Herbicide action in the severity of diseases in cultivated plants¹

Ação herbicida na severidade de doenças em plantas cultivadas

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Abstract - The severity of diseases in plants can be affected by practices such as the application of herbicides for the control of weeds. Therefore, we aimed to present and discuss the relationship between the use of herbicides and the severity of diseases of cultivated plants. After contact, the pathogens try to infect and colonize the hosts, which, in turn, use mechanisms to defend themselves from infection. The trichomes, the cuticles and the epicuticular wax are barriers against penetration. Phenolic compounds, enzymes and phytoalexins are substances synthesized by plants and toxic to the pathogens. The herbicide action influences the severity of diseases indirectly on the defense mechanisms of plants or directly when it presents toxic effects to the pathogens. The effect of adjuvants in the commercial formula of herbicides causes degradation of trichomes, epicuticular waxes and cuticles, favoring penetration of pathogens. Harmful effects on the physiology and secondary metabolism of plants, caused by herbicides, affects the synthesis of the above mentioned defense compounds. However, the herbicide molecules may inhibit the germination of spores and the growth of fungal hyphae, which results on the reduction of the infection and colonization. Necrotic points resulting from herbicide action may make plants less prone to the occurrence of rust, mildews and powdery mildews, caused by biotrophic fungi. Herbicides also have secondary effects such as population reduction of alternative host plants and vector insects. The relationship between herbicides and the severity of diseases in crops is an important tool for the phytosanitary management in an integrated way.

Keywords: leaf anatomy; fungi; chemical management of weeds; defense mechanisms; secondary metabolism

Resumo - A severidade de doenças em plantas pode ser afetada por práticas, como a aplicação de herbicidas para o controle de plantas daninhas. Por isso, objetivou-se apresentar e discutir a relação do uso de herbicidas na severidade de doenças de plantas cultivadas. Após contato, os patógenos tentam infectar e colonizar os hospedeiros, enquanto que estes também utilizam de mecanismos para se defender da infecção. Os tricomas, a cutícula e as ceras epicuticulares são barreiras à penetração. Compostos fenólicos, enzimas e fitoalexinas são substâncias sintetizadas

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pelas plantas e tóxicas aos patógenos. A ação herbicida influencia a severidade de doenças de maneira indireta sobre os mecanismos de defesa das plantas ou de forma direta, quando apresenta efeito tóxico aos patógenos. O efeito de adjuvantes presentes na formulação comercial dos herbicidas provoca degradação dos tricomas, das ceras epicuticulares e da cutícula, favorecendo a penetração dos patógenos. Efeitos prejudiciais na fisiologia e metabolismo secundário das plantas, provocados por herbicidas, afeta a síntese de compostos de defesa supracitados. Entretanto, as moléculas herbicidas podem inibir a germinação de esporos e crescimento de hifas fúngicas, o que implica na redução da infecção e colonização. Pontos necróticos decorrentes da ação herbicida podem tornar as plantas menos propensas à ocorrência de ferrugens, míldios e oídios que são causadas por fungos biotróficos. Os herbicidas ainda possuem efeitos secundários como a redução populacional de plantas hospedeiras alternativas e de insetos vetores. A relação entre herbicidas e severidade de doenças em culturas figura como importante ferramenta para o manejo fitossanitário de forma integrada.

Palavras-chaves: anatomia foliar; fungos; manejo químico de plantas daninhas; mecanismos de defesa; metabolismo secundário

Introduction

Plants are subject to biotic diseases, caused by pathogens, during their whole life cycle. This relationship between pathogen and the host is very intimate, and is the same as a battle in which the involved parties developed attack and defense mechanisms (Amorim and Pascholati, 2011). External factors, common in cultivated areas and their surroundings, such as the use of herbicides for the handling of weeds, can interfere negatively or positively on plants diseases.

The adjuvants in the commercial formula of herbicides can cause degradation of trichomes, epicuticular waxes and cuticles (Tuffi-Santos et al., 2009; Santos et al., 2015), affecting the natural barriers of plants against penetration of pathogens. Additionally, herbicides can alter the synthesis of products coming from the secondary metabolism, among them the phytoalexins, which are composed with the antimicrobial properties (Rizzardi et al., 2003), just like increasing the activity of enzymes connected to the plants' defense mechanism (Pereira et al., 2009).

Besides the indirect effect on the plants' defense mechanisms, the herbicides can present an inhibition effect in the germination of spores and/or *in vitro* growth of several fungi (Larson et al., 2006; Soares et al., 2008; Rosa et al.,

2010; Tuffi-Santos et al., 2011). Therefore, in this review, we aimed to present and discuss the relationship between the use of herbicides and the severity of diseases of cultivated plants.

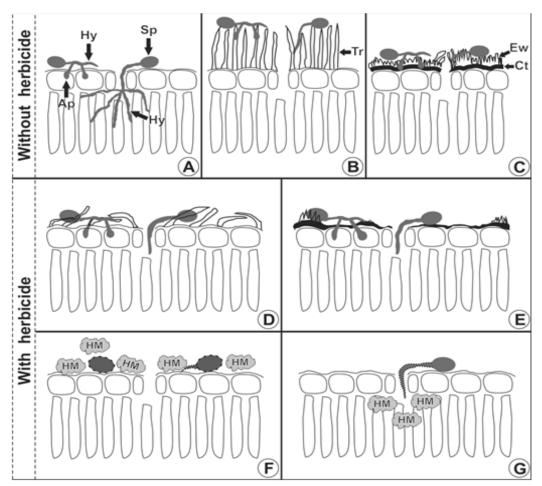
Pathogen-host Relationship and Plants Defense Mechanisms to Pathogens

After contact with the plants surface, the pathogen tries to infect and colonize the host tissues, which, in turn, use mechanisms to defend themselves from infection (Amorim and Pascholati, 2011). The plants defense mechanisms to pathogens are classified as structural and biochemical. Among the several defense mechanisms there are those which were already present in the plants before contacting the pathogen and others that are stimulated responding to the infection. When they are in the host tissues, before infection, we say they are part of the pre-formed mechanisms; and when they are produced in response to the infection they are called post-formed (Stangarlin et al., 2011).

Regarding the ways they penetrate their hosts, the fungi are the most versatile pathogens because they can penetrate directly through the formation of appressorium, through natural openings or wounds (Amorim and Pascholati, 2011). Bacteria penetrate through natural openings and injury, while virus through injury



or vectors (Amorim and Pascholati, 2011). The leaf surface is the first obstacle found by pathogens. Leaves with great amount of trichomes may involve fungal spores and prevent them from reaching the other epidermal cells and penetrate the host even after germination (Figure 1B). In that respect, bean plants with higher density of trichomes have higher tolerance to anthracnose (Jerba et al., 2005). After trichomes, the presence of a thicker cuticle associated with the deposition of epicuticular wax, especially in the stomatal cavity region (Figure 1C), are highly important barriers to penetration (Reina-Pinto and Yephremov, 2009). Stomata are the main way of penetration through natural openings (Amorim and Pascholati, 2011). Thus, the stomatal density may be another important defense mechanism of plants against penetration (Paz-Lima et al., 2010).



A. Host without structural defense mechanisms; Spores (Sp) germinate and hyphae (Hy) penetrate directly through the appressorium (Ap) or through natural openings. **B.** In the presence of trichomes (Tr), in some cases, even if the spores do not germinate and cannot reach the epidermis and penetrate the host tissues. **C.** Thick cuticle (Ct) and epicuticular was (Ew), forming barriers to penetration. **D.** Adjuvant effect of the herbicides can damage the trichomes and favor penetration. **E.** Adjuvant effect degrades the cuticle and promotes degradation of the epicuticular wax, enabling penetration. **F.** Herbicides molecules (MH) inhibiting germination of fungal spores. **G.** Herbicides molecules in the inside of the lead inhibiting the growth of fungal hyphae. *The information on the interactions plant-pathogenherbicide used to carry out the present scheme was extracted from the following papers: Wyss and Müller-Schärer (2001); Larson et al. (2006); Tuffi-Santos et al. (2009); Rosa et al. (2010); Amorim and Pascholati (2011); Stangarlin et al. (2011); Tuffi-Santos et al. (2011); and Santos et al. (2015).

Figure 1. Indirect herbicide action in the structural defense mechanisms of plants to pathogens (B-E) and direct in the germination of spores or hyphae growth (F-G).



As an answer to the infection, the following stand out: formation of halos, papillae, areas of lignification or abscission and tylose. The papillae are projections of epidermal cells with the purpose of increasing the space between the cellular wall and the membrane in the exact place where the pathogen makes pressure for penetration (Stangarlin et al., 2011). The resistance to *Fusarium culmorum* in wheat is associated to the formation of papillae (Kang and Buchenauer, 2000). Areas of lignification or abscission layers are crucial to avoid progression of the disease. After the infection, the areas of lignification isolate the pathogen in a certain already contaminated area and makes the flow of water and nutrients difficult from the host to the fungus and the transit of toxins and enzymes of the pathogen for the host (Stangarlin et al., 2011).

In the pathogen-host relationship, the biochemical mechanisms produced by the plants are more versatile. Among the pre-formed we have: chlorogenic substances. acid. protocatechuic acid and catechol, α-tomatina, avenacinas, tulip osídeos, phenolic glycosides, protein inhibitors and vegetable defense enzymes. Among the defense compounds formed after infection, we can mention the reactive forms of oxygen, nitrous oxide and phytoalexins (Stangarlin et al., 2011). Resulting from secondary metabolism, the phytoalexins have antimicrobial properties and are produced by plants in response to physical, chemical or biological stress (Amorim and Pascholati, 2011; Stangarlin et al., 2011).

The pathogen-host relations in plants' diseases are dynamic and can be influenced by external factors, just as it happens when there is contact between the plant and the herbicide.

How do Herbicides Affect the Severity of Diseases?

Herbicides can interfere indirectly in the pathogen-host relations on the plants' defense mechanisms to pathogens (Figure 1D and E) and, directly, in the germination of spores and/or in the growth of fungal hyphae (Figure 1F and G). In both cases, they can affect positively or negatively the severity of diseases in cultures.

When herbicides selective to the crops are used, usually the applications are done in a total area, since the product does not cause economic problems to the plants grown. However, when the herbicides are not selective, the applications must be done directed at the weeds, avoiding undesirable contact with the culture. Even when that is done, part of the product may be dragged by wind and reach the crops by drifting. In the first case, although there is no toxic effect for the crop, adjuvants in the commercial formula of the herbicides may cause loss of turgor and/or degradation of trichomes, degradation of the cuticle and the epicuticular wax, damage to the stomatal complex and degradation to the epidermis (Rawlinson et al., 1978; Tuffi-Santos et al., 2009; Santos et al., 2015; Tuffi-Santos et al., 2015). In the second case, in addition to the adjuvant effect, the product will also cause an herbicide effect, which, in several cases, results on the alteration of the physiology and in the metabolism of the cultivated plants (Machado et al., 2010; Langaro et al., 2014).

The effects of the adjuvants in the preformed structural defense mechanisms may favor the penetration of pathogens to the host. When promoting loss of turgor and degradation of trichomes, the space between the fungal spore and the leaf surface is reduced, enabling the penetration of the host by the fungal hyphae after germination (Figure 1D). Moreover, the erosion of the cuticle and the epicuticular wax, resulting from the adjuvant effect, facilitates penetration (Figure 1E). Sprays in leaves of Brassica napus with dalapon, carbutamide and propyzamide reduced the amount and altered the shape of epicuticular wax, which favored the infection by Cylindrosporium concentricum (Rawlinson et al., 1978).

The herbicide effect is more complex and depends on the action mechanism of the used herbicide. Glyphosate is the most used and studied herbicide in Brazil and in the world, so



most papers found in the literature correspond to this molecule. In 63945 papers, involving herbicides, available at the Science Direct data base, most are of this herbicide (12%). The influence of herbicides on the severity of diseases is very little studied, and it is related to only 0.03% of the total published. From these papers, around 58% used glyphosate and 42% other herbicides. Most of the researchers evaluated the direct effects of the herbicides on the pathogens. Only 20% of the papers studied the indirect influence, especially on the structural defense mechanisms of the plants.

Glyphosate is a non-selective herbicide and its action mechanism affects the route of shikimic acid, forerunner involved in the defense of plants to pathogens, standing out: tannins and anthocyanins, salicylic acid, lignin, flavones, coumarins and isoflavones (Buchanan et al., 2000; Srivastava, 2001). Therefore, sensitive plants, previously treated with glyphosate can become more vulnerable to the attack of pathogens.

On the other hand, glyphosate can direct harmful effects on the present germination of fungal spores (Figure 1F) or growth of fungal hyphae (Figure 1G). The shikimate pathway, which is affected by glyphosate, is also present in fungi and bacteria (Richards et al., 2006). Hence the importance of this herbicide the handling in of phytopathogenic agents. Several studies reported direct action of glyphosate in germination and in vitro fungal growth. Larson et al. (2006) saw that glyphosate inhibited in vitro growth of Fusarium oxysporum and Rhizoctonia solani, fungi that cause root yellowing and rot, respectively. This inhibiting effect is possibly related to the glyphosate action mechanism. When inhibiting enzyme 5enolpyruvylshikimate-3-phosphate (EPSP), the herbicide prevents the production of aromatic amino acids tryptophan, phenylalanine and tyrosine (Jaworski, 1972). These amino acids are crucial for mycelial growth in fungi (Rosa et al., 2010).

Glyphosate inhibited the growth of Rhizoctonia solani, Ceratocystis fimbriata, Cryphonectria cubensis, Phytophthora capsici, Macrophomina phaseolinea, Sclerotium rolfsii, Fusarium oxysporum and Mirothecium roridum, which cause important diseases in bean plants, mango trees, eucalyptus, pepper and tomato (Rosa et al., 2010). Tuffi-Santos et al. (2011) also saw the inhibiting effect of this herbicide in the germination and *in vitro* growth of Puccinia psidii which causes rust in eucalyptus. In another study, 2,4-D, glyphosate and linuron reduced germination and inhibited in vitro growth of Puccinia lagenophora (Wyss e Müller-Schärer, 2001).

Another important group of herbicides (protoporphyrinogen inhibit PROTOX oxidase). When inhibiting the synthesis of this enzyme, there is accumulation of protoporphyrinogen IX, which spills from the chloroplast to the cytoplasm of the cell and, in the presence of light and oxygen, results in the formation of reactive forms of oxygent (Silva and Silva, 2012). These compounds are important in the defense of plants to the pathogens, once they are part of the post-formed biochemical mechanisms (Stangarlin et al., 2011). In turn, sensitive plants treated with these herbicides have necrotic spots on the leaves because, in the presence of light, these reactive forms of oxygen cause lipid peroxidation, resulting in the cellular death by degradation of the plasmatic membranes. This fact reduces the infection by biotrophic pathogens, which must have an alive host tissue in order to install and reproduce (Bedendo, 2011).

Besides the direct effect in the pathogen or indirect in the defense mechanisms of plants to pathogens, secondary influence of the herbicide action on the severity of diseases also stands out. The weeds can be potential hosts for a wide range of phytopathogen agents (Ntidi et al., 2012; Altinok, 2013; Gonçalves et al., 2015). In this sense, the use of herbicides for the handling of weeds represents great importance in the appearance and severity of some diseases. To handle root rot caused by *Rhizoctonia solani*



and *Rhizoctonia oryzae* in crops of barley, the control of weeds is the main disease management strategy (Babiker et al., 2011). In three years of field experiments, the authors observed that, when chemical handling of weeds with glyphosate was employed, there was significant reduction in the severity of disease in the following crop (Babiker et al., 2011). However, the herbicide action promotes reduction of the vegetable biodiversity in the cultivated areas due to the control of weeds; and the presence of a species cultivated in isolation may favor certain diseases (Altiere, 1999).

Another important point is the influence of herbicides in the insects that, many times can act as vectors, especially of diseases caused by virus (Gutiérrez et al., 2013; Mauck et al., 2015). Egan et al. (2014) saw reduction of three species of herbivorous and the increase of a species of pest when exposed to dicamba herbicide.

Herbicides Alter the Severity of Diseases

The reduction of severity of some diseases resulting from the use of herbicides is reported in the literature, especially the rust caused by biotrophic fungi. Tuffi-Santos et al. (2011) mentioned a smaller severity of eucalyptus rust in previously treated seedlings with glyphosate through a smaller leaf area affected by pustules, the smaller number of urediniospores per pustule and smaller number of urediniospores per leaf area.

Preventive applications of glyphosate, besides reducing the severity of rust in soy plants cultivated in greenhouse, have a harmful effect in the germination of spores of *Phakopsora pachyrhizi* (Soares et al., 2008). Rosa et. al. (2010) also reported the efficacy of glyphosate in the healing power of important diseases in soy crops such as Asian rust (*Phakopsora pachyrhizi*), Soybean powdery mildew (*Erysiphe diffusa*) and the Myrothecium spot (*Mirothecium roridum*). Transgenic soy plants that are resistant to glyphosate were

inoculated with pathogenic agents mentioned previously and treated with glyphosate three days after inoculation, resulting in a significant reduction of the severity of diseases (Rosa et. al, 2010).

Somac and Foster-Hartnett (2012), when evaluating the handling of leaf diseases in alfalfa resistant to glyphosate, saw that a previous treatment with glyphosate was efficient in the control of alfalfa rust caused by *Uromyces striatus*. However, it was not as efficient in the reduction of anthracnose symptoms (*Colletotrichum trifolii*) and stem black spot (*Phoma medicaginis*). The authors also affirmed the healing effect of glyphosate for alfalfa rust because, when applied 10 days after inoculation of *U. striatus*, the herbicide significantly reduced the severity of the disease.

On the other hand, several results confirmed the increase in severity of the diseases after the application of herbicide. Despite the inhibiting effect on the mycelial growth *in vitro* of *Pythium arrhenomanes*, causative agent of root rot in sugar cane, the herbicides glyphosate, pendimethalin and terbacil promoted an increase in the severity of the disease in the field (Dissanayake et al., 1998). In bean plants, previously treated with glyphosate, Descalzo et al. (1998) observed an increase in the populations of *Pythium ultimum* and *P. coloratum*, as well as the increase in the severity of *damping off*.

Experiments in the field developed by Sanogo et al. (2001) revealed that the application of herbicides acifluorfen, glyphosate and imazethapyr promoted an increase in the levels of the sudden death syndrome (*Fusarium solani* f. sp. glycines) in soy plantations. The authors associated the increase in the severity of the disease with the state of crop stress caused by the application of herbicides.

Bradley et al. (2002), when studying root rot and soybean hypocotyl rot, disease caused by *Rhizoctonia solani*, confirmed that the herbicides dimethenamid+metribuzin, pendimethalin, acifluorfen and imazethapyr



increased the severity of the disease in soy plants cultivated in greenhouses.

Final Remarks

Due to the different action mechanisms of the herbicides and the peculiarities in the pathogen-host interactions in several diseases, it is not possible to predict safely the real effect of the herbicide action in the severity of the disease.

The study of the interaction between the herbicides and the severity of diseases in plants is an important tool for phytosanitary management in an integrated way, especially due to the focus of metabolic and/or physiologic alterations resulting from the use of these phytosanitary products. It is worth mentioning that the related substances to the biochemical defense mechanism of the plants result from secondary metabolism, which is directly or indirectly affected by the use of herbicides in the crops.

Studies on the interaction between the herbicide and the pathogens must be intensified and deepened to allow advances in knowledge regarding this important topic for world agriculture.

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