Title

Forage production and nutritional characteristics of buckwheat as affected by maturity and conservation method

Running title

FORAGE YIELD AND NUTRITIONAL VALUE OF BUCKWHEAT

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Abbreviations: ADF, acid-detergent fibre; ADL, acid-detergent lignin; ANOVA, analysis of variance; CP, crude protein; DM, dry matter; NDF, neutral-detergent fibre; NFC, non fibrous carbohydrate; RFV, relative feed value; TDN, total digestible nutrient.

Key words: *buckwheat, hay, silage, wilting, L. plantarum*.

SUMMARY - Two experiments were carried out in 2013/ 2014 in order to evaluate the forage yield and the nutritional value of the fresh, hayed and ensiled common buckwheat. Two varieties were harvested at the Green and Brown achenes stages. The silage was produced in experimental mini-silo. The need for wilting the forage (at 35% DM) and for the addition of *L. plantarum* as inoculum were evaluated. From Green to Brown achenes, the dry matter (DM) yield, the relative feed value (RFV) and the total digestible nutrient (TDN) increased (respectively from 3 to 4 t/ha, from 136 to 152 and from 56 to 59%) while the crude protein (CP) decreased (from 14 to 10%). Compared with the fresh forage, haymaking resulted in a marked decrease of CP, RFV and TDN, while ensiling did not change the CP and slightly decreased RFV and TDN. Forage wilting worsened the silage quality, while the inoculation improved it. Quali-quantitative differences between varieties were detected.

INTRODUCTION. - Buckwheat (*Fagopyrum esculentum* Moench.) is an annual dicotyledon herb belonging to the family Polygonaceae. The stem is erect with a variable branching, bearing one, rarely two, leaves per node. Inflorescences develop in the leaf axils and at the end of stem and branches (MARSHALL, 1980). Each plant produces a lot of flowers, although only a low proportion develop into dark-hulled triangular achenes, containing one starch-filled seed (HALBRECQ *et al.*, 2005).

For many centuries buckwheat has been an important crop in various parts of the world, especially in the central and northern regions of eastern Europe, Canada and the United States. In the period following the last World War, the area cultivated with buckwheat has undergone a drastic reduction due to multiple causes, such as changing eating habits of populations and the lack of selected varieties. In Italy buckwheat is cultivated in limited areas in the Alps and the Apennines,

especially in the provinces of Sondrio, Bolzano and Lucca, with production limited to local consumption (BRUNORI *et al*., 2006; TALLARICO *et al*. 2008).

In recent years there is a novel interest in buckwheat cultivation, driven by the rising demand for its products, primary the gluten-free and high biological value flour (KAUR *et al*., 2015). The entire plant also contains several compounds that can be used for the production of nutraceutical preparations and functional foods (AHMED *et al*., 2014).

The crop cycle of buckwheat is quite short, lasting 9-12 weeks in dependence on environmental conditions (AHMED *et al*., 2014). This species grows best at cool and humid conditions and the optimal temperatures for plant growth are 18-23 °C (CAWOY *et al*., 2009). In temperate climates these conditions are often achieved in mountain areas where it is traditionally sown in May-June and harvested in August-September. However, due to short crop cycle, in flat areas of the Mediterranean region, buckwheat could be sown in April and harvested in June, thus avoiding the summer drought.

Although the importance of buckwheat grain as food is known and well documented (ALVAREZ-JUBETE *et al*., 2010), buckwheat as whole plant is not commonly used in ruminant feeding. However, its suitability as a diet component has been demonstrated in dairy cows (AMELCHANKA *et al*., 2010), despite some irritating skin disorders described on light-coloured animals when continuously exposed to sunlight (DE JONG, 1972). In addition, when fed to dairy cows, the buckwheat forage was found to promote the transfer of α-linoleic acid from feed to milk (KÄLBER *et al*., 2011) and it can also contribute to mitigate ruminal methane production (LEIBER *et al*., 2012).

The optimal time to harvest buckwheat forage is not well defined, although some authors generally indicate the flowering stage (KÄLBER *et al*., 2012). Indeed, buckwheat plants are indeterminate in growth habit and flowering pattern and produce flowers essentially continuously, from three weeks after sowing to the end of the cycle (QUINET *et al*., 2004).

Obviously, in this long period changes in plant composition and structure occur, which, in turn, can influence conservation processes and the nutritive value of the forage (GOERING *et al*., 1972). Thus, the optimal time of buckwheat harvest in order to obtain the highest quali-quantitative production remains unclear. Moreover, information about the best conservation methods for buckwheat forage, like hay or silage, is scarce. In the ensiling of grasses, it is quite common to use biological additives or to wilt the forage before ensiling, so to improve the fermentation quality, increase the feeding value and reduce the production of effluent from silage (HENDERSON, 1993; DAWSON *et al*., 1999). Considering the low DM content of buckwheat harvested for forage, wilting could be considered as an option, but the changes on the chemical composition and the nutritional characteristic of silage are unknown.

Starting from above, we assessed the possibility to introduce the cultivation of buckwheat for forage production into flat Mediterranean regions with the specific aim to: i) identify the optimal phenological stage for harvest that can maximize the quantity and quality characteristics of the forage; ii) identify the influence of different conservation methods, hay or silage, on the qualitative and nutritive characteristics of the forage; iii) assess the need of wilting or the addition of bacterial inoculum to improve the quality and nutritive characteristics of the silage. In order to do that, we tested the performance of two commercial varieties for fresh, hayed and ensiled forage production in response to different maturity stages.

MATERIALS AND METHODS**. -** This paper reports two related experiments. The first one (Experiment 1) investigated the effect of year, maturity stage at harvest, and variety on the yield and chemical composition of fresh and hayed forage of field cropped buckwheat. The second experiment (Experiment 2) was carried out in the laboratory and aimed to determine the effect of year, maturity stage at harvest, variety, wilting pre-treatment and bacterial inoculation on the chemical composition of buckwheat silage.

Experiment 1. – Field trials were carried out in the 2013 and 2014 growing seasons at the Enrico Avanzi Interdepartmental Centre of Agro-Environmental Research (CIRAA) of the University of Pisa. Main soil physical and chemical properties were 43.4 % sand, 38.8 % silt, 17.8 % clay, 7.5 pH, 21.1 g kg^{-1} organic matter (Walkley and Black method), 1.71 g kg^{-1} total nitrogen (Kjeldhal method), 6.6 mg kg^{-1} available P (Olsen method), 128.1 mg kg^{-1} available K (ammonium acetate test method).

In each year treatments were two buckwheat (*Fagopyrum esculentum* Moench) varieties and two stages of maturity at harvest. The experiment was set in a split-plot design with three replicates. Harvest stage was the main plot and variety the sub-plot. Two commercial varieties were utilized, Bamby and Lileja, chosen for their wide cultivation throughout Europe. Each plot was 240 m^2 area. Buckwheat was sown on April 24th 2013 and April 17th 2014, with a 14-cm row spacing at a density of 250 viable achenes per m^2. Nitrogen, phosphorous and potassium fertilizers were applied at rates of 40, 44 and 83 kg ha^{-1}, respectively as urea, triple superphosphate and K_2SO_4. Nitrogen was applied just before seeding, while P and K before tillage.

Forage harvest was performed at the beginning of the Green achene and Brown achene stages (HALBRECQ *et al*., 2005). These stages were chosen because they fall slightly before and after the peak of flowering, which is considered corresponding to the maximum accumulation of biomass in stems and leaves (CAWOY *et al*., 2009), and have the advantage of being easily detected even in a species with an indeterminate growth such as buckwheat, where both flowers and achenes at different maturity stages are almost always present simultaneously. These stages were reached about 9 and 11 weeks after sowing, respectively.

Crop harvest was performed at 5 cm cutting height, using a sickle-bar mower. Fresh weight yield was determined in a swath of 1- by 5-m cut through the center of each plot. One forage sample of 1 kg was collected from the swath, separated into leaves, stems and inflorescences (flowers plus achenes), dried at 65° C to constant weight, and weighed to determine dry matter (DM) yield of the

fresh forage. A part of the remainder forage was immediately collected and a part was thinly spread on black plastic for 24 h (35% DM, approximately) in order to obtain, respectively, unwilted and wilted forage to use in Experiment 2. Another swath, close to the first one, was used to prepare the hay (85% DM, approximately), following the conventional haymaking technique used in the area. A 0.5 kg sample of buckwheat hay was collected for chemical analysis.

All hay and silage samples were analyzed to determine DM concentration, crude protein (CP), fat, ash, neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL), according to the methods of MARTILLOTTI *et al*. (1987). The non-fibrous carbohydrate (NFC) was estimated as follow:

$$NFC = 100 - (\%NDF + \%CP + \%Fat + \%Ash)$$

To estimate fibre quality the RFV (Relative Feed Value) was calculated. This index expresses the nutritional value of forage compared with a full bloom alfalfa, that has RFV 100. RFV is calculated from the estimates of Dry Matter Intake (DMI) and Digestible Dry Matter (DDM), as follows (ROHWEDER *et al*., 1978):

$$RFV = (DMI \times DDM)/1.29, \text{ where}$$

$$DMI \text{ (Dry Matter Intake, \% of body weight)} = 120/NDF\%$$

$$DDM \text{ (Digestible Dry Matter)} = 88.9 - (0.779 \times ADF).$$

The TDN (Total digestible nutrients) was estimated as suggested by NRC (2001).

The CP and TDN yield per unit area were calculated by multiplying the yield per hectare and the CP and TDN concentration.

Data were statistically analyzed by analysis of variance (ANOVA), using CoStat statistical package (version 6.4, CoHort Software, CA, USA). For dry weight of plant parts and relative chemical analysis, the main effect of year, maturity stage, variety and their interactions were tested. Significantly different means were separated at the 0.05 probability level by the least significant difference test (STEEL *et al*., 1997)

Experiment 2. - The ensiling experiment was carried out comparing two stages (Green and Brown achenes), two varieties (Bamby and Lileja), two wilting treatments (unwilted and wilted), two biological additives (uninoculated and inoculated). Each combination of treatments was replicated three times, resulting in 48 silages per year.

The unwilted and wilted forage was chopped into 2–3 cm pieces with a laboratory chopper and ensiled in laboratory mini-silo of 1 L capacity. Each mini-silo contained the forage at about 300 kg DM m^{-3} density. The inoculated treatment was obtained by adding to the silage 4 bacterial strains of *Lactobacillus plantarum* applied at a rate of 10^6 colony forming units (CFU) per gram of fresh matter. All 48 silages were stored at 20 °C for five months.

On silages, the same chemical-bromatological analysis, RFV and TDN performed on fresh forage and hay were carried out. In addition, the pH (aqueous silage extract), the concentrations of lactic, acetic, propionic and butyric acid and the concentration of ammonia nitrogen ($N\text{-}NH_3$ as % of total N) were determined. The lactic and monocarboxilic acids (acetic, propionic and butyric) were determined by HPLC according to the method of CANALE *et al*. (1984). The ammonia nitrogen was determined according to the method of WALL and GEHRKE (1981).

Data were statistically analyzed by analysis of variance (ANOVA), using CoStat statistical package (version 6.4, CoHort Software, CA, USA). For all characters the main effect of year, maturity stage, variety, wilting, additives and their interactions were tested. Significantly different means were separated at the 0.05 probability level by the least significant difference test (STEEL *et al*., 1997).

RESULTS AND DISCUSSION. - Because the main effects of year and its interactions with other treatments were never significant, data reported are the means of the two years. The absence of a significant year effect on the growth and nutritive characteristics of buckwheat was probably consequence of the quite similar climatic conditions during the 2013 and 2014 growing seasons.

Indeed, total rainfall from April to June was 151 mm in 2013 and 145 mm in 2014 and, in both years, the ten-days mean temperature varied from 13 to 23 °C, and the mean temperature of the entire cycle was 18° C.

Experiment 1. - Table 1 shows the ANOVA for the production and the chemical characteristics of the buckwheat forage. Buckwheat reached Green and Brown achenes stages 63 and 77 days after sowing, respectively, without any appreciable varietal difference. In this period the DM percentage of the fresh forage changed from 16 to 25% (data not shown).

The forage DM yield of buckwheat (aerial part), as the average of the two years and the two varieties, increased by about 45% from Green to Brown achenes stage, reaching a value of about 4 t/ha (Table 2). From the first to the second harvest, the DM increased by 8% in the stems and, more markedly, in the inflorescences (+226%), while it did not change appreciably in the leaves. Productions obtained in the present research were slightly lower than those reported by KÄLBER *et al*. (2012) in Switzerland (4.4 t/ha), where buckwheat was sown in the summer and suggests that, probably, sowing in April was slightly limiting due to low temperatures. Even though, it seems the only sowing time that can ensure a sufficient rainfall in the Mediterranean plain.

Averaged of the two stages, the forage production was by about 20-30% higher for the variety Bamby than for the Lileja, both considering the entire aerial part and separate organs (Table 2).

The proportion of DM occurring as different plant organs was amended by the means effects of maturity stage and varieties. From Green to Brown achenes the proportion of leaves within the forage decreased from 30 to 19% and that of stems from 52 to 39%, whereas that of inflorescences increased from 18 to 42% (Fig. 1). Varieties differed slightly in the proportion of leaves that was higher in Lileja (26% of the entire aerial part) rather than in Bamby (23%) (data not shown).

The chemical characteristics of buckwheat forage were primary affected by the maturity stage at harvest and, to a lesser extent, by variety (Table 3). From the stage of Green to Brown achenes there was a significant reduction in the concentration of CP, fat, ash, NDF, ADF and cellulose and a

significant increase in NFC. The highest changes occurred in CP (-28%), probably due to the decrease in the proportion of leaves previously reported.

Quite surprisingly the fibre concentration (NDF and ADF) of buckwheat decreased from Green to Brown achenes by about 10%, while RFV and TDN values increased by 12 and 4%, respectively. GIRMA *et al*. (2011) reported that, generally, the herbage yields increase with crop maturity, while their nutritive value decreases. However, we observed a different trend in buckwheat, probably because of the indeterminate growth habit, according to which flowers, green grains and mature grains are present on the plants at the same time (CAMPBELL, 1983). In particular, the decrease of the stems proportion from Green to Brown achenes, was probably responsible for the decrease in the fibre concentration and for the increase of the RFV. On the other hand, the marked increase in the inflorescences, mainly due to starch filling of the achenes, was probably responsible for the increase in the NFC and TDN values.

The RFV value of forage observed at the stage of Brown achene (152) makes it suitable for use in dairy cow feeding in the first stage of lactation (UNDERSANDER, 2003).

Averaged over the two years and the two harvest stages, significant differences between the two varieties, were detected just for some parameters (Tab. 3). The variety Lileja, compared to Bamby, presented a higher NFC value, while a lower concentration of NDF and hemicellulose, which resulted in a higher RFV value (+10%).

The yield per unit area of CP and TDN differed between harvests, and was 384 and 1518 kg/ha at the Green achenes and 404 and 2292 kg/ha at the Brown achene stages (data not shown). Thus, at the more advanced phenological stage we observed also an increase of CP and TDN per unit area. For the two varieties we detected a higher yield for Bamby than for Lileja, both for the CP (443 *vs* 345 kg/ha) and the TDN (2130 *vs* 1680 kg/ha).

As observed for the DM of the fresh forage, also in the hay the amount of crude protein, NDF, ADF and cellulose decreased from Green to Brown achenes, while the NFC increased (+25%)

(Table 4). These changes affected the RFV and TDN values, which increased by about 16 and 6%, respectively. Considering the low protein content and the high NDF content, the quality of the hay was comparable to a medium-quality mixed hay, at both phenological stages. Accordingly, the buckwheat hay could be compared to a mid maturity/mature grass hay (NRC, 2001).

Compared with the fresh forage, haymaking resulted in a decrease of the chemical and nutritional characteristics at both stages. Consequently, the RFV and TDN were adversely affected: from fresh forage to hay the RFV and TDN decreased by about 26 and 7%, respectively.

Experiment 2. – As expected, wilting increased the DM percentage of the forage, leading it to about 35% at both Green and Brown achenes stages (data not shown). The interactions between the maturity stage at harvest and the crop wilting treatment changed the pH and lactic acid concentration of the buckwheat silage (Fig. 2). Both parameters did not show appreciable variations between the two maturity stages in the unwilted crop (pH 3.8 and 14 g/kg lactic acid), while in the wilted crop pH values increased up to 4.2 and lactic acid decreased to 12.5 g/kg. Probably, the wilting of the crop, causing lower water content, reduced the access of microorganisms to the carbohydrates that they were accumulated in the brown achenes, thus causing sub-optimal fermentation that resulted in a lower acidification.

Variations depending on wilting, averaged over the other treatments, were also found in the concentrations of acetic acid and ammonia nitrogen (Fig. 2). The first increased by 33% and the second by 150%, and both trends were probably related to the higher pH.

The addition of *L. plantarum* inoculum resulted in significant changes in the chemical characteristics of the silage (Fig. 3), with no interaction with the other treatments (Table 5). The inoculum had a positive effect on the characteristics of silage, resulting in a significant decrease of pH (-0.2 point), an increase of lactic acid (+5.5 g/kg) and a decrease of both acetic acid (-0.5 g/kg) and $N\text{-}NH_3$ (-0.5%). Similar results were obtained by FILYA (2003), using the *L. plantarum* as inoculum in ensiling cereals. This is probably because the inoculants of *L. plantarum*, that is a

facultative homofermentative species, produce only lactic acid from the fermentation of hexoses (KLEEREBEZEM *et al*., 2003).

Considering the sum of all the volatile fatty acids and the lactic acid present in the buckwheat silage, in all cases it resulted that the lactic acid represented the large majority, about 90%, on average. Both butyric and propionic acid were practically absent in the buckwheat silage, with a maximum concentrations up to 0.1 g/kg, and without any change dependent on the imposed treatments (Table 5).

The main nutritive characteristics of buckwheat silage are shown in Table 6. As the average of the other treatments, from Green to Brown achenes, we recorded a reduction in the CP (-29%), ash (-17%), NDF (-11%), ADF (-9%), hemicellulose (-12%), cellulose (-11%) and an increase of NFC (+ 38%) and fat (+ 38%) concentration. These changes resulted in an improvement of the fibre quality (RFV +15%) and nutritional value (TDN +10%).

The chemical characteristics of silage were also affected by variety: in Lileja, compared to Bamby, we observed a lower concentration of NDF (-6%), ADF (-5%) and hemicellulose (-11%), while the concentration of CP (+ 6%) and ash (+ 11%) and RFV (+ 9%) increased.

The ensiling process did not change appreciably the CP and ash concentration compared to the fresh forage, while it mainly affected the NFC, probably because of the fermentation of carbohydrates, which decreased by 14% at the Green and by 9% at the Brown achenes stage. On the other hand, structural carbohydrates (ADF and ADL) increased. These changes led to a slight worsening of RFV and of the nutritional value (TDN). The hemicellulose concentration decreased from the fresh to the ensiled forage (by 11-19% in the two growth stages), probably because hemicellulose was broken into simple carbohydrates by enzymes and microorganisms under the acidic environment. The simple carbohydrates are then more easily utilized by microorganisms (MCDONALD, 1981; MATSUOKA *et al*., 1997).

CONCLUSIONS. - The research, carried out in the field and in the laboratory, has highlighted the main productive and nutritional characteristics of the fresh, hayed and ensiled forage of buckwheat, in relation to the growth stage of the plants and the varieties used.

Results showed that the shift of buckwheat harvest from the Green to the Brown achenes stage allowed to obtain a marked increase in the forage yield, mainly because of the increase in inflorescences (flowers plus achenes). This also modified the chemical and nutritional composition of the forage, which showed a reduction in the protein and fibre fractions and an increase in the non-fibrous carbohydrates and, therefore, in the nutritional value. Best yield were almost 4 t/ha DM, 400 kg/ha CP and 2300 kg/ha TDN.

Varietal differences appeared minor. However, in general, in our experiment the variety Bamby was more productive than the Lileja, while the Lileja showed a slightly higher quality, probably because of its higher leafiness.

Forage conservation caused a reduction in its nutritional quality. The greater reductions, compared to fresh forage, occurred in the hay, and concerned especially the concentration of proteins (losses of about 30%), of non-fibrous carbohydrates (-23%) and to a lesser extent of TDN (-6%). In silage, indeed, losses were not practically verified in the protein concentration, and were low for both non-fibrous carbohydrates and in the nutritional value.

The experimental design concerning ensiling, planned to evaluate the need of wilting the forage at 35% DM and the addition of *L. plantarum* as inoculum, showed that wilting generally worsened the nutritional characteristics of silage, while the addition of the inoculum improved it.

In conclusion, the forage obtained from buckwheat showed satisfactory nutritional characteristics compared to other forages in the same condition. Moreover, the silage presented low pH and good nutritional properties in terms of high levels of lactic acid, low levels of butyric acid and moderate ammonia concentrations, resulting in acceptable intake levels by ruminants.

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TABLES

TABLE 1. - *ANOVA of the forage yield and chemical analysis of buckwheat*.

	Source of variation: year (A) x stage (B) x variety (C)†	
Character	**Stage (B)**	**Variety (C)**
	Forage yield	
Leaves	ns¶	*
Stems	*	**
Inflorescences	**	*
Aerial part	*	**
	Chemical analysis on fresh forage	
Crude protein	**	*
Fat	*	ns
NFC	*	*
Ash	**	ns
NDF	*	**
ADF	*	ns
ADL	ns	ns
Hemicellulose	ns	**
Cellulose	*	ns
RFV	*	**
TDN	*	ns
	Chemical analysis on hay	
Crude protein	**	ns
Fat	*	ns
NFC	*	ns
Ash	*	ns
NDF	ns	ns
ADF	**	ns
ADL	ns	ns
Hemicellulose	*	ns
Cellulose	**	ns
RFV	*	ns
TDN	*	ns

† Only source of variations with statistically significant effects are presented.

¶ ns: not significant; *: significant for $P \leq 0.05$; **: significant for $P \leq 0.01$.

TABLE 2. - *Dry weight (g m^{-2}) of leaves, stems, inflorescences and aerial part of buckwheat, as affected by maturity stage and variety.*

Treatment	Leaves	Stems	Inflorescences	Aerial part
	——————————— Stage ———————————			
Green achenes	79.4 a†	140.3 a	50.9 A	270.6 a
Brown achenes	74.8 a	151.8 b	165.7 B	392.3 b
	——————————— Variety ———————————			
Bamby	84.4 b	165.9 B	124.1 b	374.4 B
Lileja	69.8 a	126.2 A	92.5 a	288.5 A

† in a column and mean effect values followed by the same letter are not statistically different. Capital letter for $P \leq 0.01$; small letter for $P \leq 0.05$.

TABLE 3. - *Chemical composition (% DM), TDN (% DM) and RFV of fresh forage of buckwheat, as affected by maturity stage and variety.*

Treatment	Crude protein	Fat	NFC	Ash	NDF	ADF	ADL	Hemicellulose	Cellulose	RFV	TDN
					Stage						
Green achenes	14.4 B†	2.2 b	30.3 a	12.0 B	45.2 b	29.7 b	6.2 a	15.6 a	23.5 b	135.7 a	56.3 a
Brown achenes	10.3 A	1.7 a	39.4 b	9.6 A	41.8 a	26.7 a	6.8 a	15.1 a	19.9 a	152.2 b	58.5 b
					Variety						
Bamby	12.0 a	2.0 a	33.2 a	10.8 a	45.4 B	28.6 a	6.4 a	16.8 B	22.1 a	137.1 A	56.9 a
Lileja	12.7 b	1.9 a	36.5 b	10.9 a	41.6 A	27.8 a	6.5 a	13.8 A	21.3 a	150.9 B	57.9 a

† in a column and mean effect values followed by the same letter are not statistically different. Capital letter for $P \leq 0.01$; small letter for $P \leq 0.05$.

TABLE 4. - *Chemical composition (% DM), TDN (% DM) and RFV of hay of buckwheat, as affected by maturity stage.*

Stage	Crude protein	Fat	NFC	Ash	NDF	ADF	ADL	Hemicellulose	Cellulose	RFV	TDN
Green achenes	9.3 B†	1.2 a	25.6 a	10.4 b	56.1 a	37.3 B	7.3 a	18.8 a	30.0 B	99.3 a	51.9 a
Brown achenes	7.2 A	1.9 b	30.2 b	8.6 a	54.1 a	28.6 A	7.4 a	25.5 b	21.2 A	114.7 b	54.9 b

† in a column values followed by the same letter are not statistically different. Capital letter for $P \leq 0.01$; small letter for $P \leq 0.05$.

TABLE 5. - *ANOVA of the ensiled forage of buckwheat*.

	Source of variation: year (A) x stage (B) x variety (C) x wilting (D) x additive (E)†				
Character	**B**	**C**	**D**	**B x D**	**E**
pH	*¶	ns	**	*	*
Lactic acid	Ns	ns	**	*	**
Acetic acid	Ns	ns	*	ns	**
NH_3	*	ns	**	ns	*
Crude protein	**	**	ns	ns	ns
Fat	**	ns	ns	ns	ns
NFC	**	*	ns	ns	ns
Ash	**	**	**	ns	ns
NDF	**	**	ns	ns	ns
ADF	**	*	ns	ns	ns
ADL	ns	ns	ns	ns	ns
Hemicellulose	**	**	ns	ns	ns
Cellulose	**	ns	ns	ns	ns
RFV	**	**	ns	ns	ns
TDN	**	ns	ns	ns	ns

† Only source of variations with statistically significant effects are presented.

¶ ns: not significant; *: significant for $P \leq 0.05$; **: significant for $P \leq 0.01$.

TABLE 6. - *Chemical composition (% DM), TDN (% DM) and RFV of ensiled forage of buckwheat, as affected by maturity stage and variety*.

Treatment	Crude protein	Fat	NFC	Ash	NDF	ADF	ADL	Hemicellulose	Cellulose	RFV	TDN
	Stage										
Green achenes	14.2 B†	1.3 A	26.0 A	12.1 B	49.1 B	35.2 B	8.9 a	13.9 B	26.3 B	117.2 A	50.1 A
Brown achenes	10.1 A	1.8 B	35.7 B	10.1 A	44.2 A	32.0 A	8.7 a	12.2 A	23.3 A	135.3 B	55.1 B
	Variety										
Bamby	11.8 A	1.5 a	30.2 a	10.5 A	48.2 B	34.4 a	8.9 a	13.8 B	25.4 a	121.1 A	52.4 a
Lileja	12.5 B	1.6 a	31.6 b	11.6 B	45.1 A	32.8 a	8.6 a	12.3 A	24.2 a	131.4 B	52.7 a

† in a column values followed by the same letter are not statistically different. Capital letter for $P \leq 0.01$; small letter for $P \leq 0.05$.

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FIGURES

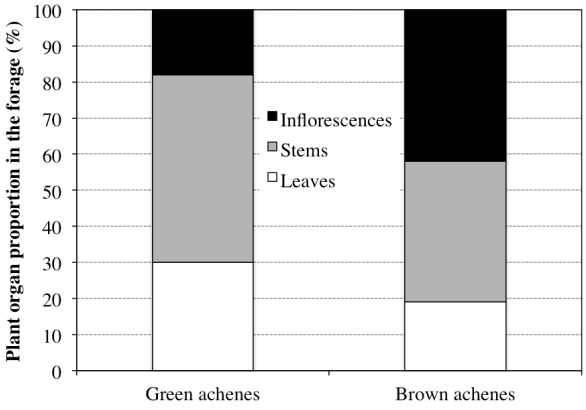

FIG. 1. - Proportion of the leaves, stems and inflorescences in the forage of buckwheat, as affected by maturity stage. All values in green and brown achenes stage are different for $P \leq 0.05$.

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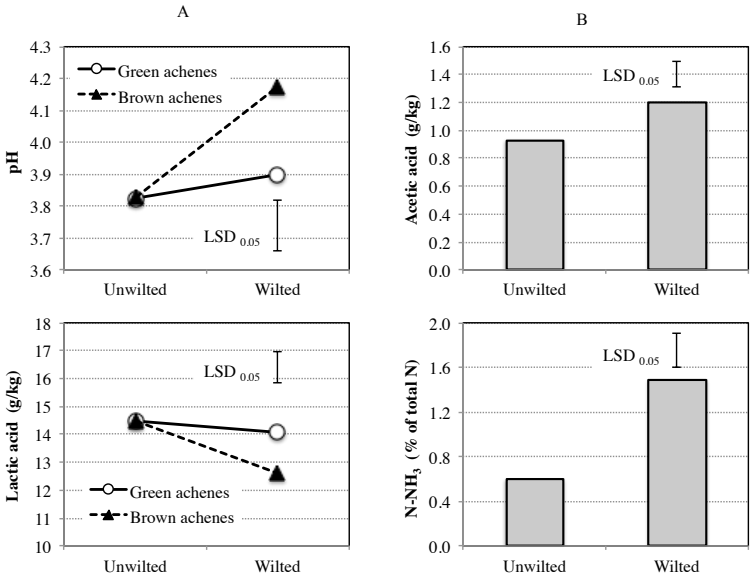

FIG. 2. - pH, lactic acid, acetic acid and N-NH_3 as affected by the interaction wilting x maturity stage (column A) and by the mean effect of wilting (B). Vertical bars represents LSD for $P \leq 0.05$.

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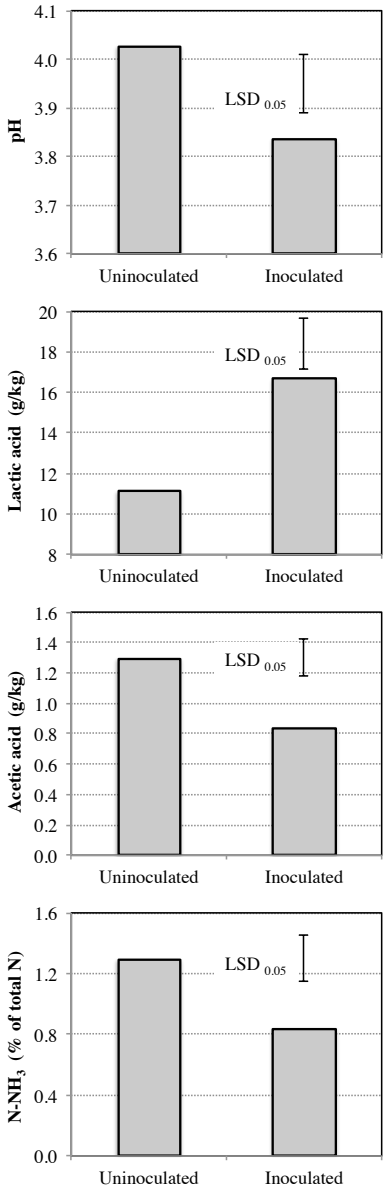

FIG. 3. - pH, lactic acid, acetic acid and N-NH_3 as affected by the mean effect of inoculation. Vertical bars represents LSD for $P \leq 0.05$.

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