ha⁻¹ forage dry matter when sown in early 10 spring in rainfed conditions, and close to 6 t ha⁻¹ when sown in late spring with the aid of 11 irrigation. The highest grain yield, 2.2 t ha⁻¹, was obtained with the early spring sowing, 12 13 irrespective of irrigation treatment. Forage yield was higher than obtained in central Europe 14 (Kälber et al. 2012; Kalinova and Dadakova 2013) when irrigated, but slightly lower when 15 rainfed. Otherwise, grain yield was approximately 25% lower than the best performance of 16 this crop (FAOSTAT 2016), but in line with maximum values obtained in central Europe 17 (Schulte et al. 2005; Kalinova and Dadakova 2013), in Iran (Sobhani et al. 2014), in Japan 18 (Murayama 2001) and in hilly regions in Italy (Brunori et al. 2006). Our results suggest that 19 buckwheat cultivation could be profitably introduced into Mediterranean climate regions, 20 however limited water availability and high temperatures play a crucial role in determining 21 the best sowing time for both grain and forage production. In addition, shifts in sowing time 22 expose plants to a variety of day lengths, which also influence plant growth and phasic 23 development (Michiyama et al. 2005).

While the length of the entire growth cycle varied by less than 7% in response to sowing time, the thermal time was 20% higher in LSp. The higher value, 1200 °Cd, matches that reported by Edwardson (1995) for buckwheat sown in North Dakota (USA) in May. The

1 lower thermal time required by ESp and LSu plants confirms the findings of Quinet et al. 2 (2004) that short days cause early apical senescence in buckwheat, and indicates that Bamby 3 and Lileja are sensitive to the photoperiod, showing reduced plant growth in short days. Since day length increased from 13:07 to 15:27 h in ESp, ranged between 14:01 and 15:27 h with 4 5 both increasing and decreasing trends in LSp, and decreased from 13:06 to 9:12 h in LSu, this 6 suggests that buckwheat plants required a longer growth period and more thermal time when 7 they were grown for the entire crop cycle with a longer day length than 14 h and were 8 exposed to 15 h day length at initial growth stages (Arduini et al. 2016).

9 In addition to short days, in ESp and LSu, also low temperatures could have contributed 10 to the reduced plant growth. Indeed, averaged over years, mean temperatures were below the 11 optimal range for buckwheat growth of 18-23 °C (Cawoy et al. 2009) for more than half the 12 cultural crop cycle in ESp and LSu, but only for approximately one week in LSp. Thus, late 13 spring and summer sowings match the best photothermal conditions for buckwheat forage 14 production in a Mediterranean environment. However, the amount of rainfall received by the LSp plants was markedly lower than 90 mm, which is the threshold for obtaining an 15 16 acceptable forage yield of buckwheat (Marshall and Pomeranz 1982), thus the forage yield was higher in ESp than in LSp, in rainfed conditions. 17

18 Irrigation positively affected stem elongation, with increments of up to 40 cm in plant 19 height, and increased leaf and inflorescence dry matter more than twofold and increased that 20 of stems over threefold. Irrigation slightly increased forage yield also in LSu, despite the 21 higher rainfall than in ESp. This result indicates that soil moisture at planting, which is much 22 higher in spring than in late summer, is important to sustain buckwheat growth in the initial 23 stages, and that early growth influences final vegetative biomass. Since rainfall patterns in the 24 two years of the research were close to the 10-year average, the present results suggest that 25 irrigation support should be planned when buckwheat is sown in late spring or late summer.

1 With all sowing dates, plants needed approximately two weeks to pass from the first 2 green to the first brown achene stage. During this period, forage production increased by 3 approximately 75% in ESp and in irrigated LSp, which was a key factor in terms of the high forage yield obtained with these treatments. Primarily inflorescences and secondly stems 4 5 contributed to the yield increase between harvests, whereas leaf biomass increased only in irrigated LSp. At the brown achenes stage however, forage had a higher proportion of 6 7 inflorescences and a lower proportion of leaves, which could influence its nutritional and 8 nutraceutical value. It has in fact been reported that the concentration of total digestible 9 nutrients and polyphenolic substances differs in flowers and leaves of buckwheat (Bystricka 10 et al. 2014; Mariotti et al. 2015), and also changes within plant parts according to growth 11 stage and sowing date (Baumgertel et al. 2010; Sobhani et al. 2014). The nutritional value of 12 buckwheat forage obtained from an early spring sowing was found to be higher at the first 13 brown achene stage than at the first green achene stage, whereas the content in crude protein 14 was lower (Mariotti et al. 2015).

In the present research, grain yield responded differently from forage yield to photothermal conditions and water regime, since the highest achene yield was obtained with ESp, and the lowest achene yield was obtained with LSp, irrespective of irrigation treatments. Yield reductions in LSu and LSp were the result of a combination of adverse photothermal conditions and water stress, since they were only partly alleviated by irrigation.

In buckwheat, flower production greatly exceeds seed set (Kinet *et al.* 1985), indicating that reproductive development from flower initiation up to seed maturity is critical for grain yield determination. In our research, the analysis of yield components indicated that the primary cause of poor yield was the low number of seeds per plant, which could be attributed almost entirely to a strong reduction in the seed-set ratio, since inflorescence biomass at the first green achene stage was similar across sowing dates in rainfed conditions and even higher in LSp when irrigated. Arduini *et al.* (2016) also found that the number of inflorescences per

1 plant was almost double in plants sown in late spring compared to those sown in early spring, 2 and Quinet et al. (2004) and Kalinova and Dadakova (2013) reported that long days increased 3 flower production. Flower failure has usually been attributed either to internal factors, such as competition between organs for available resources, or to unfavourable external conditions 4 5 and, in buckwheat, high temperatures and water stress have both been suggested as important 6 factors regulating seed set (Taylor and Obendorf 2001). Slawinska and Obendorf (2001) and 7 Cawoy et al. (2009) found that temperatures exceeding 25 °C, which occurred for several 8 days during the LSp crop cycle, caused flower withering and fruit desiccation, while a 3-day 9 water deficit stress at the beginning of flowering reduced the seed set by up to 50%. Marshall 10 and Pomeranz (1982) also reported that a limited water supply induced early embryo abortion 11 and lighter mature seeds.

In our research, LSp plants produced more flowers and achenes when irrigated, however the achenes were very light, suggesting that water supply positively affected the flower and seed set, but not seed filling. Thus, we can state that, in Mediterranean climates, summer temperatures severely limit the achene yield of buckwheat sown in LSp, and that prolonged flowering induced by long days are only partially effective. In fact, according to Slawinska and Obendorf (2001), seeds initiated late in the flowering period fail to fill with seed storage reserves in the embryo and endosperm and scarcely contribute to yield.

19 The lower grain yield obtained with LSu compared to ESp was probably due to the 20 detrimental effect of low temperatures on flowering and, especially, on fruit ripening 21 (Funatsuki et al. 2000; Arduini et al. 2016). Cawoy et al. (2009), reported delayed and 22 reduced flowering for temperatures lower than 15 °C, and fruit abortion for less than 10 °C, 23 and in both years of our research these were the conditions experienced by LSu plants during 24 the reproductive phase. It is worth noting however that neither resources availability nor 25 photothermal conditions appeared to limit seed filling in this season, since we found that 26 mean seed weight was higher in LSu than in ESp plants.

1 All summarized, the present research highlights that buckwheat is suitable for cultivation 2 in plain regions of Mediterranean Europe for the production of both forage and grain, 3 however the choice of sowing date is crucial for acceptable yields. Early spring was found to 4 be the best sowing time for both forage and grain production in rainfed conditions. However, 5 while soil water from autumn and winter rainfall proved to be sufficient to sustain plant 6 growth in this period, low temperatures and short days can limit vegetative growth and forage 7 yield. To increase forage production, buckwheat should be sown at the end of spring, however 8 irrigation is necessary for crop growth.

9 Our results clearly indicate that, irrespective of water supply, late spring sowings are not 10 suited to grain production in a typical Mediterranean environment, because of the negative 11 effect of high temperatures on flower fertilisation and seed filling. In order to escape high 12 temperatures and drought, buckwheat can also be sown at the end of summer, however short 13 days and low temperatures considerably reduce forage production, while grain yield is 14 acceptable. For late summer sowings, we also suggest that irrigation support should be 15 planned in order to sustain initial plant growth, especially in years with a prolonged summer 16 drought.

In Mediterranean climate regions, buckwheat could thus be profitably introduced as a minor summer crop, in early spring, for grain production, and late spring for forage production. Considering that buckwheat has limited requirements in regard of tillage, it could be sown in late spring to obtain a second forage crop after the harvesting of a forage winter cereal. Alternatively, buckwheat could also be sown as a second crop at the end of summer, for grain production.

The two varieties that we tested - Bamby and Lileja - responded similarly to treatments, but differed slightly in size and harvest index, so that Bamby might be more suitable for forage, and Lileja for grain. Finally, with all treatments forage yield was higher when '1-2 brown achenes are visible at the base of the first inflorescence developed on the main stem'

1	(stage 85, Arduini scale), which could, therefore, be taken as a reference stage for the harvest
2	of buckwheat forage.
3	
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23	

- 1 Tables
- 2

3

Table 1. Duration (days), and accumulated thermal time (°Cd) and rainfall (mm) from

- 4 sowing to harvests of buckwheat, as affected by sowing time in the two years of the
- 5

research.

			Harvest stage		
Year	Sowing time	Variable	Green achenes	Brown achenes	Maturity
2012	Early spring	date	06 June	19 June	11 July
	(17 April)	days	50	63	85
		thermal time	525	704	1048
		rainfall	114.0	129.4	132.0
	Late spring	date	02 July	16 July	16 August
	(24 May)	days	39	53	84
		thermal time	556	777	1259
		rainfall	21.8	22.8	26.0
	Late summer	date	12 October	25 October	30 November
	(4 September)	days	38	51	87
		thermal time	548	709	988
		rainfall	100.8	137.2	425.8
2013	Early spring	date	27 May	11 June	5 July
	(8 April)	days	49	64	88
		thermal time	523	701	1044
		rainfall	119.0	148.0	150.6
	Late spring	date	4 July	19 July	17 August
	(27 May)	days	38	53	82
		thermal time	506	745	1211
		rainfall	31.6	33.2	44.8
	Late summer	date	10 October	23 October	27 November
	(3 September)	days	37	50	85
		thermal time	527	689	1032
		rainfall	144.2	204.2	309.0

 1
 Table 2. Dry weight of leaves, stems and inflorescences (g m⁻²) at the first green
 2

 2
 and first brown achenes stages, as affected by the sowing time x irrigation x stage

 3
 of harvest interaction. Data are the means-±SD of two years, two varieties and

 4
 three replicates.

 5
 For each plant part, values followed by the same letter are not statistically

different for $P \le 0.05$.

			G : /:	
_			Sowing time	
Harvest stage	Irrigation	Early spring	Late spring	Late summer
			Leaves	
Green achenes	Rainfed	65.2 <u>±5.3</u> c	47.2 <u>±2.7</u> b	43.6 <u>±3.5</u> ab
	Irrigated	66.5 <u>±5.9</u> c	77.3 <u>±8.3</u> d	43.5 <u>±2.2</u> ab
Brown achenes	Rainfed	62.5 <u>±4.7</u> c	48.0 <u>±2.9</u> b	38.9 <u>±1.9</u> a
	Irrigated	63.8 <u>±7.4</u> c	94.9 <u>±7.4</u> e	46.7 <u>±3.3</u> b
			Stems	
Green achenes	Rainfed	133.9 <u>±15.2</u> d	99.9 <u>±7.3</u> bc	70.3 <u>±7.9</u> a
	Irrigated	137.9 <u>±8.5</u> d	212.0 <u>±23.4</u> e	78.0 <u>±6.6</u> ab
Brown achenes	Rainfed	205.6 <u>±12.8</u> e	104.8 <u>±9.6</u> c	70.3 <u>±2.9</u> a
	Irrigated	203.5 <u>±25.3</u> e	349.2 <u>±32.3</u> f	95.1 <u>±6.1</u> bc
		Inflorescences		
Green achenes	Rainfed	21.5 <u>±1.7</u> a	27.6 <u>±2.3</u> a	32.8 <u>±3.9</u> ab
	Irrigated	20.6 <u>±1.2</u> a	43.7 <u>±4.3</u> b	27.4 <u>±3.1</u> a
Brown achenes	Rainfed	101.5 <u>±7.1</u> d	59.1 <u>±5.2</u> c	113.2 <u>±7.7</u> d
	Irrigated	117.2 <u>±11.1</u> e	149.1 <u>±27.7</u> g	133.4 <u>±5.7</u> f

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1	Table 3. Dry weight of stems and inflorescences, as affected by the
2	stage of forage harvest x variety interaction. Data are means <u>±SD</u>
3	of two years, three sowing times, two irrigation treatments and
4	three replicates.
5	Within a column, values followed by the same letter are not
6	statistically different for $P \le 0.05$.

Harvest stage	Variety	Stems (g m ⁻²)	Inflorescences (g m ⁻²)
Green achenes	Bamby	129.6 <u>±22.6</u> b	30.9 <u>±4.1</u> a
	Lileja	113.8 <u>±20.3</u> a	27.3 <u>±3.8</u> a
Brown achenes	Bamby	185.3 <u>+43.3</u> d	104.7 <u>±17.0</u> b
	Lileja	159.2 <u>±36.1</u> c	114.6 <u>±15.8</u> c

7

8

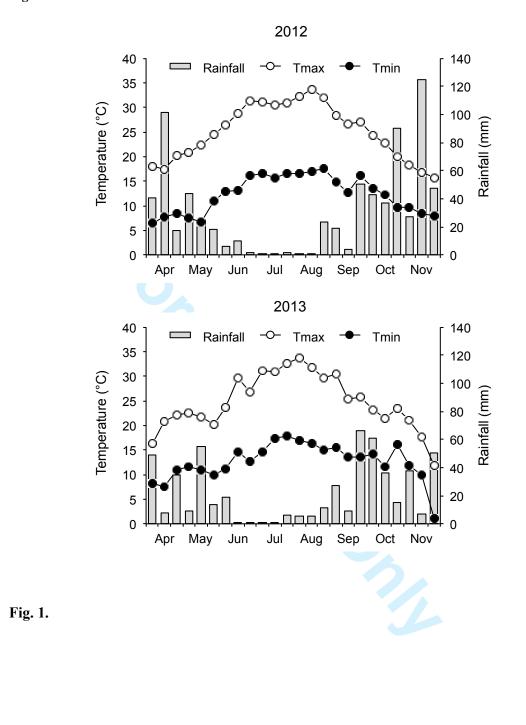
1	Table 4. Mean achene weight and harvest index of buckwheat at
2	maturity, as affected by the mean effects of sowing time and
3	variety. Data are means <u>±SD</u> of two years, two irrigation
4	treatments, two varieties or three sowing dates, and three
5	replicates.
6	Within a mean effect and column, values followed by the same letter
7	are not statistically different for $P \le 0.05$.

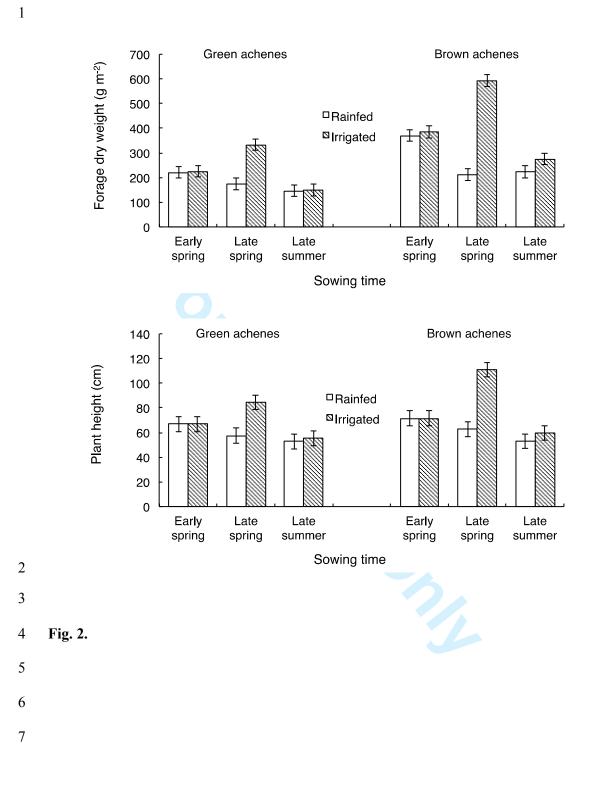
Treatment	Mean achene weight (mg)	Harvest index (%)
	Sowing time	
Early spring	22.7 <u>±0.5</u> a	48.8 <u>±2.1</u> a
Late spring	10.6 <u>±1.1</u> b	12.9 <u>±1.2</u> b
Late summer	25.6 <u>±0.9</u> c	52.4 <u>±1.4</u> c
	Variety	
Bamby	18.7 <u>±2.6</u> a	35.8 <u>±6.7</u> a
Lileja	20.3 <u>±2.6</u> b	40.7 <u>±7.0</u> b

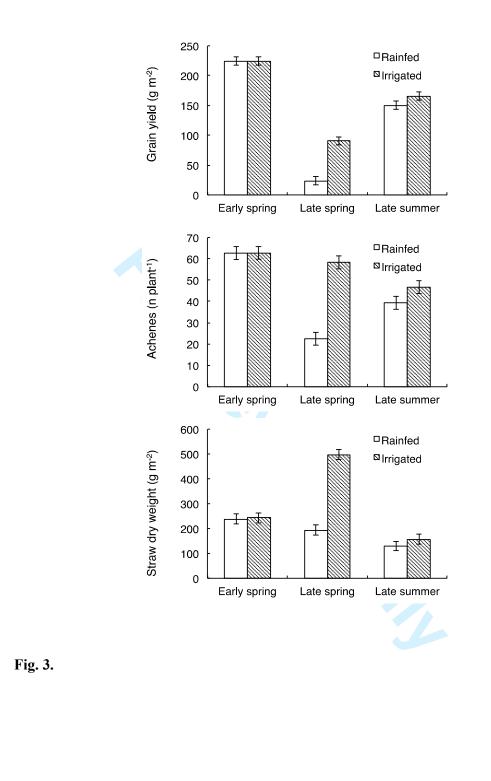
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Figures







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2	
3	Figure captions
4	
5	Fig. 1. Decadic rainfall, and maximum and minimum temperatures over the research periods:
6	April-November 2012 and 2013.
7	
8	Fig. 2. Forage dry weight and plant height of buckwheat at the first green and first brown
9	achenes stages, as affected by the sowing time x irrigation x stage of harvest interaction. Data
10	are the means of two years, two varieties and three replicates. Vertical bars represent LSD for
11	$P \le 0.05.$
12	
13	Fig. 3. Grain yield, number of achenes per plant and straw dry weight of buckwheat at
14	maturity, as affected by the sowing time x irrigation interaction. Data are the means of two
15	years, two varieties and three replicates. Vertical bars represent LSD for $P \le 0.05$.
16	