Characterization of Infection and Colonization of Strawberry Crowns by *Colletotrichum acutatum*

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Keywords: anthracnose, colonization, infection process

Abstract

Strawberry anthracnose, caused by *Colletotrichum acutatum* J.H. Simmonds, is one of the most important diseases of this crop in Huelva (southwestern Spain). Lesions can occur on all parts of the plant but anthracnose crown rot is specially severe leading to wilt and death of plants. In this work, the infection and colonization process of *C. acutatum* on strawberry plants was studied. 'Camarosa' strawberry plants (highly susceptible to *C. acutatum*) were inoculated by applying 100 µl droplets of a conidia suspension of the fungus on crowns, on buds and between second and third stipules. Plants were evaluated for response over a 30 days period. The severity of disease, estimated as percentage of plants showing symptoms on aerial parts, and the amount of infected crown tissues were determined at 2, 5, 10, 15, 20, 25 and 30 days after inoculation. Depending on the inoculation site the severity of anthracnose crown rot varied between plants. Moreover, an ultrastructural study of infected plants exhibiting anthracnose crown rot was carried out by transmission electron microscopy. The colonization of the crown tissues by intracellular and intercellular hyphae in the cortex, medulla and vascular system was observed resulting in cell colapse and necrosis.

INTRODUCTION

Strawberry anthracnose, caused by *Colletotrichum acutatum*, is an important disease in production areas in Huelva, southwestern Spain. Several species of *Colletotrichum* have been described causing strawberry anthracnose (Howard et al., 1992) *C. acutatum* being the most common species in Europe (Denoyes et al., 1996). Lesions can occur all over the plant, including roots, leaves, flowers, stolons, and fruits but is most devastating when the fungus invades the crown because it causes wilt and sudden death of the plant (Howard et al., 1992). Plants with anthracnose crown rot (ACR) may die in the nurseries or after being transplanted into fields. Crown rot is difficult to detect on plants at the moment of transplant because infections are in the initial stages before red streaks have developed in crowns. Plant death depends on the stage of infection at the moment of transplanting, and climatic conditions (Beraha and Wright, 1973; Howard et al., 1992; Leandro et al., 2003). These environmental conditions are common in Huelva causing plants losses of up to 80%.

Host colonization and pathogenesis are well characterized for several species of *Colletotrichum* (Dickman, 2000). Also, the ultrastructure of the early stages of infection of starawberry petioles by *C. acutatum* describing the infection structures during the penetration phase have been reported by Arroyo et al. (2005). However, few studies have investigated the infection process of *C. acutatum* in strawberry crowns. Milholland (1982) described the invasion and colonization of crown tissues by *C. fragariae* using light microscopy. The aim of this study was to characterize the infection and colonization of strawberry crowns by *C. acutatum* in order to elucidate some aspects of anthracnose crown rot that remain poorly understood.

MATERIALS AND METHODS

'Camarosa' strawberry plants, highly susceptible to *C. acutatum* (De los Santos,

1998), were planted in 10.5 × 13.5 cm plastic pots containing sterilised peat (Klansmann-Deilmann, Geeste, Germany) and grown for 30 days, before inoculation with *C. acutatum*, in a greenhouse maintained at 25ºC day / 15ºC night ±5ºC.

C. acutatum isolate CECT-20240 was used in this study. The fungus was grown on potato dextrose agar (PDA, DIFCO) for 7 d at 25ºC under continuous fluorescent light. Conidial suspensions for inoculation were prepared by flooding the culture plates with 4-5 ml of sterile distilled water, scraping the colony surface with a scalpel, and filtering the suspension through sterile cheesecloth. The concentration was adjusted to 1×10^6 conidia ml^{-1} using a hemocytometer. Strawberry plants were inoculated on three different sites: directly on crowns, on buds and between second and third stipules by applying 100 µl droplets of conidial suspension. For each type of inoculation eight plants were used. Inoculated plants were enclosed in plastic bags for 48h to maintain high relative humidity and were incubated in a growth chamber at 25ºC, with a 16h photoperiod beneath fluorescent light. Control plants were treated with 100 µl of sterile distilled water. Disease incidence was evaluated daily. Also, the presence of pathogen in crown tissues was quantified by isolating the fungus from inoculated crowns at different times. Furthermore, the infection process of *C. acutatum* into crown tissues was studied by using light and electron microscopy. Material was prepared as described by Arroyo et al. (2005).

RESULTS AND DISCUSSION

ACR symptoms were observed at 5 days post inoculation (dpi) in plants inoculated directly on crowns affecting to 50% of the plants. The percentage rise to 100% at 10 dpi (Table 1). Wilting and death of the plants inoculated in this way was observed at 10 dpi affecting to 25% of them. The percentage of dead plants rose to 50% at 20 dpi and to 75% at 30 dpi (Table 2). Small flecks or lesions of 3 to 5 mm length developed on the inoculated area. Longitudinal sections of infected crowns revealed reddish brown lesions similar to the those reported by Arroyo (2004). However, in plants inoculated on buds and between stipules, ACR symptoms were detected at 30 dpi affecting to 25% of the plants (Table 1). In plants inoculated on buds, brown dark lesions were observed causing foliar deformation. In plants inoculated between stipules, orange lesions were detected on the inoculated area. No wilting or plant death was observed in plants inoculated on buds and between stipules (Table 2). The percentage of infected crown was higher in plants inoculated directly on crowns than in plants inoculated on buds or between stipules (Table 3). This means that the invasion of the crown tissues by the fungus was much more advanced in these plants and it can be related to visible symptoms on aerial parts.

Germination and penetration of crown tissues occurred after 12-24 hpi (Arroyo et al., 2005). The invading hyphae grew intercellularly in the cortex causing collapse of the cell walls. At 3 dpi, several cortex layers had collapsed with hyphae inside the cells. The fungus spread both intercellularly and intracellularly throughout the parenchyma. At 5 dpi, necrosis of the cortex was extensive and the vascular bundles had been invaded. The phloem and xylem vessel elements were filled with intracellular hyphae, but there was no breakdown of xylem vessel walls. The fungus progressed into the crown tissues invading the vascular tissue and pith. The colonization and invasion of vascular tissue could explain the wilting of the aerial parts of the plant and eventually, necrosis and collapse of cells could cause the death of the plant.

CONCLUSIONS

Anthracnose crown rot is difficult to detect because it shows a slow development or progress. Therefore, the wilting and sudden death of the strawberry plant in a short time after being transplanted, less to 30 days, occurs in those plants whose crowns are just infected when they are planted. However, if the infection by *Colletotrichum acutatum* comes from the buds or from the stipules the ACR symptoms are displayed at least at 30 dpi. These symptoms are very reduced and no wilt or plant death was recorded in this period.

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Tables

Table 1. Average percentage of 'Camarosa' strawberry plants showing ACR symptoms after 30 days. CD, plants inoculated directly on crowns; CB, plants inoculated on buds, and CE, plant inoculated between 2nd and 3rd stipules.

Inoculation sites	Days post-inoculation						
	2	5	10	15	20	25	30
CD	0	50	100	100	100	100	100
CB	0	0	0	0	0	0	25
CE	0	0	0	0	0	0	25
Control	0	0	0	0	0	0	0

Table 2. Average percentage of dead plants by ACR inoculated with *C. acutatum*. CD, plants inoculated directly on crowns; CB, plants inoculated on buds, and CE, plant inoculated between 2nd and 3rd stipules.

Inoculation sites	Days post-inoculation						
	2	5	10	15	20	25	30
CD	0	0	25	25	50	75	75
CB	0	0	0	0	0	0	0
CE	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0