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EFFECT OF ETHYLENE ON NATURAL RESISTANCE OF CITRUS FRUIT TO STEM-END ROT CAUSED BY *DIPLODIA NATALENSIS* AND ITS RELATION TO POSTHARVEST CONTROL OF THIS DECAY

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Abstract. **Ethylene degreening of early season citrus fruit for marketing purposes is a common practice in Florida. This practice enhances stem-end rot caused by *Diplodia natalensis*, but the mechanisms for this increase in decay are largely unknown. A study to determine the influence of ethylene on the natural resistance of 'Valencia' oranges to this decay was conducted. After fruit were de-buttoned and subjected to ethylene at 0, 5, and 50 ppm for 60 hours, fruit were then inoculated with *D. natalensis* at the stem cavity and incubated at 21 °C for 2 weeks. Fruit treated with ethylene at 0, 5 and 50 ppm showed a decay incidence of 10.0, 33.3 and 73.3%, and a disease severity index of 0.3, 0.8, and 2.5, respectively. Ethylene appeared to reduce the natural resistance of fruit tissues to *D. natalensis*, and the degree of tissue susceptibility was positively correlated to the ethylene concentration. Evaluation of chemical control for this disease showed that a fruit drench with thiabendazole (TBZ) or Imazalil before fruit degreening was much more effective than packingline applications of these chemicals after degreening treatment. Therefore, to effectively control Diplodia stem-end rot on early season fruit at postharvest stage, fruit should be drenched with TBZ or Imazalil prior to degreening, and ethylene concentration and duration of degreening should be the minimum necessary to achieve successful degreening of the fruit.**

Stem-end rot caused by *Diplodia natalensis* Pole-Evans is an important postharvest disease on Florida citrus fruits. The disease is more prevalent on ethylene degreened early season fruit. Ethylene degreening of early harvested citrus is a common commercial practice that improves fruit coloration for marketing purposes, but this practice significantly enhances the incidence and severity of Diplodia stem-end, and causes significant economic losses (Barmore and Brown, 1985). Recommended degreening conditions for Florida citrus use five ppm of ethylene, 28-29 °C and 90-96% relative humidity for various periods of time depending on fruit varieties and degree of fruit color break (Wardowski, 1996). Diplodia stem-end rot can develop rapidly during and after excessive degreening, and can be observed in fruit at the packinghouse, and is often seen at market arrival or shortly thereafter (Ismail and Zhang, 2004).

Mechanisms for the stimulation of Diplodia stem-end rot by ethylene are still not fully understood. Brown and Wilson (1968) reported that entry of *D. natalensis* into healthy tissue of the button and fruit are prevented by the cuticle and wound periderm which separates necrotic and healthy tissue. *D. natalensis* does not enter the fruit until openings develop in the separation layer between the button and fruit at the time of abscission. After entry, the fungus rapidly colonizes starch-laden cells and protoplasm between the button and fruit. Brown and Burns (1998) reported that the activities of 'Valencia' fruit abscission enzymes, polygalacturonases and cellulases was enhanced by high ethylene level (55 ppm) compared to that of low ethylene level (2 ppm). However, Barmore and Brown (1985) further reported that the enhanced Diplodia stem-end rot could not be solely explained by abscission development. Therefore, it is still not clear if ethylene affects not only fruit abscission, but also alters the natural resistance of fruit peel to Diplodia stem-end rot.

In citrus groves, *D. natalensis* colonizes the surface of a fruit's button and does not cause decay before harvest. Since ethylene treatment can significantly reactivate pathogen infections and decay development, the more effective way to control this disease should be to eliminate or reduce the pathogen population size on the fruit surface before ethylene degreening. There are two common commercial steps to control this decay before ethylene degreening, fungicide applications by preharvest spraying or postharvest fruit drenching. Packingline application of fungicides after degreening treatment is also a common commercial practice for this decay control, but it might be less effective compared to the applications of fungicides before degreening treatment.

The objectives of this study were to determine the effect of ethylene treatment on the natural resistance of citrus fruit peel to Diplodia stem-end rot, and to compare the effectiveness of fungicides (thiabendazole and Imazalil) for Diplodia stem-end rot control by postharvest drench before degreening or packingline drip after degreening treatment.

Materials and Methods

Influence of ethylene on Diplodia stem-end rot incidence of 'Valencia' fruit with their buttons attached. Mature 'Valencia' fruit were obtained from a local packinghouse (Hunt Brothers, Lake Wales, Fla.). Fruit were brought to the Citrus Research and Education Center's packinghouse, University of Florida, Lake Alfred, Fla., and were divided into three groups of approximately 150 fruit each. Each group was then divided into 3 replicates. The three groups of fruit were then subjected to ethylene treatments at 0, 5 and 50 ppm, respectively, at 30 °C and 92 to 96% relative humidity (RH) for 60 hrs. Ethylene concentrations were adjusted and monitored using gas chromatography. After ethylene treatment, fruit were maintained at 21 °C and 92 to 95% RH for 2 weeks, and the incidence of Diplodia stem-end rot was recorded weekly.

Effect of ethylene on natural resistance of fruit tissue beneath the button to Diplodia stem-end rot after removing buttons. 'Valencia' fruit were obtained from a local packinghouse as described previously. On the same day, fruit were treated with ethylene at 5 ppm at 30 °C and 92-96% RH for 24 hrs. The buttons of each fruit were then forcibly removed with a probe. Debuttoned fruit were divided into three groups with 90 fruit each. Each group of debuttoned fruit was then separated into three replications with 30 fruit each. The three groups of the fruit were subjected to ethylene degreening at 0, 5 and 50 ppm, respectively, at 30 °C and 92 to 96% RH for 60 hrs. After degreening treatment, the stem-cavity of each fruit was inoculated with 0.1 ml of an aqueous suspension containing 1% blended *D. natalensis* mycelia. Inoculated fruit were incubated at 21 °C for 2 weeks, and the incidence and severity of Dipolodia stem-end rot were recorded. Decay severity ratings for Diplodia stem-end rot were defined as follows: 0—no decay; 1—decay area was less than 25% of fruit surface; 2—decay area was between 26 to 50% of fruit surface; 3—decay area was between 51 to 75% of fruit surface; and 4—decay area was between 76 to 100% of fruit surface.

Diplodia stem-end rot control using drench applications of fungicides before degreening treatment. To simulate the commercial drench application of Florida packinghouses, a small drench system was constructed and used in the test. The system consisted of a solution container, showerhead, two pumps, tubes, and a plastic support frame. The two pumps circulated the drench solutions throughout the system. The showerhead delivered the drench solutions to the fruit. About 40 L of the solution were used in a drench cycle. Flow rate was approximately 20 L min^{-1}. 'Ambersweet' oranges were purchased from a local packinghouse and placed in plastic crates (60 × 40 × 30 cm). Three crates were stacked on top of each other as a treatment to simulate a commercial drench procedure. Fruit were drenched with TBZ or Imazalil solution (1,000 ppm) for 3 min, and drained for 4 min (commercial standard practice) prior to ethylene degreening. Fruit drenched with water served as controls. Drenched fruit were subjected to ethylene (10 ppm) degreening at 30 °C and 92-96% RH for 72 hrs. Three replications were used for each treatment. Each replicate had about 70 'Ambersweet' oranges. Ethylene degreened fruit were stored at 21 °C and 92-96% RH, and incidence of decay was recorded weekly.

Diplodia stem-end rot control using packingline application of fungicides after degreening treatment. 'Valencia' fruit were obtained from a local packinghouse as described previously. Fruit were degreened with ethylene (10 ppm) for 72 h at 30 °C and 92-96% RH, then washed, dried, and randomized through a packingline. Fruit were then dripped with Imazalil or TBZ at 1,000 ppm on a simulated commercial packingline. The contact time between fruit and fungicides on the wet brush bed of the packingline was 15 to 20 s. Treated fruit were dried at approximately 52 °C for 1-2 min, then stored at 21 °C for 2 weeks. Fruit dripped with water and incubated at 21 °C served as controls. Four replicates were used for each treatment, and each replicate had about 60 fruit. Disease incidences were recorded weekly.

Data analysis. Analysis of variance of data was performed using the Statistica program (StatSoft, Tulsa, Okla.). Percentage decay data were transformed to arcsine values before analysis. Treatment means were compared using the Duncan multiple range test ($P = 0.05$).

Results

Effect of ethylene treatment on Diplodia stem-end rot on fruit with their buttons attached. After 'Valencia' oranges (buttons not removed) were subjected to ethylene degreening at 0, 5 and 50 ppm, respectively, for 60 h and stored at 21 °C for 2 weeks, the incidence of Diplodia stem-end rot on the fruit treated with 0, 5 and 50 ppm ethylene were 3.3, 20.0 and 66.7%, respectively (Fig. 1). The incidences of Diplodia stem-end rot of 50-ppm ethylene treated fruit were 3.3 and 20.2 times higher than that of 5-ppm ethylene treated fruit and control fruit, respectively.

Influence of ethylene on natural resistance of fruit tissue underneath button to D. natalensis infection. After 'Valencia' oranges were de-buttoned and subjected to ethylene treatment for 60 h, fruit were then inoculated with *D. natalensis* at the stem cavities and incubated at 21 °C for 2 weeks. Fruit treated with ethylene at 0, 5 and 50 ppm showed Diplodia stem-end rot incidences of 10.0, 33.3 and 73.3%, and severity indexes of 0.3, 0.8, and 2.5, respectively (Fig. 2A and 2B). Incidence and severity of Diplodia stem-end rot of 50-ppm ethylene treated fruit were significantly higher ($P = 0.05$) than those of 5-ppm ethylene treated fruit or control oranges.

Control of Diplodia stem-end rot using fungicides before or after degreening treatment. When 'Ambersweet' oranges were drenched with the suspension of Imazalil or TBZ at 1,000 ppm before ethylene degreening (10 ppm for 72 h at 30 °C and 92-96% RH), Imazalil and TBZ reduced Diplodia stem-end rot by 81.8 and 95.9%, respectively, compared to that of control fruit after 3 weeks of storage at 21 °C (Fig. 3A). TBZ performed significantly better than Imazalil for decay control. Untreated fruit showed 81.7% Diplodia stem-end rot.

After 'Valencia' oranges were degreened with ethylene (10 ppm) for 72 h, and then washed, dried and dripped with

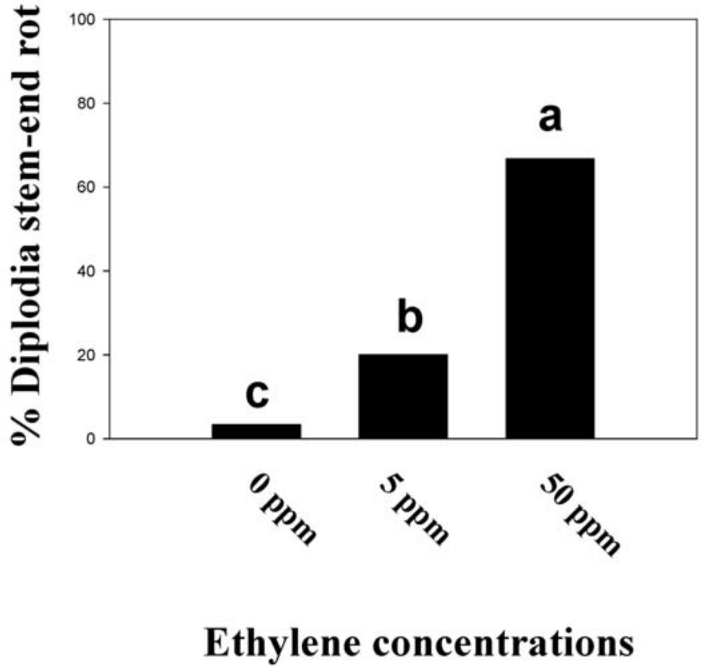

Fig. 1. Effects of ethylene concentrations on stem-end rot caused by *Diplodia natalensis* using intact 'Valencia' oranges. Fruit were treated with ethylene for 60 hrs, and then stored at 21 °C for 2 weeks. Means with the same letter on each bar are not significantly different ($P = 0.05$) based on Duncan's multiple range test.

Imazalil or TBZ at 1,000 ppm through a simulated commercial packingline, TBZ, but not Imazalil significantly reduced the incidence of Diplodia stem-end rot after 3 weeks of storage at 21 °C (Fig. 3B). However, TBZ only reduced decay by 44.4% over the control.

Discussion

D. natalensis survives on deadwood of citrus trees in groves, and water disseminates the spores of the fungus to the necrotic tissue of the button of immature fruit, causing latent infections (Brown, 1971). After harvest these latent infections can reactivate and invade mature fruit through the abscission zone when button abscises (Brown, 1971). Commercial ethylene degreening treatment of early season citrus could greatly enhance the occurrence of Diplodia stem-end rot (Brown and Lee, 1993; Ismail and Zhang, 2004). Test results from the current study were consistent with other previous reports (Barmore and Brown 1985, Brown, 1986; Brown and Lee, 1993). The mechanisms for the ethylene stimulation of Diplodia stem-end rot are still unclear. Brown and Wilson (1968) reported that *D. natalensis* does not enter the fruit until natural openings develop in the separation layer between the button and fruit at time of abscission. Ethylene is the most effective abscission agent known, and apparently stimulates the formation of abscission, and leads to increased Diplodia stem-end rot. Further study on the interactions between ethylene concentration, button abscission and decay incidence by Barmore and Brown (1985) indicated that abscission of the button does not ensure that the decay will develop. However, the penetration of rind by *D. natalensis* from the button was more rapid after ethylene treatment at 50 ppm than at 1 or 10 ppm (Barmore and Brown, 1985). This implies that ethylene might also affect susceptibility of rind tissue to pathogen infection and penetration.

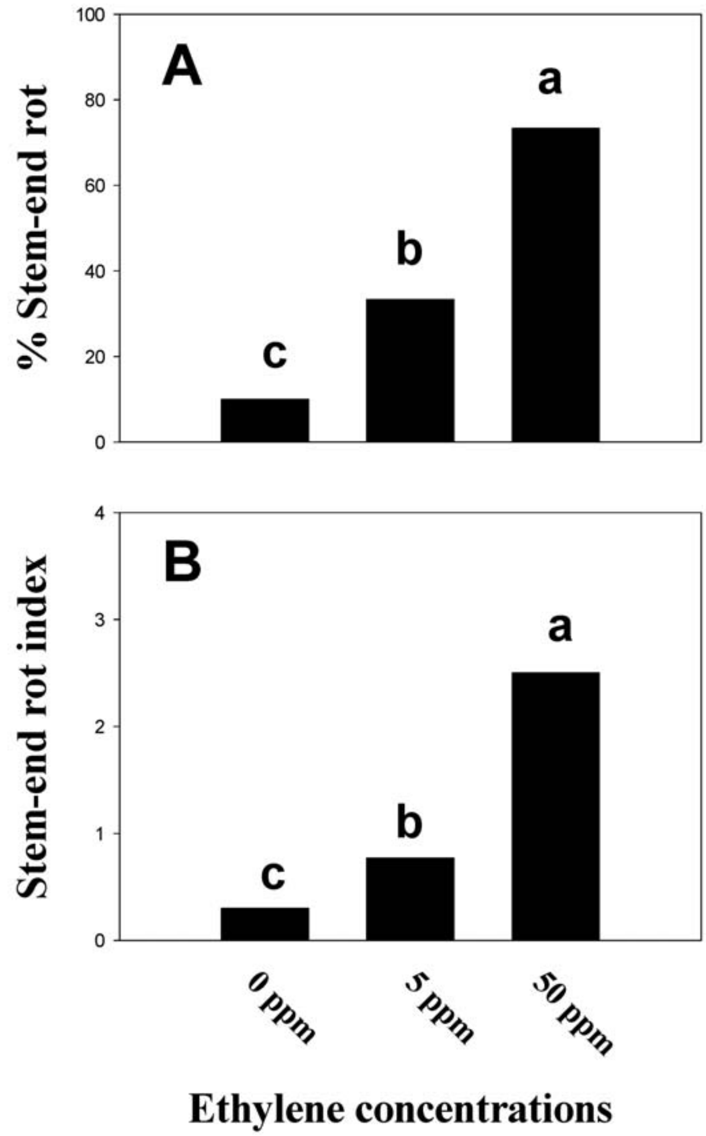

Fig. 2. Effects of ethylene concentrations on the susceptibility of fruit tissues underneath button to stem-end rot caused by *Diplodia natalensis* using debuttoned and inoculated 'Valencia' oranges. Fruit were debuttoned, and then treated with ethylene for 60 hrs before inoculation. Treated fruit were stored at 21 °C for 2 weeks. Decay incidence (A) and severity (B) were recoreded. Means with the same letter on each bar are not significantly different ($P = 0.05$) based on Duncan's multiple range test.

In the current study, to exclusively determine the effect of ethylene on the susceptibility of the peel tissue, especially the tissues underneath the button, to *D. natalensis* infection and penetration, the buttons of fruit were removed before ethylene treatment. This approach excluded the influence of abscission formation (natural openings) on pathogen infections. After ethylene degreening, stem cavities of debuttoned fruit were inoculated with pathogen mycelia, providing a uniform pathogen inoculum potential. The results of the current study showed that the pathogen infected and penetrated the peel tissue underneath the buttons on 50-ppm ethylene treated fruit much more rapidly than on 5-ppm ethylene treated fruit or untreated fruit. Disease also developed more rapidly on 5-ppm treated fruit than on control fruit. This further

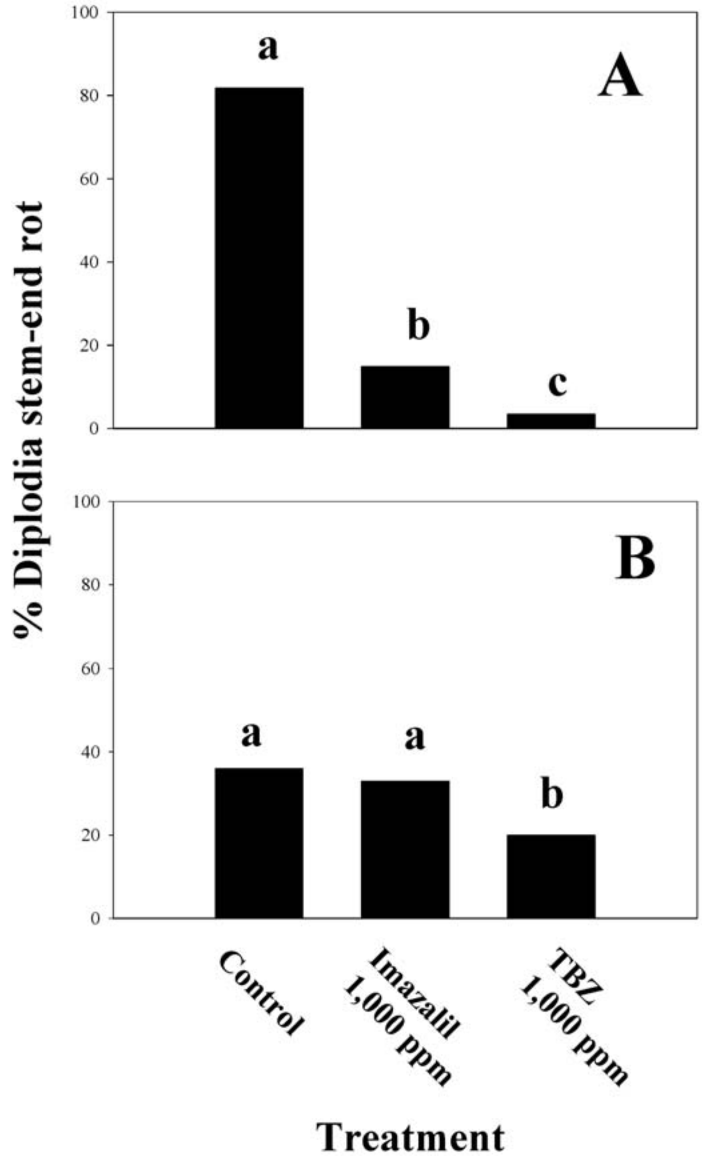

Fig. 3. Effects of fungicides on stem-end rot caused by *Diplodia natalensis* using drenching before ethylene degreening (A) or packingline drip after degreening (B). Treated fruit were stored at 21 °C for 3 weeks. Means with the same letter on each bar, for each experiment, are not significantly different ($P = 0.05$) based on duncan's multiple range test.

demonstrated that ethylene not only affects the formation of an abscission layer between button and fruit to promote pathogen infection, but also directly affects the physiology and biochemistry of peel tissues, especially for the tissues underneath the fruit button, leading to a reduced natural resistance of rind tissues to *D. natalensis* infections, penetration and disease development. Further study is needed to determine how ethylene physiologically and chemically affects the peel tissues to reduce the peel's natural resistance to the pathogen.

As discussed previously, the pathogen remains on the surface of button and fruit before an abscission layer forms to provide natural openings for the pathogen's ingression. Ethylene degreening of fruit is a common practice in packinghouses, and it promotes the formation of abscission between the button and fruit, and reduces the natural resistance of fruit rind to *D. natalensis*. Therefore, a better strategy for Diplodia stem-end rot control would be to eliminate or reduce pathogen population size before ethylene degreening treatment. Drench application of fungicides after harvest is a good step to control this decay before degreening in Florida packinghouses. The drench application of fungicides in the current study demonstrated that both Imazalil and TBZ provided effective control of Diplodia stem-end rot.

In commercial packinghouses, after ethylene degreening, fruit are subjected to the packing process which includes washing, sizing, drying, grading, waxing, fungicide application, storage and shipping. Although packingline application of Imazalil and TBZ is a common practice for postharvest control of stem-end rot and molds in Florida packinghouses, ethylene degreening treatment of fruit could greatly reduce the efficacy of fungicides for Diplodia stem-end rot control. In the current study, when Imazalil or TBZ at 1,000 ppm was applied to excessively ethylene degreened 'Valencia' fruit through a packingline, TBZ reduced Diplodia stem-end rot by 44.4%, but Imazalil only reduced the decay by 8.3% compared to control fruit.

In conclusion, fruit degreening using ethylene, especially excessive ethylene concentrations (more than 5 ppm) and prolonged degreening duration, can greatly stimulate Diplodia stem-end rot of citrus fruit. Mechanisms for the enhancement of Diplodia stem-end rot by ethylene degreening appear to be not only related to the stimulation of abscission which forms natural openings, but also to be related to the reduction of natural resistance of fruit rind to pathogen infection and penetration. Postharvest drenching of fruit with fungicides is an effective approach to control Diplodia stem-end rot.

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