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Ethylene inhibitor aminoethoxyvinylglycine on glomerella leaf spot in apple cultivar 'Royal Gala'

Inibidor de etileno aminoetoxivinilglicina sobre a mancha foliar de 'Glomerella' na cultivar de macieira 'Royal Gala'

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ABSTRACT

Aqueous solution of Aminoethoxyvinylglycine (AVG) has been commercialized in Brazil as ReTainTMC, 15% mainly as a potent inhibitor of ethylene biosynthesis by prevention of pre-harvest abscission and ripening of apple fruits. The effect of the product was evaluated during the 2007-08 and 2008-09 crop seasons in edafoclimatic conditions of Santa Catarina upland. Plants of 'Royal Gala' cultivar were sprayed with ReTainTMC, four weeks before the first commercial harvest at doses of 0; 62,5; 125, and 250mg a.i L⁻¹. The incidence and severity were quantified weekly in 100 leaves distributed in four branches with 12 replications and assessed the area under the incidence -I and severity -S disease progress curve (AUIDPC and AUSDPD) of Glomerella leaf spot (GLS). The AUIDPC and AUSDPD were significantly higher after AVG application and although there was no significant difference between 125 and 250mg a.i L⁻¹ doses in both 2007-08 and 2008-09 crop seasons. In general, the symptoms of GLS (e.g. chlorosis, necrosis) increased between the fourth and eighth week after application.

Key words: *Malus domestica*, growth regulator, *Colletotrichum gloeosporioides*, incidence, severity, area under disease progress curve.

RESUMO

Solução aquosa de Aminoetoxivinilglicina (AVG) tem sido comercializada no Brasil como ReTainTMC, 15%, principalmente como um potente inibidor da biossíntese do etileno na prevenção da abscisão e maturação de frutos em macieira. O efeito do produto foi avaliado durante as safras 2007-08 e 2008-09 nas condições edafo-climáticas do planalto Catarinense. Plantas de maçãs 'Royal Gala' foram

pulverizadas com o ReTainTMC, quatro semanas antes da primeira colheita comercial, nas concentrações de 0;62,5; 125 e 250mg i.a L⁻¹. A incidência e a severidade foram quantificadas semanalmente em 100 folhas, distribuídas em quatro ramos por plantas, com 12 repetições e calculado a área abaixo da curva do progresso da incidência -I e severidade -S da doença (AACPID e AACPSD). A AACPID e AACPSD foram significativamente maiores após a aplicação do AVG e não houve diferença significativa entre as doses de 125 e 250mg i.a L⁻¹ nos dois anos de cultivo avaliados. Em geral, os sintomas típicos de clorose e necrose da Mancha da Glomerella aumentaram entre a quarta e oitava semana após a aplicação.

Palavras-chave: *Malus domestica*, regulador crescimento, *Colletotrichum gloeosporioides*, incidência, severidade, área abaixo da curva progresso da doença.

INTRODUCTION

Brazil is one of the largest apple (*Malus domestica* Borkh.) producers in South America. Apple is one of the most important agricultural products of Santa Catarina State and represents the second most important temperate-zone fruit in Brazil. More than 1.4 million tons were produced in Brazil in 2008, and Santa Catarina State was responsible for more than 51% of this production (ICEPA, 2009). However, the production is based on imported cultivars susceptible to many fungal diseases. Glomerella leaf spot (GLS) caused by the fungus *Colletotrichum gloeosporioides* (Penz.)

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Sacc., is one of the most important summer diseases of apple orchards in southern Brazil and severe damages may occur every year (BOGO et al., 2010). Under favorable conditions, GLS can reach 75% of defoliation and reduction yield (CEREZINE et al., 1992). Additionally, GLS has been observed in others important commercial cultivars in Brazil, such as Granny Smith and Pink Lady (SUTTON & SANHUEZA, 1998).

Aminoethoxyvinylglycine (AVG - [S]-*trans*-2-amino-4-(2-aminoethoxy)-3-butenic acid hydrochloride) is a potent inhibitor of ethylene biosynthesis. In Southern Brazil, AVG is commonly used in apple orchards to prevent pre-harvest abscission and ripening of fruits, which is the greatest threat to crop yield (BRACKMANN et al., 2004). AVG has been used to study the participation of ethylene synthesis in bud break of *Mangabeira* (*Hancornia speciosa* Gomez), a native specie from Brazilian Northeast (PEREIRA-NETTO, 2001); dry-matter partitioning in rice (*Oryza sativa* L.) (MOHAPATRA et al., 2000); fruit ripening (LAYNE et al., 2002) and fungal pathogenesis (KIM & HWANG, 2000; ROBISON et al., 2001; DIAZ et al., 2002). There are many recent studies regarding pre- and post-harvest applications of AVG to enhance the production and quality attributes of no climacteric and climacteric fruits (KIM & HWANG, 2000; LAYNE et al., 2002; AMARANTE et al., 2010). However, there are not sufficient data regarding the effects of AVG on tropical and subtropical fruits diseases and most of the reports are with horticultural crops (ROBISON et al., 2001; SALTVEIT, 2005). Some preliminary data from one year of research of AVG effects on GLS were published by AMARANTE et al. (2010), but the real role of AVG as an ethylene inhibitor and in promoting or preventing plant disease development remains unclear. Most of the reports are with ethylene effect on diseases.

Volatile substances like ethylene, which can be inhibited by AVG, are produced by avocado (*Persea americana* Mill.), and banana (*Musa acuminata* Colla) fruits, inhibiting germination and appressorium formation in *C. gloeosporioides*, *C. musae* (Berk & Curt.) von Arx., but not in other *Colletotrichum* strains (ROBISON et al., 2001). Therefore, the effect of AVG on fungal pathogenesis appears to be a plant-like response.

Transgenic orange (*Citrus aurantifolia* L.) fruits, incapable of producing ethylene, required exogenous ethylene to induce multiple appressorium formation of *Colletotrichum* sp. and consequently diseases lesions. These results strongly suggest that these fungi must have coevolved to develop a mechanism that uses ethylene as a signal to

differentiate into multiple infection structures and thus time the infection process (SALTVEIT, 2005).

A retarding effect of ethylene on disease development has previously been reported on McIntosh apples infected by *Gloeosporium album* (LOCKHART et al., 1968). On the other hand, ethylene has been reported to stimulate disease development or spore germination of certain postharvest fungi suspension. (BROWN, 1978).

Fresh avocado fruits exposed to ethylene increased concentrations of epicatechin wax and an antifungal diene in the peel. This high level of epicatechin was required for maintenance of concentrations of the antifungal diene sufficient to provide decay resistance during avocado fruit ripening (ARDI et al., 1998). On the other hand, ethylene induced a transient increase in the levels of the antifungal diene, without affecting decay development (PRUSKY et al., 1996). Imbalance of growth regulators such as auxin, ethylene, and jasmonic acid plays an essential role in postbloom fruit drop of citrus incited by *C. acutatum* J. H. Simmonds (CHEN et al., 2006).

In many pre- and post-harvest fruit diseases, fungi remain latent until the fruit ripens and how the fungus manage its infection at ripening of the host is not known. Like many other inhibitors, AVG may affect more metabolic pathways than those attributed to its mode of action. Despite of some previous results of ethylene action on fruit disease, the effect of AVG, as an ethylene inhibitor, on fungal growth or disease development is still unclear. The following research was conducted to study the effects of different doses of AVG sprayed four weeks before commercial harvest during two crop seasons on the area under the incidence -I and severity -S disease progress curve of GLS on 'Gala' cultivar apples.

MATERIALS AND METHODS

The experiments were conducted in a twelve-year-old commercial apple orchard located in Lages, Santa Catarina State, Southern Brazil during the 2007-08 and 2008-09 crop seasons. The orchard was localized at approximately 1.250m above sea level with a predominantly humid mesoterm clime (Cfb), according to the Köppen classification, with mild winter and summer.

The orchard density was 667 plants ha⁻¹ with 1.340 plants distributed in a row (planted at 5.0x3.0m) of 'Royal Gala' grafted on EM-7 rootstock in alternate row with 'Fuji'. The temperatures, relative air humidity and pluviometric total regime average during the two crop seasons were 16,4°C, 84% e 688mm, respectively (Figure 1) which were considered favorable to GLS

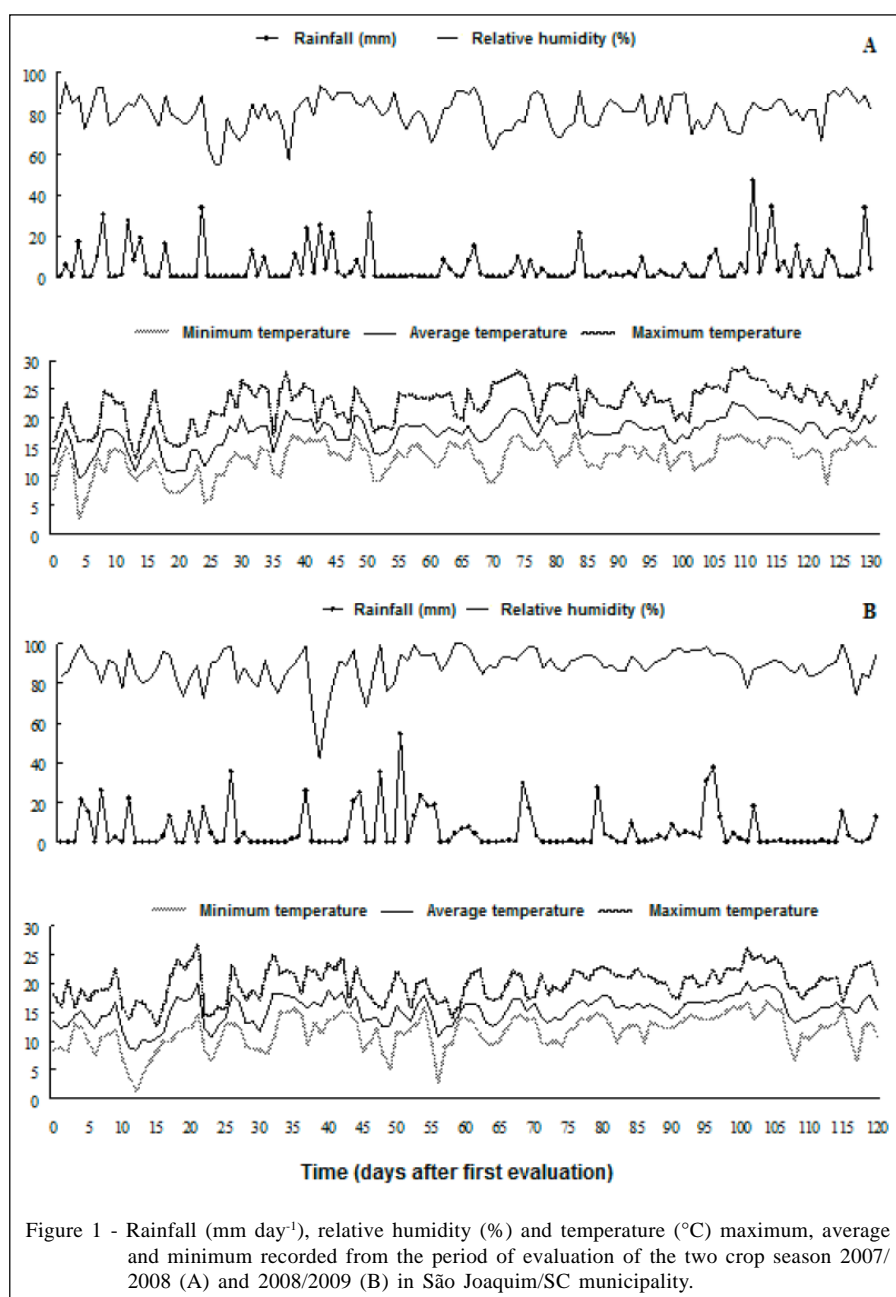
disease development. Ditiocarbamates fungicides for GLS control were suspended 20 days before AVG application, according to the fungicide residual period.

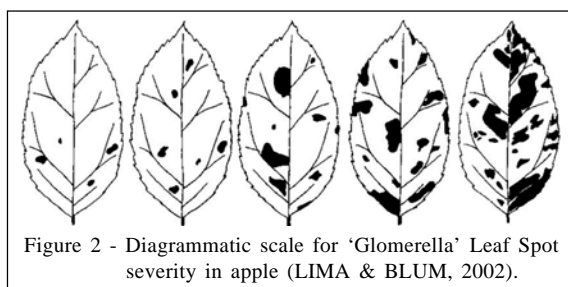
'Royal Gala' apple trees were sprayed from the top down up to dripping with aminoethoxyvinilglycine (AVG; ReTain™C, 15% a.i. p/p, Abbott Laboratories Inc. Agricultural Products, IL, USA) at doses of 0; 62,5; 125, or 250mg a.i L⁻¹ four week before the first commercial harvest in January 2007 and 2008.

Incidence and severity were evaluated in twenty-five leaves per branches totaling one-hundred

leaves in 4 branches per plants during 8 weeks. The incidence was determinate by number of leaves with GLS symptoms in relation to total evaluated leaves and the severity was quantified by a specific diagrammatic scale (Figure 2). The data were used to calculate the area under incidence-*I* and severity-*S* disease progress curve (*AUIDPC* and *AUSDPC*) by trapezoidal integration value of incidence and severity (CAMPBELL & MADDEN, 1990), using *t* test (*P*<0.05).

The experiments followed a completely randomized block design with twelve replicates. Whole





trees were blocks and single-scaffold limbs per tree were the experimental units. Four branches in the medium part of each tree were labeled, and each branch was separated from the surrounding branches by plastic sheets and sprayed with the ReTainTMC solutions containing an organo-silicon surfactant (0.1% v/v Silwet L-77) until dripping. The treatments were applied in a random distribution on separate branches of each tree.

RESULTS AND DISCUSSION

The ReTainTMC (AVG) application, as an ethylene inhibitor, increased the incidence and severity of GLS in both the 2007-08 and the 2008-09 crop seasons (Table 1). The GLS intensity was affected by doses above of 125mg a.i L⁻¹ of AVG. The *AUIDPC* and *AUSDPC* were significantly higher after application of 125 and 250mg a.i L⁻¹ doses of AVG and although there was no significant difference between 125 and 250mg a.i L⁻¹ doses in both crop seasons (Table 1). In general, the symptoms of GLS (e.g. chlorosis, necrosis) began to increase after the fourth week of application until the end of the eighth week. Progression of the disease was significantly rapid after the fourth week under the weather conditions, and led to advanced leaf necrosis of most plants by the end of the eighth week.

Similarities between the effects of AVG and many disease symptoms (chlorosis, necrose, leaf abscission) have led many researchers to hypothesize that the large quantity of AVG that is involved in the plants during compatible plant-disease interactions may contribute significantly to disease symptom development. The present results support this model and corroborate with others results from similar experiments with compatible plant-disease systems in which ethylene synthesis was prevented either chemically or through manipulation of biosynthetic pathway (ROBINSON et al., 2001; SALTVEIT, 2005).

AVG is an important ethylene inhibitor, and as an enzyme inhibitor, requires pyridoxal phosphate as an activator (CAPITANI et al., 2002) and can compromise the enzyme activities involved in some plant tissue defense mechanisms, increasing the disease intensity, as occurred in this research with GLS. Ethylene can functions as a signal in many biotic and abiotic stress situations and pathogen-plant interactions (BLEECKER & KENDE, 2000). Therefore, the increase of GLS incidence and severity in the doses of 125 and 250mg a.i L⁻¹ of AVG corroborate with the interpretation that AVG, as an ethylene inhibitor, can affect the pathogenic process in the inductor role of the pathogen's post-infection sites (TORNERO et al., 1997; ROBINSON et al., 2001; SALTVEIT, 2005).

Production of ethylene can be induced by pathogen invasion, by fungal toxins, by race-specific and as well as endogenous elicitors. Ethylene may affect plant defense related with processes such as production of phytoalexins, pathogenesis-related proteins (RODRIGO et al., 1993; TORNERO et al., 1997). Therefore, ethylene has been a target for the study of susceptibility or resistance mechanisms in the last decades.

Table 1 - Area under incidence-*I* disease progress curve (*AUIDPC*) and Area under severity-*S* disease progress curve (*AUSDPC*) of *Glomerella* leaf spot, caused by *Colletotrichum gloeosporioides*, according to different doses of Aminoethoxyvinylglycine (AVG; ReTainTMC sprayed in 'Royal Gala' apple. Lages/SC-Brazil, 2007-08 and 2008-09 crops seasons.

AVG rates (mg a.i L ⁻¹)	----- <i>AUIDPC</i> *-----		----- <i>AUSDPC</i> *-----	
	2007-08	2008-09	2007-08	2008-09
0	8,82Ba*	7,71Ba	2,94Ba	3,10Ba
62.5	11,14Ba	9,33Ba	4,15Ba	5,05Ba
125	29,07Aa	22,19Aa	13,76Aa	15,33Aa
250	28,21Aa	25,30Ba	10,23Aa	12,42Aa
C.V. (%)**	21,04	27,41	14,18	16,92

*Means followed by the same capital letter on the columns and small letter on the rows are not significantly different by *t* test (*P*<0.05).

*Area calculated by trapezoidal integration value of incidence-*I* and severity -*S* (CAMPBELL & MADDEN, 1990).

**coefficient of variation.

The different results regarding the role of ethylene in plant defense could reflect its involvement in multiple physiological processes. Ethylene can accelerate senescence in leaves and ripening in fruits (BLEECKER & KENDE, 2000; AMARANTE et al., 2010) but this might predispose the tissue for development of disease caused by some, mostly necrotrophic, pathogens. In this particular case, AVG affected the *C. gloeosporioides* pathogenic process either in pre or post infection sites, increasing GLS incidence and severity.

The 'Gala' cultivar produces high quantities of ethylene (MASIAS et al., 1998) and, therefore, can be highly susceptible to AVG action in the process of GLS disease resistance, pre-harvest fruit drop, rapid maturation on the tree and, consequently, substantial commercial benefits may be achieved with the use of AVG. However, in this research it has a negative effect on the increase of GLS intensity in Santa Catarina upland conditions.

CONCLUSION

Experiments conducted in a twelve-year-old commercial apple orchard with 'Royal Gala' grafted on EM-7 rootstock showed that application of doses above of 125mg a.i L⁻¹ of ReTain™C increased the area under incidence-I and severity-S disease progress curve of GLS in both 2007-08 and 2008-09 crop seasons in edafoclimatic conditions of Santa Catarina upland. The symptoms of GLS began to increase after the fourth week of application until the end of the eighth week. Progression of the disease was significantly rapid after the fourth week under the weather conditions, and led to advanced leaf necrosis of most plants by the end of the eighth week. Commercial benefits like fruit abscission prevention and fruit size are already proved with the use of AVG, but it has a negative effect on the increase of GLS intensity in these experiments conditions.

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