Effect of forage aqueous extracts on *Glycine max* L. Merr., *Zea mays* L. and *Bidens pilosa* L.

Efecto de extractos acuosos de forraje sobre *Glycine max* L. Merr., *Zea mays* L. y *Bidens pilosa* L.

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Abstract

The aim of the present study was to investigate the potential allelopathic effects of isolated or intercropped aqueous forage extracts on the physiological performance of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) seeds, as well as their phytotoxicity to the weed species *Bidens pilosa* L. Aqueous extracts were prepared at a concentration of 5%, and the tests with soy and corn were conducted by wetting the germination papers with different treatments and performing procedures within the standards of the Brazilian Rules of Seed Analysis. Tests with the cover plant extracts on the seeds of *B. pilosa* used BOD-type repetitions. Fifty weed seeds were placed in a gerbox with filter paper moistened with the different treatments with five repetitions. Distilled water was used as the control in all tests. Extracts of *Avena strigosa* Schreb and *Raphanus sativus* L. (radish), cultivated individually and in combination, increased the seed germination percentage (%G) and the length of soybean seedlings. Similarly, extracts of *Pennisetum glaucum* (L.) R.Br and *Crotalaria spectabilis* Roth, in individual and combined cultivation, provided gains in corn %G. Furthermore, all tested extracts suppressed germination and decreased the speed of the germination index of the hairy beggarticks when compared to the control, with the radish extract showing the greater reduction effect on the %G of the weed plant *B. pilosa.*

Keywords
allelopathy • stimulating effect • weed suppression

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Resumen

El objetivo del presente estudio fue investigar la posible influencia de la alelopatía de los extractos acuosos de cultivos forrajeros, aislados o en consorcio, sobre rendimiento fisiológico de las semillas de soja [*Glycine max* (L.) Merr.] y de maíz (*Zea mays* L.), además de su fitotoxicidad en la hierba *Bidens pilosa* L. Se prepararon extractos acuosos a una concentración del 5%. Los estudios con soja y maíz se llevaron a cabo humedeciendo los papeles de germinación correspondientes a los diferentes tratamientos y se realizaron dentro de los estándares de las Reglas de Análisis de Semillas de Brasil. Estudios con los extractos en las semillas de *B. pilosa* se llevaron a cabo en una cámara de tipo B.O.D. Se colocaron cincuenta semillas de malezas en caja gerbox conteniendo papel de filtro humedecido con los diferentes tratamientos en cinco repeticiones. El agua destilada se utilizó como control en todas las pruebas. Los extractos de *Avena strigosa* y *Raphanus sativus* L., cultivados tanto en forma aislada como en consorcio, aumentaron el porcentaje de germinación de semillas (%G) y la longitud de las plántulas de soja. Asimismo, los extractos de *Pennisetum glaucum* (L.) R.Br. y *Crotalaria spectabilis* Roth, cultivados aislados y en consorcio, proporcionaron ganancias en el %G de maíz. Además, todos los extractos evaluados suprimieron la germinación y redujeron la velocidad del índice de germinación de la maleza en comparación con el control, siendo el extracto de *R. sativus* el que más redujo el % G de *B. pilosa*.

Palabras clave
alelopatía • efecto estimulador • supresión de malezas

Introduction

The choice of cover plant is important for the success of crop rotation, with species used individually or in a consortium. The benefits provided by these plants include improvements in the physical properties of the soil, reduction of erosion, incorporation of nutrients, and suppression of weeds (12). However, plant coverage can also have an allelopathic effect that suppresses germination and inhibits the initial growth of plants of agricultural interest that are subsequently planted in the site (2, 6).

In southern Brazil, intercropping of cover crops such as grasses and legumes is a common and recommended practice. The consortium approach allows an intermediate carbon/nitrogen (C/N), which is not always possible with isolated species, and also results in a lower rate of decomposition than that of isolated legumes, thus providing longer soil coverage and synchronization between the supply and demand of nitrogen (N) for crops (25).

Among the cover crops used in summer, pearl millet (*Pennisetum glaucum* (L.) R.Br.) promotes strong nutrient accumulation and high phytomass production over a short period of time (19). *Crotalaria* (*Crotalaria spectabilis* Roth) is a legume recommended in combination with pearl millet that has a main advantage of reducing nematode populations (1). In the winter, black oats (*Avena strigosa* Schreb), a rustic plant with rapid growth, is the most cultivated in southern Brazil owing to its high dry matter yields and low N release speed from wastes. When this plant is applied in combination with leguminous or Brassica species, improved vegetation coverage in the soil and greater N fixation or recycling often occurs (7). The Brassicaceae forage radish (*Raphanus sativus* L.) has a high capacity for N extraction from deep soil layers, rapid initial development, and high dry matter yield (27), which makes it suitable for intercropping with black oats.

Crotalaria juncea and millet plants have been shown in the past decades to exert strong suppression of invasive plants while promoting high soil coverage and canopy light capture (8, 10, 16). In addition to the physical factors, chemical factors are also involved in the suppression of weeds by cover plants. For example, Hagemann *et al.* (2010) verified that the use of extracts from the aerial parts of white and black oats caused a reduction in the germination and root and hypocotyl growth of ryegrass and peanuts. Furthermore, studies on the use of allelochemical aqueous leaf extracts have been performed to mimic what occurs in nature (15, 22).

Considering the above, our aim was to investigate the potential allelopathic influence of intercropped or non-intercropped aqueous cover plant extracts on the physiological performance of soybean [*Glycine max* (L.) Merr.] and corn (*Zea mays* L.) seeds, as well as their phytotoxicity to the hairy beggarticks (*Bidens pilosa* L.) weed.

Material and methods

The cover crops used were pearl millet [*Pennisetum glaucum* (L.) R. Br.], cultivar BRS 1501; *Crotalaria* (*Crotalaria spectabilis* Roth), black oats (*Avena strigosa* Schreb) cultivar EMBRAPA 29; and forage radish (*Raphanus sativus* L.) cultivar IPR 116. The seeds were purchased from a commercial supplier.

Plants were cultivated in a field in a rural area in the municipality of Terra Roxa, Parana State, Brazil (24°13'54.32" S, 53°58'46.90" W, altitude 303 m) to obtain the vegetal dry masses for aqueous extract preparation. The sowing period and quantity of seeds used followed the protocol described by Calegari (2016). Fertilization and weed, crop disease, and pest management had not yet been conducted.

The size of all production parcels was 10 m^2, and planting was performed by haul. The fall/winter cover plants, black oat and forage radish, were planted in combination and in isolation at the beginning of April 2018. When combined, 30 g (2068 seeds) of black oat + 5 g (455 seeds) of forage radish were used, while individually, 66 g (4550 seeds) of black oat and 16.2 g (1117 seeds) of radish were employed. The green mass was harvested 86 d after emergence, when both species were in the phenological stage of flowering.

Similarly, the spring/summer coverage crops *Crotalaria* and pearl millet were cultivated in consortium and individually at the beginning of October 2018. When in consortium, 10 g (570 seeds) of *Crotalaria* + 8 g (2000 seeds) of millet were used; when isolated, 20 g (5000 seeds) of millet and 15 g (857 seeds) of *Crotalaria* were utilized. During the phenological stage of flowering, 82 days after emergence, the green mass was harvested.

After biomass collection, the material was dehydrated in the shade with continuous passage of wind. The dry mass was then crushed in a blender and distilled water was added to obtain aqueous extracts at a concentration of 5%. Subsequently, the mixture was strained using a sieve.

To simulate the context of the first soybean crop and the second corn crop, aqueous extracts from the black oat and forage radish plants were used with the soybean seeds, and extracts from the *Crotalaria* and millet plants with the corn seeds.

The aqueous extract (5%) treatments for soybean seeds (Monsoy 6410) were as follows: black oat, forage radish, and their combination. Distilled water was used as the control. The test was conducted in accordance with the standards of the Rules for Seed Analysis (RSA) (17), consisting of eight replicates of 50 seeds. The evaluations were performed on the fifth and eighth days of the experiment to determine germination percentage and seedling length (cm).

Correspondingly, for corn (Pionner 3380), the aqueous extracts (5%) treatments were *Crotalaria*, pearl millet, and their combination. Distilled water was used as the control. The test was conducted in accordance with the standards of RSA (17), consisting of eight replicates of 50 seeds. On the fifth and eighth days of the experiment, germination percentage and seedling length (cm) were determined.

All aqueous extracts were assessed for germination of the hairy beggarticks (*Bidens pilosa* L.) weed. The tests were performed with five replicates of 50 seeds each. The seeds were placed on two Germitest papers in Gerbox type plastic boxes, which were stored in a BOD-type chamber at 25°C with a 14 h photoperiod. The Germitest papers were moistened with the extract in a proportion of 2.5 times the dry paper weight. Germination counts were performed simultaneously each day, with radicle emission by the seed as the determining factor. The germination percentage and germination speed index were calculated following the protocols of the Ministry of Agriculture, Livestock and Food Supply (MAPA), Brazil (2009) and Maguire (1962).

The results were analyzed using SISVAR software (9). Data were subjected to analysis of variance, and when significant differences were found, the Tukey test was used to compare the averages at 5%.

Results and discussion

The black oat and forage radish extracts cultivated both individually and in combination promoted gains in germination and length of soybean seedlings (table 1). This suggests that there was no undesirable chemical effect of straw (dry biomass) on the initial growth of the soybeans. This system is common in southern Brazil. Our results corroborate those of Krenchinski *et al.* (2018), who verified that the use of black oats, either isolated or intercropped with radish, had the potential to increase soybean yield.

Table 1. Seed germination percentage (%G) and seedling length (SL) of soybean in the presence forage aqueous extracts.

Tabla 1. Porcentaje de germinación de semillas (% G) y longitud de plántulas (SL) de soja en presencia de extractos acuosos de forraje.

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Treatments	Variables	
	% G	SL (cm)
Control	93 b	14.68 c
Black oats extract	95 ab	17.51 b
Forage radish extract	97 a	17.88 b
Consortium extract	97 a	21.86 a
CV (%)	1.74	3.10

The effect of straw has also been reported on *Zea mays*, an important crop in Brazil, which is the second largest producer globally. Before harvest, a green manure system has also been proposed for some crops.

All cover plant extracts tested with corn promoted gains in seed germination; however, the extracts of isolated pearl millet and *Crotalaria* + millet combined reduced the length of the seedlings. Treatment with the isolated *Crotalaria* extract increased the growth of corn seedlings (table 2). According to Nunes *et al.* (2014), *Crotalaria* extract (*Crotalaria juncea*), compared to the other plants studied (*Brassica napus* L., *Crambe abyssinica* Hochst, *Linum usitatissimum* L., and *Raphanus sativus* L.), contributed the most to the increase in the germination percentage and growth of lettuce, cucumber, and soy seedlings. The positive effect of *Crotalaria* on the initial growth of corn indicates increased vigor of the plants. Carvalho *et al.* (2004) investigated *Crotalaria* grown in the spring and observed that this cover plant provided an 18.5% increase in corn productivity, compared to that of the fallow area. This increase in productivity may be correlated with a strong initial development of corn seedlings in the field.

Table 2. Seed germination percentage (% G) and seedling length (SL) of corn in the presence of forage aqueous extracts.

Tabla 2. Porcentaje de germinación de semillas (% G) y longitud de plántulas (SL) de maíz en presencia de extractos acuosos de forraje.

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Treatments	Variables	
	% G	SL (cm)
Control	92 b	14.63 b
Pearl millet extract	95 a	12.93 c
Crotalaria extract	96 a	19.05 a
Consortium extract	97 a	13.69 c
CV (%)	1.57	4.23

All tested extracts suppressed germination and decreased the speed of germination index (SGI) of the hairy beggarticks when compared to the control (tables 3 and 4). Treatment with millet extract reduced the germination percentage by 47% and induced a considerable delay in germination. This extract also resulted in the shortest initial length of corn (table 2, page 73), which is likely related to the allelopathic effect of the cover plant, although *B. pilosa* was more sensitive to the presence of allelopathic compounds. The radish extract was the most phytotoxic for weed seeds, reducing germination by 89%.

Table 3. Percentage of germination (% G) and speed of germination index (SGI) of hairy beggarticks in the presence of cover plants aqueous extracts (pearl millet and crotalaria).

Tabla 3. Porcentaje de germinación (% G) y velocidad del índice de germinación (SGI) de la hierba *B. pilosa* en presencia de extractos acuosos de forraje (mijo perla y crotalaria).

Treatments	Variables	
	% G	SGI
Control	79 a	17.66 a
Pearl millet extract	42 c	6.87 d
Crotalaria extract	62 b	11.75 b
Consortium extract	49 c	8.66 c
CV (%)	4.25	5.60

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Table 4. Percentage of germination (% G) and speed of germination index (SGI) of hairy beggarticks in the presence of cover plants aqueous extracts (black oats and forage radish).

Tabla 4. Porcentaje de germinación (% G) y velocidad del índice de germinación (SGI) de la hierba *B. pilosa* en presencia de extractos acuosos de forraje (Avena negra y rábano forrajero).

Treatments	Variables	
	% G	SGI
Control	78 a	17.39 a
Black oats extract	66 b	12.84 b
Forage radish extract	8 d	1.68 d
Consortium extract	48 c	8.63 c
CV (%)	8.13	8.59

Different letters in the columns differ statistically by the Tukey test at 5% significance.

Las diferentes letras en las columnas difieren estadísticamente según la prueba de Tukey con una significancia del 5%.

Studies demonstrating the chemical and physical effects of cover crops of different species on invasive plants have been recurrent (5, 18, 21, 23, 26). Sturm *et al.* (2018) claimed that knowledge of the proportions of allelopathic and competitive effects could lead to the development of cover crop consortia with optimal weed suppression characteristics in the field.

Taken together, the results obtained herein demonstrate the benefit of using cover crops, a strategy that not only contributes to the integrated management of weeds, but also improves the production system by increasing the initial growth of soy and corn.

Conclusion

The aqueous leaf extracts of the two investigated plant consortia and the isolated cover plants favored the germination of the crops of interest, soy and corn, and were phytotoxic to noxious beggarticks. Forage radish extract most effectively reduced the germination of hairy beggartick seeds.

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