MPMI Vol. 9, No. 9, 1996, pp. 864-868. Publication no. M-1996-1101-02N.

Research Note

Effect of Ethylene on Activation of Lesion Development from Quiescent Infections of *Colletotrichum gloeosporioides* in Avocado Fruits

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26 March 1996. Accepted 16 August 1996.

Exposure of *Colletotrichum gloeosporioides* conidia placed on avocado wax and on intact avocado to 45 µl of ethylene per liter stimulated spore germination, appressorium formation, and proliferation. Exposure of immature cultivar Fuerte avocado fruits inoculated with *C. gloeosporioides* to 45 µl of ethylene per liter enhanced appressorium proliferation and an early climacteric but did not affect lesion growth during 20 days following the treatment. During this period fungitoxic concentrations of the antifungal diene in the fruit peel remained at concentrations higher than 2,260 µg/g fresh weight. Overmature cv. Reed avocado fruits inoculated with *C. gloeosporioides* and exposed to 45 µl of ethylene per liter showed enhanced germination, single and multiple appressorium formation, and earlier fruit ripening. Lesion development by *C. gloeosporioides* occurred in ethylene-treated fruits in parallel to the development in untreated fruits. Levels of the antifungal diene from the second day after harvest decreased in parallel in ethylene treated and untreated fruits. We conclude that exposure of avocado fruits to exogenous ethylene treatment induces multiple appressorium formation and fruit ripening, but it does not activate lesion development by *C. gloeosporioides*.

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. in Penz. is the most destructive postharvest pathogen of avocado and other tropical fruits. The pathogen infects tropical fruits throughout the period of fruit growth, but remains quiescent for weeks or months while the fruit is immature. Conidia of the fungus germinate on the peel of avocado fruit, producing appressoria, a process induced by fatty alcohols present in avocado wax (Podila et al. 1993; Prusky and Saka 1989). The appressoria produce infection pegs that breach the wax layer and come to rest on the underlying epidermal cells (Prusky et al. 1991). Upon harvest and fruit ripening, quiescent infections are activated, causing extensive damage to the fruit (Prusky and Keen 1993).

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Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 1543-E, 1995 series.

Quiescent infection appears to be a fungal response to adverse physiological conditions temporarily imposed by the host. The lack of available nutrients, the presence of preformed antifungal compounds, and the lack of enzymatic potential have been suggested as possible causes of quiescent fungal infections on unripe fruits (Swinburne 1993; Prusky 1996). The quiescence of *C. gloeosporioides* in unripe avocado fruit (*Persea americana* Miller var. *drymifolia* (Schldl. and Cham.) S.F. Blake 'Fuerte') has been attributed to the presence of high concentrations of the antifungal compound, 1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (Prusky et al. 1982). In addition, epicatechin, a natural flavone present in the peel, inhibits fungal pectolytic enzymes such as pectate lyase and polygalacturonase, which might be involved in fungal attack (Wattad et al. 1994). Concentrations of the antifungal diene and epicatechin decrease during fruit ripening and were accordingly suggested as major factors in permitting activation of quiescent infections.

Recently, Flaishman and Kolattukudy (1994) reported that the hormone ethylene stimulates appressorium proliferation of *Colletotrichum* at concentrations over 1 µl/liter. They suggested that ethylene generated by the host during ripening would be a signal for the activation of quiescent fungal infections on the fruit surface. In the present work we confirm the effects of ethylene on conidium germination and appressorium proliferation of *C. gloeosporioides* in an in vitro system and on avocado fruits. However, our data suggest that the development of lesions initiated from quiescent appressoria of *Colletotrichum* on unripe fruits is dependent on the decrease in concentration of antifungal diene present in the peel (Prusky and Keen 1993).

Effect of ethylene on conidium germination and *C. gloeosporioides* appressorium formation in vitro and on avocado fruits.

Ethylene was applied to germinating conidia of *C. gloeosporioides* incubated in petri dishes with disks covered with avocado wax (Prusky et al. 1991). A rapid increase in conidium germination and single appressorium formation was induced during the first 3 to 7 h of incubation on avocado wax by 5 µl of ethylene per liter (Fig. 1A and B). Ethylene at 45

µl/liter showed a similar effect on conidium germination and appressorium formation (results not shown). When *C. gloeosporioides* germinating conidia on avocado fruits were exposed to a stream of 45 µl of ethylene per liter a significant enhancement of multiple appressorium formation was observed (Fig. 2).

Ethylene does not activate lesion development of quiescent *C. gloeosporioides* infections on immature fruits.

Freshly harvested immature avocado fruits (60 days before natural harvest) were inoculated and exposed during 24 h to 0 and 45 µl of ethylene per liter. Endogenous ethylene evolution and levels of the antifungal diene in fruit peel were followed in uninoculated fruits. The ethylene-treated fruits did exhibit an earlier climacteric peak (Fig. 3). One week after exposing the fruits to 45 µl of ethylene per liter, the antifungal diene levels were 3,240 ± 780 and 3,630 ± 510 µg/g fresh weight in treated and untreated fruits. Seventeen days after treatment, the concentrations of the diene decreased to 2,540 ± 180 and 2,700 ± 190 µg/g fresh weight in ethylene treated and untreated fruits, respectively. No symptoms of decay were observed in ethylene treated and untreated fruits during the first 20 days of storage following the treatment (Fig. 4). Two days later, the concentration of the diene was still fungitoxic (2,260

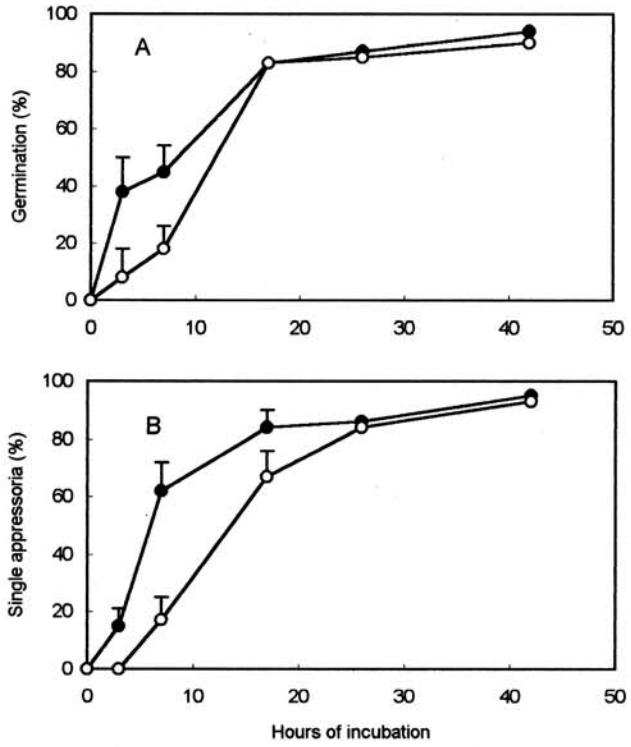

Fig. 1. Germination **(A)** and single appressorium **(B)** formation of *Colletrotrichum gloeosporioides* conidia (isolate Cg-14) on avocado wax exposed to ethylene. Conidia were exposed ● or not ○ to ethylene. Conidia were harvested from a 7- to 10-day-old PDA culture, suspended in 10 ml of water, filtered through Miracloth, washed once by centrifugation, and brought to the required concentration (10^6 spores/ml). Conidia were spotted on 13-mm nitrocellulose discs (Millipore, HAWP 13 mm diameterr 0.45-µm pore size), some of which were covered with 5 mg of avocado wax. The disks were incubated over a glass slide in a moist petri dish and exposed to a stream of 100 ml/h of 0 (air) or 5 µl/l of ethylene for 24 h at 20°C as described previously (Prusky et al. 1991). Air leaving the jars passed to the outside of the store room which was maintained at 20°C. At different times of incubation, the disks were sampled and conidial development was terminated by staining with cotton blue in lactophenol. Germination and appressorium formation from 300 conidia on four different individual discs (1,200 conidia total) were examined microscopically. Experiments were repeated three times.

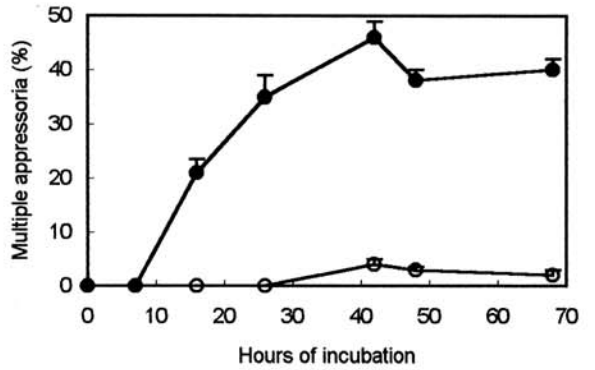

Fig. 2. Effect of ethylene on multiple appressorium formation from conidia *of Colletrotrichum gloeosporioides* on avocado fruit cv. Fuerte exposed to 45 µl/l ethylene. ●, ethylene-treated, ○, air-treated. Freshly harvested avocado fruits were spot-inoculated with *C. gloeosporioides* and incubated at 20°C, while exposed to air or an ethylene flow of 45 µl/l for 24 h. Following the treatment, fruits were transferred to normal atmospheric conditions. Spot inoculation was carried out by placing 1 to 2 µl of aqueous suspension of conidia (5×10^7/ml) obtained from a 10-day-old plate on the pericarp of freshly harvested intact fruits at nine points along the longitudinal axis of the fruit. Ten fruits were inoculated per treatment (90 inoculations per treatment). Fruits were then incubated under high humidity at 20°C for up 3 days. At different times after initiation of incubation slices of inoculated avocado pericarp, approximately 0.5 mm thick, were sampled and conidial development was terminated by staining with cotton blue in lactophenol. Percent of conidia with multiple appressoria was calculated using as 100% all the conidia that formed appressoria. A total of 300 conidia from four different individual pericarp slices (1,200 conidia total) were examined microscopically. Experiments were repeated three times.

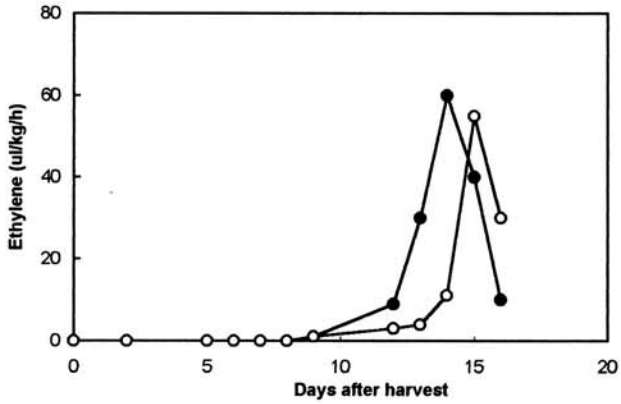

Fig. 3. Effect of exposure of avocado fruit cv. Fuerte to exogenous ethylene on the evolution of endogenous ethylene during fruit ripening. Fruits were harvested 60 days before maturation (60 days before regular harvesting), and exposed to 45µl/l ethylene for 24 h at 20°C. Fruits were then stored at 20°C. Ethylene evolution of four individual fruits from ethylene (●) and air (○) treatment was measured at 1- to 3-day intervals. Fruits were weighed and placed in 1-liter glass jars. The jars were closed for 1 h and head-space gas samples were taken for measurements of ethylene. Ethylene was detected by gas chromatography with a flame ionization detector, alumina column, and N_2 as the carrier gas. The data are means of four measurements calculated on the basis of fresh weight of each fruit.

± 120 µg/g fresh weight) and only superficial disease symptoms, less than 1 mm diameter, were observed. Similar superficial symptoms were reported by Coates et al. (1993) in unharvested avocado fruits inoculated with *C. gloeosporioides.*

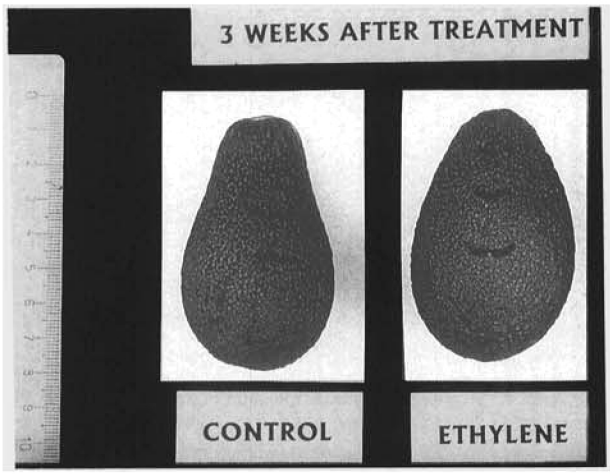

Fig. 4. Lack of symptom expression of *Colletotrichum gloeosporioides* on avocado fruits cv. Fuerte 20 days after exposure to ethylene. Immature fruits were spot inoculated at the longitudinal axis of the fruit and exposed to 45 µl/l ethylene for 24 h at 20°C.

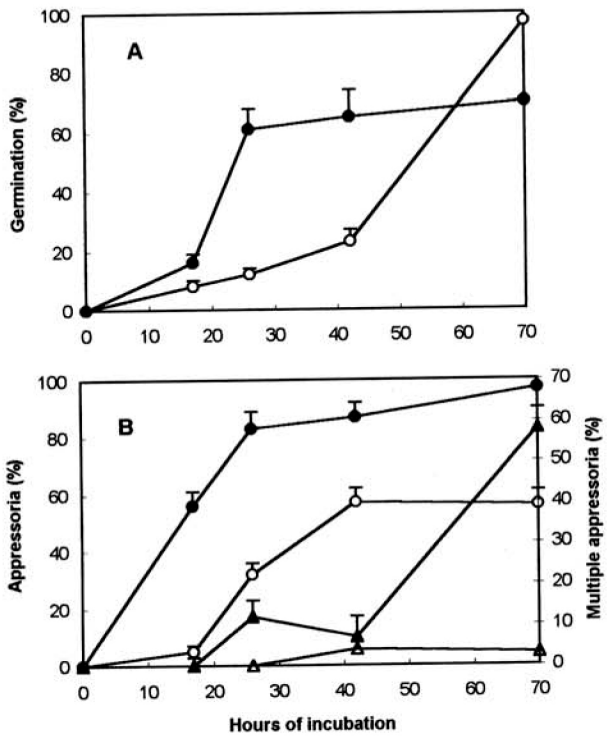

Fig. 5. Effect of ethylene on germination, single and multiple appressorium formation of *Colletotrichum gloeosporioides* on overmature avocado fruits cv. Reed. Overmature (3 mon after the regular harvest), freshly harvested fruits were spot-inoculated with *C. gloeosporioides,* incubated at 20°C and exposed for 24 h to 45 µl/liter ethylene. **A,** germinated conidia, and **B,** single (O,O) and multiple appressoria (Δ,Δ). ΔO, ethylene-treated; ΔO, air-treated. Spot inoculation was carried out as described before (Fig 2).

Effect of ethylene on the activation of lesion development of *C. gloeosporioides* infections on overmature avocado fruits.

Germination as well as single and multiple appressorium formation were induced on the peel of freshly harvested overmature fruits cv. Reed (120 days after normal harvesting) by exposure to 45 µl of ethylene per liter for 24 h (Fig. 5). Ripening of overmature avocado cv. Reed avocado fruits occurred over 7 days. Ethylene caused a significantly earlier decrease in fruit firmness, indicating that the climacteric peak of avocado fruit occurred earlier (Biale 1950); however, fruit ripening did not affect decay development by *C. gloeosporioides* compared to untreated fruits (Fig. 6). The initial concentration of the antifungal diene in the peel of overmature Reed fruits was 660 ± 130 µg/g fresh weight. Ethylene treatment enhanced a transient increase in the level of the antifungal diene during the first day after exposure (1,300 ± 220 µg/g fresh weight in ethylene treated as compared to 480 ± 60 µg/g in untreated fruit). The levels of diene subsequently decreased to similar subfungitoxic concentrations (330 ± 20 µg/g fresh weight) 5 days after harvest in both ethylene treated and control fruits. Two days later symptoms of decay were observed.

In the present work we have confirmed the effect of ethylene on the enhancement of conidium germination and on single and multiple appressorium proliferation. Flaishman and Kolattukudy (1994) carried out their experiments on sonicated wax samples (approximately 1 µg/cm^2) and at concentrations of ethylene starting at 0.5 µg/liter. In our experiments non-sonicated wax samples at higher concentrations (1 mg/cm^2 Millipore disk) and ethylene at 45 µl/liter were used. In spite of the different methods, similar conclusions were obtained

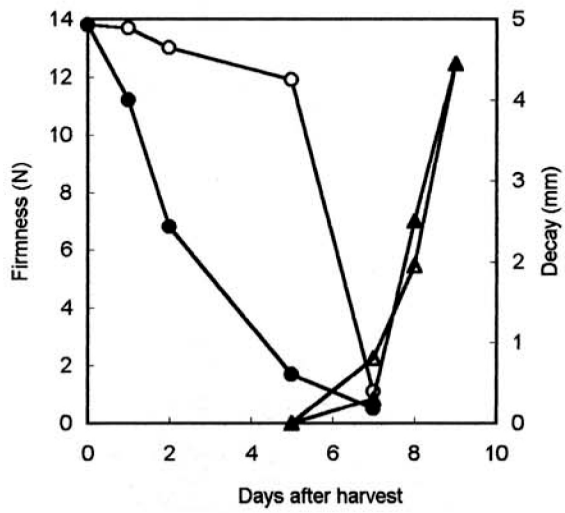

Fig. 6. Effect of ethylene on decay development caused by *Colletotrichum gloeosporioides* and changes in fruit firmness in overmature avocado fruits cv. Reed. Avocado fruit 120 days after regular harvest were spot inoculated by placing 1- to 2-µl aqueous suspension of conidia (5 ×10^7 spore/ ml) on the pericarp of freshly harvested intact fruits at nine points along the longitudinal axis of the fruit. Ten fruits were inoculated per treatment (90 inoculations per treatment), and incubated at high humidity at 20°C until symptom development occurred. Fruit firmness (N = Newton, a parameter of fruit ripening) was determined with a Penetro Meter (Chatillon, USA) on three fruits of each treatment at each sampling period. Experiments were repeated three times. Firmness OO, Decay development ΔΔ ; OΔ, ethylene-treated, (45 µl/liters for 24 h); OΔ, air-treated.

concerning enhanced germination and single and multiple appressorium formation from conidia of *C. gloeosporioides* by ethylene treatment. However, we have been able to differentiate the effect of ethylene on the climacteric process and avocado ripening from the process of initiation of lesion growth from quiescent appressoria of *C. gloeosporioides*. Ethylene treatment at 45 µl/liter applied to immature or overmature fruits enhanced multiple appressorium formation and avocado ripening, but it did not trigger earlier lesion development. This suggests that enhanced evolution of ethylene production during ripening is not a signal for activating disease development in avocado fruits. Former results (Prusky et al. 1991; Coates et al. 1993) suggest that appressoria become quiescent after germination. The possibility that ethylene produced during fruit ripening could become a signal for lesion development of an appressorium that has already germinated and become quiescent in the field several weeks earlier, is of extreme importance to attempts to modulate postharvest diseases. However, no data have been presented on the effect of ethylene on the growth of germinated appressoria. The present results support previous reports demonstrating that the activation of germinated appressoria during avocado ripening is related to a decline of the preformed diene to subfungitoxic concentrations in the peel (Prusky et al. 1982).

Although fruit ripening, ethylene production, and decay development may occur concomitantly, lack of correlation between ethylene level and fungal invasion was observed in several other cases: (i) decay symptoms occurred in immature and overmature avocado fruits in the field under a high incidence of inoculum (Coates and Gowanlock 1992). In both cases symptoms of decay were noted on the fruits but ethylene evolution was not detected in the orchard; (ii) treatment of avocado fruits with 0.2 M $CaCl_2$ delayed ripening as well as ethylene evolution, but did not delay decay development (Prusky et al. 1982); (iii) mesocarp tissue of unharvested unripe avocado fruits is susceptible to *C. gloeosporioides* since most the antifungal diene is compartmentalized and not free in the tissue (Kobiler et al. 1993); (iv) differential susceptibility of avocado cultivars to *C. gloeosporioides* was not related to the ripening process and ethylene evolution but to the rate of epicatechin decrease in the peel (Prusky et al. 1988); and (v) mango fruits do not produce more than 0.2 µl of ethylene per liter during the climacteric period (Biale 1950), (a concentration that does not enhance appressorium proliferation, according to Flaishman and Kolattukudy 1994) and are still very susceptible to postharvest disease caused by *C. gloeosporioides*. This indicates that although ethylene is produced during fruit ripening of climacteric fruit, there is no direct implication of its involvement in lesion growth.

The effect of ethylene on climacteric or nonclimacteric fruits on the enhancement of lesion development should be tested in relation to the natural mechanism(s) of resistance of each fruit. The avocado fruit seems to be a system where the effect of ethylene in the activation of quiescent appressoria can easily be observed since a mechanism of resistance has been described (Prusky and Keen 1993). The induction of lesion development by *Colletotrichum* after exposure to ethylene in citrus and in transgenic tomato (a nonclimacteric fruit and a climacteric fruit incapable of producing ethylene, respectively) might also be related to biochemical changes in the fruit. Exposure of oranges to ethylene induces fruit degreening and triggers a decrease in the antifungal activity present in the flavedo, specifically of citral (Ben Yehoshua et al. 1995). Also, when ethylene is applied to transgenic tomatoes it may reverse the inhibitory effect resulting from the presence of an antisense copy of the 1-aminocyclopopane 1-carboxylic acid synthase gene, enhancing biochemical changes like softening, color, and aroma (Oeller et al. 1991) and a reduction of α-tomatine as occurs in ripe fruits (Verhoeff and Liem 1975). Thus, activation of quiescent appressoria of *Colletotrichum* may be dictated by a series of changes in the host rather than a single signal produced by the host which affects the pathogen. Ethylene may be involved in increasing the potential of fungal attack by increasing appressorial proliferation but not the capability of attack. For fungal pathogenicity, other factors such as the decrease in fruit resistance, are possibly involved in the activation of lesion development in ripening fruits.

ACKNOWLEDGMENTS

This research was partially supported by Binational US-Israel Fund for Agriculture Research and Development, US-Israel Cooperative Development Research Program, and Germany-Israel Agricultural Research Agreement.

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