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Non-thermal plasma application improves germination, establishment and productivity of Gatton panic grass (*Megathyrsus maximus*) without compromising forage quality

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ABSTRACT

Megathyrsus maximus (Gatton panic) is a tropical grass highly valued both for its use as forage and for its biofuel potential. A major constraint in establishing pastures of this cultivar is the low viability and germination of seeds and the poor initial seedling establishment. We used non-thermal plasma (NTP, partially ionised gas) as a novel technology to treat seeds of this grass, aiming to improve their quality (i.e. germination traits). We also followed the performance of seedlings grown from NTP-treated seeds under field conditions by assessing seedling establishment, biomass production and forage quality during the first regrowth period, which is the critical period for pasture establishment. Two NTP treatments were performed through dielectric barrier discharges employing N_2 as carrier gas. Non-treated seeds served as the control. Results showed that the viability of NTP-treated seeds was, on average, 1.5-fold higher than the control, and that germination energy and germination percentage of treated seeds was superior to the control by 2.1-fold and 2.2-fold, respectively. A field experiment showed that seedling establishment parameters (dynamics of cumulative emergence, emergence coefficient, and weighted average emergence rate) and pasture early productivity (represented by shoot dry matter) were enhanced by NTP treatment (phenolic sheet–polyester film barrier and 3 min exposure), showing 1.4–2.6-fold higher values than the control, confirming the results of the laboratory assays. Although NTP markedly increased the shoot dry matter production of the pasture, which was related to higher tiller population density and greater tiller weight, it did not affect the forage quality of the plants grown in the field. We conclude that NTP technology is suitable to improve seed germination of Gatton panic, in turn leading to improvements in seedling establishment and biomass production under field conditions without compromising forage quality.

Keywords: early establishment, forage quality, germination, *Megathyrsus maximus* ‘Gatton Panic’, non-thermal plasma, pasture productivity, seed quality, yield components.

Introduction

Rearing of bovine livestock ranks sixth among the main economic activities in Argentina because of high export earnings (INDEC 2021). Moreover, Argentinian consumption of beef in 2020 was ~50 kg per capita (Treboux and Terré 2021), the highest in the world. Since the 1990s, livestock enterprises, initially in the central-south of Argentina (Pampean ecoregion), were gradually displaced to the north-central part of the country (Chaco Seco ecoregion) as the natural grasslands of the Pampean ecoregion were progressively replaced by annual crops (Viglizzo *et al.* 2011; Cáceres 2015; Gasparri *et al.* 2015; Piquer-Rodríguez *et al.* 2018). The Chaco Seco ecoregion is a large plain with forest and xerophytic shrub, with patches of grasslands as native vegetation (Boletta *et al.* 2006; Giménez 2016). The soils are deep, silty-loam in texture and generally fertile. The mean temperature ranges between 18°C and 24°C, and monsoon rains vary between 400 and 1000 mm/year (Minetti *et al.* 1999; Olson *et al.* 2001; Hijmans *et al.* 2005). In general, the development of large-scale livestock enterprises has been conducted by removing native vegetation through heavy machinery and leaving relicts of the tallest trees, followed by the sowing of high-yielding, exotic C_4 grasses (Cáceres 2015; Grau *et al.* 2015; Clausen *et al.* 2020). Together with changes in herd management, this process has increased beef production 4.5-fold (Fumagalli *et al.* 1997; Fumagalli and Kunst 2002; Ferrando *et al.* 2005). The grass primarily incorporated in this productive scheme is *Megathyrsus maximus* (Jacq.) B.K.Simon & S.W.L.Jacobs (syn. *Panicum maximum* Jacq.) (Gatton panic) (Volante *et al.* 2016; Clausen *et al.* 2020; Fernández *et al.* 2020; Gaitán *et al.* 2021), owing to its tolerance to shading (exerted by the relict trees), good adaptation to edaphoclimatic conditions, and higher biomass production than native C_4 grasses (Kunst *et al.* 2006; Descheemaeker *et al.* 2014; Murray *et al.* 2016; Baldassini *et al.* 2018). Nevertheless, Gatton panic has some productivity issues related to low seed germination, poor seedling establishment and slow initial growth, which can hinder pasture establishment. Seeds of Gatton panic are frequently reported with 5–20% germination, whereas seed viability (as detected by tetrazolium test) can remain at 70–90%, suggesting that the low germination is related to seed dormancy (Song and Kalms 2007).

Commercial seed production of Gatton panic is complex because of asynchronous flowering and premature seed abscission (Hopkinson and English 1985; de Oliveira and Humphreys 1986). Freshly harvested seeds of almost all cultivars usually show low germination percentages due to uneven maturation, seed shattering and seed dormancy (Harty *et al.* 1983). Some embryos may be too immature to germinate immediately after the harvest, and must undergo a further maturation phase to reach full maturity viability (Harty *et al.* 1983; Hopkinson and English 1985; Adkins *et al.* 2002). According to Hopkinson and English (1985), immaturity and dormancy are the main reasons for the low quality of seed and are the primary cause of quality variation. Dormancy is the temporary inability of viable seeds to germinate under the same external environmental conditions triggering germination when the restrictive state ends (Cabrera *et al.* 2020). Although dormancy enables the seed to sense the environment and germinate when environmental conditions are likely to support seedling establishment, it can be a problem for commercial production, where uniform seedling establishment is desired (Adkins *et al.* 2002). Seeds of most crops possess little or no dormancy, having been selected for rapid and uniform germination through long periods of domestication. By contrast, the seed dormancy characteristics of most species of warm-season grasses have not been substantially altered by plant breeders. Therefore, dormancy can cause uneven seedling emergence in the field, resulting in an irregular stand of plants, and this might slow pasture establishment in the presence of competitive weeds (Lacerda *et al.* 2010). Therefore, dormancy-breaking technologies are needed to provide farmers with a tool to achieve the higher germination rate required for field-scale conditions.

Non-thermal plasmas (NTP) are partially ionised (quasi-neutral) gases (Randeniya and de Groot 2015) that are currently being assessed for their potential to improve seed quality, plant growth and yield (Holubová *et al.* 2020). This novel technology can control crop pathogens and enhance plant resistance to fungal infections and abiotic stresses (Feng *et al.* 2018; Pérez-Pizá *et al.* 2018, 2019, 2021). When ambient air or a similar gas mixture is used as the plasma gas, NTP consists of highly reactive species of nitrogen, oxygen and hydrogen (RNS, ROS and RHS, respectively) (Hertwig *et al.* 2018). In Gatton panic, seed dormancy appears to be related to physical constraints imposed by the seed tissues enclosing the embryo (Richard *et al.* 2016; Cabrera *et al.* 2020), which likely derive from the existence of a lock-like structure fixing the lemma and palea together (Who *et al.* 1991). Breaking of seed dormancy is considered one of the possible biological applications of NTP (Šerá and Šerý 2018; Cui *et al.* 2019) because it can provoke etching and erosion of the seed coat, thereby attenuating the seed dormancy (Šerá *et al.* 2009).

On this basis, we aimed to evaluate the effects of NTP on Gatton panic germination, seedling establishment, biomass productivity and forage quality during the early growth phase of the pasture under field conditions. After exposing seeds to different NTP treatments, seed viability, germination energy, and germination percentage were measured. Field experiments were then conducted to assess the establishment, biomass productivity and forage quality of plants grown from NTP-treated versus non-treated seeds.

Materials and methods

Non-thermal plasma source

The volume dielectric barrier discharge used for seed treatment consisted of a needle-array power electrode and a ground plate electrode covered by a dielectric barrier of either two polyester films (100 μm thick, commercial name Mylar; DuPont Teijin Films, Chester, VA, USA) or an arrangement of a thin phenolic sheet (2.5 mm thick, commercial name Pertinax; Wuxi Chifeng Metal Products, Wuxi, Jiangsu, China) with two polyester films. The gap between the upper surface of the seeds and the tip of the needles was fixed to 6 mm during the experiments. The power supply was a high-voltage sinusoidal waveform power supply (0–35 kV) operating at 50 Hz. Nitrogen gas (purity >99.5%) was injected into the discharge active region at a measured gas flow rate of 6 NL (normal air litre)/min.

The experimental setup was similar to that described in detail in Pérez-Pizá *et al.* (2018). The electrical parameters of the discharge were monitored by using a four-channel oscilloscope (TDS 2004C; Tektronix, Beaverton, OR, USA) with a sampling rate of 1 GS (gigasamples)/s and an analog bandwidth of 70 MHz. The discharge voltage was measured by using a high-impedance voltage probe (Tektronix P6015A, 1000×, 3 pF, 100 MΩ). The power consumption in the discharge was measured by the Lissajous method (Pipa *et al.* 2012), inserting a 2 μF capacitor in series with the discharge. Charge–voltage characteristics of the discharge for the two arrangements used are shown in Fig. 1*a*, *b*. As expected, the measured discharge power (175 W) for the two-film polyester barrier (Fig. 1*a*) was significantly higher than that for the composite barrier (60 W) (Fig. 1*b*) because of the increase in the discharge capacitance. The temperature of the discharge walls during the seed treatments was measured with an infrared handheld thermometer to ensure that it never exceeded 40°C.

Plant material and plasma treatments

Commercial seeds of Gatton panic were utilised for the experiments (mature caryopses provided by Oscar Pemán & Asociados, Córdoba, Argentina). NTP treatments were performed on these seeds following the methodology described in Pérez-Pizá *et al.* (2018). Seeds were treated with plasma exposures of 1 or 3 min depending on the dielectric barrier, constituting plasma treatments MN1 (Mylar dielectric barrier + nitrogen as carrier gas + 1 min exposure) and PMN3 (Pertinax–Mylar dielectric barrier + nitrogen as carrier gas + 3 min exposure). Non-treated seeds were used as control. Half of the seeds of each treatment were used for the laboratory assays, with the other half retained for the field experiment (Fig. 1*c*).

Laboratory experiments to assess seed viability and germination traits

Seed viability (i.e. a measure of the ability of the embryo to germinate) was evaluated on four replicates of 100 seeds,

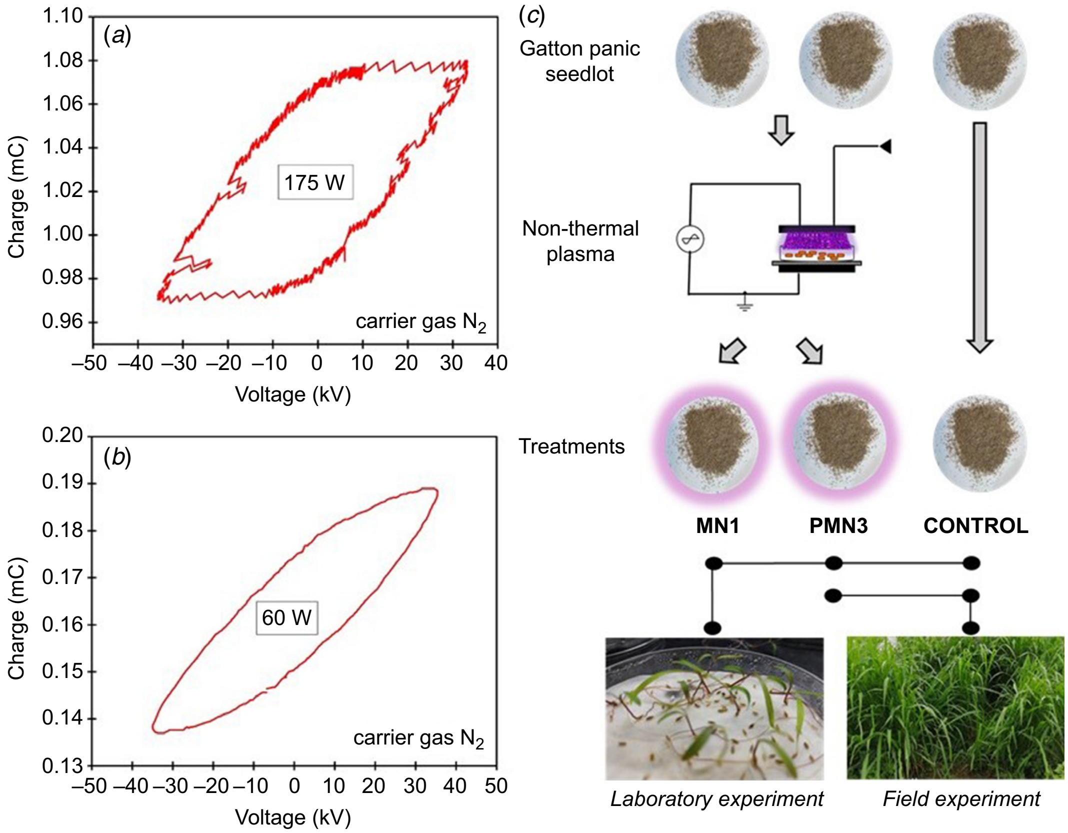

Fig. 1. Charge–voltage characteristics of the dielectric barrier discharge for the conditions used in the experiments: (*a*) two-film polyester (Mylar) barrier, and (*b*) barrier of phenolic sheet (Pertinax) with two polyester films (Mylar). (*c*) Schematic representation of the application of non-thermal plasma treatments (MN1, Mylar barrier + nitrogen as carrier gas + 1 min exposure; PMN3, Pertinax–Mylar barrier + nitrogen as carrier gas + 3 min exposure) on Gatton panic seeds and their use for laboratory and field experiments.

according to the tetrazolium test proposed by ISTA (2019) for *Panicum* spp. Four replicates of 100 seeds were analysed for germination energy (the proportion of seeds that have produced normal seedlings 15 days after sowing, with high values indicating vigorous seeds) and germination percentage (the proportion of seeds that have produced normal seedlings) following the Top of Paper germination test for *Panicum maximum* proposed by ISTA (2019); seeds were placed in Petri dishes on top of filter paper moistened with a 0.2% potassium nitrate (KNO_3) solution, and the dishes were placed in a germination cabinet under an alternating temperature regime (20°C for 16 h and 30°C for 8 h).

Field experiments to assess seedling establishment, pasture growth, yield components and forage quality

Considering the similar performance of the two NTP treatments in terms of germination improvement in the laboratory assay, only one treatment (PMN3) was selected for the field experiment. The field experiment was conducted at Quimilí (EEA INTA Quimilí), Santiago del Estero (27°38′S, 62°24′W; 137 m a.m.s.l.), in Argentina. The soil was loamy-silty, with little pedogenetic development, classified as Entic Haplustol (USDA Soil Taxonomy; Soil Survey Staff 2010), and class $\mathrm{III_{ec}}$ (risk of water and wind erosion, temperatures and lack of humidity limit its use) of the 'Land-capability classification' (Klingebiel and Montgomery 1961).

The experimental plots were arranged in a randomised complete block design with four replicates. Seeds exposed to PMN3 and the non-treated control were sown on plots of 25 m^2 (5 m by 5 m), with a distance between rows of 0.2 m, a sowing depth of 1–3 mm and a sowing density of 13.7 kg/ha (1561 filled seeds/m^2). Chemical control of weeds and hand-weeding were performed one month before sowing. After sowing, hand-weeding was performed to reduce competition from undesirable weeds and assist pasture establishment. During the experimental period, precipitation was recorded and effective precipitation (i.e. the fraction of the total precipitation that is available for crop use) was calculated according to Soil Conservation Service (1972) (Supplementary material Fig. S1). Daily minimum and maximum air temperatures were recorded and used to calculate the growing degree-days (GDD), employing a base temperature of 15.4°C (Berone 2016).

Seedling establishment evaluations were performed at 15, 17 and 22 days after sowing (DAS) (Brown and Mayer 1988; Ranal and de Santana 2006). At each date, the number of emerged seedlings per m^2 (coleoptile above the soil surface) was evaluated in 12 transects of 0.6 m, and the dynamics of cumulative emergence (cumulative number of emerged seedlings per m^2) was determined. The emergence coefficient (relationship between number of emerged seedlings and number of filled seeds) was calculated as:

$$\text{Emergence coefficient} = \frac{S_k}{X} \times 100 \qquad (1)$$

where S_k is the cumulative number of seedlings per m^2 at date k (the last date of evaluation during the emergence period), and X is the number of filled seeds sown per m^2 (i.e. 1561 seed/m^2). The daily emergence rate (average number of seedlings that emerged per day during the emergence period) was calculated as:

$$\text{Daily emergence rate} = \frac{S_k}{t_k} \times 100 \qquad (2)$$

where S_k is as above, t_k is the time interval (days) between the start of emergence and the last date of evaluation during the emergence period (k). The weighted average emergence rate (average of partial emergence speeds) was calculated as:

$$\text{Weighted average emergence rate} = \frac{\sum_{i=1}^{k} S_i/t_i}{N} \qquad (3)$$

where S_i is the number of seedlings per m^2 at date i (not the accumulated number but the number corresponding to the ith evaluation date), t_i is the time interval (days) between the start of emergence and the ith evaluation date, N is the number of moments of evaluation during the emergence period, and k is as above.

Pasture growth and yield components were assessed by determining the shoot dry matter (DM) and number of tillers at 193 GDD (34 DAS), 368 GDD (49 DAS), 490 GDD (65 DAS) and 643 GDD (83 DAS), counted from the beginning of emergence to the beginning of flowering. Samples were obtained by cutting the grass biomass at soil level in an area of 1 m^2, with a hedge trimmer. Care was taken to maintain a distance of at least 0.5 m between the last sampled areas and the new ones. Tillers were counted to obtain the tiller population density (tillers/m^2) and samples were dried at 60°C to constant weight to determine DM (g/m^2). Tiller weight (g/tiller) was calculated by the ratio between total shoot DM (g) and number of tillers. Weed DM and senescent material DM were both quantified, but due to their low proportion of the total DM (weeds <3%, senescent material <6%), they were not considered in the calculations.

Forage quality was evaluated by analysing the digestible DM, crude protein, neutral detergent fibre (NDF) and acid detergent fibre (ADF) on shoot DM samples (n = 4). Crude protein (nitrogen (N) content, including both true protein and non-protein N; total N × 6.25) was analysed by the micro-Kjeldahl technique (Bateman 1970). NDF (proportion of DM composed of hemicellulose, cellulose and lignin) and ADF (proportion of DM composed of cellulose and lignin) were determined according to Goering and Van Soest (1970) with an ANKOM Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Digestible DM, defined as the portion of DM in a feed that is digested by animals so they can use it to satisfy their nutrient requirements (Habermann *et al.* 2019), was estimated as:

$$\text{Digestible DM} = 88.9 - (\text{ADF} \times 0.779) \tag{4}$$

Statistical analyses

Statistical analyses were performed using the software package InfoStat version 3.1.2 (2014; https://www.infostat.com.ar/). Data obtained from laboratory experiments were analysed by one-way analysis of variance after testing for the assumption of normal distribution (Shapiro–Wilk test) and homogeneity of variances (Levene's test). Fisher's least significant difference (l.s.d.) tests were performed for multiple comparisons (at $P = 0.05$) of groups by means. For field data, means were compared through a mixed model and Fisher's l.s.d. tests ($P = 0.05$), where treatments were considered fixed factors and blocks were regarded as random ones. Linear regression was used to study the relationship between shoot DM and (1) digestible DM, (2) crude protein, (3) NDF or (4) ADF. Slope tests were done to compare the relationships of the NTP treatment and the control; when slopes and intercepts of regressions did not differ between groups, data were pooled, and a single regression was adjusted.

Results

Germination parameters of Gatton panic as affected by NTP treatments

All assessed germination traits (seed viability, germination percentage and germination energy) were considerably enhanced by both NTP treatments (MN1 and PMN3) (Fig. 2). Representative seedlings grown from NTP-treated seeds and the non-treated control (Fig. 2*a*) indicate an improvement in seedling length (especially of the root) in response to NTP treatment. Seed viability (Fig. 2*b*) was significantly ($P = 0.002$) increased by NTP treatment, being 1.47- and

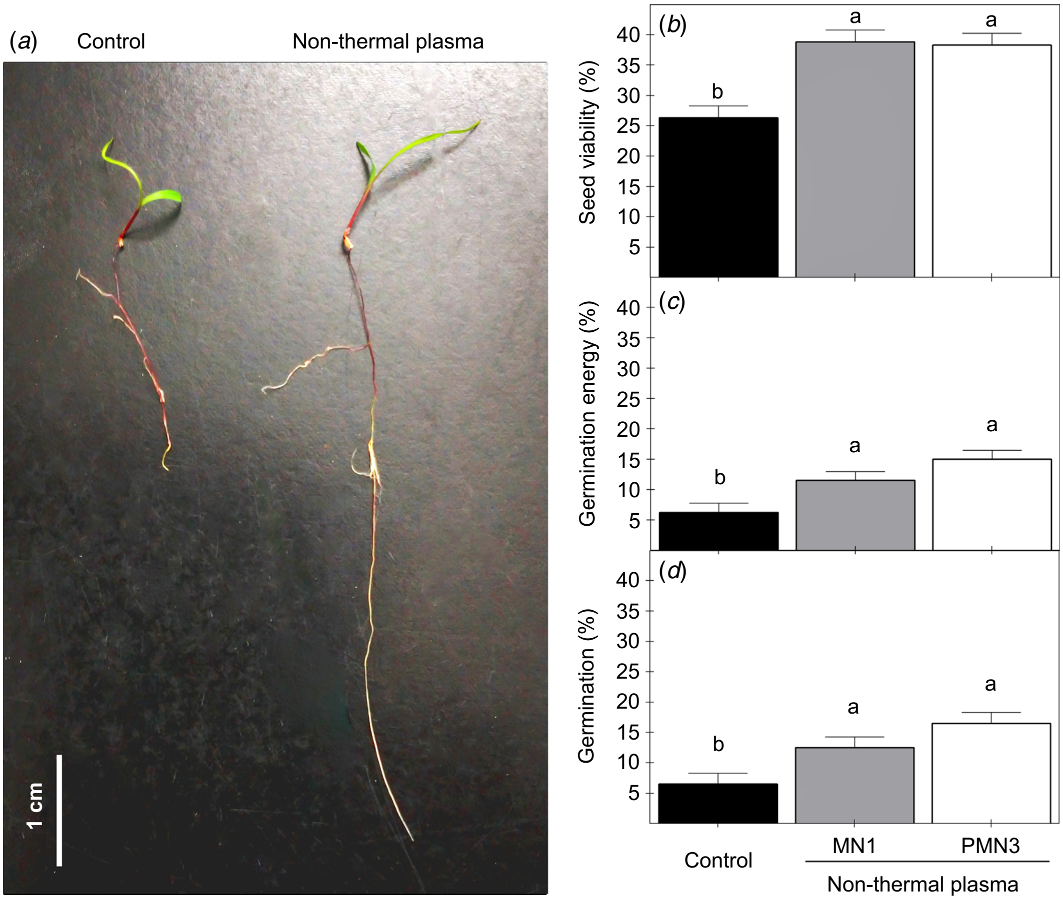

Fig. 2. Laboratory assay evaluating germination traits of Gatton panic seeds treated with non-thermal plasma (MN1 and PMN3 treatments) against non-treated controls: (*a*) representative seedlings showing growth improvement in response to seed treatment with non-thermal plasma, (*b*) seed viability, (*c*) germination energy, and (*d*) germination percentage. Capped lines indicate standard error ($n = 4$). Treatment means with the same letter are not significantly different (Fisher's l.s.d. test at $P = 0.05$).

1.46-fold higher in MN1 and PMN3 groups, respectively, than the control. Compared with the non-treated control, the germination energy of seeds increased 1.82- and 2.38-fold in MN1 and PMN3 groups, respectively ($P = 0.007$; Fig. 2*c*), and seed germination percentage increased 1.92- and 2.53-fold ($P = 0.010$; Fig. 2*d*).

Seedling establishment of NTP-treated Gatton panic seeds in the field

Seedling emergence started at 15 DAS, following cumulative effective precipitation of 50 mm from 9 to 14 DAS (Fig. S1). Three of the pasture establishment parameters (dynamics of cumulative emergence, emergence coefficient, and weighted average emergence rate) were considerably enhanced by seed exposure to NTP treatment (PMN3); only the daily emergence rate was not affected (Table 1). The number of seedlings per m^2 (represented by the dynamics of cumulative emergence) in plots planted to PMN3-treated seeds was 2.6-fold higher than in control plots at 15 DAS, and 1.5-fold higher at 17 and 22 DAS (Table 1). Measurements of the number of seedlings per m^2 finished at 22 DAS because after that, it was not possible to distinguish tiller origin (seeds vs buds) during the tillering phase. The stability observed between 17 and 22 DAS for this parameter could be explained by the soil condition: lack of moisture in the topsoil (due to low rainfall between 14 and 22 DAS; Fig. S1), and scarce stubble or crop coverage.

The emergence coefficient, which represents the percentage of full-seeded seeds that reached the emergence, was 1.5-fold higher in PMN3 treatment plots than the control (Table 1), indicating that NTP treatment led to a greater number of emerged seedlings. The speed of emergence, as indicated by the daily emergence rate, showed no significant differences between PMN3 and the control (Table 1); however, the daily emergence rate does not account for whether the emergence occurs earlier or later in the emergence period. To deal with this limitation, the weighted average emergence, which is the average of partial emergence speeds, was calculated. This new variable was considered more suitable for detecting and evaluating differences between PMN3 treatment and the control regarding the speed of emergence, because it acquires a greater value not only when the final number of seedlings is higher but also when seedlings emerge at early stages. Results showed that this variable was 1.8-fold higher in PMN3 treatment plots than in control plots, indicating that, besides the greater number, seedlings coming from NTP-treated seeds emerged earlier than those from untreated control seeds.

Shoot growth and yield components of NTP-treated Gatton panic seeds in the field

Pasture shoot growth, measured as shoot DM, was positively affected by plasma. Although the shoot DM harvested at 193 and 368 GDD did not show significant differences between PMN3 and control plots ($P > 0.07$), the cuts performed at 490 and 643 GDD showed significantly more shoot DM in PMN3 plots than the control (2.3 and 1.7 times, respectively; both $P < 0.008$, Fig. 3*a*). The results for yield components (tiller population density and tiller weight; Fig. 3*b* and *c*) revealed that this greater shoot DM in PMN3 plots at 490 and 643 GDD was related to significantly higher tiller population density (both $P < 0.040$) and greater tiller weight (both $P < 0.013$). Yield components of PMN3 plots did not differ from the control at 368 GDD, in accord with shoot DM results (tiller population density $P = 0.564$, tiller weight $P = 0.663$). Data for yield components at 193 GDD are not available.

Forage quality of Gatton panic as affected by NTP treatment

Significant linear relationships between shoot DM production and forage quality parameters were found (all $P < 0.05$). The parameters of these relationships were similar in both treatments as indicated by the slope and intercept tests

Table 1. Dynamics of cumulative emergence, coefficient of emergence, daily emergence rate, and weighted average emergence rate in plots of Gatton panic from non-thermal plasma treated seeds (PMN3) and from non-treated control seeds.

Variable		Treatments		*P*-value
		Control	PMN3	
Dynamics of cumulative emergence (no. of seedlings/m^2)	15 DAS	60.0 ± 13.0b	153.5 ± 17.6a	0.0275
	17 DAS	125.0 ± 18.0b	193.5 ± 13.5a	0.0401
	22 DAS	135.0 ± 8.0b	205.0 ± 21.7a	<0.0001
Coefficient of emergence (%)		8.6 ± 0.5b	13.1 ± 1.4a	0.0001
Daily emergence rate (no. of seedlings/day)		44.7 ± 26.6	61.4 ± 17.1	0.6110
Weighted average emergence rate (no. of seedlings/day)		39.2 ± 8.2b	71.3 ± 5.5a	0.0294

Values are mean ± standard error ($n = 4$). Within rows, means followed by different letters are significantly different (Fisher's l.s.d. test at $P = 0.05$).
DAS, days after sowing.

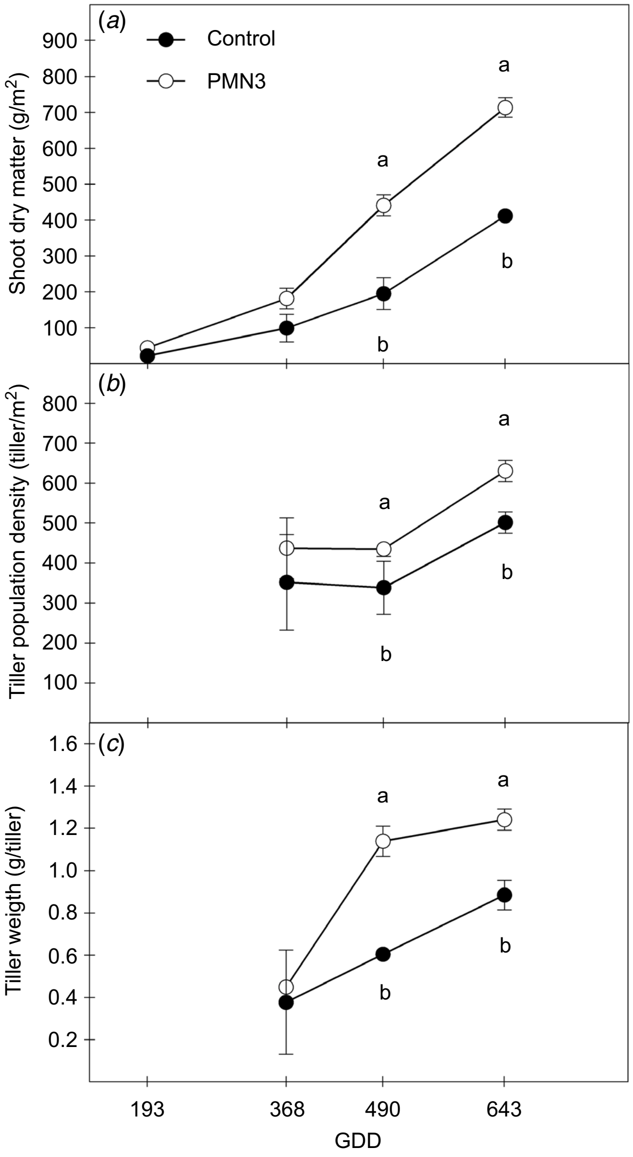

Fig. 3. Shoot growth and yield components of Gatton panic in plots sown to non-thermal plasma treated seeds and non-treated control seeds: (*a*) shoot dry matter, (*b*) tiller population density, and (*c*) tiller dry weight. GDD: cumulative thermal time with a base temperature of 14.5°C. Capped lines indicate standard error ($n = 4$). For each time, means with different letters are significantly different (Fisher's l.s.d. test at $P = 0.05$).

performed (PMN3 vs control: slope $P > 0.408$, intercept $P > 0.064$, in all cases). Therefore, the PMN3 treatment promoted shoot DM production relative to the control (see previous sections, Fig. 3) but did not change any of the analysed parameters of forage quality (DM digestibility, crude protein, NDF and ADF). Because there were no differences between treatments, their data were pooled to obtain a single linear regression for each forage quality parameter as a function of shoot DM. As expected, DM digestibility and crude protein concentration were negatively related to shoot DM production (Fig. 4*a*, *b*; $P = 0.0088$ and < 0.0001, respectively), whereas NDF and ADF were positively associated with the increase in shoot DM production (Fig. 4*c*, *d*; $P = 0.0012$ and 0.0027, respectively). Therefore, the increase in forage production, as long as the phenology of the pasture advanced, was related to an increase in NDF and ADF, and a concomitant decrease in crude protein content and DM digestibility.

Discussion

Studies were conducted to assess the effects of NTP on Gatton panic seed germination, employing two plasma treatments that differed by the type of dielectric barrier covering the ground electrode and the exposure time of seeds to plasma (i.e. MN1 and PMN3). Both treatments similarly enhanced viability, germination energy, and germination percentage compared with the control, indicating their utility in stimulating seeds to germinate faster, potentially leading to faster seedling emergence, which is crucial to reduce seed exposure to biotic and abiotic stresses in the soil. According to Cabrera *et al.* (2020), Gatton panic seeds present a type of dormancy that is strongly associated with seed covers. Holloway *et al.* (2021) found that the coleorhiza prevents root emergence of dormant caryopses; therefore, it forms part of the covering layers that control dormancy and germination in grasses. It has been suggested that seed dormancy due to seed covers can be alleviated through conventional pre-germination treatments such as physical scratching or scarification, and heat and chemical treatment (Hopkinson 1993; Adkins *et al.* 2002; Dutra *et al.* 2015). In this sense, the potential ability of NTP to produce erosion and cracks on the seed coat and to increase its hydrophilicity (Ling *et al.* 2014; Randeniya and de Groot 2015; Stolárik *et al.* 2015; Kriz *et al.* 2017; Puligundla *et al.* 2017) might explain the improvements observed in Gatton panic germination traits. Previous studies by our group back up these ideas, showing that plasma application can even stimulate the germination of non-dormant seeds such as soybean (Pérez-Pizá *et al.* 2018, 2019, 2021). These studies show that NTP improves the wettability and imbibition of seeds by eroding their coat. They also demonstrate that plasma-generated ROS and RNS can positively regulate phytohormones involved in germination. In this sense, treatment of Gatton panic seeds with NTP could have increased the permeability of seed covers by modifying the physical or biochemical properties, mimicking what a conventional treatment (e.g. physical cover removal, scarification) achieves. ROS and RNS are also known to work as potential signal molecules for seed embryo transition from the dormant to the non-dormant state (Cui *et al.* 2019). Accordingly, it can be proposed that plasma-generated ROS and RNS contributed to alleviation

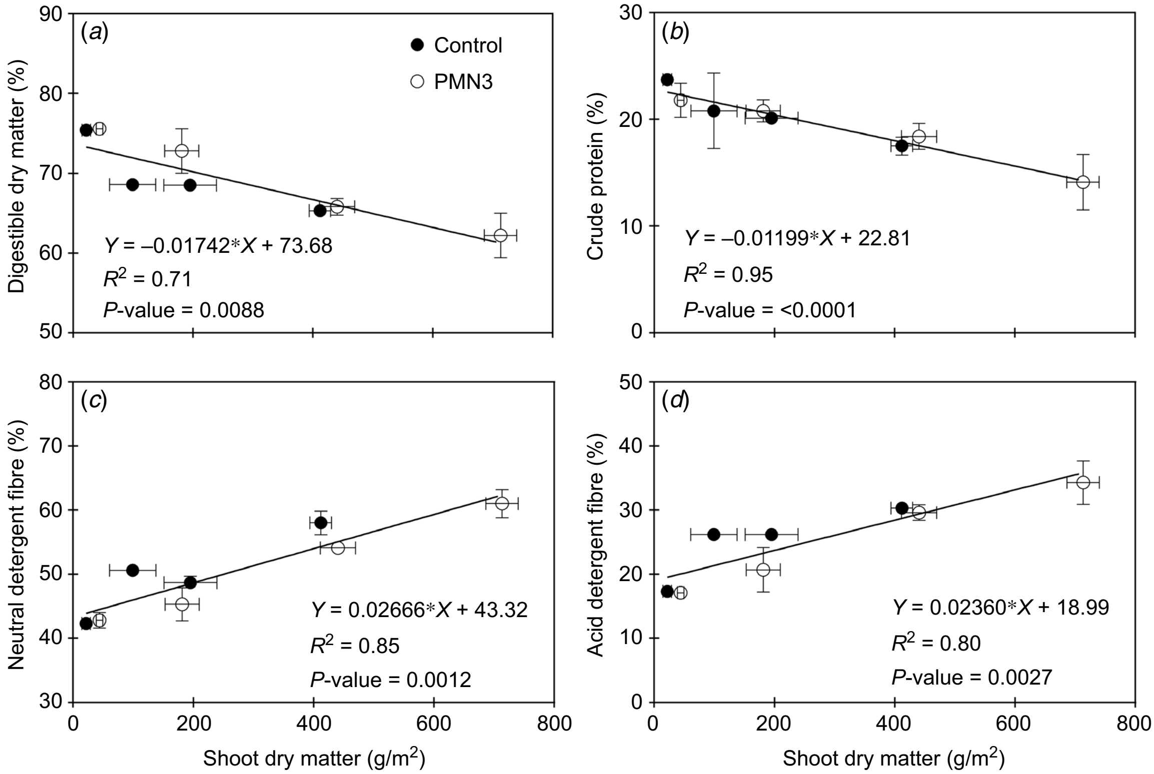

Fig. 4. Forage quality of Gatton panic in relation to shoot DM production in plots sown to non-thermal plasma treated seeds (PMN3) and non-treated control seeds: (*a*) digestible dry matter, (*b*) crude protein, (*c*) neutral detergent fibre, and (*d*) acid detergent fibre. Solid lines represent significant regressions, and the corresponding R^2 and *P*-values are presented. Capped lines indicate standard error ($n = 4$).

of Gatton panic seed dormancy, as shown in *Chenopodium album* (Šerá *et al.* 2009) and *Arabidopsis thaliana* (Cui *et al.* 2019). Regarding the improvement observed in seed viability, plasma components might have enhanced the ability of seeds to restore the integrity of cell membranes within the imbibition as suggested previously for soybean seeds (Pérez-Pizá *et al.* 2019). Another contribution of NTP to enhancing seed viability and germination could be its ability to remove pathogens from seeds, resulting in significant improvement in seed health (Pérez-Pizá *et al.* 2018, 2021). Further studies are needed to understand the mechanisms underlying the effects of NTP on seed germination traits.

Because only non-significant differences were found in the effectiveness of the two NTP treatments on Gatton panic seeds in the laboratory tests, we randomly chose just one of them (PMN3) to guide the field experiment. Nonetheless, it should be noted that the exposure time of the MN1 treatment was shorter than PMN3 treatment by a factor of 3. This is consistent with the increase in power measured for the dielectric barrier discharge (by nearly the same factor, see Fig. 1), due to the corresponding rise in the discharge capacitance. In practice, the MN1 plasma treatment could offer some advantages over PMN3, in that it allows the seed processing rate to be considerably increased, thus favouring the upscaling of this technology for the industry. A pilot-scale plasma prototype (operating at atmospheric pressure) is being validated by our group, and finding the optimal configuration of the experimental arrangement that maximises positive effects on seeds is crucial. This prototype was designed to treat ~100 kg seed/h and is aimed to be transferred to industry as an environmentally friendly processing tool for seed treatment.

In the field experiment, NTP treatment showed promising results, with seedling establishment parameters significantly improved, confirming the laboratory assay finding of a positive effect on the predisposition of seeds to germinate. This promotion of germination determined a faster seedling emergence and a greater number of seedlings per m^2 in the field. Although opportunities for comparison are minimal because there are no available data about NTP effects on grass species, we suggest that the observed improvement in seedling establishment may be related not only to enhanced germination (this contribution) but also to the already known positive effect of NTP on root growth promotion, as seen in wheat (Jiang *et al.* 2014; Dobrin *et al.* 2015) and soybean (Pérez-Pizá *et al.* 2019, 2021).

Regarding shoot growth, our results showed that NTP promoted biomass production and that this was related to an enhancement of yield components (tiller density and weight). Plasma has been shown to improve plant early growth in several species in the laboratory, but few studies have evaluated growth improvement under field conditions. Li *et al.* (2016), Kriz *et al.* (2017), and Kumar *et al.* (2017) show that plants grown from plasma-treated seeds of *Brassica napus*, *Abelmoschus esculentus* and *Arachis hypogaea* (respectively) grow faster, achieve flowering and maturity earlier, and yield more than plants grown from non-treated seeds. Those authors highlight the enhancement effect of NTP on root growth and suggest that this might be increasing the ability of plants to uptake water and nutrients from the soil.

The NTP treatment had no negative impact on the quality of the high-yielding forage produced by the pasture in the field. This finding is of particular interest, because the impact of seed treatment with plasma on the biomass quality of the resulting plants has rarely been explored. As expected from the literature, the increased shoot DM production during advanced stages of pasture growth was correlated with higher NDF and ADF values and lower crude protein and digestible DM (Callow *et al.* 2003; Donaghy *et al.* 2008; Agnusdei *et al.* 2012; Chapman *et al.* 2014). This is typical in most forage species and particularly in C_4 grasses because plants produce thickening lignification of the walls and show a reduction of cell content as they mature (Cano *et al.* 2004; Schnellmann *et al.* 2020).

Our results concerning NTP applied to Gatton panic seeds are of interest for the seed industry because plasma treatment would allow poor-quality seeds coming from the field to reach the minimum germination values required by national institutions for sale (e.g. in Argentina 15% and 25% for germination percentage and viability, respectively, quantified in pure seeds; INASE 2021), ensuring an innovative and competitive offer in the seed market. Farmers would also observe the benefits of the technology through sowing smaller volumes of seeds, which would reduce the associated costs (seeds, fuel, operating time, etc.). Furthermore, given that plasma enhances the emergence of seedlings and the supply of aerial biomass, the success of pasture establishment, which is associated with animal receptivity and, therefore, with economic indicators (Kunst *et al.* 2012), would be guaranteed by the application of this technology. Additionally, because the persistence of a perennial pasture largely depends on its early establishment (Descheemaeker *et al.* 2014; Moore *et al.* 2014; Dutra *et al.* 2015), NTP might also indirectly improve this critical parameter. Future studies will evaluate the effects of seed treatment with NTP on the performance of the pasture in successive cuts and on its persistence.

Conclusion

This study allows the conclusions that NTP technology applied to seeds of Gatton panic can effectively alleviate seed dormancy and greatly improve germination. Under field conditions, we proved that NTP applied to seeds can speed up seedling emergence and increase tiller density and shoot DM without producing a negative impact on the quality of the forage produced. With this eco-friendly technology, there is potential to minimise establishment failures, thus reducing the costs of sowing additional seeds. Future studies should address the physiological responses underlying the promoting effects of NTP and the benefits of this technology to pasture productivity and persistence.

Supplementary material

Supplementary material is available online.

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Data availability. The datasets generated during the study are available from the corresponding author on reasonable request.

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