COMMUNICATIONS IN PLANT SCIENCES

RESEARCH ARTICLE

Incidence of anthracnose in auxin-treated soybean plants and seedlings in laboratory and greenhouse conditions

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The objective was to evaluate the incidence and severity of anthracnose on soybean plants and seedlings treated with coco-grass extract and 2,4-D. The samples of *Colletotrichum* sp. were distributed into petri dishes containing BDA medium. After replicate, the dishes were sealed with a plastic film and placed for growth in the BOD chamber at 27 ºC for one week, then performed a new replication. Ten soybean seeds were put into plastic boxes fully filled with autoclaved sand, using six replicates. Treatments consisted of spraying a solution of coco-grass extract or 2,4-D at concentrations of 0%, 25%, 50%, 75% and 100% on soybean seedlings and plants, under laboratory and greenhouse conditions. An increase in the number of injured plants according to the increase of the concentration of the extract, where a smaller but not insignificant number of plants injured in the treatment 25% of coco-grass and the maximum value of injured plants in the highest concentration (100%) of this treatment.

Highlighted Conclusion
Auxin derived from coco-grass extract causes a greater susceptibility of soybean to anthracnosis.

Soybean is largely cultivated on all regions of the world, it reached a production of 334,894,085 Mg in the crop year of 2016, being United States, Brazil and Argentina the largest producers in the world (FAO 2018). The role of soybeans in the Brazilian industry is also recognized, according to MAPA (2015), about 5.8 million Mg of edible oil are produced per year, 23.5 million Mg of protein meal, out of a total of 30.7 million tons of soybean that the industry transforms in byproducts.

The search for alternative methods that may increase productivity in some way, such as investment in soil fertility, use of crop rotation, use of irrigation, control of pathogens and use of plant regulators are essential for the continuity of the agricultural market, however, there are cases where the use of plant regulators makes the plant more susceptible to the action of the pathogen in its cell.

The use of auxin in a plant, whether in biological or synthetic form, is an example of this, its use cases a series of effects, such as cell elongation and differentiation, membrane permeability impairment, increase in respiration process, increase in synthesis of messenger RNA and increase in the synthesis of proteins, such factors are responsible for the better development of the plant (Agrios 1997). However, these processes make the cell wall more susceptible, helping the fungus to penetrate the plant cells more efficiently, since it is no longer so hard.

Anthracnose is one of the major fungal diseases of economic interest of soybeans, directly attacking the pods, causing them to fall and consequently a fall in production (Kimati 2005). It is known that some pathogens are responsible for increasing the concentrations of auxin in plants, which in turn leads to a greater susceptibility of the cell wall of the plants to the enzymes that the pathogens produce, thus facilitating their contamination (Llorente et al. 2008).

Indirect effects in plants may be caused by the presence of auxin such as cell wall structure, root morphology and stomatal pattern. For example, rice plants treated with Indol Acetic Acid (IAA) obtained a decrease in the resistance to *Xanthomonas oryzae* pv. *oryzae* probably due to the activation of the biosynthesis of the cell walls that loosened the walls, facilitating the development of pathogens (Ding et al. 2008).

The hypothesis is that plants treated with auxin, whether in biological or synthetic form, will present a higher incidence of anthracnose under them. Thus, the objective was to evaluate the incidence and severity of anthracnose on soybean plants and seedlings treated with coco-grass extract and 2,4-D Amine 72.

MATERIAL AND METHODS

The present work was carried out in the laboratory of phytopathology and in greenhouse of the Pontifical Catholic University of Paraná, *campus* Toledo, with coordinates 24°43'S and 53°46'W. First, the sample containing *Colletotrichum dematium*. was provided by the UFPR (Universidade Federal do Paraná), Palotina campus and then distributed into 20 petri dishes containing 20 ml of BDA medium only. For the experiment the cultivar "BMX RR Potencia" was chosen, which has a high size, good resistance to bedding, high yield potential, medium to high demand of soil fertility and accepted by the agricultural zoning for the western region of Parana (Brasmax 2015), being the seeds obtained from a rural property located in the municipality of Matelandia – PR.

Each petri dish, together with the culture medium and the other materials were autoclaved at 120 ºC and 1 Atm for 15 minutes and the placed in drying oven at 50 ºC for 12 hours. The fungus distribution was performed in a laminar flow chamber with ultraviolet light. It was cleaned with 70% alcohol and then the ultraviolet was turned on for 15 minutes to cause the germicidal effect to act on any remaining organism.

After the replicate, the plates were sealed with a plastic film and placed for growth in the Biochemical Oxygen Demand (BOD) chamber at 27 ºC for one week, then performed a new replication. In the first attempt of replication there was contamination of the plates, being necessary to discard all the material and then carried out a new inoculation through a new inoculum collected from bean plants. The material replication was by the identification of the fungus in a magnifying glass, and the material was cut and sanitized in sodium hypochlorite for one minute, followed by 70% alcohol for 30 seconds and then triple washed in distilled and autoclaved water.

After washing, the material was then spread into 20 petri dishes containing solid BDA culture medium for fungus growth, after seven days were discarded the contaminated plants and performed another replication of the non-contaminated petri dishes. Then, after another seven days, another 20 petri dishes were replicated, repeating the process and discarding the contaminated ones until 60 viable petri dishes were obtained.

For the preparation of the coco-grass extracts, were collected the aerial part of these plants, washed with deionized water and then weighted according to the treatments, then the plants were grinded with distilled water in a blender for five minutes. After grinded, the extract was sieved to remove the excesses. The laboratory experiment had 2 different parameters tested (coco-grass extract and 2,4-D diluted) which consisted in: 100% (1 g of green extract for each 10 mL of deionized water), 75% (0.75 g of green extract for each 10 mL of deionized water), 50% (0.5 g of green extract for each 10 mL of deionized water), 25% (0.25 g of green extract for each 10 mL of deionized water) and 0% (deionized water only) for the coco-grass. For the 2,4-D essay the dilutions were made with a 1 mL pipette and 5 beakers containing deionized water, forming the following treatments: 100% (1.0 mL of 2,4-D to 1.0 liter of water), 75% (0.75 mL of 2,4-D to 1.0 liter of water), 50% (0.5 mL of 2,4-D to 1.0 liter of water), 25% (0.25 mL of 2,4-D to 1.0 liter of water) and 0% (deionized water only).

Ten soybean seeds were put into Gerbox® boxes fully filled with autoclaved sand with six replicates per treatment. Seven days after sowing, the treatments were applied with the help of a 500 mL hand spray, spraying about 20 mL of each treatment per box.

At 14 days after sowing was performed the inoculation of 10^6 spores previously counted in a Neubauer chamber. To prepare the inoculum containing the fungus, a petri dish completely colonized by the fungus was diluted with 10 mL of deionized water. From this dilution was removed 0.1 mL which was laid up on the Neubauer chamber with a coverslip, and then were counted the four largest quadrants in a microscope with a 100-fold increase, then made and average of the number of spores with the following formula and, as the formula gives us only a factor of 10^4 a rule of 3 has been made until the required factor 10^6 was obtained.

After that, in a greenhouse, 8 seeds were sowed, per pot, in polypropylene pots with a capacity of 11 liters of soil, filled with commercial substrate and autoclaved sand in the 2:1 proportion preciously mixed in a tarp on the ground, so that there was a better distribution between sand particles and substrate. When the emergence occurred the thinning was done, leaving only 4 plants per plot.

The application of the previously formulated coco-grass and 2,4-D extracts in the same concentrations as the laboratory test were carried out in the V5 stage of the culture, being the inoculation of the spores in the concentration of 10^6 carried out in the R1 stage, in both cases a hand spray for application was used.

The whole plants were collected at the R4 stage, where the following factors were evaluated: aerial part fresh weight, aerial part length, percentage of powdery mildew in the plants, root fresh weight, root length, percentage of anthracnose in plants, aerial and root dry weight.

The weighting was carried out with a precision scale, the drying of the material was carried out in a vertical forced circulation oven at 65 ºC for seven days, the percentages of anthracnose and powdery mildew were estimated with the aid of a diagrammatic scale and the length measurement with a measurement tape.

For the laboratory test all the results were gathered at 21 days after sowing and at the end of the data collected from the greenhouse, the statistical analysis was performed using the Tukey test at the 5% significance level between the treatments (Coco-grass and 2,4-D), and the quadratic regression analysis was performed using the SISVAR statistical software (Ferreira 2010).

RESULTS AND DISCUSSION

Soybean seedlings treated with 2,4-D were less injured than ones treated with coco-grass extracts (Table 1). In addition, Figure 1 shows an increase in the number of injured plants according to the increase of the concentration of the extract, where a smaller but not insignificant number of plants injured in the treatment 25% of coco-grass and the maximum value of injured plants in the highest concentration (100%) of this treatment. In relation to the 2,4-D treatment, it was possible to observe that all the plants presented an injury in the control, and also a reduction in the quantity of injured plants since the increase in the concentration caused the mortality of the plants (Figure 1).

Table 1. Statistical analysis of the uninjured variable for soybean seedlings inoculated with coco-grass and 2,4-D carried out in the laboratory through the Tukey test at the 5% level of significance.

Uninjured (%)	
Treatment	Averages*
Coco-grass	6.33 a
2,4-D	2.33 b
CV (%) = 176.76	
Average mean: 4.33	

* Means followed by the same letter in the column did not differ statistically at the 5% level of significance.

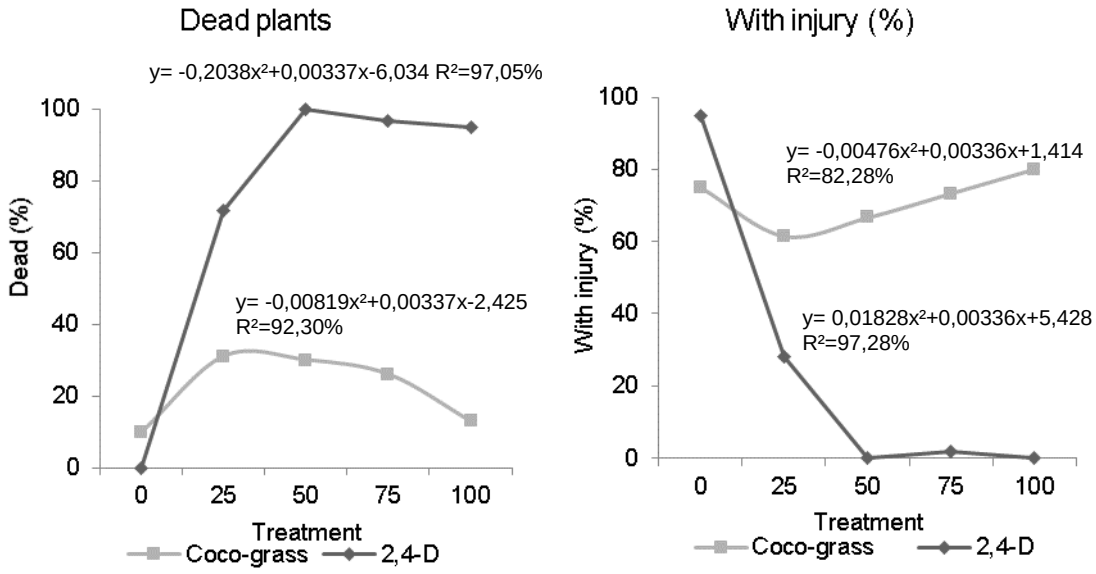

Figure 1. Quadratic regression analysis performed in the laboratory for extracts of coco-grass and 2,4-D in different concentrations for the variable Dead plants and plants with injury.

Correlating the data in the Table 1 and in the Figure 1, it can be noted that the statistical difference between coco-grass and 2,4-D in Table 1 is related to the quantity of dead plants in the treatment with 2,4-D, since this was higher as the concentration of the extract increased and for the coco-grass, it presented a lower mortality rate as the concentration increased. The disparity of plants that did not present an injury to the 2,4-D treatment and the high percentage of plants that presented an injury in the treatment of coco-grass contributed to the increase of such a high coefficient of variation.

Plants of *Arabidopsis* when treated with auxin while inoculated with *Pseudomonas syringae* showed a meaningful increase in the development of the disease to not treated plants, since *P. syringae* has the ability to produce an effector protein AvrRpt2 that is not recognized by the *Arabidopsis* plants, leading to auxin production, which might be one mechanism used by pathogens to infect the plants (Chen et al. 2007).

In Figure 2 it is possible to notice that the plants, in general, presented a better development in the concentration of 25% of the coco-grass treatment, and higher concentrations of this treatment resulted in a decrease in the values of the tested variables.

In Figure 3 it is possible to note a correlation of the data of the dry matter of the treatments with the green matter, since that concentrations higher than 25% of coco-grass also caused a decrease in the aerial and root weight. This result is not consistent with Lorenzi's (2000) claim, according to the author, coco-grass presents a high content of indole-butyric acid, which leads to a greater development of the roots.

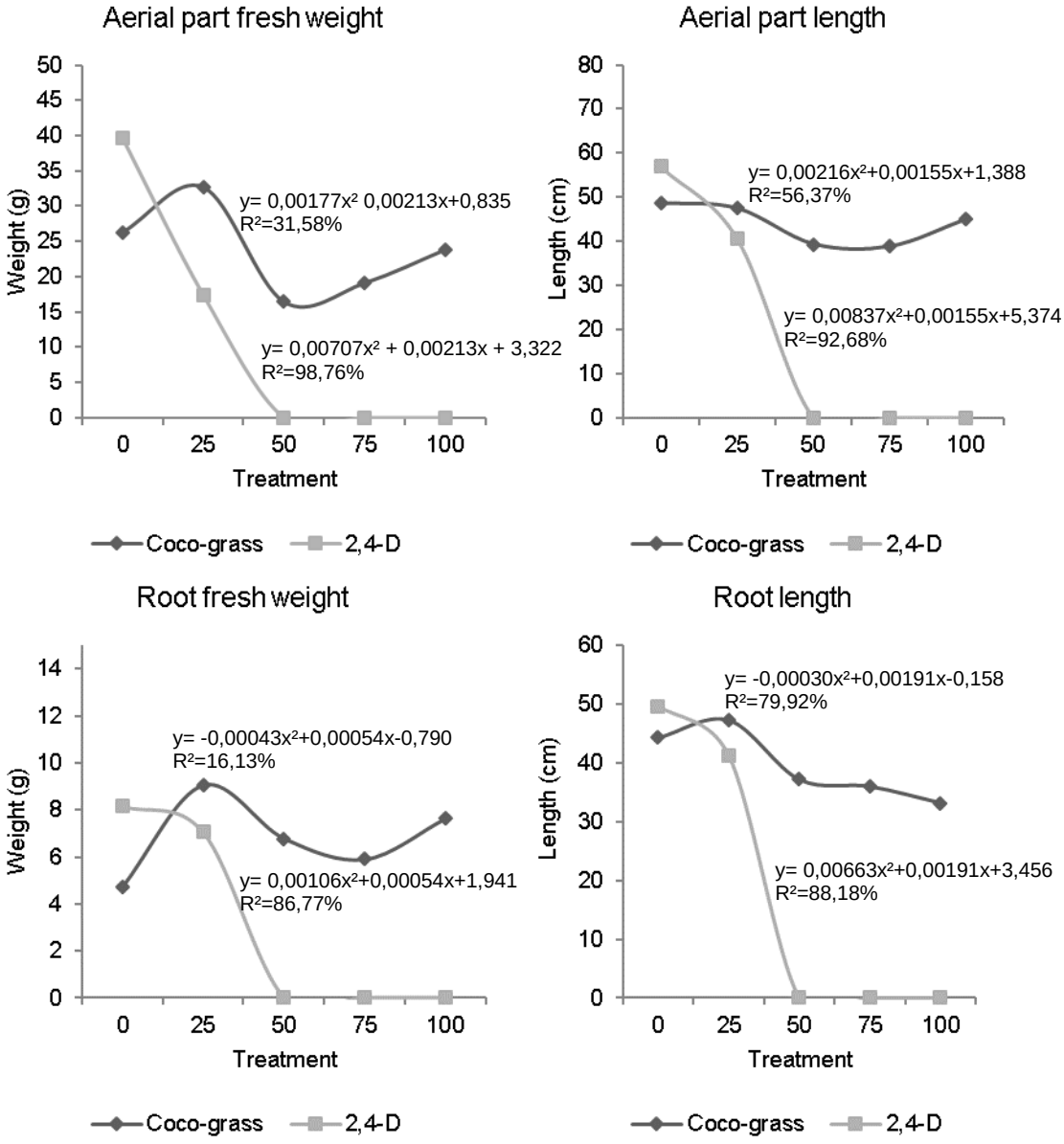

Figure 2. Quadratic regression analysis performed in greenhouse for the extracts of coco-grass and 2,4-D in different concentrations for the variable Aerial part fresh weight, Aerial part Length, Root fresh weight, Root length.

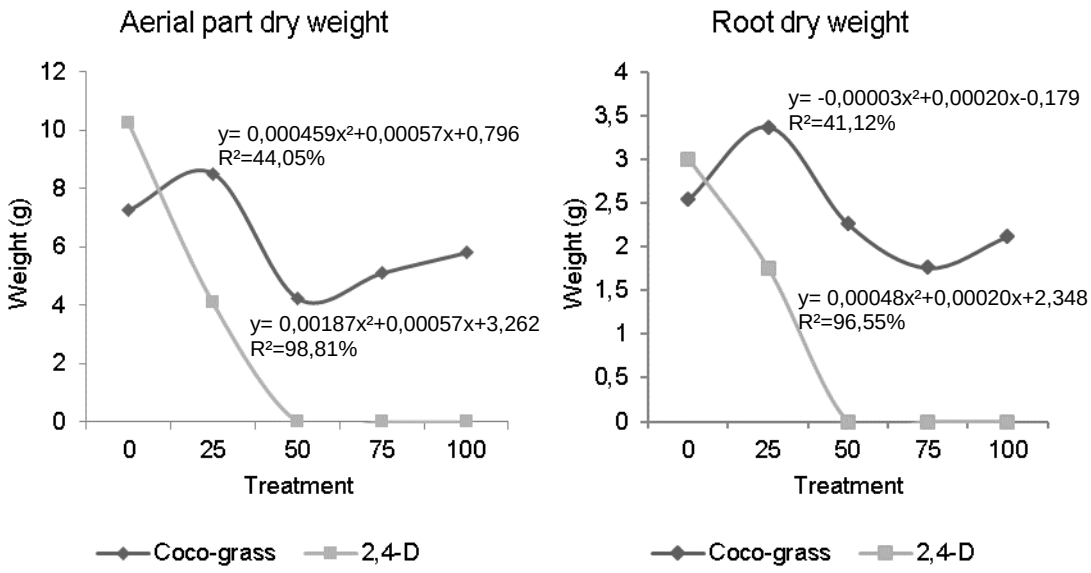

Figure 3. Regression analysis performed in greenhouse for the extracts of coco-grass and 2,4-D in different concentrations for the variable Aerial part dry weight and Root dry weight.

The reduction in the development of the plants may be related to a negative allelopathic effect on them caused by the coco-grass extract. Muniz et al. (2007) found that the extract of roots of *Cyperus rotundus* in the concentration of 100 g/L in soybean seeds resulted in an inhibition in germination, being this value twice the maximum dosage tested in this experiment.

In Tables 2 and 3 is observed a high value of the coefficient of variation, being this result possibly due to the irregularity of plants that had these diseases within the various treatments tested, since in all tests the treatment 2,4-D caused the death of the plants in concentrations above 25% in the essay carried out in greenhouse (Figures 3 to 8), making it impossible to determine which of the treatments provided a greater susceptibility of the soybean to the disease.

Table 2. Percentage of incidence of powdery mildew in soybean plants inoculated with coco-grass extract and 2,4-D in greenhouse, analyzed by Tukey test at the 5% level of significance.

Powdery mildew	
Treatment	Averages
Coco-grass	24,88 a*
2,4D	3,08 b
CV = 107,15 %	
Average mean: 13,98	

* Means followed by the same letter in the column did not differ statistically at the 5% level of significance.

Table 3. Percentage of anthracnose incidence in soybean plants inoculated with extract of coco-grass and 2,4-D in greenhouse, analyzed by the Tukey test at the 5% level of significance.

Anthracnose (%)	
Treatment	Averages
Coco-grass	9,87 a*
2,4-D	6,02 a
CV = 134,11%	
Average mean: 7,98	

* Means followed by the same letter in the column did not differ statistically at the 5% level of significance.

Testing the interaction between auxin and *Macrophomina phaseolina,* Mah (2011) found that concentrations between 5 nM and 50 nM of the substance promoted an increase in the resistance of *Medicago truncatula* to the fungus and made the plants healthier. Comparable results were found with *Agrobacterium tumefaciens* (Yamada, 1993), *Pseudomonas syringae* pv. tomato DC3000 (Chen et al. 2007, Navarro et al. 2006) and *Pseudomonas savastanoi* (Yamada 1993) when plants received exogenous auxin. In addition, Thilmony et al. (2006) found that *Pseudomonas syringae* pv. tomato DC3000 has the ability to change the auxin physiology and movement in plants of Arabidopsis. This way, auxin can cause disease susceptibility in plants and that a reduction in the auxin signaling could cause increased resistance in plants.

The disruption of auxin signaling in *Arabidopsis* mutant plants let to an increase in the resistance of this plant to the fungus *Fusarium oxysporum* (Kidd et al. 2011). Lloente et al. (2008) found that the presence of auxin helped regulate the immunity of the axr2-1 and axr1-1 mutants of *Arabidopsis*, making these more susceptible to *B. cinereal* and *Plectosphaerella cucumerinado* than wild plants of this species.

Kimura et al. (1973) reported that a phytotoxin produced by the fungus *Colletotrichum lagenarium* has the anti-auxin function, that is, it inhibits the production of auxin in the leaves. This factor may have contributed to the low percentage of plants with anthracnose. However, Robinson et al. (1998), Chung et al. (2003), Maor et al. (2004) and Tsavkelova et al. (2012) say that *Colletotrichum gloesporioides* f. sp. *aeschynomene, Colletotrichum acutatum* possess enzymes capable of synthesizing auxin when in plants.

Some viral pathogens can cause diseases by the manipulation of auxin signaling components, such as the interaction between tobacco mosaic virus replicase and Aux/IAA proteins that harms the transcriptional activation of auxin-responsive genes, promoting symptoms of disease in tomatoes and Arabidopsis (Padmanabhan et al. 2005, 2006, 2008).

Changing the signaling of a particular hormone may result in imbalance of another hormone. Thus, the imbalance between the quantity of hormones such as auxin, abcisic acid and salicylic acid can cause an imbalance in the resistance of plants to natural attackers. The presence of salicylic acid in *Arabidopsis* plants can activate the resistance of this plant to powdery mildew (*Erysiphe difusa*) and *P. syringae* (Tsuda et al. 2008, Vlot et al. 2009).

In a certain way, the application of auxin in the form of coco-grass extract caused a greater susceptibility of these plants to the diseases, where all the plants had symptoms of anthracnosis in the laboratory essay and anthracnosis and powdery mildew in the greenhouse essay for all tests performed.

Concentrations above 25% in the 2,4-D treatment caused the plants to die which makes it hard to define which treatment promoted higher levels of disease. For the coco-grass extract, the concentration of 25% promoted higher values of Aerial part fresh weight, Aerial part Length, Root fresh weight, Root length, Aerial part dry weight and Root dry weight.

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