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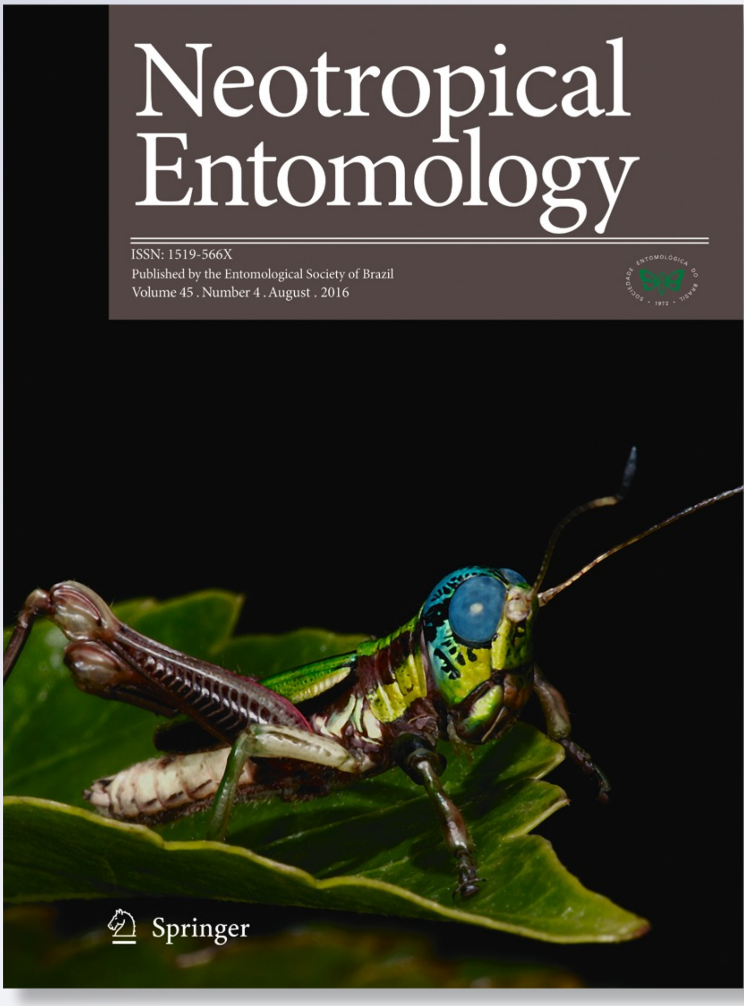
Neotropical Entomology

ISSN 1519-566X
Volume 45
Number 4

Neotrop Entomol (2016) 45:361-368
DOI 10.1007/s13744-016-0377-y



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ECOLOGY, BEHAVIOR AND BIONOMICS

Assessment of Injuries Caused by *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) on the Incidence of Bunch Rot Diseases in Table Grape

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Keywords

Botrytis cinerea, fruit damage, grapes, South American fruit fly

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Edited by André L Lourenção – IAC

Received 27 June 2014 and accepted 28 January 2016
Published online: 24 February 2016

Abstract

Anastrepha fraterculus (Wied.) is the main insect pest of table grapes (*Vitis vinifera*) in the Southern Region of Brazil. In this study, we aimed to investigate the effect of fruit puncturing by adult females and larval infestation by *A. fraterculus* on the occurrence of bunch rot disease in the grape (cultivar "Itália") by evaluating grapes (a) punctured for oviposition by females of *A. fraterculus*, sterilized in laboratory with novaluron (40 mg L^{-1}) and further spray-inoculated separately with *Botrytis cinerea* (1×10^6 conidia mL^{-1}), *Glomerella cingulata* (1×10^6 conidia mL^{-1}), and bacteria and yeast that cause sour rot (1×10^5 cells mL^{-1}), (b) grapes punctured for oviposition by non-sterilized females with pathogen spraying, (c) grapes with mechanical wounds and pathogen spraying, (d) grapes with no wounds and with pathogen spraying, (e) grapes punctured for oviposition by *A. fraterculus* chemically sterilized in laboratory with novaluron, (f) grapes punctured for oviposition by *A. fraterculus* non-sterilized in laboratory with novaluron, (g) grapes with mechanical wounds, and (h) grapes with no sterilization or pathogen spraying. Our data indicated that the mechanical and oviposition wounds caused by *A. fraterculus* increased the percentage of grapes infected by *B. cinerea*, *G. cingulata*, and microorganisms of acid rot. The grape puncturing by *A. fraterculus* and the mechanical wound allows the penetration of *B. cinerea* and microorganisms leading to acid rot. We conclude that the fruit fly *A. fraterculus* may facilitate phytopathogens penetration leading to bunch rots in the table grape Itália.

Introduction

One factor limiting production of table grapes in Southern Brazil is the incidence of plant pathogens that cause bunch rot (Garrido & Sônego 2004), such as *Botrytis cinerea*, *Glomerella cingulata*, and a complex of bacteria and other yeasts that can cause full production losses in vineyards (Garrido *et al* 2008). The control of these pathogens in table grapes is based on the systematic application of chemical fungicides (Freire *et al* 1991, Genta *et al* 2010). Despite the control measures taken, several factors such as the shrinking of the bunches, varietal susceptibility, decreased plant vigor due to overfertilization (Marois *et al* 1986, Gallotti & Grigoletti 1990, Garrido & Sônego 2005), inadequate fungicide application, mechanical damage on the berries from grape thinning scissors, and the occurrence of fungicide-resistant isolates (Chardonnet *et al* 2000, Latorre *et al* 2002, Kim & Xiao 2010) can still occur.

One poorly understood factor that might drive fungal damage is the occurrence of vector insects that could increase fungal infection by spreading rot pathogens between grapevine bunches (Botton *et al* 2003). In other countries, it is known that the bunch-moth *Lobesia botrana* Denis & Schiffermüller (Lepidoptera: Tortricidae) is primarily responsible for the dispersal of *B. cinerea* and *Aspergillus* spp. in vineyards, leading to severe losses (Fermaud & Le Menn 1992, Mondy *et al* 1998, Cozzi *et al* 2006), but other examples of insects vectoring disease in grapes are also available (Botton *et al* 2005, Hickel & Schuck 2005, Ringenberg *et al* 2006, Bisotto-De-Oliveira *et al* 2007, Morandi Filho *et al* 2007).

In recent years, there has been an increase in the occurrence of the South American fruit fly *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) in vineyards of fine table grapes, namely the cultivar "Itália" and cultivars of the "Moscato" group (Chavarria *et al* 2009, Formolo *et al* 2011) in production areas of Southern Brazil. The wounds caused by *A. fraterculus* in grapes—oviposition punctures and/or larvae fruit pulp damages—are difficult to detect (Botton *et al* 2003, Zart *et al* 2010). The oviposition wounds, besides inducing green grapes to drop prematurely, may also serve as a gateway to pathogens and in this way lead to additional production losses.

An increase in the incidence of the bitter rot caused by *G. cingulata* in apple fruits wounded by the ovipositor of *A. fraterculus* has been reported (Berton *et al* 2005), and additional work with the fungus pathogen *Botryosphaeria dothidea* indicated that disease incidence was increased in ovipositor wounded fruits by *A. fraterculus* as compared with mechanical wounded fruits (Santos *et al* 2008). During the host fruit selection process, females of *A. fraterculus* are known to evaluate fruit suitability for oviposition by inserting their ovipositor into the fruit even when no eggs are laid (Sugayama *et al* 1997, Zart *et al* 2011). These "bite proof" events, combined with high mobility of the flies throughout vineyards and the presence of pathogen repositories in alternative *A. fraterculus* hosts near in these areas, have been suggested to indicate a link between *A. fraterculus* and fungal bunch rot diseases in Southern Brazil (Zart *et al* 2009, Nachtigal *et al* 2010, Formolo *et al* 2011). This hypothesis highlights the need for studies on the secondary effect of fruit fly attacks on the incidence of diseases in fruit.

This study examines the effect of the oviposition wounds caused by *A. fraterculus* on grapes of the cultivar Itália (*Vitis vinifera*) compared to mechanical wounds, on the penetration of *B. cinerea*, *G. cingulata*, and bacteria and yeasts that cause acid rot, under laboratory conditions.

Material and Methods

Grape bunches

Grape bunches of the cultivar Itália were collected from the field (29°07′13″S; 51°14′16″W) and cultivated under an impermeable polyethylene cover to the phenological 38th stage of the Eichhorn & Lorenz (1984) scale, or the 16.1°Brix. The vineyard was maintained following Nachtigal *et al* (2010), and no fungicides were sprayed for 21 days before sampling. Bunches of grapes were collected, stored in thermal box (23 ± 2°C), transported to the laboratory, and washed in running distilled water. Samples were then surface-disinfected in a 70% ethanol and 1.5% NaOCl solutions for 1 min, washed, and dried at 24 ± 2°C. Finally, the grapes with their pedicels were collected with sterilized scissors.

Treatments

The treatments examined were the following: SF + P, Grape berries punctured for oviposition by females of *A. fraterculus*, sterilized in laboratory with novaluron (40 mg L^{-1}) and subsequently spray-inoculated separately with *Botrytis cinerea* (1×10^6 conidia mL^{-1}), *Glomerella cingulata* (1×10^6 conidia mL^{-1}), and bacteria and yeast that cause acid rot (1×10^5 cells mL^{-1}); F + P, Grape berries punctured for oviposition of non-sterilized females with pathogen spraying; MW + P, grape berries with mechanical wounds and pathogen spraying; P, grape berries with no wounds and with pathogen spraying; SF, grape berries punctured for oviposition by *A. fraterculus* chemically sterilized in laboratory with novaluron; F, grape berries punctured for oviposition by *A. fraterculus*; MW, grape berries with mechanical wounds; and C, grape berries with no sterilization or pathogen spraying (control).

The experimental trials were performed in a completely randomized factorial 3 × 8 (pathogens × wound type) with ten replicates of 20 grapes per treatment. The experiment was repeated twice (replicates). Our experimental unit was defined as a 100-mL white plastic cup holding one berry. Each cup was covered at the bottom with a rubber mat (4 × 4 cm) with 0.5-cm grid squares, cleaned and disinfested. Data were analyzed by ANOVA, and the means were compared by the Tukey's post hoc test at a 0.05 significance level

Insects used in the experiments.

In vitro *A. fraterculus* cultivation was a source of adult insects for experimental trials. The cultivation was kept in the Laboratory of Entomology of Embrapa Grape & Wine, Bento Goncalves, RS, Brazil (Machota *et al* 2010) with mean daily relative humidity ranging from 70 to 80%, under fluorescent lamps with maximum photosynthetic photon flux density (PPFD) of 250 μmol m^{-2} s^{-1} and 12-h photoperiod. The sterilization of the flies was carried out in a solution of the insecticide novaluron (Rimon 100 EC®, 40 mg $L^{-1}$of active ingredient (Milenia Agrociências S.A.) + food attractant (hydrolysed protein BioAnastrepha, 50 mL L^{-1}, BioControle Métodos de Controle de Pragas Ltda.).

The solution containing insecticide + food attractant was offered ad libitum for 96 h prior to the placement of grapes for oviposition. The solution was presented as 4-mm wide drops that were deposited at the bottom of the rearing cages. A solid diet mixture of soy extract, wheat germ, and brown sugar (ratio 3:1:1, *w/w/w*) and water was also supplied in the same cages used in the trials. Prior to the introduction of grapes for oviposition, the insecticide syrup was removed.

Obtaining grapes with mechanical punctures and wounds

Punctures were obtained by placing grapes in rearing cages (41 × 29.5 × 30 cm) containing approximately 150 couples of *A. fraterculus* for 4 h. The grapes were removed, transferred to plastic containers (300 mL), and covered with voile fabric (Zart *et al* 2010). For each pathogen, 1200 grapes for oviposition were homogeneously arranged in the bottoms of 12 rearing cages. After of oviposition, grapes were held for approximately 48 h to allow oxidation of the oviposition site (Zart *et al* 2011), and only the grapes that had 3 to 10 punctures were selected for the further assays. The same procedure was applied for sterilized and non-sterilized flies. To confirm the presence of insect larvae in grape samples from non-sterilized females, we held 20 grapes from each rearing cage with or without novaluron (control) after 12 days. The inhibition of the larval hatching was assessed as a response to the supply of insecticide in rearing cages.

The mechanical wound on the grape cuticles was performed with an entomologic "micro" pin (15 mm length and diameter of 0.20 mm). Each grape was wounded with just one mechanical puncturing made at the central part of the skin (1 to 2 mm deep), that is a similar value to the average size of the ovipositor of *Anastrepha* spp. (Zucchi 2000).

Pathogens

The pathogens *Botrytis cinerea* (CNPUV145), *Glomerella cingulata* (CNPUV380), and bacteria and yeasts (CNPUV220) were supplied from the Collection of Microorganisms of Agro-Environmental Interest (CMIA) of Embrapa Grape & Wine, Bento Gonçalves, Rio Grande do Sul State (RS), Brazil. The three pathogens were isolated from bunches of the cultivars Itália (CNPUV145), "Cabernet Sauvignon" (CNPUV380) and "Moscato Embrapa" (CNPUV220) from vineyards at Vale dos Vinhedos, Bento Goncalves, RS. The microorganisms were cultivated in potato dextrose agar (PDA) medium in a growth chamber at 25°C and 12-h photoperiod for morphological identification and inoculum production. The inocula were prepared from colonies after 10–15-day incubation, and the suspensions were set to 1.0×10^5 conidia mL^{-1} for fungal pathogens (Gabler *et al* 2003, Viret *et al* 2004, Tavares & Souza 2005) and 1.0×10^5 cells mL^{-1} for bacteria and yeasts (Machado & Bettiol 2010).

For experimental trials, 100-mL white plastic cups were used to hold the berries. The cups were covered at the bottom with a rubber floor mat (4 × 4 cm) with 0.5-cm grid squares. The cups and bottoms were cleaned and disinfected by immersion in an alcohol solution 70% and sodium hypochlorite 1.5% for 1 min and washed for 30 s in distilled water three times (Menezes & Assis 2004). The cups were dried at 24 ± 2°C in a chamber with laminar air flow under UV radiation (50 W) for 15 min (Alexandre *et al* 2008). The rubber floor was used to keep the area around the grapes moistened, with no contact with the humid plastic surfaces.

We inoculated the grapes by spraying them (Romanazzi *et al* 2002, Viret *et al* 2004, Camili *et al* 2010) with inocula suspensions until runoff. We kept the inoculated grapes in a closed container with a plastic lid placed inside a humid chamber (90–100% RH) for 7 days to allow germination and penetration of the pathogens into the host tissue.

Infection assessment

Seven days after application, the plastic containers were opened and the grapes were evaluated every 48 h by observing the incidence of symptoms and signs of diseases (criterion of "presence or absence" of reproductive structures) for 12 days after the first assessment.

Statistical analysis.

For the statistical analysis, data were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk 1965) and homogeneity of variance by Bartlett (Bartlett 1937). In all experiments, data that did not conform to a normal distribution or homogeneity of variance were transformed via arcsin $\sqrt{(x/100)}$. Means were compared by Tukey's test at 5% probability using the SAS software (SAS Institute 2003).

Results

Our experiment was repeated twice with similar results. A significant interaction ($p < 0.01$) among all the factors analyzed was detected, indicating that the disease severity in grapes depended on the pathogen and the type of wound performed on the grapes of the cultivar Itália.

We observed stunted larval development in treatments with Novaluron (40 mg L^{-1}). A similar response has been observed for other insecticides with a role on disruption of the chitin synthesis in *Anastrepha ludens* (Loew 1873) (Martinez & Moreno 1991, Moreno *et al* 1994) and *Ceratitis capitata* (Wied. 1824) (Diptera: Tephritidae) (Budia & Vinuela 1996, Casaña-Giner *et al* 1999, Navarro-Llopis *et al* 2004).

Assessment of infection by Botrytis cinerea

The highest percentage of grapes infected by *Botrytis cinerea* (93.0%) occurred in the treatment with artificial (mechanical) wounding ("MW + P"). The wounds caused by the puncture of *A. fraterculus*, sterilized or not ("SF + P" and "F + P"), showed a significant percentage of infected berries also, reaching 51.5 and 32.5%, respectively. There was no significant difference in the percentage of infected grapes between sterile flies ("SF") or not sterile ("F") in the treatments, seeming to indicate that the oviposition wound alone would act as a gateway for *B. cinerea* infection (Fig 1).

When *B. cinerea* conidial suspension was sprayed on the surface of intact grapes ("P"), 16.0% of the grapes were infected compared to control ("C"), with no application of the pathogen.

Assessment of infection by Glomerella cingulata

We observed that F + P (97.0%) and SF + P (94.0%) treatments revealed the highest severity levels, with no significant differences between the puncturing wounds caused by *A. fraterculus* that had been sterilized or not (Fig 2).

Berries inoculated with the pathogen P (59.5%) did not show differences when compared to MW + P treatments. It is remarkable that *G. cingulata* produced higher levels of symptoms in berries than *B. cinerea* infected ones in the absence of natural or mechanical wounds. This result corroborates the findings of Alfenas & Mafia (2007). Those authors reported that the *Colletotrichum gloesporioides* germinated conidia have specific mechanisms for penetration into the tissues of the plant by means of a strong and melanized appressoria placed under the leaf infection sites, that seems to be effective regardless of the existence of wounds or natural openings as points of entry (Ludwig *et al* 2014). The treatments "SF," "F," and "MW" did not differ from the C, with 10.0, 8.5, 6.0, and 5.5% of infected grapes, respectively, showing that the wound caused by the *A. fraterculus* or mechanical wound without the inoculation of the pathogen did not enhance significantly the infection levels of *G. cingulata*.

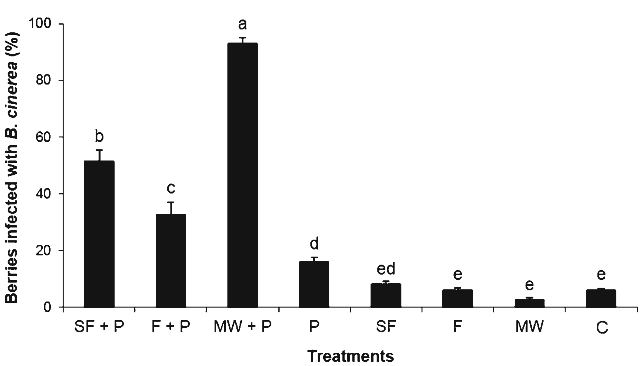

Fig 1 Percentage (%) of grapes of cv. "Itália" (*Vitis vinifera*) infected by *Botrytis cinerea* after wound caused by *Anastrepha fraterculus* and mechanical wound in laboratory. *SF + P* sterilized flies + pathogen, *F + P* flies + pathogen, *MW + P* mechanical wound + pathogen, *P* pathogen, *SF* sterilized flies, *F* flies, *MW* mechanical wound, *C* control. Means that followed the same *small letters* between the treatments did not differ in the Tukey's test at 5% of significance.

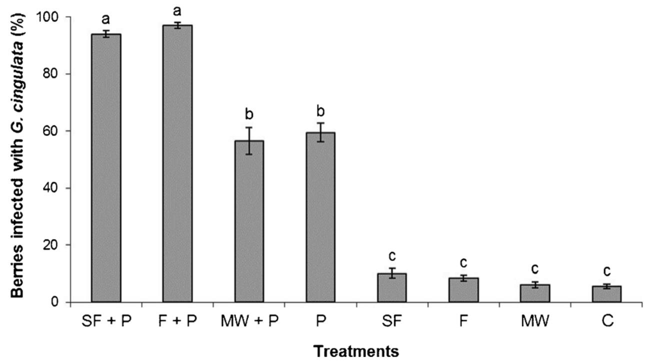

Fig 2 Percentage (%) of grapes of cv. "Itália" (*Vitis vinifera*) infected by *Glomerella cingulata* after wound caused by *Anastrepha fraterculus* and mechanical wound in laboratory. *SF + P* sterilized flies + pathogen, *F + P* flies + pathogen, *MW + P* mechanical wound + pathogen, *P* pathogen, *SF* sterilized flies, *F* flies, *MW* mechanical wound, *C* control. Means that followed the same *small letters* between the treatments did not differ in the Tukey's test at 5% of significance.

Assessment of infection by sour rot

The highest percentage of infected grapes after inoculation of bacteria and yeasts that cause sour rot were recorded in the treatments F + P and MW + P, with 25.0 and 24.0% of grapes infected, respectively (Fig 3). This result shows that there was a higher incidence of this disease in grapes with wounds, regardless of wound origin. No differences were observed in the larval development caused by the puncturing wounds when compared with the mechanical wounds. Treatment P showed a lower incidence of 10.0%, not differing from the control C (Fig 3).

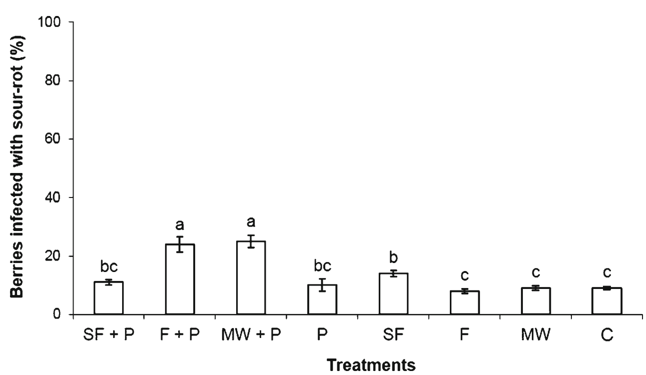

Fig 3 Percentage (%) of grapes of cv. "Itália" (*Vitis vinifera*) infected by sour-rot after wound caused by *Anastrepha fraterculus* and mechanical wound in laboratory. *SF + P* sterilized flies + pathogen, *F + P* flies + pathogen, *MW + P* mechanical wound + pathogen, *P* pathogen, *SF* sterilized flies, *F* flies, *MW* mechanical wound, *C* control. Means followed the same *small letters* between the treatments did not differ in the Tukey's test at 5% of significance.

In treatments with the application of the pathogens *B. cinerea*, *G. cingulata*, and bacteria and yeasts of sour rot, the response to wounds caused by the puncture of sterilized *A. fraterculus* differed from those non-sterilized, in a way that SF + P treatment showed a significant increase in the incidence of grapes with *B. cinerea*. The same responses seemed to not occur for sour rot berries.

When wounded grapes punctured by the ovipositor of *A. fraterculus* were compared with the mechanically wound ones, it was observed that MW + P had a higher incidence of grapes with gray-mold and sour rot than those only punctured by oviposition wounds. With respect to *G. cingulata*, SF + P, and F + P the treatments showed a higher penetration percentages than the MW + P ones. These findings indicate that the occurrence of penetration in grapes of the cultivar Itália by *G. cingulata* was slightly favored by mechanical wounds over punctures caused by oviposition of *A. fraterculus*, sterilized or not.

Discussion

Assessment of infection by Botrytis cinerea

Several studies have shown that in the presence of an inoculum, besides the infection of flowers, *B. cinerea* has the ability to penetrate grapes even in the absence of wounds (Marois *et al* 1992, Comménil *et al* 1999, Coertze *et al* 2001), since they are capable of penetrating directly through cuticle or though stomata and micro-fissures in the grape berry skin (Pucheu-Planté & Mercier 1983). However, in this study, wounds significantly increased the level of pathogenic infection. The incidence of *B. cinerea* in grapes in the control group (no pathogen application) was attributed to the presence of the fungus in the latent stage (Pearson & Goheen 1990). In these cases, the puncturing for oviposition of *A. fraterculus* in grapes with the disease in the latent stage could be an additional factor for the acquisition and dispersal of the pathogen in vineyards.

For *B. cinerea*, it is known that wounds on grape skin facilitate the infection processes (Bulit & Dubos 1990, Coertze *et al* 2001). In vineyards, the symptoms of *B. cinerea* gray mold rise with the increasing of maturity and concentration of sugars in the grapes, contributing to the fungal development (Perez 1998). In this case, it must be taken into account that the presence of *A. fraterculus* is greater in the vineyards near the harvesting period of the fruit (Nondillo *et al* 2007, Chavarria *et al* 2009).

This study demonstrated that for the cultivar Itália, the mechanical wound allowed more *B. cinerea* invasion than wounds caused by ovipositors or by oviposition and larval growth. However, it was observed that the presence of wounds caused by the insect did increase the incidence of the disease. Indeed, *A. fraterculus* significantly contributes to the increase in the incidence of the pathogen in the culture as occurred with *Lobesia botrana* and *Botrytis cinerea* in Europe (Fermaud & Le Menn 1992, Mondy *et al* 1998, Cozzi *et al* 2006).

Assessment of infection by glomerella cingulata

Denardi *et al* (2003) and Berton *et al* (2005) studied the incidence of *G. cingulata* in fruits of different apple cultivars, with and without wounds. These authors found that the pathogen established itself more quickly in fruits with wounds. In this study, there was no difference in the incidence of disease in fruits with wounds from sterile or fertile *A. fraterculus*. This can be explained, at least in part, by the ecology and morphology of the fungus as a specialized plant pathogen. *Colletotrichum* species possess conidial spores capable of germinating and developing highly melanized penetration appressoria when conidia become attached on a compatible host tissue and develop their germ tubes (Münch *et al* 2008). Moreover, melanin by itself acts as determinant of pathogenicity in many plant diseases. As phenol-based polymer, it plays a role in the formation of a thickened ring surfacing the "melanized appressorial ring structure" (MARS) located around the penetration pores (Ludwig *et al* 2014) that holds the penetration hyphae (penetration peg). Also, melanins were shown to be associated with secreted hydrolases and may serve to concentrate cell wall degrading enzymes at the penetration site (Hignett *et al* 1979).

Some researchers argue that *Colletotrichum* melanized appressoria are the support or the emergence of the penetration peg, and the further penetration through the plant cuticle and cell wall probably involves a combination of mechanical force, produced by high turgor pressure and enzymatic degradation (Perfect *et al* 1999). In fact, *Colletotrichum*/*Glomerella* genuses appear to have an enhanced competitive advantage regarding the ability to producing their own puncture wounds to penetrate host tissues.

Assessment of infection by sour rot

Our results show that bacteria and yeasts that cause sour rot need wounds to start infection processes, confirming results of Alfenas & Mafia (2007). According to Hespanhol-Viana *et al* (2007), the grape cultivar Itália has a weakness in the region where the grape intersects with the pedicel. This weakness may be related to the occurrence of wounds in this region at the time of separating the grapes from the pedicel with the use scissors, which would explain the incidence of sour rot in the control.

The bacterial symbiotic association with Tephritid flies occurs in all stages of the insect development, showing regions of the digestive tract anatomically adapted to host these microorganisms (Christenson & Foote 1960, Bateman 1972, Aluja 1994). During oviposition, the bacteria present at the end of the digestive tract come in contact with the surface of the eggs, penetrating through the micropyle and lodging in the gastric caeca of the embryo (Christenson & Foote 1960, Bateman 1972). Thus, these microorganisms degrade the tissues of the punctured fruit (Murillo *et al* 1990, Aluja 1994). In the present study, we hypothesize that the increase in incidence of *B. cinerea* could be explained by the decrease in competition for substrate for fungal growth due to the sterilization of *A. fraterculus* in SF + P, reducing the activity of microorganisms that might maintain mutualistic relations with the larvae (Perez 1983).

Grapes that inoculated only with pathogen displayed less disease than punctured grapes. Those responses generally indicate that the presence of punctures in grapes significantly enhances the incidence of diseases, indicating that the management practices could preserve the integrity of the grapes and may protect bunches against rot pathogens. Analyzing the wounds of sterilized *A. fraterculus* with subsequent spraying of the pathogens SF + P, the wounds did not indicate significant differences ($p > 0.05$) in the incidence of *G. cingulata* when they are compared to F + P treatments. However, SF + P-treated grapes showed an increased incidence of sour rot and gray mold. Different pathogens did result in distinct losses (percentage of infected grapes) such that the average of infected grapes used in the treatments with bacteria and yeasts that causing sour rot (17.5%) was lower than those inoculated with *B. cinerea* (48.2%) and *G. cingulata* (76.7%).

Under field conditions, the development of infections caused by other pathogens, such *as B. cinerea*, can occur simultaneously with the sour rot on the same bunches, but not on the same grapes, because the acetic acid produced in grapes infected by acid rot inhibits spore germination and fungal growth (Gravot *et al* 2001). Similarly, the increased incidence of acid rot in F + P in relation to the SF + P is probably due to the existing population of microorganisms already existing in the digestive tract of *A. fraterculus*, including yeasts and bacteria (Bateman 1972, Drew *et al* 1983, Murillo *et al* 1990, Martinez *et al* 1994, Kuzina *et al* 2001) that contribute to the tissue breakdown and result in increased infections of grapes by sour rot.

The finding that wounds caused by sterile female punctures (SF) increase the incidence of these diseases is significant because *A. fraterculus* sometimes punctures the grapes without laying eggs when they perform a "bite proof" in plant tissues (Zart *et al* 2011). This fact argues against the use of insecticides and growth regulators as deadly toxic agents in baits for inhibiting larval development as a strategy to control the culture, since they do not inhibit oviposition (Navarro-Llopis *et al* 2007, 2010, Alemany *et al* 2008).

Comparing the wounds caused by sterile or fertile *A. fraterculus* with mechanical wounds, it was observed that the wounds played similar roles in the incidence of diseases with no significant difference between "M," "ME," and "FA" for the pathogens of *B. cinerea* and *G. cingulata*. Although these treatments did not differ from C, it is worth noting that in cultivars of table grape near the maturity stage, mainly those of white pulp and clear skin, the wounds caused by *A. fraterculus* are visible and affect the commercial value of the fruit for the *in natura* market or industrial processing (Haji *et al* 2001, Habibe *et al* 2006, Chavarria *et al* 2009, Zart *et al* 2009). In the case of grapes for industrial processing, it is generally considered that the cultivars in this group are not damaged by *A. fraterculus* (Botton *et al* 2003).

In this study, characteristics such the compactness of the bunches, proximity of the grapes, and length of pedicel, and size and weight of the grapes were not evaluated; however, these features also influence the infection processes and development of bunch rot diseases in vineyards (Garrido & Sônego 2005, Hespanhol-Viana *et al* 2007). Field experiments using methods similar to those employed in this experiment would be useful to further analyze these factors.

The results of this study suggest that, besides the direct wound, pathogens may increasingly penetrate through the wounds caused by the ovipositor or other body parts of *A. fraterculus*. The high incidence of *A. fraterculus* in vineyards in the Southern Brazil (Nondillo *et al* 2007, Chavarria *et al* 2009, Zart *et al* 2009) along with the harvesting season of the cultivar Itália suggests that it might be suitable to adopt an integrated management procedures for the pests and diseases, although there is availability of fungicides for the pathogenic control of fungal rots associated to grape bunches (Latorre *et al* 2002, Kim & Xiao 2010). Regarding the sour rot disease, however, the use of fungicides is not an available tool; therefore, the management of causal agents of diseases to reduce damages in bunches appears to be essential to avoid higher economic losses in grape production.

Acknowledgments The authors thank Mrs Renata Gava, research analyst of Laboratory of Plant Pathology of Embrapa Uva e Vinho, for the aid in laboratory procedures, Mr Lucas da R Garrido (Embrapa Uva e Vinho), Mr Flávio RM Garcia (UFPel), and Mr Marcus AK Almança (IFRS) for the suggestions during the preparation of the manuscript, and the National Council for Scientific and Technological Development (CNPq) for the financial support to this project.

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