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# Infection time by *Dothiorella* sp. and symptom onset of guava end rot

V. Bravo[1], D. Rodríguez[2], M.E. Sanabria[2], M. Marín-Larreal[3], R. Santos[4], E. Pérez[5], L. Sandoval[6]

[1]División de Estudios para Graduados, Programa de Fruticultura, [3]Dpto. de Botánica, [4]Dpto. Fitosanitario, [6]Instituto de Investigaciones Agronómicas, Facultad de Agronomía. Universidad del Zulia. Apdo. 15205. Maracaibo, ZU 4005. Venezuela
[2]Posgrado de Fitopatologia, Decanato de Agronomia, Universidad Centroccidental "Lisandro Alvarado" (UCLA). Apdo. 400. Cabudare, estado Lara, Venezuela
[5]Centro Frutícola del Zulia-CORPOZULIA. Km. 27, carretera vía El Mojan., municipio Mara, estado Zulia. Venezuela

## Abstract

To determine the time of initial infection by *Dothiorella* sp. (Teleomorph: *Botryosphaeria dothidea*) in guava (*Psidium guajava* L.) fruits and the onset of disease symptoms, a field trial was installed three times (from December 1999 to July 2000). During 16 weeks, the following treatments were established weekly: 40 fruits covered and inoculated (FCI) with 3 ml/fruit of a conidia suspension, 40 fruits non covered and non artificially inoculated (FSCSI), and 40 fruits covered and non inoculated (FCSI), the latter used as a treatment control. Seven days after inoculation, 20 fruits from each treatment were taken to the laboratory, disinfected, the pericarp cut in small segments, planted in agar V8-Juice and incubated for one month. The remaining 20 fruits of each treatment were left on the plants until the end of the trial, to observe the onset of disease symptoms. Significant differences ($P<0.01$), with regard to infection, were found among treatments and fruit age. Initial infection was observed at week 7 of fruit age, with 2.39% for FCI, and 19.05% for FSCSI. Infection increased progressively up to 100% (FCI) and 42.46% (FSCSI) in week 10. A low infection throughout the trial period (4.7 to 7.2%) was obtained in the control treatment (FCSI), due to contamination inside bags. Symptoms were firstly

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[1]Author's email: cristibu@cantv.net, rdorian@ucla.edu.ve mesanabria@ucla.edu.ve

observed in week 11, in treatment FCI (20%), and in week 12, in FSCSI (15%) and FCSI (5%), increasing in the following weeks. Results show the critical stages in fruit and fungal development, which should be taken into account for disease prevention.
**Key words:** Infection age, *Botryosphaeria dothidea*, macroscopic symptoms.

## Introduction

End rot caused by the fungus *Dothiorella* sp. (Teleomorfo: *Botryosphaeria dothidea*) is one of the most important illnesses of guava fruit (*Psidium guajava* L.) (7, 18).

In guava, the fungus produces an initial symptom that consists on reddish brown dot in the apical area of the fruit, around the floral residues, which extends until covering them completely. In the dead tissues, globular black picnidia are produced, which have hyaline macroconidium (7, 18).

The production of picnidia in guava fruits with end rot, favored with temperatures between 28 and 32ºC, relative humidity between 55 and 100%, and with periods of 12 hours of light and darkness (7) or continuous fluorescent light (4). Likewise, these conditions have been catalogued as optimum by *B. dothidea* (13, 17).

In apple and peach, symptoms of the illness caused by *B. Dothidea* have been observed 72 hours after the inoculation (5), while in vine happened three months later. Apple fruits are sensitive to this fungus seven weeks after the fall of petals, but require from 1 – 1.5 months after the infection for the appearance of symptoms (17).

In researches done by Sitterly and Shay (20), was detected that the resistance to the damage of white end rot in immature fruits of apple caused by *B. dothidea*, was due to the physiological factor or induced factors at the time that the fruit approached to maturation. On the other hand, Kohn and Hendrix (13), determined in apple observable values of the illness appeared from 6 to 8 weeks before the crop. Drake (9), on the other hand, determined that in this crop some infections are seen once the fruit is formed.

Likewise, it has been determined that apple fruits are more sensitive to *B. Dothidea* at the time that increases the content of sugar in the fruit (11). Similarly, was determined that symptoms of end rot caused by this fungus appeared when solid total soluble levels were of approximately 10.5%, with incidence levels of the illness of 100% when solid levels reached 13.8% (13). In guava, fruits in a physiological ripening status (98 days) reached values of total soluble solids between 11.83 and 15.74 ºBrix (3, 14).

In guava fruits, end rot is an illness which is widely distributed in producer regions of Venezuela, which reduces production and productivity, as well as quality of the fruit which causes the rejection of it, for the fresh

consumption as well as for the industrial. Nowadays, lost estimated by this illness, plus the regressive death caused by *Meloidogyne incognita* (8), are very high and have caused the disappearance of a big number of plantations in the region.

Investigations done in this field and in the laboratory have not been enough to reduce the illness, therefore it is required that besides knowing the crop, the climatologic behavior of the area, practices of handle and the identification of the causal agent, it is necessary further information about the epidemiology of the illness and on the development phase of the fruit, where the initial infection happens in order to establish rational conditions of the handle of the illness (4). Therefore, this investigation was carried out with the purpose of determining the age of the guava fruit where the initial infection by *Dothiorella* sp. happens, as well as the appearance moment of the symptoms of the illness.

## Materials and methods

**Location of the essay:** The study was carried out at the commercial production unit "San Onofre de las Margaritas", located at Km 12 of "Nueva Lucha" area, Mara municipality, Zulia state, Venezuela. For this, the cultivated surface was selected with *P. guajava,,* a part of six-year-old plants, with a plot design of 7m x 7m.

**Climatic characteristics:** Mara municipality is located on Maracaibo plain, Zulia state; area that characterizes by having an annual average temperature of 28ºC, the potential evapotranspiration reaches values from 2000 to 2200 mm annual and relative humidity of 75% (18). In this area, hydric and soil resources are limited for the production, that is the reason underground water has been used (15). Precipitation is from 500 to 600 mm, with irregular rain distribution, bimodal type, which peaks of maximum precipitation occurred in May and October, and the minimum precipitation in December, January and July – august (15).

**Preparation of the inoculum:** For the preparation of the inoculum *Dothiorella* sp. fruits with symptoms of end rot were selected, lightly washed for 3 min with current water and parts of the sick tissue were cut, which were disinfected by immersion in sodium hypochlorite at 0.525% (neutral pH) for 1 min, subsequently, were washed three times in sterile distilled water for 1 min and let dried with sterile absorbent paper.

The sick tissue was isolated in Petry plates which had nutritive medium of agar-juice-V8 (JV8) clarified and acidified with lactic acid (pH-4) at 25% (JV8-CA) (14). Later, plates were incubated at 27 ± 1ºC, 55% hr and continuous fluorescent light for three weeks (4). Plates were filled with 10 ml of sterile distilled water, and a spatula to scrape off the surface

of the crop to obtain a suspension of mycelium/conidia, which was processed using the modified Cobb method (21). The resultant solution was filtered using four layers of sterile gauge to remove fragments of mycelium.

The spores' concentration of the solution was determined with a Neubauer camera, and the concentration was adjusted to $1x10^5$ conidia per ml with sterile distilled water (4, 10).

**Inoculation:** In order to determine the initial moment of infection of *Dothiorella* sp. in guava fruits, 30 6-year-old trees were selected, where remained two weeks after the differentiation of buds (pre-flowering) (2, 6, 14), was proceeded to select at the same time 150 reproductive buds per tree by means of their appearance and uniform size, for a total of 4500 buds, due to the high percentage (62%) of fruits fall (14) distributed in four quadrants of the plant (north, south, east and west).

From the total of selected reproductive buds, 100 were covered at random (2 buds per bag), with doubled bags of white paper, which were strongly tied to the branch in its upper part with a thread, and the union was closed with Parafilm®. Bags were opened in their lower area to facilitate the inoculation process and subsequently, were folded and stapled until the time of the harvest (figure 1). The rest 50 buds were market, but were not put inside bags.

Three treatments were distributed in the following way: covered and inoculated fruits (FCI), covered fruits without inoculation (FCSI), fruits without any covering and inoculated naturally (FSCSI). Treatments initiated in week number two, and were applied each week of age of the fruits, until the end of their development (16 weeks). Once covered, in treatment FCI 40 fruits were inoculated every week approximately (20 bags) with 3 ml/fruit of the prepared suspension of conidia using a manual sprinkler, later bags were closed. Likewise, 40 fruits without inoculation (FCSI) and 40 fruits without any covering and without artificial inoculation (FSCSI) were selected.

Seven days after each inoculation 20 buds or fruits were collected from each of treatments. The same number of fruits per treatment were let in the plant until the end of the experiment (week 16), to establish the appearance moment of the symptoms of the illness. This procedure was carried out weekly until the end of the grow cycle (119 – 120 days) (29. The essay was installed three times (from December 1999 to July 2000). Due to the depletion on the quantity of fruits, the first essay was carried out until week 7, the second essay until week 11 and the third until week 16.

Fruits collected every week were taken to the laboratory properly refrigerated, where were washed with current water for 5 min, and disinfected following the previously described procedure. Later, it was proceeded to put sections of fruits in Petri plates with clarified agar JV8, and these were incubated for a month under the same conditions already mentioned, until the detection of the

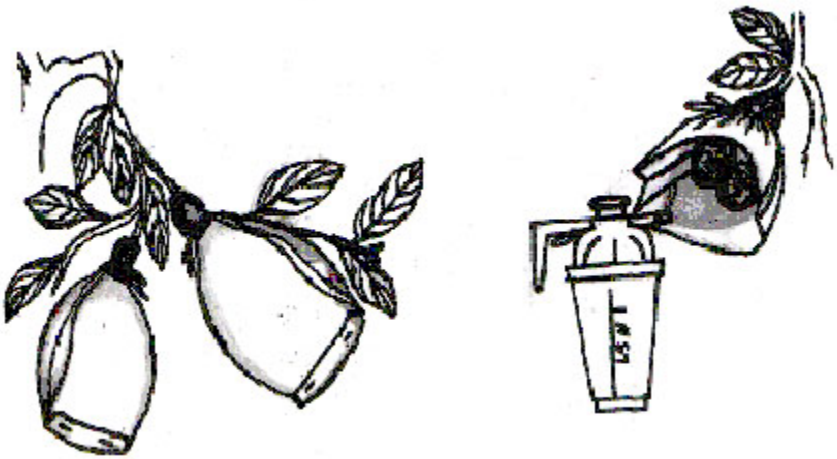

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**Figure 1. Package technique of guava fruits used to determine the beginning of the infection by *Dothiorella* sp. Package detail (cover) and inoculation of fruits.**

mycelium growth and the sporulation.

Fruits let in the plant every week were collected at the end of the experiment, having fruits of a week and even of sixteen weeks of inoculation. These were also taken to the laboratory, where it was proceeded to identify the common symptomatology produced by *Dothiorella* sp. In those fruits where symptoms were not observed yet, were disinfected according to the previously described process, and were put on a humid chamber to induce the appearance of symptoms and to discharge any mistake in the results.

**Evaluation and statistical analysis:** The experiment was planned using a randomized sample design, which experimental unit was represented by the fruit. It was evaluated the initial of infection (days) and of appearance of symptoms (days). The variance analysis was done to establish the significance of treatments and the age of fruits, using the GLM procedure of SAS (19). Also, the mean comparison test of Duncan was done. Likewise, for the information of the appearance age of symptoms a frequency analysis was done, and treatments were compared using the square-chi tests.

## Results and discussion

**Moment of the infection beginning of fruits**

All guava fruits inoculated with *Dothiorella* sp. showed symptoms of end rot (figure 2). Essays installed in the three dates mentioned had similar results in relation to the beginning of the infection. The information of the

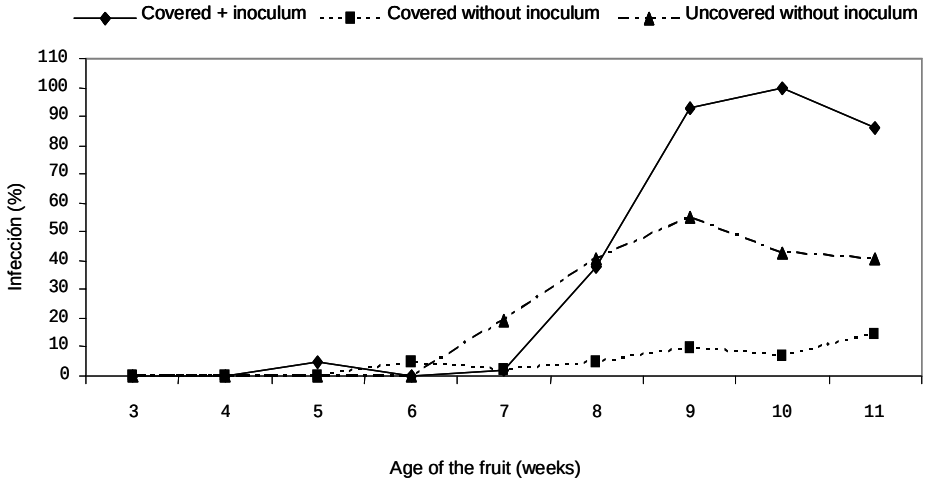


**Figure 2. Infection by *Dothiorella* sp. of guava fruits *Dothiorella* sp. under three treatments.**

second and third install date was combined until week 11, and were submitted to a statistical analysis. The variance analysis showed significant differences ($P<0.01$) between treatments, age of the fruit and the interaction per age, except for the interaction treatment per repetition (table 1). Mean comparisons of treatments showed a significantly higher infection for covered and inoculated fruits, followed by fruits without covering and without artificial inoculation (table 2).

Analyzing the infection in the different ages of the fruits and combining treatments, was found that the infection started in week 5 in covered fruits with a very low percentage (1.6%), and statistically the same to previous weeks; due to probably the weather created inside the bag, where were included leaves and small branches unavoidably, which might had in epiphytic way, conidia of Dothiorella that infected the fruits. This is proved due to the low infection percentage observed. It is in week 7, when the infection rises (8%) and makes statistically different, increasing in the following weeks until reaching 52.4% (table 3).

On the other hand, it was also observed a higher incidence of infected fruits in the inoculated covered; reaching values of 100% due to high concentration of used inoculum, compare to the natural inoculum in those uncovered, where was found a maximum of incidence of the illness of 55%. In relation to the covered fruits without inoculation, the infection kept low during all the period under study, obtaining a maximum of 14% in week 11.

In table 4, the infection analysis by age is shown for each of

treatments. It is observed that in covered fruits, either inoculated or without inoculation, the infection initiated in the fifth and sixth week respectively; meanwhile in fruits exposed to air and without artificial inoculation, the infection started in week 7.

These results indicate that the infection by Dothiorella sp. started in the seventh week of the fruit's development, which corresponds to phase I of the development curve of the fruit described by Laguado *et al.* (14), Araujo *et al.* (2) and Caraballo (6).

Similar results were found by Kohn and Hendrix (13), using different levels of *Botryosphaeria dothidea* inoculum on apple, determining that the average of lesions was higher (2,2) in the highest level of inoculum used ($10^5$ conidia/ml) and lower (1.37) in the lowest level of inoculum ($5x10^3$ conidia/ml). Contrary to these results, Moraes *et al.* (16) did not find differences in relation to the incidence and symptoms of gumming in branches of three citric species, inoculated naturally and artificially with *Dothiorella gregaria*.

**Appearance moment of symptoms**

The test of $c^2$ showed significant differences ($P<0.01$) for treatments as well for age of fruits (table 5). Symptoms began to appear after week 11, that is, seven weeks after the first infection detected in 20% of inoculated and covered fruits; in week 12 in 15% of fruits without cover and without artificial inoculation, and in 5% in covered fruits without inoculation (figure 3). The appearance of symptoms began to increase in the following weeks (figure 3) until reaching 65% for covered and inoculated fruits, 35% for fruits without any cover and without artificial inoculation and 15% for covered fruits without inoculation. This proves that the pathogen infected the fruit and kept latent until week 14, when environmental conditions were favorable, or the fruit was on a ripening phase that allowed the development of symptoms (1).

Similar results were mentioned by Brown and Britton (5), who observed the appearance of symptoms

**Table 1. Variance analysis for the age of guava fruits (*Psidium guajava* L.) and the infection moment with *Dothiorella* sp., combining the results of two experiments until week 11.**

| Variance source | G. of L. | S. C. | C.M | F | Pr>F |
|---|---|---|---|---|---|
| Treatment[1] | 2 | 7734.37 | 3867.18 | 68 | 0.0001 |
| Age | 8 | 32272.44 | 4284.05 | 75.33 | 0.0001 |
| Treat. x Age | 16 | 13281.89 | 830.11 | 14.59 | 0.0001 |
| Error | 54 | 3071.20 | 56.87 | | |
| Error | 80 | | | | |

[1]Treatments: Covered and inoculated fruits; covered without inoculation fruits; fruits without any covered and without inoculations

**Table 2. Infection percentage of guava fruits (*Psidium guajava* L.) inoculated with *Dothiorella* sp. for the three treatments.**

| Treatments | Infection(%) |
|---|---|
| Covered and inoculated fruits | 42,6 [a] |
| Covered fruits without inoculation | 19 [c] |
| Fruits without any covered and without inoculation | 31,3 [b] |

Means with different letters differ statistically (P<0.01).

caused by *B. dothidea* on fruits of apple and peach, 72 hours after the inoculation, while in vine was observed three months later. Likewise, Parker and Sutton (17) and Kim *et al*., (12) mentioned that in apple fruits inoculated with *B. dothidea*, might infect within seven weeks after the fall of petals, and require from 1 to 1.5 months after the infection for the development of symptoms. Kohn and Hendrix (13) determined that in apple there were not observable levels of *Botryosphaeria dothidea* just until the fruit aged from 6 to 8 weeks before the crop. Likewise, determined that in var. Red Delicious apples inoculated with *B. dothidea*, 75% of fruits were infected three weeks after the fall of petals. Thus, the production peak of spores of the pathogen agreed to the phase near the ripening of the fruit, where this was more sensitive to fungus *B. dothidea*. Similarly, Moraes *et al*. (16) detected symptoms of gumming in citric caused by *Dothiorella gregaria*, 56 days after the

**Table 3. Infection of guava fruits (*Psidium guajava* L.) by *Dothiorella* sp., combining treatments of covered and inoculated fruits, covered fruits without inoculation, and fruits without any covered and without inoculation.**

| Age(Week)) | Infection(%) |
|---|---|
| 3 | 0[a] |
| 4 | 0[a] |
| 5 | 1.6[a] |
| 6 | 1.6[a] |
| 7 | 8[b] |
| 8 | 28[c] |
| 9 | 52.4[d] |
| 10 | 50[d] |
| 11 | 47[d] |

Means with different letters differ statistically (P<0.01).

**Table 4. Infection of guava fruits (*Psidium guajava* L.) by *Dothiorella* sp., submitted to three treatments in different ages.**

| Age of the fruit(Weeks) | Treatment | | |
|---|---|---|---|
| | FCI | FCSI % of infection | FSCSI |
| 3 | 0.00 | 0.00 | 0.00 |
| 4 | 0.00 | 0.00 | 0.00 |
| 5 | 4.77 | 0.00 | 0.00 |
| 6 | 0.00 | 4.77 | 0.00 |
| 7 | 2.39 | 2.39 | 19.05 |
| 8 | 38.10 | 4.77 | 40.43 |
| 9 | 92.86 | 9.53 | 54.77 |
| 10 | 100.00 | 7.15 | 42.46 |
| 11 | 85.72 | 14.29 | 40.48 |

FCI: Covered and inoculated fruits, FCSI: Covered fruits without inoculation
FSCSI: Fruits without any covered and without inoculation.

inoculation.

Week 14 (98 days) corresponds to phase III of the development of guava fruit, where the content of total soluble solids reaches values from 11.83 to 15.74 ºBrix, mainly formed by saccharose, which is formed by the glucose (3, 14). In apple fruits, was found that symptoms of end rot caused by *B. dothidea* appeared when levels of total soluble solids were of approximately 10.5%, reaching the incidence levels of the illness to 100%, when total soluble solids reached 13.8% (13).

The increment of sugar during the ripening causes a reduction on the firmness of fruits as a result of changes in the structure and composition of cell walls (17), which increases the susceptibility of fruits toward the attack of the fungus and the appearance of symptoms (11, 18).

**Table 5. Values of $c^2$ for the symptoms appearance of the illness caused by *Dothiorella* sp., in relation to the age of guava fruits (*Psidium guajava* L.) and the used treatments.**

| | $\chi^2$ | Probability |
|---|---|---|
| Treatments | 26.44 | 0.001 |
| Age | 211.40 | 0.001 |

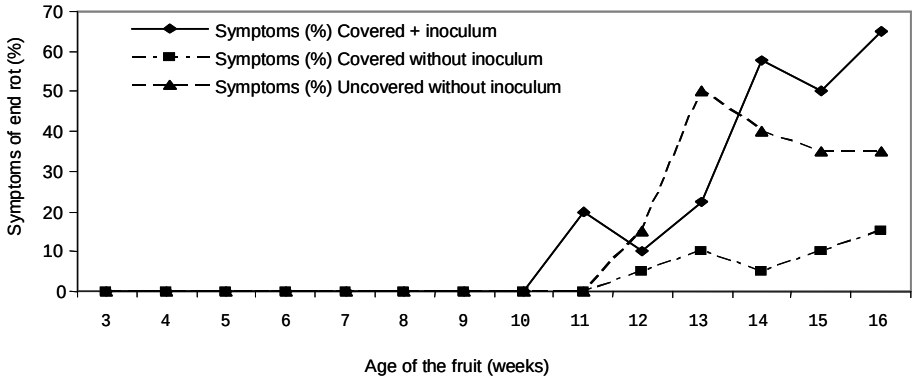


**Figure 3. Appearance moment of symptoms of end rot caused by *Dothiorella sp.* in guava fruits (*Psidium guajava* L.).**

## Conclusions

The infection of guava fruits (*P. guajava* L.) with *Dothiorella* sp. initiates within seven weeks of the fruit's age, correspondent to the phase I of the development curve of the fruit.

The symptoms of end rot under field conditions, appear 14 weeks later, with a long incubation period (seven weeks), which might be influenced by the ripening phase of the fruit (phase III, 91 – 119 days).

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