ORIGINAL ARTICLE

Potential implications to wheat establishment due to negative effects of *Eragrostis plana* in rhizospheric soil

Henrique von Hertwig Bittencourt[1]*, Lisandro Tomas da Silva Bonome[1],
Edison Ferreira dos Santos Júnior[1], Alexandre Monkolski[2], Roger Paulo Mormul[3],
Michelangelo Muzell Trezzi[4], Jakeline Galvão de França Monkolski[2]

[1] Agronomy, Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Brazil

[2] Biology, Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Brazil

[3] Biology, Universidade Estadual de Maringá, Maringá, Brazil

[4] Agronomy, Universidade Tecnológica Federal do Paraná, Pato Branco, Brazil

Abstract

Exotic plants, such as *Eragrostis plana* in southern Brazil, may cause significant problems in agriculture. This study aimed to elucidate the influence of *E. plana* rhizosphere soil on wheat germination and initial development. Bioassays with soil sampled from an infested agroecosystem were carried out using wheat as the target species. A factorial design was used, crossing soil from the horizontal and vertical distances from the *E. plana* tiller base and considering seasons as a blocking factor. The interaction between season and vertical and horizontal soil positions influenced normal wheat seed germination, with the lowest values (69%) observed in the winter bottom and intermediate soil positions. The highest abnormal seedling percentage (17.6%) was recorded in the summer middle vertical soil position. Dormant wheat seeds were higher (7%) in the spring bottom and distal soil positions. The season was the most important factor for germination, but hypocotyl, radicle, and total wheat seedling length also varied according to soil position. Shorter hypocotyls and seedlings were registered in the summer soil surface, while shorter radicles were observed in the proximal horizontal soil position in the same season. This variable response of wheat germination and seedling development to the infested soil demonstrated *E. plana* seasonality. The influence also varied according to the distance from the plant tiller base. These findings may be used to improve *E. plana* management in infested fields and to understand the mechanism of action of its allelochemicals in future research.

Keywords: introduced species, invasive plant, South African lovegrass, weed ecology

Introduction

Agriculture has suffered negative impacts from biological invasions with varying threats among countries. Crop losses due to invasive species and pathogens are estimated at billions of US dollars per year and can compromise food security (Pimentel *et al.* 2005). Therefore, it is crucial to understand how invasive species can influence crops, which in turn can directly affect society.

The invasive species *Eragrostis plana* Nees, native to southern Africa, was introduced into South America in the 1950s. Since its introduction, *E. plana* has spread from southern Brazil to Uruguay and Argentina, becoming a highly invasive and dominant species in grassland plant communities. It was used by ranchers as forage in the 1970s until the Brazilian Ministry of Agriculture banned the spread of seeds and propagules in 1979. Despite measures taken to control its dispersion, in 2007 it was estimated that one million hectares in the Brazilian state of Rio Grande do Sul were infested by *E. plana* (Medeiros and Focht 2007).

The highly invasive potential of *E. plana* can be related to the generation of seeds with high viability (Bittencourt *et al.* 2017), a high rate of plant carbon assimilation (Botha 1992), and fast initial growth (Schefer-Basso *et al.* 2016). Moreover, *E. plana* has a high resistance to trampling, frost, and drought (Medeiros and Focht 2007) and has low palatability for cattle to graze (Quattrocchi 2012), making it a difficult weed to control.

Among the invasive features of *E. plana*, its allelochemical production (Bittencourt *et al.* 2018b), which boosts its invasion performance, can be highlighted. This mechanism, described as a novel weapon hypothesis, usually occurs in terrestrial habitats (Callaway and Ridenour 2004).

The production and release of allelochemicals depend on environmental conditions and plant phenology. Also, the amount of allelochemicals from *E. plana* in the soil depends on the distance from the tiller base (Bittencourt *et al.* 2018b). Moreover, previous studies have shown that the presence of *E. plana* can have negative impacts on germination of *Paspalum notatum*, *P. regnellii*, *Megathyrsus maximus* e *Setaria sphacelata* (Ferreira *et al.* 2008) and seedling development (Bittencourt *et al.* 2020) of palisade grass, alfalfa and wheat.

Therefore, we carried out a factorial experiment on wheat germination and seedling development using soil collected at different horizontal and vertical distances from the *E. plana* tiller base with four temporal repetitions (seasons). Our objective was to identify how the *E. plana* rhizospheric soil position and yearly seasons affect wheat germination and seedling development.

Materials and Methods

The experiment was carried out in a factorial design, crossing soil from the horizontal and vertical distances from the *E. plana* tiller base and considering seasons as a blocking factor. Soil, classified as humic cambisol (Embrapa 2004) from an infested field, was sampled in each season (autumn, winter, spring, and summer) in the municipality of Abelardo Luz, Santa Catarina State, Brazil (–26.527820, –52.255808). The soil samples which were taken considering the vertical distance from the *E. plana* tiller base were labeled as superficial (s) – 0 to 5 cm deep; medium (m) – 5 to 10 cm deep; and bottom (b) – 10 to 15 cm deep. Similarly, we considered the horizontal distance from the *E. plana* tiller base as: proximal (p) – 0 to 5 cm; intermediate (i) – 5 to 10 cm; and distal (d) – 10 to 15 cm. This generated nine different *E. plana* rhizosphere soil positions (Fig. 1).

The sampled soil was dried using an oven with forced air circulation at 40°C until reaching constant mass. Then, it was smashed and sieved in 2 mm stainless steel mesh before it was transferred to glass containers and stored in the dark at ~24°C until the bioassay was carried out with the soil from all seasons at the same time. Finally, we arranged 30 g of soil and 12 ml of distilled water on germitest paper with four replications. This approach guaranteed experimental units' independence, totalling 36 experimental units for each season.

After finalizing the soil disposal, 25 wheat seeds were added. The paper with the seeds, soil, and water was then disposed in the form of rolls and packed in transparent plastic bags for incubation at 20°C for 7 days in a Mangelsdorf

Fig. 1. Map with the sampling scheme crossing the horizontal and vertical distances from the *Eragrostis plana* rhizospheric soil taken in Abelardo Luz, Santa Catarina State, Brazil and soil processing steps to bioassay. Vertical position: s – surface, m – middle, and b – bottom. Horizontal position: p – proximal, i – intermediate, and d – distal

chamber with a 24 h photoperiod. The bags were sealed to avoid exchanging moisture and gases with the environment and external contamination of microorganisms.

Normal, abnormal, dormant, and dead seeds were counted at the end of the incubation period and converted into percentages per experimental unit. Also, the hypocotyl, radicle, and total seedling length of normal wheat seeds were measured using a digital calliper at the end of the incubation period and converted into a mean value per experimental unit. These measurements were used as our response variables in the statistical analysis.

Shapiro-Wilks test was used to verify normality, while equality of variances was checked using Levene's test. Then, a permutational analysis of variance was applied to all response variables, considering the horizontal and vertical distances from the *E. plana* rhizosphere as the main categorical factors and the seasons as a blocking factor. All analyses were performed in the R software, considering *p*-values <0.05 as significant results.

Results and Discussion

We found that seasons *per se* affected significantly only abnormal seedlings, with the highest mean in the summer soil (14.3%). Vertical and horizontal soil position *per se* did not influence the percentage of normal germination, dormant or dead seeds (Table 1).

The differences in the responses of some of the germination-related variables according to the seasons in which the rhizospheric soil was collected indicated the seasonality of *E. plana* in influencing the germination process of neighboring plants. In addition, the *E. plana* population may benefit from the germinating difficulty of other species.

However, the interaction between season and vertical and horizontal soil positions also significantly affected normal seed germination (Table 1, Fig. 2). The highest normal seedling percentage occurred during

Table 1. Statistical results for the effects of the treatments on the percentage of normal, abnormal, dormant and dead wheat seed germination

Source of variation	df	Normal		Abnormal		Dormant		Dead	
		F	p	F	p	F	p	F	p
Season (Se)	3	1.90	0.10	3.84	**<0.01**	1.43	0.92	2.11	0.06
Vertical (Ve)	2	0.66	0.24	1.85	0.15	0.23	0.84	1.15	0.36
Horizontal (Ho)	2	1.50	0.38	2.21	0.10	0.62	0.66	0.13	0.79
Se*Ve	6	3.46	**<0.01**	3.58	**<0.01**	0.80	0.78	0.95	0.56
Se*Ho	6	1.25	0.17	0.73	0.64	0.90	0.56	1.31	0.22
Ve*Ho	4	0.43	0.77	0.30	0.82	2.19	**0.04**	1.37	0.20
Se*Ve*Ho	12	2.17	**0.02**	1.00	0.39	2.95	**<0.01**	1.18	0.37
Residuals	108	–	–	–	–	–	–	–	–

Bold numbers represent significant results
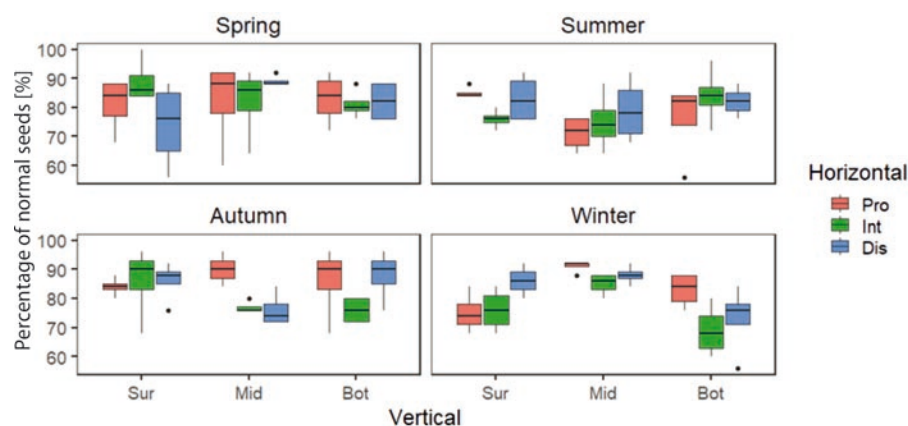
Fig. 2. Boxplots of means and standard deviations for the percentage of normal seed germination according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)

winter, in the mid-vertical position and proximal horizontal soil position (91%). In contrast, the lowest occurred during winter, in the bottom vertical and intermediate horizontal soil position (69%).

We also found that the interaction between season and vertical soil position significantly affected abnormal seedlings (Table 1, Fig. 3), with the highest percentage occurring during summer in the middle vertical soil position (17.6%). In comparison, the lowest occurred during winter, also in the middle vertical soil position (4.3%).

As there are reports of *E. plana* aqueous extracts influencing the speed of germination of target species (Bittencourt *et al.* 2020), future work could evaluate the mechanism of action of these compounds. This could elucidate how these compounds act, either by increasing the time required for seed germination or by boosting seed dormancy. In summer, the higher percentage of abnormal seedlings tends to result in fewer adult plants capable of breeding, consequently lowering yield.

As with normal seedling percentage, the interaction between season and vertical and horizontal soil positions significantly affected dormant seeds (Table 1, Fig. 4).

The highest percentage of dormant seeds occurred during spring, in the bottom vertical and distal horizontal soil positions (7%). In comparison, the lowest (0%) occurred in the pool of seven combinations between season and vertical and horizontal soil positions (mostly related to warmer seasons: spring/summer). Dormant seeds may decrease a wheat plant stand and favor the exposure of non-germinated seeds to ageing, degradation and predation by natural enemies (Dalling *et al.* 2011).

If the lowering in the number of normal seedlings and the increment in dormant seeds and abnormal seedlings that hindered wheat happens to another species, it may also decrease its frequency in the plant community in the long run (Eriksson and Ehrlén 2008). This is a significant result, given that the seed bank in southern Brazil grasslands invaded by *E. plana*
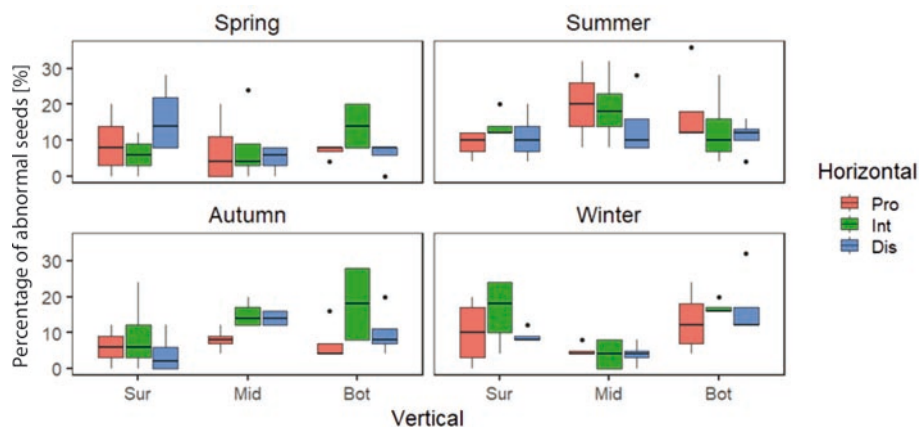
Fig. 3. Boxplots of means and standard deviations for the percentage of abnormal seed germination according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)
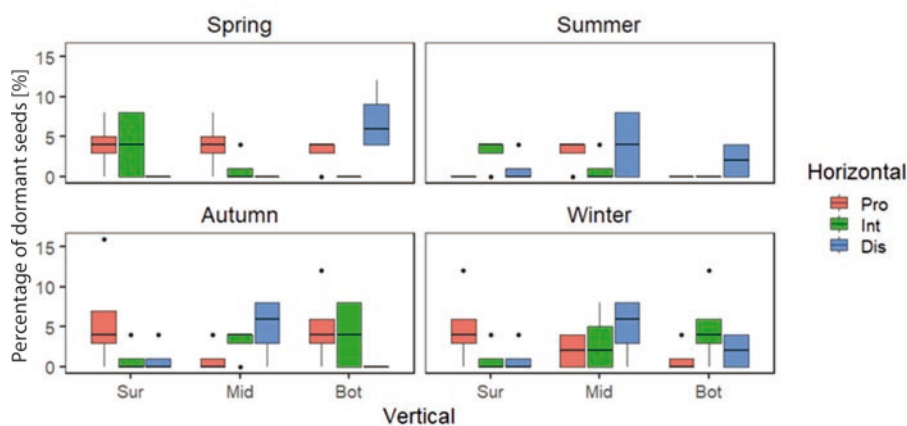
Fig. 4. Boxplots of means and standard deviations for the percentage of dormant seeds according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)

is composed mainly of warm-season grasses (Garcia 2009), which tend to be greatly influenced by the recruitment process during summer. This assumption is supported by the observations of farmers who usually report a reduction in the density of forage species in the pastures invaded by *E. plana*.

The spatial influence of *E. plana* rhizospheric soil observed on wheat germination is also important for understanding the dynamics in which the interaction with neighboring plants occurs. The season and spatial variations observed for some wheat variables may occur as a function of when and how substances biosynthesized by *E. plana* are released into the environment and their behaviour in the soil. Plant allelochemicals can enter the environment either by being released by exudation and volatilization or through tissue degradation and may be altered in the soil after being released by donor plants to be more or less toxic to the target plants (Zimdahl 2018).

Our results indicate that more than one of these allelochemical releasing processes occur during seasons, due to the spatial differences in wheat germination and initial seedling development suppression. These differences may also be related to *E. plana* physiological age since these plants synthesize different secondary metabolites throughout their phenological cycle (Scheffer-Basso *et al.* 2019).

The observed inhibition or delay in wheat germination may be related to the presence of phenolic compounds, such as ferulic, caffeic, and *p*-coumaric acids. These compounds are known for inhibiting the synthesis and action of gibberellins (Chen *et al.* 2022). They also influence the genetic expression of the amylases in plants (Singh *et al.* 2009), delaying the mobilization of the seed reserves without directly compromising structures such as cells or organelles (Levitt *et al.* 1984). Future research can explore the interaction

between *E. plana* allelochemicals and genetic expression in target plants to reveal the action mechanisms of these substances.

Regarding dead seeds, a marginal difference was registered (Table 1, Fig. 5), with the highest dead seed percentage occurring in spring soil (6.2%).

Wheat seedling development was influenced by treatments and their interactions. The season was the most critical factor affecting seedling development, with significant effects on hypocotyl, radicle, and total seedling length (Table 2).

The decrease in growth of the wheat seedlings may also be associated with the activity of *E. plana* allelochemicals previously identified in the soil solution (Bittencourt *et al.* 2018b). Additionally, the aqueous extract of the *E. plana* has already demonstrated the ability to suppress wheat's initial development, reducing seedling size compared to the control with distilled water (Bittencourt *et al.* 2020).

Results obtained by several authors allowed Gniazdowska and Bogatek (2005) to highlight the interference of allelochemicals in processes that demand energy from plant cells. Among phenolic acids, the allelochemical action in the target plant is the alteration of ion absorption, such as P (Lyu *et al.* 1990), which can compromise plant development. The reduction in ion absorption may result from decreased respiration and ATP synthesis by the cells of the radicle (Gniazdowska and Bogatek 2005). These disorders can possibly occur in target plants that get *E. plana* allelochemicals and may be the subject of further research.

However, the effect of seasons on hypocotyl length depends on the vertical soil position, with longer hypocotyls occurring during spring in the bottom vertical soil position (95 mm) and shorter
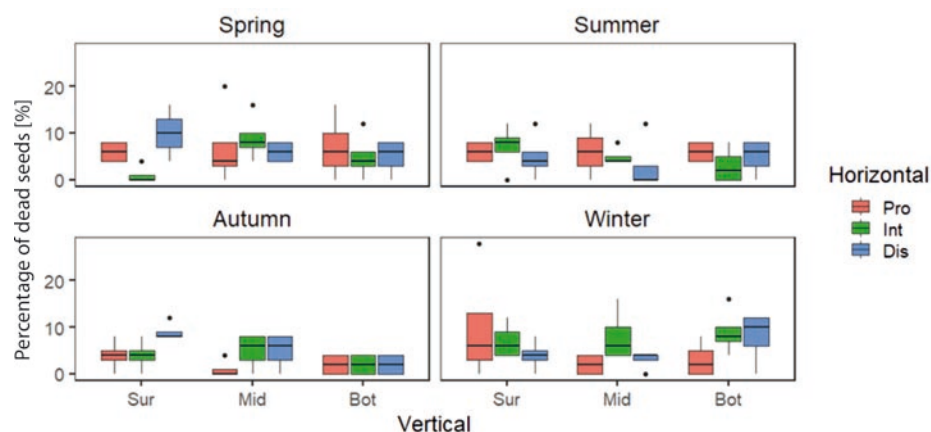
Fig. 5. Boxplots of means and standard deviations for the percentage of dead seeds according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)

Table 2. Statistical results for the effects of the treatments on the length of hypocotyl, radicle and total wheat seedlings

Source of variation	df	Hypocotyl length		Radicle length		Total length	
		F	p	F	p	F	p
Season (Se)	3	99.10	**<0.01**	6.67	**<0.01**	31.76	**<0.01**
Vertical (Ve)	2	0.56	1.00	1.80	0.11	1.78	0.29
Horizontal (Ho)	2	2.17	0.13	0.18	0.94	0.34	0.64
Se*Ve	6	18.49	**<0.01**	5.66	**<0.01**	12.72	**<0.01**
Se*Ho	6	1.92	0.06	3.68	**<0.01**	1.97	**0.04**
Ve*Ho	4	2.26	**0.02**	1.09	0.39	0.54	0.83
Se*Ve*Ho	12	1.46	0.22	0.81	0.58	1.11	0.39
Residuals	108	–	–	–	–	–	–

Bold numbers represent significant results

hypocotyls occurring during winter in the bottom vertical soil position (75 mm, Fig. 6). Also, vertical and horizontal soil positions significantly affected hypocotyl length independent of the season (Table 2). Longer hypocotyls occurred on the vertical surface and in the intermediate horizontal soil position (90 mm), while shorter hypocotyls occurred in the surface and proximal soil positions (86 mm, Fig. 6).

The wheat radicle and hypocotyl length were identified as variables sensitive to the influence of *E. plana* rhizospheric soil, mainly in summer. It is assumed that this seasonal effect is related to the *E. plana* phenological cycle. In addition to these hypothetical statements, *E. plana* senescence was observed in fields between March and April, when the biosynthesis of allelochemicals in the different parts of the plant should drop considerably. Omezzine *et al.* (2014) observed in *Trigonella foenum-graecum*, which is recognized for its allelopathic properties, that the bioactive compounds were primarily produced during the stages of growth and reproduction, decreasing significantly with senescence.

Maybe for this reason, the effect of seasons depends on the vertical soil position to affect radicle length (Table 2), with longer radicles occurring in winter on the soil surface (152 mm) and shorter radicles occurring during summer on the soil surface (140 mm, Fig. 7). Moreover, the season also interacted with the horizontal soil position. In this case, longer radicles occurred in winter in the proximal soil position (151 mm), and a shorter radicle occurred in summer in the proximal soil position (139 mm).

Considering that our sample window includes both the *E. plana* senescence (autumn and winter) and the active phases (spring and summer), temporal fluctuations in the production and release of allelochemicals in the environment were expected. Further research can explore how and which *E. plana* allelochemicals are synthesized and released into the environment at different stages of plant development and tissues.
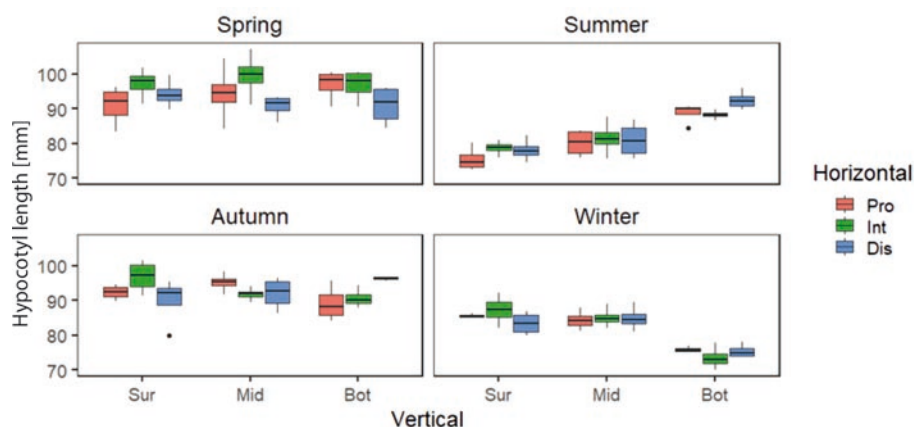
Fig. 6. Boxplots of means and standard deviations for the seedling hypocotyl length according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)
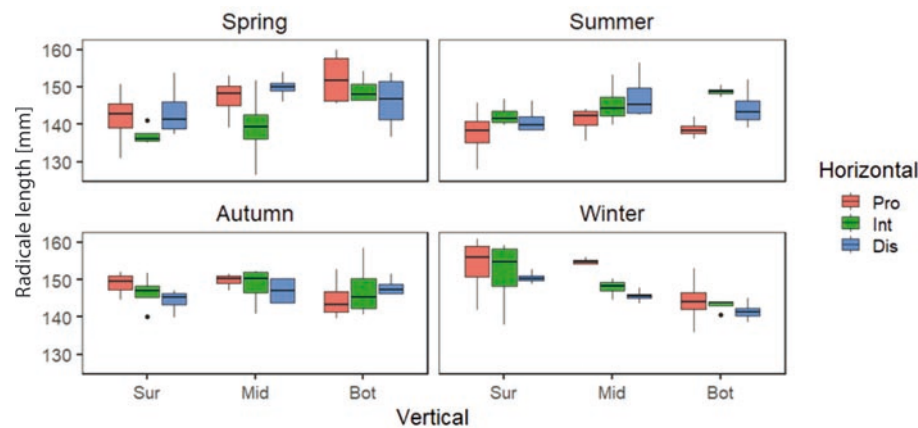
Fig. 7. Boxplots of means and standard deviations for the seedling radicle length according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)
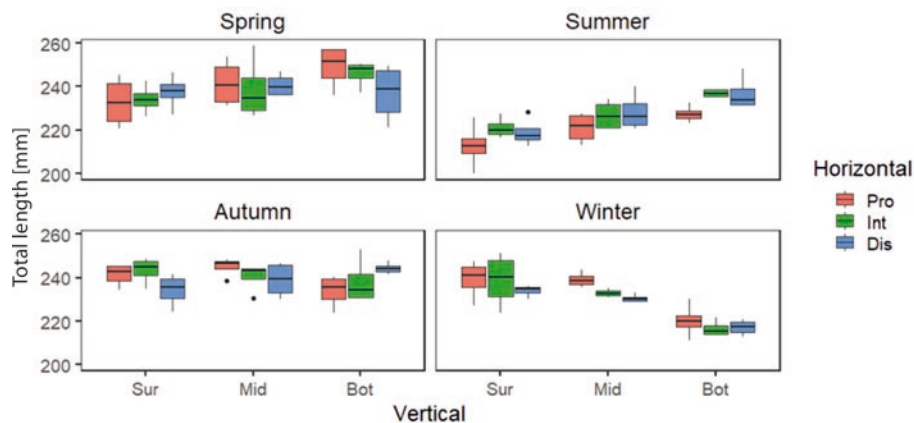
Fig. 8. Boxplots of means and standard deviations for the seedling total length according to vertical and horizontal soil position in each season. Vertical positions: Sur – Surface (0–5 cm deep), Mid – Middle (5–10 cm), and Bot – Bottom (10–15 cm). Horizontal position: Pro – Proximal (0–5 cm far from the *Eragrostis plana*), Int – Intermediate (5–10 cm), and Dis – Distal (10–15 cm)

Finally, we found that season interacted with vertical soil position and significantly affected total seedling length (Table 2). Longer seedlings occurred during spring in the bottom vertical soil position (244 mm), and shorter seedlings occurred during summer on the soil surface (217 mm, Fig. 8). Similar to radicle length, the season also interacted with horizontal soil position to impact the total seedling length. In this case, longer seedlings occurred in spring in the proximal soil position (241 mm), and shorter seedlings occurred in summer in the proximal soil position (220 mm).

Besides *E. plana* allelochemical production and release, we believe that the soil type and climatic phenomena can also modulate the differences observed in wheat germination and initial seedlings development. The humic cambisols of our experiment are characterized by their high-water storage and cation exchange traits, which probably support the maintenance of toxic

compounds released by *E. plana* in the soil solution. In addition, the presence and concentration of other organic and inorganic compounds already present in the soil may interact with the allelochemicals to determine their toxicity on the target plants (Blum 2011). These hypotheses regarding allelochemical interaction with the soil are endorsed by the results of previous studies that recorded an increase in the phytotoxicity of *E. plana* aqueous extract to wheat when the plant dry aerial part was mixed with soil and incubated before extraction in comparison to plant dry aerial part incubated without soil (Bittencourt *et al.* 2018a).

Meteorological data from a meta-analysis study by Buffon and Binda (2013) from the 1960s to the 1990s in the city where the soil was sampled showed that the rainfall fluctuates considerably between seasons. The autumn period is characterized by rainfall ranging from 140 to 150 mm, followed by an arid winter when

the average rainfall is below 143 mm. The beginning of spring is characterized by an increase in rainfall to 210 mm, followed by a drop and a further increase in rainfall in the summer, reaching 180 mm. Thus, the leaching and translocation processes likely influence compounds such as terpenes, alkaloids, phenolic compounds, steroids, long-chain fatty acids, and unsaturated lactones. Also, some *E. plana* allelochemicals, such as polyphenols and terpenoids, might respond differently to these fluctuations of environmental factors.

The substances produced by *E. plana* alternating soil positions throughout the year are probably driven by how allelochemicals are biosynthesized and released into the environment, adding to the effects of rainfall and soil water balance. Farmers, extensionists, and researchers may consider seasonality to manage *E. plana* and cultivated populations to prevent adverse impacts in agriculture from plant invasion processes. The conditions observed in the region where the soil was sampled seem to favour *E. plana* dominance. This hypothesis makes it prudent to try modifying the structure of the agricultural plant community through species diversification over time and space, aiming to break the alien plant perennialization and consequent dominance in the agroecosystem plant community.

Conclusions

Temporal and spatial variation was verified in the harmful interference of *E. plana* on the germination and initial growth of wheat seedlings through the soil, with results showing greater susceptibility in the summer. Although wheat is not grown at this time of the year in the region where the soil was sampled, it can contribute to the suppression of other species grown during that period and whose relationship is encouraged to be part of future research.

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