Postharvest Biological Control of *Botrytis cinerea* on Kiwifruit by Volatiles of 'Isabella' Grapes

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ABSTRACT

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The potential of volatile substances emitted by 'Isabella' grapes (*Vitis labrusca*) to control gray mold (*Botrytis cinerea*) on 'Hayward' kiwifruit (*Actinidia deliciosa*) was studied. The closed Mariotte system was used as a bioassay method to analyze quantitatively the biological action of these volatiles on *B. cinerea* growth. In vivo experiments compared the effects of volatiles from 'Isabella' grapes versus volatiles from 'Roditis' grapes (*V. vinifera*) and a *B. cinerea* control on the growth and disease development of *B. cinerea* on kiwifruit. The effect of the volatiles on the

growth of *B. cinerea* was tested at various temperatures and times of inoculation after the wounding of kiwifruit, as well as using various weights and developmental stages of the grapes. The 'Isabella' volatiles limited the incidence of infection by reducing both the inoculum density and the activity of the pathogen. The weight and developmental stage of the grapes were important in the degree of inhibitory action of the 'Isabella' volatiles. The inhibitory action was more pronounced at 21°C irrespective of the inoculation time after wounding. The study shows the potential for successful biological control of *B. cinerea* on kiwifruit by volatiles from 'Isabella' grapes.

Additional keywords: postharvest gray mold.

Fungi of the genera *Botryosphaeria*, *Cryptosporiopsis*, *Penicillium*, *Diaporthe* (Phomopsis stem-end rot), *Colletotrichum*, *Alternaria*, *Sclerotinia*, and *Phoma* all cause postharvest rots of kiwifruit (10,27,28,29,34). However, the most serious pathogen on kiwifruit in storage is *Botrytis cinerea* Pers.: Fr. (gray mold) (10,23,24,27,28). In extreme cases, *B. cinerea* losses of over 20% in California, 30% in New Zealand (23), and 50% in kiwifruit grown in Italy have been recorded (28). However, direct fruit losses of 0.2 to 2% are more common (23). In Greece, losses caused by gray mold on kiwifruit in storage are estimated at 1 to 1.5% (C. Thanassouloupoulos, unpublished data).

Fungicides are the primary means of controlling postharvest diseases, including that caused by *B. cinerea* on kiwifruit (10,24). Nevertheless, disease reduction achieved by fungicide applications may not be economical because the cost of materials and application may exceed the loss from rot (24). In addition, frequent applications of fungicides increase the risk of selecting resistant strains of the pathogen (9,24,36). Furthermore, under optimal conditions for disease development, control by fungicides is often ineffective (39). More importantly, the pronounced oncogenic risk of fungicides (26) creates an urgent need for new and effective means of controlling postharvest diseases that pose less risk to human health and the environment (40–43). Biological control is an exciting alternative. According to Baker's broad definition of biological control (4), among the several promising approaches (41) is the possibility of using natural plant products.

Studies have shown that natural plant volatiles have an inhibitory effect on the growth of pathogenic fungi, including *B. cinerea* (1–3,11,16,22,30–33,35,38,39). Kulakiotu et al. (20) found that volatile substances emitted from 'Isabella' grapes (*Vitis labrusca* L.) act as biocontrol agents of *B. cinerea* in in vitro and in situ

studies, in the latter case on 'Roditis' grapes (*V. vinifera* L.). In the present work, we study the effect of volatiles emitted from grapes of the 'Isabella' cultivar on the growth of *B. cinerea* in vivo on the 'Hayward' kiwifruit cultivar (*Actinidia deliciosa* [A. Chev.] C. S. Liang et A.R. Ferguson). The objective of this study was to verify the biological action of 'Isabella' volatiles on *B. cinerea* on a host other than grapes. The closed Mariotte system, used as a bioassay method to analyze quantitatively the biological action of 'Isabella' volatiles, provided the possibility of studying this action on the growth of the fungus. The effect of 'Isabella' volatiles on the growth of *B. cinerea* on kiwifruit was tested at various temperatures and times of inoculation after wounding of the kiwifruit, as well as at various weights and developmental stages of the grapes. The 'Isabella' volatiles limited the incidence of infection by reducing both the inoculum density and the activity of the pathogen. The study confirms the action of 'Isabella' volatiles as biocontrol agents of *B. cinerea*. Preliminary reports have been published elsewhere (18,19).

MATERIALS AND METHODS

The fungus. *B. cinerea* was isolated from a naturally infected grape berry from the vineyards of the Aristotle University of Thessaloniki. The isolate of the fungus belongs to an intermediate sclerotial and sporulating morphotype (17). The fungus was grown on potato dextrose agar (PDA, Oxoid Ltd., Hampshire, UK) medium in petri dishes. Pure cultures were obtained by transferring mycelium, 2 mm in diameter, from the margins of 4-day-old actively growing colonies. Kiwifruit were inoculated with mycelium on PDA disks 2 mm in diameter. Inoculation with mycelium was preferred because the process of infection from mycelium is essentially the same as that from germ tubes, but the mycelium has a nutrient-providing saprophytic base (PDA disks). The inoculum potential of mycelium is therefore much greater than that of germinating conidia, and is less dependent on the external environment (17). *B. cinerea* mycelium was transferred

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from the margins of actively growing colonies and placed upside down on wounded areas of kiwifruit. In order to prevent the variability of cultures, inoculum was taken from the periphery of the colonies at the same distance from the center.

Plant material. Clusters were collected of resistant cv. *Isabella* (*V. labrusca*) from a vineyard in the area of Stavros in the Pieria prefecture (first, second, and fourth experiments), and of susceptible cv. *Roditis* (*V. vinifera*) from a vineyard in the area of Orfani in the Kavala prefecture, both located in northern Greece, in the Province of Macedonia. The ripening stage, expressed in soluble solids content (SSC), was 21.2 and 17.1% for 'Isabella' and 'Roditis' grape berries, respectively. In the third experiment, 'Isabella' grape clusters were collected from a vineyard in the area of Siviri, in the Halkidiki prefecture of northern Greece. 'Hayward' kiwifruit were collected from the kiwi berry vineyards of the Aristotle University. The ripening stage, expressed in SSC, was 5.8% for the 'Hayward' kiwifruit. Clusters of grapes and kiwifruit were randomly selected from all parts of the vineyards. Grapes and kiwifruit uniform in color and free of physiological irregularities and mycological, entomological, or other infections were selected. The grape clusters were randomly separated into groups of smaller clusters. The kiwifruit were brushed to eliminate the flower remnants and then randomly separated into groups.

Bioassay method. The modified closed Mariotte system (18,20) was used in all experiments. The closed Mariotte system permitted the concentration of O₂ to be maintained at 20% and the removal of CO₂, while preventing the exchange of gases.

Antifungal bioassay. Glass vessels (3 liters in the first and fourth experiments, 5 liters in the second experiment, and 2 liters in the third experiment) were used as desiccators in the modified closed Mariotte system. A small vessel containing 50 ml of 4 N NaOH was placed on the base of each vessel to trap the respiratory CO₂. Sterile filter papers (one [first, third, and fourth experiments] or two [second experiment] 5 by 5 cm, Whatman Chromatography 3 MM paper) placed on the base of the vessels were moistened with 0.7 ml of sterile water in order to provide high humidity. 'Isabella' or 'Roditis' grape clusters were also placed at the bottom of the vessels. Sterile wire mesh screens allowed fruit to be placed on the small vessels with NaOH. Kiwifruit were placed in the vessels on the clusters. The kiwifruit were wounded at two or four diametrically opposite points around the pedicel. The fruit were wounded with a cork borer 2 mm in diameter to a depth of 2 to 3 mm. Four-day-old *B. cinerea* mycelium on 2-mm-diameter PDA disks were placed in each wound. The number of (i) infected kiwifruit and (ii) kiwifruit on which fungal fruiting bodies had appeared was recorded. The latter was performed with a dissecting microscope (×4). The effect of the 'Isabella' volatiles on the *B. cinerea* thallus was determined by microscopic examination (×40) of mycelial hyphae on slides mounted in lactophenol blue.

In order to evaluate the effect of the 'Isabella' volatiles on the ability of *B. cinerea* to cause decay, the depth of rotted tissue per wound was estimated. Each fruit was cut into four quarters, along the vertical axis, with the two cuts intersecting the wounded and inoculated areas. The depth of decay was measured with calipers and expressed in millimeters, beginning at a point under the inoculum of the fungus and measuring along the main axis of the fruit. The depth of decay in the fruit was assumed to be the length of the infected tissue which internally turns dark green and is water-soaked (23).

Effect of the weight of 'Isabella' grapes. Twelve 3-liter glass vessels were used, with three replicates of each treatment, consisting of 0, 300, 400, or 500 g of 'Isabella' grapes in small clusters upon which three wounded kiwifruit, inoculated in four places with *B. cinerea*, were placed. The 12 vessels were placed in a chamber at 21°C and then connected to the closed Mariotte system. The experiment terminated on the seventh day. The effect of the 'Isabella' volatiles on the *B. cinerea* thallus was determined

by microscopic examination of mycelial hyphae on slides made by transferring hyphae from seven inoculated areas from each vessel, 84 in total.

The effect of the 'Isabella' volatiles on the ability of *B. cinerea* to sporulate in vivo on kiwifruit was determined by estimating the number of conidia per inoculated area, on seven areas from each vessel, 7 days after the beginning of the experiment. Areas were selected at random from the 12 areas that were inoculated with the fungus. A very thin piece of inoculated kiwifruit epidermis, about 20 mm in diameter, was removed with a scalpel, transferred to a beaker, washed with 10 ml of sterile water, and thoroughly stirred (for about 5 s) to separate the conidia from their conidiophores. The resulting conidial suspension was filtered through cheesecloth to remove hyphae. The density of conidia was determined by a hemacytometer in four replicates and expressed as conidia per milliliter of water.

Effect of temperature. For this experiment, 12 5-liter vessels were used. The treatments, in three replicates, were as follows: (i) six vessels with 10 kiwifruit placed on 1 kg of 'Isabella' grapes in small clusters; and (ii) six vessels with 10 kiwifruit, without the presence of grapes, used as a control. To study the effect of temperature on the action of 'Isabella' volatiles on *B. cinerea* on kiwifruit, a group of six vessels (three with grapes and three without) was placed in a chamber at 10°C, and a second group was placed in a chamber at 21°C. Each group of six vessels was then connected to the closed Mariotte system. Twenty-four hours after being placed in the vessels, the kiwifruit were wounded at two sites and then inoculated as previously described. The kiwifruit remained out of the vessels only long enough for wounding and inoculation. The experiment was terminated after 8 days at 21°C, and 12 days at 10°C, following insertion of fruit in the closed Mariotte system. Evaluation was made as described previously.

Effect of the stage of development of 'Isabella' grapes. The same Mariotte system was used with treatments in three replicates as follows: 2-liter glass vessels with two kiwifruit placed on small clusters of 200 g of (i) immature (SSC: 7.3%) 'Isabella' grapes; (ii) semimature (SSC: 11.1%) 'Isabella' grapes; (iii) mature (SSC: 18.9%) 'Isabella' grapes; and (iv) mature (SSC: 17.3%) 'Roditis' grapes. The kiwifruit were wounded, as previously described, at two sites opposite one another around the pedicel. The 12 vessels were placed at 21°C and connected to the closed Mariotte system. The wounded kiwifruit were exposed to the grape volatiles for 24 h. Then each wound was inoculated with *B. cinerea* as previously described. The experiment was terminated 6 days after placing the fruit in the closed Mariotte system and evaluated as in the two previous experiments.

Effect of inoculation time after wounding. For this experiment, 27 3-liter vessels were used. The treatments were as follows: (i) nine vessels with three kiwifruit placed on 400 g of 'Isabella' grapes in small clusters; (ii) nine vessels with three kiwifruit placed on 400 g of 'Roditis' grapes in small clusters; and (iii) nine vessels with three kiwifruit alone. All vessels were placed in a chamber at 21°C and connected to the closed Mariotte system. In all groups, the kiwifruit were wounded 24 h after being placed in the closed Mariotte system. The kiwifruit were wounded at four points, at diametrically opposite sites around the pedicel. Each wounded area was in the center of each of the quadrants of the fruit. Fruit inoculation with *B. cinerea* was as previously described and performed immediately, 24 h, and 48 h after wounding the kiwifruit. Three replicates were used for each treatment. The fruit remained outside the vessels only long enough for wounding and inoculation. On the seventh day, the small vessels with NaOH were replaced with other vessels containing new NaOH. The number of infected kiwifruit and the depth of decay were recorded after 7, 10, and 11 days for kiwifruit inoculated 0, 24, and 48 h after wounding, respectively. The effect of the volatiles on the thallus of *B. cinerea* was determined by microscopic examination as previously described. The density of conidia was

determined by measuring the number of conidia per inoculated area of fruit, in seven randomly selected inoculated areas among the 12 from each vessel, with a hemacytometer as described previously.

Data analysis. The analyses of data variance in the experiments were performed with the MSTAT (Michigan State University, version 4.00/EM) program. When the *F* values were significant, the mean comparisons were performed with the least significant difference value, at the significance level of *P* = 0.05.

RESULTS

Effect of the weight of 'Isabella' grapes. After 7 days at 21°C, sporulation of *B. cinerea* (Fig. 1) was 91.7% on the kiwifruit in the absence of the 'Isabella' grapes. In the presence of 'Isabella', the sporulation of *B. cinerea* on the kiwifruit decreased from 80.3 to 30.3% as the weight of the 'Isabella' grapes was increased from 300 to 500 g, respectively.

Fig. 1. Sporulation of *Botrytis cinerea* on kiwifruit in the absence of grapes (0 g) and in the presence of 300, 400, and 500 g of 'Isabella' grapes, 7 days after inoculation and insertion of fruit in the closed Mariotte system at 21°C (LSD_{0.05} = 18.9).

Fig. 2. Number of conidia of *Botrytis cinerea* on kiwifruit in the absence of grapes (0 g) and in the presence of 300, 400, and 500 g of 'Isabella' grapes, 7 days after inoculation and insertion of fruit in the closed Mariotte system at 21°C (LSD_{0.05} = 209.3).

Fig. 3. Depth of decay caused by *Botrytis cinerea* in kiwifruit in the absence of grapes (0 g) and in the presence of 300, 400, and 500 g of 'Isabella' grapes, 7 days after inoculation and insertion of fruit in the closed Mariotte system at 21°C (LSD_{0.05} = 0.6).

In the presence of 'Isabella' grapes, the number of *B. cinerea* conidia on the kiwifruit was significantly reduced (5.9 to 23.7 per ml) in comparison to the control (1,175.6 per ml) (Fig. 2). Although not significant, the quantity of *B. cinerea* conidia appeared to decrease (from 23.7 to 5.9 conidia per ml) in proportion to the increase in weight of the 'Isabella' grapes (from 300 to 500 g).

The depth of decay caused by *B. cinerea* (Fig. 3) was 7.1 mm on kiwifruit in the absence of 'Isabella' grapes after 7 days at 21°C. In the presence of 'Isabella', the depth of *B. cinerea* decay decreased significantly, falling progressively from 1.8 to 0.2 mm, as the weight of the 'Isabella' grapes was increased from 300 to 500 g, respectively.

Effect of temperature. No sporulation of the fungus was observed on the kiwifruit at any temperature in the presence of 'Isabella' grapes. However, in the absence of 'Isabella' grapes, at 10°C, 12 days after inoculation, *B. cinerea* sporulated on 65% of the kiwifruit, while at 21°C, 8 days after inoculation, sporulation occurred on 100% of the kiwifruit.

The depth of decay (Fig. 4) caused by *B. cinerea* was significantly lower in the presence of 'Isabella' grapes at both temperatures than that on kiwifruit in the absence of 'Isabella' grapes.

Effect of the stage of development of 'Isabella' grapes. Sporulation of *B. cinerea* (Fig. 5) was observed at a rate of 100% on kiwifruit in the presence of 'Roditis' grapes. However, the sporulation of *B. cinerea* on kiwifruit decreased in proportion to the increase in maturity of 'Isabella' grapes. No sporulation of the

Fig. 4. Effect of temperature (10 and 21°C) on the depth of *Botrytis cinerea* decay in kiwifruit, in the presence and absence of 'Isabella' grapes, 11 and 7 days after inoculation, respectively, and 12 and 8 days after insertion in the closed Mariotte system, respectively (LSD_{0.05} = 1.6).

Fig. 5. Sporulation of *Botrytis cinerea* on kiwifruit inoculated with the fungus in the presence of immature, semimature, and mature 'Isabella' grapes and in the presence of mature 'Roditis' grapes, 5 days after inoculation and 6 days after insertion in the closed Mariotte system at 21°C (LSD_{0.05} = 27.4).

fungus was observed on kiwifruit in the presence of mature 'Isabella' grapes.

The depth of decay caused by *B. cinerea* was observed at 6.6 mm on the kiwifruit in the presence of 'Roditis' grapes. The severity of the decay decreased significantly from 2.7 to 1.1 mm in the presence of 'Isabella' grapes, in relative proportion to an increase in maturity of 'Isabella' grapes (Fig. 6).

Effect of inoculation time after wounding. The production of *B. cinerea* conidia on kiwifruit in the presence of 'Isabella' grapes was very low at all inoculation times, with no significant differences among times (Fig. 7). In contrast, the number of conidia on the kiwifruit in the absence of grapes, and also in the presence of 'Roditis' grapes, decreased in relation to the increase in the time interval (from 0 to 48 h) between wounding and inoculation of the kiwifruit.

At all three times, the depth of decay caused by *B. cinerea* in kiwifruit was very low in the presence of 'Isabella' grapes, with no significant differences among times (Fig. 8). The presence of barrier-like zones was pronounced in the wounds around the inoculated areas. Barrier-like zones (calli) were well developed in the 48 h after wounding treatment. In contrast, the depth of decay in the kiwifruit, either in the presence of 'Roditis' grapes or in the absence of grapes altogether, was significantly higher than that of the above treatment at all inoculation times. The severity of decay again did not differ among the times of inoculation.

Microscopic examination. The microscopic examination of the mycelial hyphae of *B. cinerea* showed secretion of the cell protoplast without previous disruption of the cell walls, formation of large chemotopes, deformation of cell walls, and the presence of large groups of chlamydospores in those hyphae taken from kiwifruit that had been exposed to volatiles from 'Isabella' grapes. In contrast, hyphae obtained from kiwifruit either in the absence of 'Isabella' volatiles or in the presence of 'Roditis' volatiles were healthy, possessing conidiophores with abundant conidia. The above observations of the mycelium of the fungus appeared more pronounced in hyphae taken from kiwifruit in the presence of 'Isabella' grapes at the 500-g weight, at the mature stage, or at 21°C, than in hyphae taken from kiwifruit in the presence of 'Isabella' grapes at lower weights, developmental stages, or temperatures tested.

DISCUSSION

The antifungal action of volatile substances emitted from 'Isabella' grapes on the growth of *B. cinerea* has been shown on grapes in *in vitro* and *in situ* studies (20). In the present study, we have shown the strong antifungal action of 'Isabella' volatiles on the growth of *B. cinerea* on kiwifruit. The closed Mariotte system, used as the bioassay method, provided the possibility of studying the effect of 'Isabella' volatiles on the growth of the fungus *in vivo* on kiwi berry fruit. Kiwifruit inoculated with *B. cinerea*, as

Fig. 6. Depth of decay caused by *Botrytis cinerea* in kiwifruit inoculated with the fungus in the presence of immature, semimature, and mature 'Isabella' grapes and in the presence of mature 'Roditis' grapes, 5 days after inoculation and 6 days after insertion in the closed Mariotte system at 21°C (LSD_{0.05} = 1.5).

well as kiwifruit inoculated with *B. cinerea* and kept in the same containers as 'Roditis' grapes, were used as controls. This was done to study the effect of the 'Isabella' volatiles on the ability of *B. cinerea* to infect, grow, and sporulate on kiwifruit at various temperatures and times of inoculation after wounding the kiwifruit, and at varying weights and developmental stages of the grapes. Although the individual experiments were not repeated in

Fig. 7. Conidia production of *Botrytis cinerea* on kiwifruit in the presence of 'Isabella' grapes, 'Roditis' grapes, and the absence of grapes (control) when inoculated with the fungus immediately (0 h), 24, and 48 h after wounding 7, 10, and 11 days after insertion in the closed Mariotte system at 21°C, respectively (LSD_{0.05} = 338.09).

Fig. 8. Depth of decay in kiwifruit in the presence of 'Isabella' grapes, 'Roditis' grapes, and the absence of grapes (control) when inoculated with *Botrytis cinerea* immediately (0 h), 24, and 48 h after wounding 7, 10, and 11 days after insertion in the closed Mariotte system at 21°C, respectively (LSD_{0.05} = 3.37).

these studies, the major conclusions regarding efficacy of 'Isabella' grapes for control of *B. cinerea* are supported by the consistency of the responses observed from the common treatments present in multiple interrelated experiments. However, further testing may be necessary to confirm some of the specific experimental results, such as effect of maturity and inoculation time on disease control.

The wounded kiwifruit inoculated with *B. cinerea* were always infected, confirming the susceptibility of these fruit when wounded tissue is inoculated with the fungus. However, the incidence of infection decreased when the kiwifruit were exposed to volatiles from 'Isabella' grapes.

The secretion of the cell protoplast without previous disruption of the cell walls confirmed that endolysis (5,8) of the mycelial hyphae was a result of the antibiotic action of the 'Isabella' volatiles on *B. cinerea* (20). Lysis was also mentioned by Linderman and Gilbert (21), who showed that sclerotia of *Sclerotium rolfsii*, stimulated to germinate and grow by stimulatory vapors of alfalfa distillate, collapse because of depletion or lysis of the inner sclerotial contents. Bruce et al. (7) found that volatiles produced by *Trichoderma* spp. could be responsible alone for the inhibition and lysis of *Lentinus lepideus*. Apart from endolysis, the deformation of the cell walls and the presence of large groups of chlamydospores as additional effects of the action of 'Isabella' volatiles on *B. cinerea* hyphae confirmed the findings of our previous study (20). The inhibitory action of the 'Isabella' volatiles on the sporulation of *B. cinerea* and its ability to grow in the host tissue was also verified.

Important conclusions arising from this study were the following: (i) the greater the amount (weight) of 'Isabella' grapes, the stronger the antibiotic action of the 'Isabella' volatiles against *B. cinerea* on kiwifruit; (ii) the antibiotic action of the 'Isabella' volatiles against *B. cinerea* on the kiwifruit was stronger at 21°C than at 10°C; (iii) the antibiotic action of the volatiles of mature 'Isabella' grapes was stronger than that of the volatiles of the immature and semimature 'Isabella' grapes; and (iv) the antibiotic action of the 'Isabella' volatiles against *B. cinerea* was effective irrespective of the inoculation time of the kiwifruit after wounding.

The quantity of the 'Isabella' grapes was important in the expression of the antibiotic action of the volatiles emitted by them. The inhibitory action was expressed in all the weights tested, although it was more pronounced at the highest weight. The stronger antibiotic action at the highest weight of grapes was attributed to the higher concentration of 'Isabella' volatiles produced by them. Linderman and Gilbert (21) found that vapor concentrations of alfalfa hay above the level that provides maximum stimulation are inhibitory or lethal to the germination of *Sclerotium rolfsii* sclerotia. Gilbert and Griebel (14) found that microsclerotia of *Verticillium dahliae* are eliminated from soil when they are exposed to high concentrations of the volatiles from alfalfa hay. However, low concentrations produce an apparent vegetative growth response that results in a two- to fivefold increase in the number of propagules of the pathogen in soil. Mulvena et al. (25) found that the germ tube growth of *Gloeosporium musarum* spores is completely inhibited at a high concentration of the fungistat 3,4-dihydroxybenzaldehyde. Fries (13) indicated that whether volatiles are stimulating or inhibitory depends on their concentration and on the specific sensitivity of the responding fungus. The studies of other researchers (1,22,30-32,35,37) also indicate that the nature of the antifungal action of volatiles may be either fungicidal or fungistatic, depending on concentration.

The effective suppression of plant diseases by biocontrol agents is largely affected by environmental conditions such as temperature (15). The volatiles of the 'Isabella' grapes inhibited the fungus at both of the temperatures tested (Fig. 4). However, their inhibitory action was more pronounced at 21°C, although the very

same temperature is the optimum for *B. cinerea* growth (17), confirming the findings of Kulakiotu et al. (20) regarding the effect of temperature on the action of 'Isabella' volatiles on the fungus in situ on grapes.

The inhibitory action of the volatiles from mature 'Isabella' grapes was greater than that of immature grapes, because the volatiles completely inhibited the sporulation of the fungus on the kiwifruit and considerably reduced its growth. This resulted in a sharper decrease in the inoculum and in the activity of the fungus than that caused by the volatiles from 'Isabella' grapes at the other developmental stages. This suggests that the concentration and possibly the quality of the synthesis of the 'Isabella' volatiles were superior in grapes at the mature stage. This corresponds to the conclusions of other researchers. Hamilton-Kemp et al. (16), studying the effect of some natural volatiles on fungi, stated that the amounts of these compounds released depended on the stage of development of the leaves. Song et al. (35), studying the antifungal activity of hexanal vapor, indicated that the volatile biosynthesis of immature fruit is low. It could also be said that the quality and quantity of 'Isabella' volatiles vary with the growth stages of the grapes from which these volatiles are emitted.

According to Baudoin and Eckert (6), injuries in the peel of lemons become progressively more resistant to infection by *Geotrichum candidum* when inoculated 4 to 20 h after wounding. The lignin-like zones act as a defence mechanism against sour rot. The present study indicates that the inoculation time after wounding did not play an important role in the antifungal action of the 'Isabella' volatiles either on the density of inoculum or the activity of *B. cinerea* (Figs. 7 and 8). The antibiotic action of the 'Isabella' volatiles on *B. cinerea* was strong regardless of the inoculation time of the kiwifruit after wounding. However, well-developed barrier-like zones were present, especially at inoculation 48 h after wounding the kiwifruit in the presence of 'Isabella' volatiles, but absent on the kiwifruit either in the presence of 'Roditis' volatiles or in the control. This leads to the hypothesis that, beyond the antibiotic action of the 'Isabella' volatiles against *B. cinerea*, additional action of the 'Isabella' volatiles may exist which induces mechanisms of resistance in the host. This hypothesis constitutes the subject of a future study. French (12) noted that understanding the role of volatile compounds might help to explain some of the biological mysteries in germination and infectivity and contribute new techniques to the development of pest control. The identification of the components of the 'Isabella' volatiles, related to antifungal activity, and the preparation of their synthetic solutions, singly and in mixtures, are also subjects of further study. As potential fungicides, their natural occurrence as part of the human diet, their ephemeral nature, and their biodegradability suggest low toxic-residue problems (12).

This study corroborates the conclusion of Kulakiotu et al. (20) that volatiles emitted by 'Isabella' grapes act as biocontrol agents on *B. cinerea*. In this study, we show that volatiles emitted by 'Isabella' grapes control *B. cinerea* on kiwifruit by decreasing both the inoculum density and the aggressiveness of the pathogen.

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