Chapter 6

Plant Defense Enzymes Activated in Bean Plants by Aqueous Extract from *Pycnoporus sanguineus* Fruiting Body

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1. Introduction

The common bean (*Phaseolus vulgaris* L.) can be affected for more than 300 diseases caused by virus, bacteria, fungi, and nematodes. The semibiotroph *Pseudocercospora griseola* (Sacc.) Crous & Braun (sin. *Phaeoisariopsis griseola* (Sacc.) Ferraris), the causal agent of angular leaf spot, represents one of the main fungal pathogens of this crop, manifesting on the stem, leaf, and pod [1].

Traditionally, the control of the angular leaf spot has been done with the use of resistant cultivars, seeds free of pathogen and fungicides. The last one, at a short time, has it's advantages, but for a long period of time, can cause problems due the residues accumulation and environmental pollution [2]. Thus, with the objective to find new technologies, ecologically or environmentally safer, for the control of plant diseases, mainly in organic growth, alternative methods for the control of phytopathogens are been development. This kind of alternative methods are been investigated by our 'Biological and Alternative Control of Plant Diseases' research group [3].

The induction of resistance in plants involves the activation of defense latent mechanisms [4] in response to the treatment with elicitor agents, protecting against subsequent infection by pathogens. Among the non-conventional elicitors can be included the extracts of medicinal plants and essential oils [5], homeopathic drugs [6], as well as the extracts obtained from mushrooms [7-9]. Among the basidiomycetes with elicitor proprieties stands out *Pycnoporus sanguineus* (L. ex Fr.) Murr. [10], utilized since the medicine [11,12] to the alternative control of plant diseases [13,14]. These previous works had shown that the biological properties of

P. sanguineus depending of its crude or aqueous extract and not of its individual compounds, like cinnabarin, or extracts obtained from organic solvents.

Previous works had shown the potential of *P. sanguineus* components for controlling plant diseases. Aqueous extracts, obtained from liquid medium-culture filtrate (MCF) [15] and from mycelium (AEM) [16] of *P. sanguineus*, were capable to reduce in 82% and 49% to MCF, and in 93% and 50% to AEM, in greenhouse and field conditions, respectively, the severity of angular leaf spot in bean plants. However, the effect of *P. sanguineus* fruiting bodies in that pathosystem was not investigated. Against plant pathogenic bacteria, fruiting body extracts from *P. sanguineus* were efficient for the control of common bacterial blight in bean, caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith – Vauterin, Hoste, Kersters & Swings) which can occur either by direct antimicrobial activity and by resistance induction involving the activation of some pathogenesis-related proteins [17].

In another experiment, the *in situ* detection of reactive oxygen species (ROS), mainly hydrogen peroxide (H_2O_2) and superoxide (O_2^{-}), was searched in bean plants treated with aqueous extracts of the mycelium (AEM) and basidiocarps or fruiting bodies (AEB) of *P. sanguineus* and inoculated after three days with *Colletotrichum lindemuthianum* ((Sacc. & Magn.) Scrib.). It was possible to detect H_2O_2 at 48 hours after inoculation (hai) only to the treatment with basidiocarp extract. The O_2^{-} was detected mainly to the treatment with mycelium extract at 48 hai. All the treatments showed reaction for H_2O_2 and O_2^{-} in epidermal and mesophyllic cells at 192 hai, probably due the infection development. These results suggest that *P. sanguineus* extracts promote oxidative burst in bean plants, in early infection process, reducing anthracnose severity [18].

Thus, this work aimed to investigate the potential of *P. sanguineus* for controlling angular leaf spot in common bean, evaluating the *in vitro* antimicrobial activity against *P. griseola* and the induction of resistant enzymes as peroxidase, polyphenoloxidase and β-1,3-glucanase, as well as the influence on physiological mechanisms related to the energy supply, as the protein content and chlorophyll.

2. Materials and methods

Pathogen isolate: *Pseudocercospora griseola* was obtained from bean plants naturally infected, and cultivated in tomato juice (200 mL of tomato juice, 15 g of agar, 4,5 g of $CaCO_3$ and 800 mL of distillated water) for 14 days at 24 °C and dark [19].

Aqueous extract of *Pycnoporus sanguineus* **fruiting body:** This process was carried out as methodologies [7] and [13]. Fruiting bodies or basidiocarps of *P. sanguineus* were collected in western Paraná State, Brazil, and identified according [10]. To obtain the aqueous extract, dehydrated powder from basidiocarps was suspended into distilled water (14 mL g^{-1}) and, after 24 h incubation at 4ºC, the suspension was filtered through a common filter paper (8 g cm^{-2}) and centrifuged at 20,000 *g* for 25 min. The supernatant obtained, after this procedure, was considered as the crude aqueous extract.

Inhibition of conidia germination: This assay was done in microscopic slide covered with a thin layer of agar-water 1% (700 μL per slide) [20]. Aliquots of 40 μL of aqueous extracts in concentrations 0, 1, 5, 10, 15 and 20%, sterilized in autoclave or filtrated in nitrocellulose membrane (0.45 μm of pore diameter) and aliquots of 40 μL of conidia suspension of *P. griseola* ($1x10^4$ conidia mL^{-1}) obtained of a culture with 14 days old, were distributed in the surface of the slide, which were incubated in moist chamber under dark at 24 °C [21]. As control treatments were utilized fungicide (azoxystrobin: 40 mg L^{-1}) and acibenzolar-S-methyl (ASM: 75 mg L^{-1}). The percentage of the germination was determined after 24 hours with the addition of 40 μL of lactophenol cotton-blue in each slide to paralyze the conidia germination.

Inhibition of the mycelial growth and sporulation of *P. griseola***:** The extracts of basidiocarp of *P. sanguineus* were incorporated in concentrations of 0, 1, 5, 10, 15 and 20%, in tomato juice culture medium. The extracts were sterilized in autoclave and also by filtration in nitrocellulose membrane (0.45 μm of diameter of pore) [22]. As control treatments were utilized fungicide (azoxystrobin: 40 mg L^{-1}) and acibenzolar-S-methyl (ASM: 75 mg L^{-1}). For transferring *P. griseola* to Petri dishes, 100 μL of spore suspension ($1x10^4$ conidia mL^{-1}) were added to the medium and homogenized with Drygalski loop. The Petri dishes were sealed with plastic film and maintained at 24 °C and dark. Were evaluated the diameter and the number of colonies 14 days after the beginning of the experiment. At the end of the assay of mycelial growth inhibition, it was evaluated the sporulation of the fungus. For this, was prepared a suspension of conidia by the addition of 10 mL of distillated water per plate and determined the number of conidian per mL in Neubauer chamber.

Experiment in greenhouse: Two plants of common bean (cultivar IAPAR 81 – Carioca) were cultivated in plastic pots containing 5 L of a mixture of sterilized soil and sand (proportion 2:1). To resistance induction assay, were used aqueous extract of *P. sanguineus* basidiocarp at concentration of 10% and 20%. As control treatments were used water, fungicide (azoxystrobin - 40 mg L^{-1}) and acibenzolar-S-methyl (75 mg L^{-1}). The extracts and the control treatments were sprayed in the 3rd leaf (vegetative stage V4) (3 mL per leaf).

Field experiment: The experiment consisted in three randomized blocks, with five plots per block. Each plot consisted of three lines of 3 m of length, spaced 0.5 m between them, with 10 plants (cultivar IAPAR 81 – Carioca) per meter. The central line, discounting 0.5 m from the anterior and posterior borders, was considerate as useful area for evaluation. For the assay of resistance induction, were sprayed aqueous extracts of *P. sanguineus* basidiocarp at concentrations of 10% and 20%, and as control treatments were used water, fungicide (azoxystrobin: 40 mg L^{-1}) and the acibenzolar-S-methyl (ASM: 75 mg L^{-1}). The extracts (5 mL per plant) were applied twice, the first one in vegetative stage (V3) and the second in reproductive stage (R3).

Pathogen inoculation: The conidia suspension of *P. griseola* was prepared in water with Tween 20 (one drop 500 mL^{-1}), and the concentration adjusted to $4x10^4$ conidian mL^{-1}. The inoculation in the greenhouse was done three days after the application of extracts and control treatments, in the 3rd treated leaf, as well as in the 4th non-treated leaf (vegetative

stage V4), to verify a putative systemic resistance induction. After the inoculation, the plants were maintained in humidity chambers and dark at 24 °C during 48 hours and, later, maintained in greenhouse, according to methodology used by [19]. In the field, two inoculations were done, the first in vegetative stage (V3) and the second in reproductive stage (R3), both three days after the application of extracts and control treatments.

Severity evaluation: The severity of the angular leaf spot in the greenhouse was evaluated in the 3rd and 4th leaves at 8, 12, 16, 20 and 24 days after the inoculation, using diagrammatic scale prepared by [23]. In the field, the evaluations started when the first symptoms of disease appeared (seven days after the inoculation), and were obtained five evaluations on the lower middle canopy of the plant. In the second application of extracts and control treatments, the severity was evaluated as the same way that was done in the first application, but only evaluating the upper middle canopy. With the severity data was calculated the area under the disease progress curve (AUDPC) of angular leaf spot as in reference [24].

Biochemical analysis: Leaf disc with 3.46 cm^2 (three disc per sample) were collected at 48, 72, 96, and 120 hours after the inoculation (hai) and also after the symptoms appearance (144 hai). Each collected sample was immediately wrapped in aluminum foil and freeze at -20 °C. Samples were collected from the 3rd treated and inoculated leaf, as well as from the 4th non-treated but inoculated leaf, from the same plant [25].

Obtaining the protein extracts: the samples of leaves were mechanically homogenized in 2 mL of extraction buffer sodium phosphate 0.01 M (pH 6.0), in a porcelain mortar. The homogenate was centrifuged at 6.500 *g* during 10 min at 4 °C. The supernatant was considerate the enzymatic extract, for later determination of peroxidase, polyphenoloxidase and β-1,3-glucanase activities and protein content [25].

Peroxidase activity: the peroxidase activity was determined at 30 °C, by spectrophotometer at 470 nm during 2.15 min [26]. The peroxidase activity was expressed in absorbance min^{-1} g of fresh $mass^{-1}$.

Polyphenoloxidase activity: the polyphenoloxidase activity was determined according the methodology in reference [27]. The results were expressed in absorbance min^{-1} g of fresh $mass^{-1}$.

β-1,3 glucanase activity: the enzyme activity was evaluated according to [19]. The reaction was determined by colorimetric quantification of glucose released from laminarin, using *p*-hydroxybenzhydrazide. The results were expressed in μg of glucose min^{-1} g of fresh $mass^{-1}$.

The protein content: the total protein content was evaluated as [28]. The concentration of proteins, expressed in equivalent of bovine serum albumin (BSA) in one mL of sample (μg protein mL^{-1}), was determined utilizing standard curve of concentrations of BSA, varying of 0 to 20 μg mL.

The chlorophyll content: for the quantification of chlorophyll was utilized an adapted methodology [29]. The samples of plant tissue (0.1 g) were packed in glass tube with 10 mL

of acetone 80%, during 7 days in the dark at 25 °C. After this time was determined the absorbance at 663 nm and 645 nm for chlorophyll *a* and *b*, respectively. The concentration of chlorophyll *a* was obtained by the equation $(0.0127A_{663}) - (0.00269A_{645})$ and of chlorophyll *b* by the equation $(0.0029A_{645}) - (0.00468A_{663})$. The total chlorophyll content was obtained by adding the results of chlorophyll *a* and *b*. The values were expressed in mg g of fresh mass^{-1}.

Statistical analysis: The experiments were arranged in randomized blocks, with five treatments. The analyzes of variance (ANOVA) was done using the statistical program JMP (Statistical Analysis System SAS Institute Inc. USA, 1989 – 2000 version 4.0.0.), and the average compared by the Dunnett's test in level of 5% of probability.

3. Results

There was no significant effect of the concentrations of *P. sanguineus* basidiocarp extract on the spores germination, mycelial growth and sporulation of *P. griseola* (data not shown), indicating the absence of direct antimicrobial activity of these extracts on the pathogen.

However, in the greenhouse and field experiments, the area under disease progress curve (AUDPC) of angular leaf spot showed that the plants treated with *P. sanguineus* extract at 10% and 20% differ from the water-treatment, with reduction of 42% and 54% in the 3rd leaf, respectively. In the 4th leaf was observed reduction of 69% in the AUDPC for the plants treated with basidiocarp extract at 20%, not differing from ASM and fungicide control treatments (Table 1), indicating systemic resistance induction.

In the lower middle canopy, there was no statistical difference in AUDPC for basidiocarp extract when compared to water and ASM control treatments. The difference was observed just to fungicide treatment, which presented the better protection against angular leaf spot. To the upper middle canopy, was verified a reduction of 64% in the AUDPC for the treatment with basidiocarp extract at 20%, better then ASM, which is a commercial resistance inducers product. So, these results indicate the great potential of *P. sanguineus* extracts for the control of *P. griseola* in common bean, with local and systemic effects.

The biochemistry analysis reveled induction of peroxidase activity due the treatment with basidiocarp extract on the 3rd treated leaf, as well as in the 4th non-treated and inoculated leaf, demonstrating the systemic induction effect of *P. sanguineus* (Figure 1). In the 3rd leaf the basidiocarp extract at 10% reduced the activity of the enzyme when compared to the water-treatment and fungicide three days after the inoculation (DAI). To five DAI differed from ASM and fungicide, and to seven DAI from ASM. The basidiocarp at 20% presented higher activity of peroxidase at four DAI, and inhibition to three, five and seven DAI, when compared to ASM, ASM and fungicide and ASM, respectively. The 4th non-treated leaf showed the same pattern of 3rd leaf for peroxidase activity.

The polyphenoloxidase activity was influenced by treatments with extracts of *P. sanguineus*, in the 3rd leaf treated, as well as in the 4th non-treated and inoculated leaf, demonstrating systemic effect (Figure 2). In the treated leaf, the extract of basidiocarp at 10% reduced the enzyme activity when compared to the water and ASM at three DAI. To four DAI the

enzyme activity was stimulated when compared to water and fungicide, and to five DAI was smaller than ASM. The basidiocarp at 20% showed increase in activity of polyphenoloxidase in relation to fungicide at three DAI, at four and five DAI, when compared to the three control treatments and at seven DAI in relation to fungicide. The 4th non-treated leaf presented similar effects to the 3rd leaf on the activity of polyphenoloxidase.

Treatments	AUDPC			
	Greenhouse		Field	
	3rd Leaf*	4th Leaf*	LMC****	UMC****
Basidiocarp 10%	24.5[1,2,3]	5.3	51.7[3]	54.2[1,3]
Basidiocarp 20%	19.5[1,2,3]	2.7[1]	49.9[3]	32.3[1,2,3]
Water	41.9	8.8	59.4	89.0
ASM**	2.3	5.2	41.9	67.7
Fungicide***	6.7	2.4	6.3	16.1
C.V. (%)	31.4	7.3	47.5	53.9

Averages followed by a bold number differ statistically (Dunnett's test, P≤0.05) of the control treatments water (1), ASM (2) or fungicide (3);
*3rd leaf: treated and inoculated; 4th leaf: non-treated and inoculated from same plant;
**ASM: acibenzolar-S-methyl (75mg L^{-1});
***Fungicide: azoxystrobin (40 mg L^{-1});
****LMC and UMC: Lower middle canopy and upper middle canopy of the plant, respectively.

Table 1. Area under disease progress curve (AUDPC) of angular leaf spot in common bean after the application of aqueous extracts of *P. sanguineus* basidiocarp, in greenhouse and field conditions.

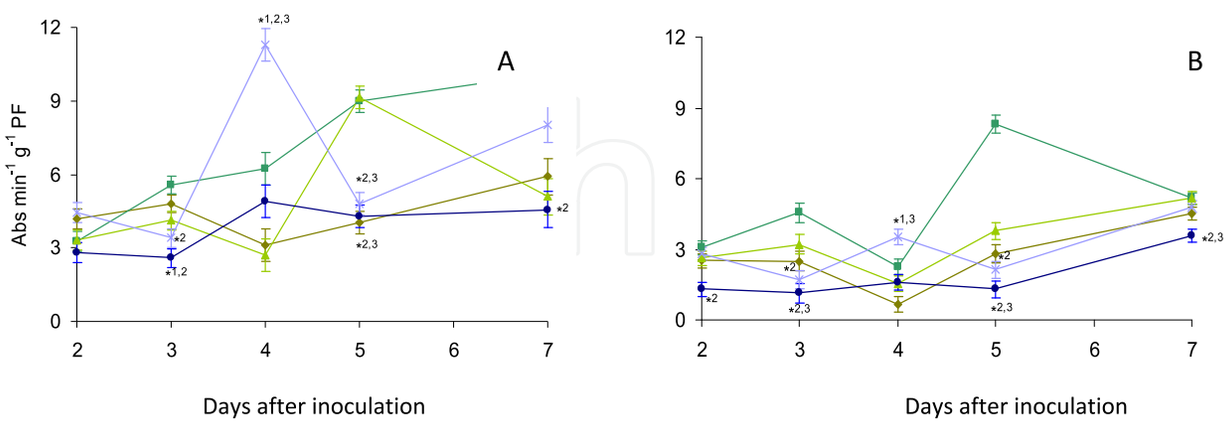

Figure 1. Peroxidase activity in bean plants inoculated with *Pseudocercospora griseola* tree days after the application of water (♦), acibenzolar-S-methyl (ASM 75mg L^{-1}) (■), fungicide (azoxystrobin 40mg L^{-1}) (▲) and the aqueous extracts of basidiocarp of *Pycnoporus sanguineus* at 10% and 20% (● and ×). **A** and **B** represent the 3rd treated and inoculated leaf and 4th non-treated and inoculated leaf, respectively. Bars indicate an average ± standard error. Average followed by * differ statistically (Dunnett's test, P≤0.05) from the control treatments water (1), ASM (2) or fungicide (3). PF: fresh mass.

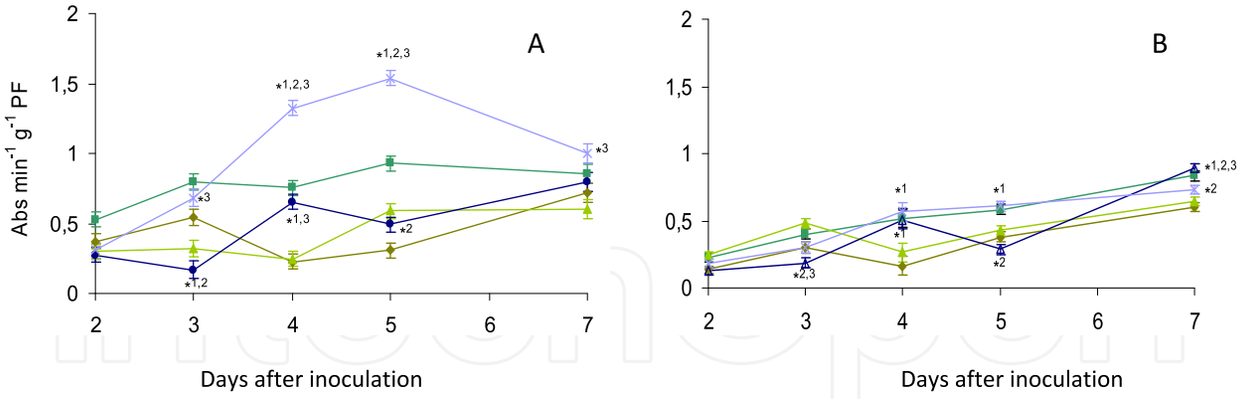

Figure 2. Polyphenoloxidase activity in bean plants inoculated with *Pseudocercospora griseola* tree days after the application of water (♦), acibenzolar-S-methyl (ASM 75mg L^{-1}) (■), fungicide (azoxystrobin 40mg L^{-1}) (▲) and the aqueous extracts of basidiocarp of *Pycnoporus sanguineus* at 10% and 20% (● and ×). **A** and **B** represent the 3rd treated and inoculated leaf and 4th non-treated and inoculated leaf, respectively. Bars indicate an average ± standard error. Average followed by * differ statistically (Dunnett's test, P≤0.05) from the control treatments water (1), ASM (2) or fungicide (3). PF: fresh mass.

The activity of β-1,3-glucanase was influenced by the treatments with extract of *P. sanguineus*, in the 3rd leaf, as well as in the 4th non-treated and inoculated leaf (Figure 3). In the treated leaf, the extracts of basidiocarp at 10% and 20% reduced the enzyme activity in relation to control treatments at five DAI, and at seven DAI for the extract at 20% when compared to water and fungicide. In the 4th non-treated leaf there was similar effect to the 3rd leaf on the activity of β-1,3-glucanase.

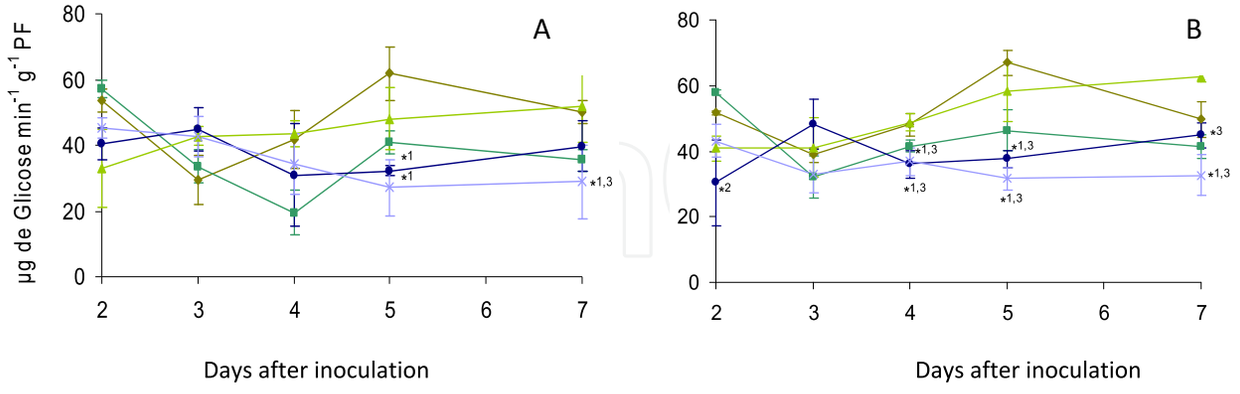

Figure 3. β-1,3-glucanase activity in bean plants inoculated with *Pseudocercospora griseola* tree days after the application of water (♦), acibenzolar-S-methyl (ASM 75mg L^{-1}) (■), fungicide (azoxystrobin 40mg L^{-1}) (▲) and the aqueous extracts of basidiocarp of *Pycnoporus sanguineus* at 10% and 20% (● and ×). **A** and **B** represent the 3rd treated and inoculated leaf and 4th non-treated and inoculated leaf, respectively. Bars indicate an average ± standard error. Average followed by * differ statistically (Dunnett's test, P≤0.05) from the control treatments water (1), ASM (2) or fungicide (3). PF: fresh mass.

The content of protein was significantly altered, both in the leaf treated with *P. sanguineus* extracts, as well as in the non-treated leaf (Figure 4). The effect of basidiocarp extract at 10% was significant at four DAI, stimulating the content of protein in relation to water and to fungicide. At five and seven DAI the effect was superior to water and to ASM. The basidiocarp at 20% was superior to water and to fungicide (three and four DAI) and at five and seven DAI was bigger than the three control treatments. This effect of *P. sanguineus* extracts on the protein content was similar for the 4th non-treated leaf.

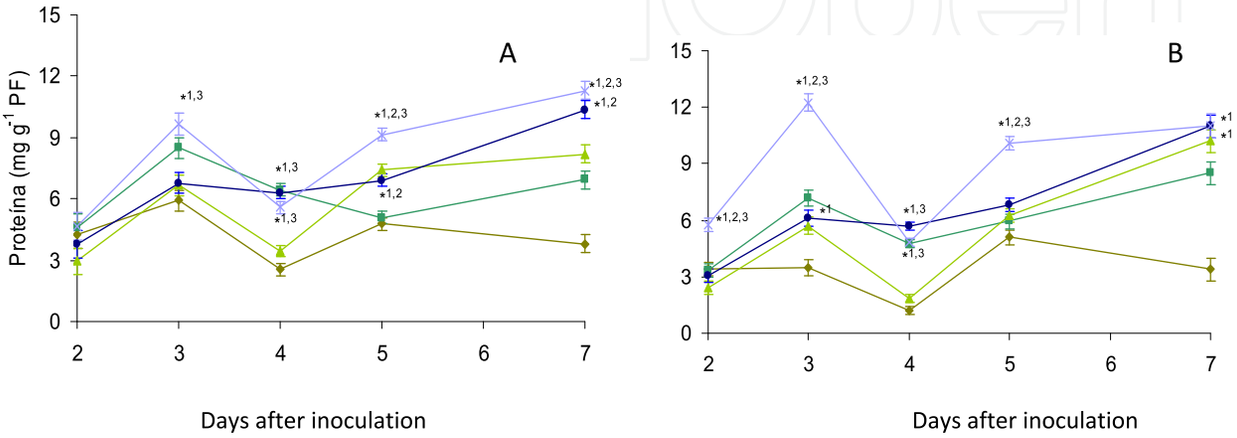

Figure 4. Protein content in bean plants inoculated with *Pseudocercospora griseola* tree days after the application of water (♦), acibenzolar-S-methyl (ASM 75mg L^{-1}) (■), fungicide (azoxystrobin 40mg L^{-1}) (▲) and the aqueous extracts of basidiocarp of *Pycnoporus sanguineus* at 10% and 20% (● and ×). **A** and **B** represent the 3rd treated and inoculated leaf and 4th non-treated and inoculated leaf, respectively. Bars indicate an average ± standard error. Average followed by * differ statistically (Dunnett's test, P≤0.05) from the control treatments water (1), ASM (2) or fungicide (3). PF: fresh mass.

The content of chlorophylls *a*, *b* and total in common bean treated with aqueous extracts of *P. sanguineus* basidiocarp and challenged with *P. griseola* was altered significantly, with increments in the level of pigments (Figure 5). It was verified a similar behavior in the chlorophyll content in the 4th non-treated leaf, emphasizing the systemic effects of *P. sanguineus* on the content of total chlorophyll in common bean.

4. Discussion

The basidiocarp extracts did not present direct antimicrobial activity on *P. griseola*. This is a satisfactory result, since for a product be considerate a resistance inductor, this should not show antimicrobial activity *in vitro* or *in vivo* assays [30].

In greenhouse and field it was observed reduction of AUDPC in plants treated with the extracts of *P. sanguineus*. In [13] the authors obtained similar results with aqueous extracts of the same basidiocarp, reducing the severity of anthracnose in common bean in a systemic way. In reference [31] was demonstrated partial reduction in the severity of anthracnose in cucumber leaf pre-treated with the fruiting bodies extracts of *Lentinula edodes* and *Agaricus blazei*, in a systemic way. The protection effect was dose-dependent and, in a minor degree,

time-dependent when it is considered the time interval between induction and pathogen inoculation.

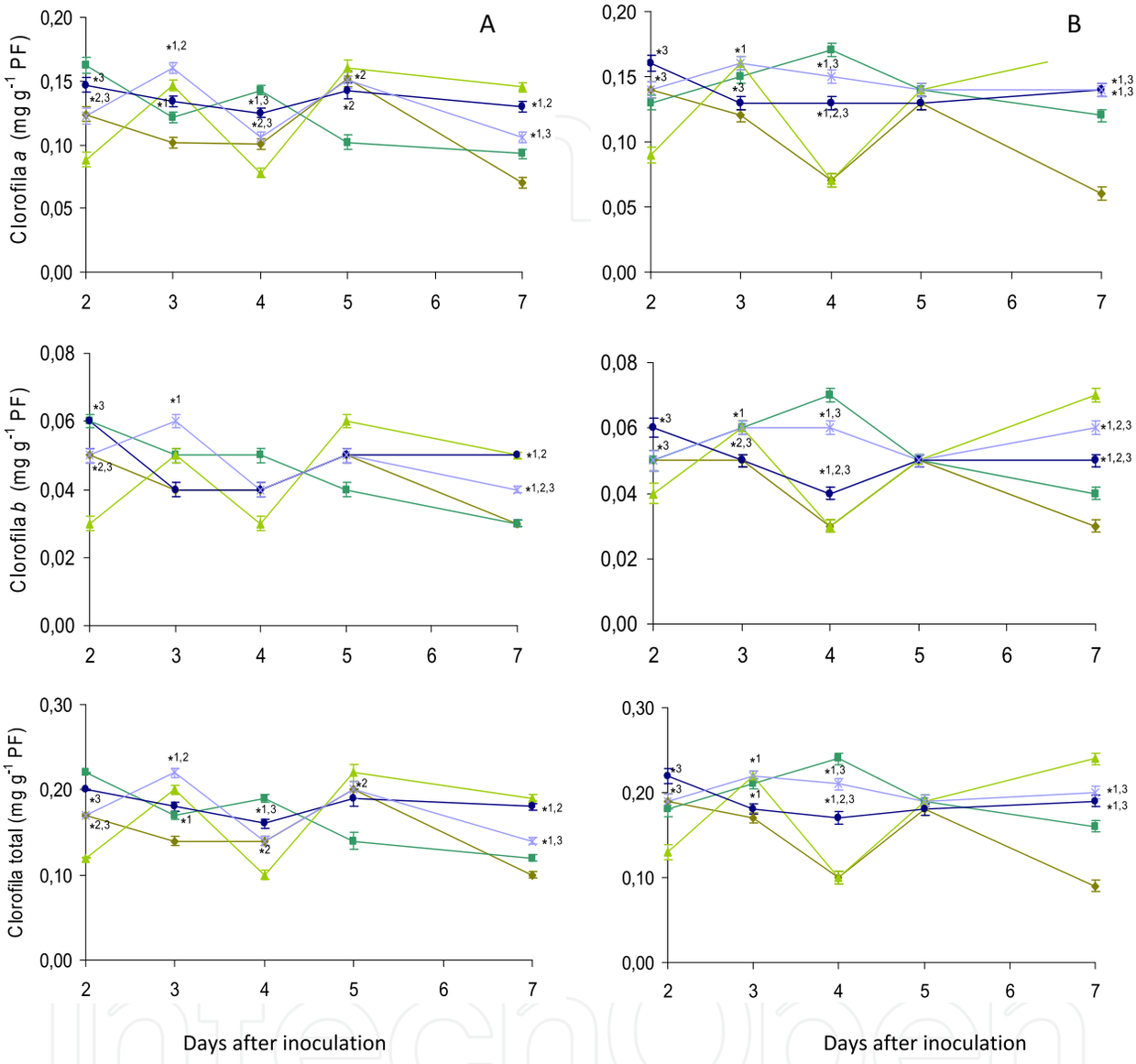

Figure 5. Chlorophylls *a, b* and total content in bean plants inoculated with *Pseudocercospora griseola* tree days after the application of water (♦), acibenzolar-S-methyl (ASM 75mg L^{-1}) (■), fungicide (azoxystrobin 40mg L^{-1}) (▲) and the aqueous extracts of basidiocarp of *Pycnoporus sanguineus* at 10% and 20% (● and ×). **A** and **B** represent the 3rd treated and inoculated leaf and 4th non-treated and inoculated leaf, respectively. Bars indicate an average ± standard error. Average followed by * differ statistically (Dunnett's test, P≤0.05) from the control treatments water (1), ASM (2) or fungicide (3). PF: fresh mass.

The activities of peroxidase, polyphenoloxidase and β-1,3 glucanase, and the content of total proteins and chlorophylls were altered in plants treated with *P. sanguineus* extract. Changes in the activities of peroxidase have being frequently correlated to the answer of resistance or susceptibility in different pathosystems. The peroxidase is responsible for the remove of atoms

of hydrogen of the hydroxyl cinnamic alcohols groups, whose radical polymerize to form the lignin. This polymer, together with cellulose and other polysaccharides occurring in the cell wall of the superior plants, works as a physical barrier to the pathogen penetration [4].

In [13] was verified peroxidase induction, local and systemically, in bean plants treated with aqueous extract of *P. sanguineus* and challenged with *C. lindemuthianum*, agreeing with the results obtained in this work. In reference [14] the authors evaluated the effects of organic extracts of *P. sanguineus* basidiocarp, and verified that the dichloromethane extract for sorghum and soybean, and ethanolic extract for soybean, inhibit the activity of peroxidase, while the hexanic extract promotes the activity for sorghum and soybean. In another work [32], the peroxidase activity in the common bean was influenced, in a time-dependent way, by the number of inducer applications. The inducer ASM promoted increase in the enzyme activity in a more accentuated way and faster than the biotic inducer *Bacillus cereus*. In [33] it was not found significant increments in activity of this enzyme in the common bean treated with *P. sanguineus* extract and inoculated with *C. lindemuthianum.* In [32] was observed that the activity of polyphenoloxidase in common bean was not altered by the treatment with *Bacillus cereus* and ASM, while in [34] was verified induction in the activity of these enzyme in tomato leaves treated with essential oil of *Cymbopogon citratus* and inoculated with *Alternaria solani.*

In the resistance induction, the increment of β-1,3-glucanase is related with the plant defense. This enzyme hydrolyzes β-1,3-glucan, which, together chitin, is the main component of fungal cell wall [35]. In another pathosystem [33] was observed increase in specific activity of β-1,3-glucanase in common bean treated with *P. sanguineus* extract and challenge with *C. lindemuthianum.* In the 1st leaf, treated and inoculated, the activity increased 28%, while in the 2nd leaf, non-treated, but inoculated, the culture filtrate at 5% and the mycelium extract at 10% increased in 331% and 1,057%, respectively, the enzyme activity.

Extracts from other basidiomycetes fruiting bodies have also induced the activity of β-1,3-glucanase. It was verified an increase in this enzyme in passion fruit inoculated with *Xanthomonas campestris* pv. *passiflorae* and treated with extracts of *L. edodes* and *A. blazei* in concentration of 20% and 40% [36]. According [19], bean plants challenged with *P. griseola* did not presented induction in the β-1,3-glucanase activity, however, when challenged with *Uromyces appendiculatus*, was verified induction of this enzyme [25]. This behavior indicates the differential interaction among the elicitors treatment and pathogens challenging, in the activation of defense mechanisms in plants. The plant could invest in the production of compounds that normally would be produced in the presence of the pathogen, however, with greater efficiency when pre-disposed to an elicitor.

In this work, the protein content was significantly altered both in the leaf treated with the *P. sanguineus* extracts as in the non-treated leaf, however, there was a faster response on protein synthesis in the 4th leaf. This result could be related to the age of the leaves in the moment of treatments application and pathogen inoculation, since the 4th leaf has probably more physiological activity than 3rd one, optimizing the protein synthesis and plant resistance response [37].

Protein synthesis could be related with the increase of the demand for substrates, necessary to the production of plant defense mechanisms induced by *P. sanguineus* treatment. Among the proteins, there are the pathogenesis related proteins (PR-proteins) which are induced in plant tissues due to inoculation with pathogens/microorganisms, systemically or local, as well as with treatments with chemical agents [38]. The activation of protein synthesis leads to a phase of plant resistance [37]. In [32] was verified reduction in protein content of bean plants when treated with *Bacillus cereus*, contrary to the treatment with ASM, demonstrating specificity in the physiological response of this host to the elicitor treatment.

The chlorophyll content *a, b* and total in bean plants treated with *P. sanguineus* extract and challenged with *P. griseola* was altered significantly, with increase in the levels of these pigments. These results suggest the need to generate energy for the synthesis of compounds involved in plant defense, considering that the chlorophyll molecules *a* and *b* constitute the two pigment systems responsible for the absorption and transference of the radiant energy [39]. The energy generated by the process of photosynthesis can, at a given time, be directed to the production of secondary metabolic compounds, as for example, in case of attack by pathogens [37]. An increase was observed in the levels of chlorophyll in regions infected with *U. appendiculatus*, which occurs both in the common bean cultivar moderately susceptible as in cultivar highly susceptible to pathogen [25].

5. Conclusions

In this work can be concluded that the extract from *P. sanguineus* basidiocarp reduce the severity of the angular leaf spot in common bean, by increasing the activity of defense enzymes peroxidase, polyphenoloxidase and β-1,3-glucanase, local and systemically. Additionally, physiological changes in the content of protein and chlorophylls were verified, probably due the apparatus energy synthesis required for plant defense mechanism involved in the reduction of this disease. In this way, the use of extracts from *P. sanguineus* fruiting bodies for the control of plant diseases in organic growth shows promising.

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