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Effects of cyproconazole, azoxystrobin and mineral oil on soybean leaf anatomy

Efeitos de ciproconazole, azoxistrobina e óleo mineral sobre a anatomia foliar da soja

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Abstract. A fungicide composed of a triazole (cyproconazole), a strobilurin (azoxystrobin) and a mineral oil (nimbus) is used for disease control, Asian Rust mainly, currently considered one of the major soybean diseases in Brazil. With the purpose of studying the effect of the fungicide and its constituents on histological characteristics of soybean (Glycine max cv. Pintado) leaves, the fungicides plus mineral oil, cyproconazole, azoxystrobin plus mineral oil and just mineral oil, were applied at R1 stage (anthesis), R3 and R5 stages. Fourteen days after the last application, central leaflet of each trifoliate leaf were harvested from five plants. Leaf anatomy variables were measured in four leaves collected in each of five plants per treatment. Histological analysis showed that the fungicide plus mineral oil increased leaf blade thickness, midrib, xylem, phloem and the width of the midrib. Azoxystrobin plus mineral oil increased the thickness of midrib, xylem, phloem and the width of the midrib. Cyproconazole increased the thickness of xylem and phloem. Finally, mineral oil reduced the thickness of leaf blade and midrib.

Keywords. Asian rust, fungicide, leaf histology

Resumo. Um fungicida composto por um triazol (ciproconazol), uma estrobilurina (azoxistrobina) e um óleo mineral (nimbus) tem sido utilizado para controle de doenças, principalmente ferrugem asiática, atualmente considerada uma das principais doenças da soja no Brasil. Com o objetivo de estudar o efeito do fungicida e seus constituintes sobre as características histológicas das folhas de soja (Glycine max cv. Pintado), fungicida + óleo mineral, ciproconazol, azoxystrobina + óleo mineral e apenas óleo mineral, foram aplicados nos estádios R1 (antese), R3 e R5. Quatorze dias após a última aplicação, as porções centrais de cada folha trifoliolada foram colhidas de cinco plantas. A anatomia de variáveis foliares foram determinadas em quatro folhas coletadas em cada uma das cinco plantas por tratamento. Análise histológica mostrou que o fungicida + óleo mineral, aumentou a espessura da lâmina foliar, nervura central, xilema, floema e largura da nervura central. Azoxistrobina + óleo mineral, aumentou a espessura da nervura central, floema, xilema, e largura da nervura central. Ciproconazol aumentou a espessura do xilema e do floema. Finalmente, óleo mineral reduziu a espessura da lâmina foliar e nervura central.

Palavras-chave. Ferrugem asiática, fungicida, histologia foliar

Introduction

In the 2010/11 Brazilian crops of soybean (*Glycine max* (L.) Merrill) amounted 75,0 million hectares, producing 60.1 million tons with 3106 kg ha^{-1} of average productivity (Conab, 2011). However, production has been affected by aggravated fungal diseases, causing considerable damage to growers, to breeding programs and therefore to the country (Sediyama et al., 2005). In Brazil, 34 types of fungal diseases in soybean have been identified (Embrapa, 2008). Among the major diseases, the Asian soybean rust caused by *Phakopsora pachyrhizi*, which has provoked great concerns among growers and technicians, mainly after the 2002/03 crop, when spread to all producing regions, posing as a threat to the culture due to damage caused and to the increased production costs generated for its control. The most widespread and effective method still used in controlling soybean rust is the use of fungicides. Among the fungicides most commonly used are mixtures of different groups of strobilurins with triazoles, which have

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proven effective in controlling the disease (Embrapa, 2008).

Characteristic effects of triazole application in plants include reduction in height, increase in stem diameter and compactness of the plant canopy. The intensity of these changes depends on the triazole, the plant specie and age, the dose and method of application of the triazole. Plants treated with triazole have smaller, but thicker leaves and thicker cuticle. The increase in leaf thickness has been correlated with the increase in the diameter of cells and/or with the presence of additional cell layers (Gao et al., 1988).

The sectional area of chloroplasts of leaves treated with triazoles, under a microscope and a spectrophotometer, is significantly larger than that of untreated leaves. In maize, a treatment with paclobutrazole did not affect the number of chloroplasts, but increased the amount of chlorophyll per chloroplast. Both paclobutrazole and uniconazole are used as growth retardants on fruit trees, ornamental plants and crops. The uniconazole was considered more active than paclobutrazole in reducing plant height, however, in soybeans, both compounds showed similar effects on plant stunting. Retardants increased the chlorophyll content and leaf thickness in soybeans but had no effect on these parameters in maize. The increase in soybean leaf thickness was due, primarily, to an increase in the thickness of palisade layer cells (Barnes et al., 1989)

In many species, treatment with triazole leads to inhibition of ethylene biosynthesis, delaying senescence and leaf abscission (Fletcher et al., 2000).

Several experiments have been carried out to examine the physiological effects of strobilurin on wheat, barley and soybeans, in which these fungicides have reduced the rate of water conductance through the stomata closure in the three crops. This resulted in lower rates of net photosynthesis, transpiration and intercellular CO_2. The water use efficiency (net photosynthesis/transpiration ratio) in well-irrigated plants is often improved by treatment with strobilurin. This is because these fungicides reduce more transpiration than photosynthesis; however, in plants under water deficit, water efficiency is not improved and is occasionally reduced after treatment with strobilurin. Strobilurin reduces photosynthesis, regardless of the effect on stomata. The effects of strobilurin are temporary, but the recovery speed of photosynthetic parameters after the application of these fungicides depends on strobilurin group. The effects of pyraclostrobin persist for at least 14 days after spraying (Nason et al., 2004).

Mineral oils, such as the nimbus, can exert some control over pests, but are also widely used as spreader-stickers. The purpose of this study was to evaluate the effect of a fungicide and its constituents on the anatomical characteristics of leaves of soybean 'Pintado' to know more about the action of the agrochemical.

Material and Methods

The soybean plants were cultivated under greenhouse conditions, irrigated near to field capacity and treated with the fungicide and the vase substract (sand, organic matter and soil at the relation 2:1:2) received the application of conventional mineral salts.

The treatments were: control (non-treated plants at anthesis stage), the fungicide (200 g L^{-1} – 20% m/v azoxystrobin + 80 g L^{-1} – 8% m/v cyproconazole + 428 g L^{-1} mineral oil), azoxystrobin (200 g L^{-1} – 20% m/v + 428 g L^{-1} nimbus – mineral oil), cyproconazole (80 g L^{-1} – 8% m/v) and mineral oil (428 g L^{-1} of mineral oil) were applied three times: at R1 stage (anthesis), R3 and R5 stages. Fourteen days after the last application, four samples of central leaflet of each trifoliate leaf were harvested from five plants.

For the anatomical study, four central leaflets were used (blade and midrib) of the third node of five adult individuals randomly chosen in each five treatments, collected at the end of the reproductive stage (R6).

Samples were fixed in FAA 70 (1:1:8 formaldehyde, glacial acetic acid and ethyl alcohol 70%) for 24 hours, dehydrated in a graded ethanol series and embedded in hydroxyl-ethyl methacrylate (Leica Historesin). Serial sections were cut on a rotary microtome (5 –10 µm thick), stained with 1% toluidine blue with 1% of sodium borate in 100 mL of distilled water (Gerrits, 1991).

Twenty measurements per treatment were taken of the following leaf anatomical variables: thickness of leaf blade, thickness of the phloem and xylem and thickness and width of the midrib.

Photomicrographs were taken with a Leica® DMLB photomicroscope equipped with a Leica® DC 300F camera. Data on leaf histological analysis were subject to variance analysis and, when significant, the means were compared using the Tukey test at 5% probability.

Results and Discussion

The mesophyll is dorsiventral type, having two layers of palisade cells (Figure 1), characteristic of the Phaseolae tribe (Lackey, 1978). The spongy parenchyma is composed of cells of various shapes and sizes with intercellular spaces quite pronounced. Lateral vascular bundles surrounded by a parenchymatous sheath of isodiametric cells, with extensions to both faces of leaf surface, were also observed along the mesophyll (Figure 1).

Below the innermost layer of the palisade, there are cells with horizontal orientation, when compared with others of mesophyll cells, called paraveinal mesophyll (Figure 1). These cells facilitate horizontal translocation of photosynthesis products (Fisher, 1967).

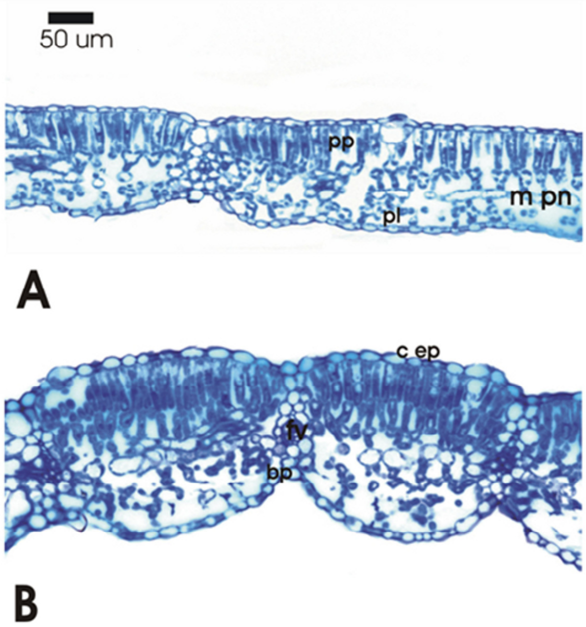

Figure 1. Cross sections of central leaflet blade of *Glycine max* 'Pintado'. (A) non-treated (control). (B) fungicide (200 g L^{-1} – 20% m/v azoxystrobin + 80 g L^{-1} – 8% m/v cyproconazole + 428 g L^{-1} nimbus – mineral oil). ep = epidermis; fv = vascular bundle; mpn: paraveinal mesophyll; pl = spongy parenchyma; pp = palisade parenchyma.

By comparing the cross sections of the leaf of control soybean 'Pintado' (Figure. 1A and 3A) with mesophyll sections of that treated with fungicide + mineral oil (Fig. 1B) and with cyproconazole (Fig. 3B), it is verified that the treatments caused an increase on the structure dimensions of the mesophyll. The herbicide tridiphane did affect the growth and structure of soybean leaf tissue too (Dionigini & Dekker, 1990). The triazole triadimefon increased the thickness of leaf in wheat and barley (Sopher et al., 1999; Asami et al., 2000).

The midrib vascular system is composed of a collateral bundle in the form of an abaxial arc and one or two smaller bundles embedded in the ground tissue (Figure 2A). Sclerenchyma fibers external to phloem and collenchyma deposited in the adaxial arc and the abaxial of midrib, are observed (Figure 2A).

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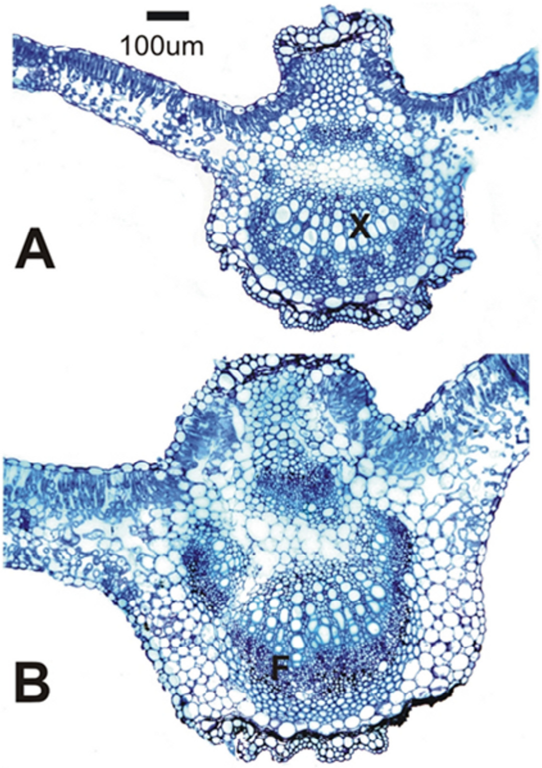

Figure 2. Cross sections of central leaflet midrib of *Glycine max* ‘Pintado’. (A) non-treated (control). (B) Fungicide (200 g L^{-1} – 20% m/v azoxystrobin + 80 g L^{-1} – 8% m/v cyproconazole + 428 g L^{-1} nimbus – mineral oil). X = xylem; F = phloem. The ground parenchyma has more cell layers in treated leaflets than in the control.

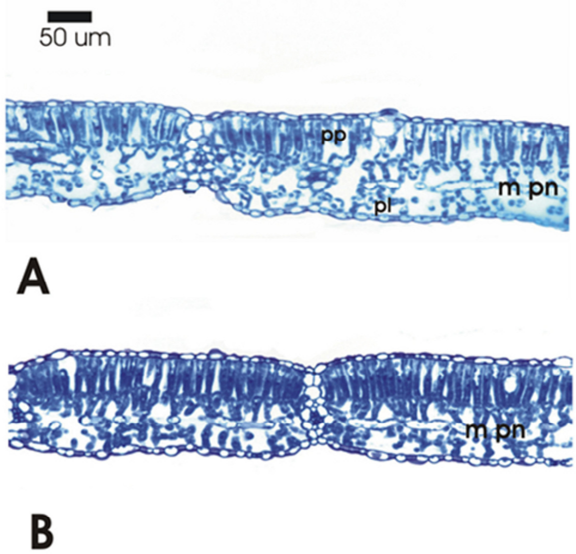

Figure 3. Cross sections of central leaflet blade of *Glycine max* ‘Pintado’. (A) non-treated (control). (B) cyproconazole (80 g L^{-1} – 8% m/v). ep = epidermis; fv = vascular bundle; mpn: paraveinal mesophyll; pl = spongy parenchyma; pp = palisade parenchyma.

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When comparing the cross sections of the midrib of the control soybean 'Pintado' (Figure. 2A and 4A) with midrib sections from leaflets treated with fungicide + mineral oil (Fig. 2B) and with cyproconazole (Figure 4B), it is observed that the treatments increased tissue constituents of the midrib. Soybean leaves treated with tridiphane presented parenchyma cells (Dionigini & Dekker, 1990), but treated unifoliate midrib tissue appeared similar to untreated midrib tissue at both sampling points (Dionigini & Dekker, 1990).

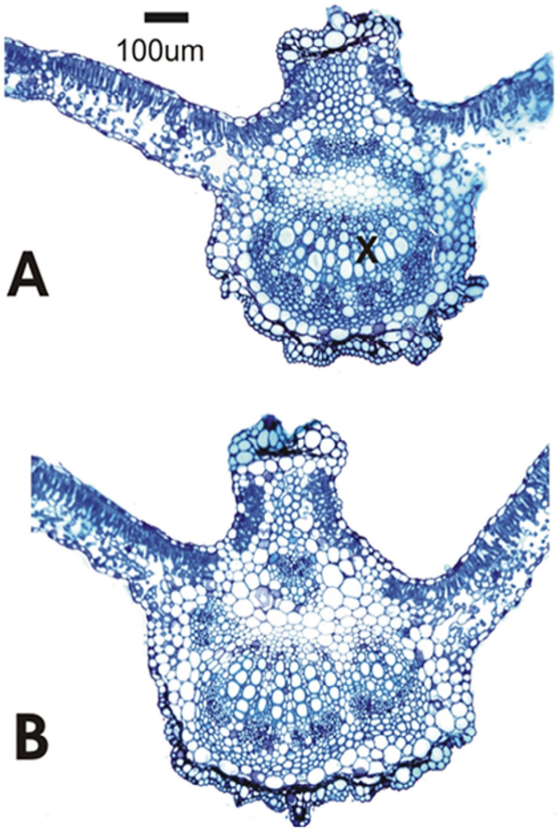

Figure 4. Cross sections of central leaflet midrib of *Glycine max* 'Pintado'. (A) non-treated (control). (B). cyproconazole (80 g L^{-1} – 8% m/v). X = xylem; F = phloem.

Fungicide + mineral oil increased leaf blade thickness, while the mineral oil reduced it.The midrib thickness was increased in treatments with fungicide + mineral oil and azoxystrobin + mineral oil, and it was reduced in plants treated with mineral oil. Treatments with fungicide + mineral oil and with azoxystrobin + mineral oil increased the midrib width of soybean leaves (Table 1).

Application of fungicide + mineral oil, azoxystrobin + mineral oil and cyproconazole increased the xylem thickness, whereas the fungicide + mineral oil, azoxystrobin + mineral oil and cyproconazole increased the phloem thickness (Table 1). In *Amophophallus campanulatus* plants, the thickness of leaf increased gradually in plants treated with triadimefon than in the control plants (Gopi et al., 2008). Triazole treatments increased the number of cells per unit area in the palisade and spongy layer of the leaf (Gopi et al., 2008). Increased mesophyll thickness, chloroplast size and level were reported in wheat with triadimefon treatment (Gao et al., 1988).

A secondary factor associated with systemic fungicides is possible hormonal causes your application in plants, influencing various physiological processes. This influence became known as 'tonic effect' and visually characterized by a greater vigor and leafiness of the plant and a shade darker green leaves. However, the exact mechanism that generates this fact is still unknown, but it is attributed to a possible hormonal effect, which indirectly influences growth by increasing the absorption of water and nutrients (Almeida & Matiello, 2000). After absorption and translocation

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through the xylem, the active ingredient settles on leaves and prolongs the photosynthetic activity. This fact is due to hormonal concentration that it has. According to Castro (2006), the molecules of the active hormonal action act in two ways. The first influences in order to activate cell membrane proteins enabling greater ionic transport, increasing the mineral nutrition of the plant. The second acts in increased enzyme activity in increase of primary and secondary metabolism, increased synthesis of amino acid precursors for the synthesis of new proteins and plant hormones. These facts justify the increased thickness of the leaf soybean leaves treated with fungicides.

Table 1. Effect of fungicide and its constituents on histological characteristics of 'Pintado' soybean leaves: blade thickness (BT), midrib thickness (NE), midrib width (NW), xylem thickness (XT) and phloem thickness (PT). Means of 20 replications.

TREATMENTS	BT (µm)	NE (µm)	NW (µm)	XT (µm)	PT (µm)
Control	169.60 b*	722.81 c	601.69 cd	126.78 c	51.63 c
Fungicide + Mineral oil	253.98 a	1023.18 a	854.29 a	186.67 a	100.08 a
Cyproconazole	177.17 b	774.99 c	630.66 c	148.37 b	66.69 b
Azoxystrobin + Mineral oil	168.32 b	885.86 b	755.33 b	182.44 a	75.16 b
Mineral Oil	133.69 c	602.88 d	530.76 d	125.29 c	45.82 c
F (treatment)	134.85 **	94.09 **	46.49 **	55.41 **	67.39 **
V. C. (%)	9.46	9.21	12.56	11.50	17.22

* Similar letters in the columns indicate that there was no difference between the means compared in the Tukey test at 5% of probability.
** Significant by the F test at 1% of probability.

Conclusion

The fungicide and its constituents applied on soybean 'Pintado' increased tissue components of the leaf mesophyll and midrib, while the mineral oil decreased tissues of these leaf structures.

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