Development of *Colletotrichum acutatum* in the Foliar Tissue of Strawberry Plants

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Abstract

Strawberry anthracnose, caused by *Colletotrichum acutatum,* is one of the most destructive disease of this crop throughout the world. Assymptomatic stages in the plant have been the aim of this work. Inoculated leaves samples were taken at different times and they were processed for scanner electron microscopy (SEM) and transmission electron microscopy (TEM). Conidial development on both surfaces leaves was determined. The ultrastructural study of fungus penetration into plant cell was characterized by the formation of vesicles over the fungus periphery and is a morphological parameter of the intense membranes traffic, also could be a evidence of a transcriptional activity and enzymatic cell secretion. Differences of symptoms on both surfaces of leaves were observed.

Keywords: anthracnose; *Colletotrichum acutatum;* latent infection; ultrastructure

INTRODUCTION

Colletotrichum spp. cause anthracnose disease or leaf blight on all significant agricultural crops and ornamental plants around the world, being one of the most destructive disease on strawberry. In fact several species of *Colletotrichum* have been reported from major areas of strawberries production crop in a large amount of countries. *Colletotrichum acutatum* J. H. Simmonds attacks aerial parts of the plant inducing high losses of seedlings and fruits. Spain is the first producer, for fresh market, after United States. Huelva in Andalucia, South-western Spain, contributes with more than 90% of the national production (DE LOS SANTOS & ROMERO MUÑOZ 1999).

In fruiting fields in Huelva *C. acutatum* cause crown rot and plant death, fruit rot, fower blight and lesions on petioles, stolons and leaves, which are light-brown with reddish color in margins (DE LOS SANTOS & ROMERO MUÑOZ 1999) and are different of black leaf spot caused by *C. fragariae* and *C. gloeosporioides* described by HOWARD and ALBREGTS (1983) in the summer nursery. Lesions on foliar tissue are not very frequent and several studies have suggested that can develop quiescent infections on strawberry plants having a epidemiological role prior to colonization of fruits and another tissues.

The initial stages of *Colletotrichum* infection is well studied and generally include conidia adhere to, and germinative on plant surfaces, produce germ-tubes which differentiate to form melanized appressoria (VAN DYKE & MIMS 1991; BYRNE *et al*. 1997). On symptomless strawberry leaves has been reported production of secondary conidia or microcyclic conidiation by *C. acutatum* and may be a significant source of inoculum for infections (LEANDRO *et al*. 2001).

The infection process of *Colletotrichum* species causing latent infection on cowpea leaves reveals that fungus colonized the mesophyll by intercellular hyphae, without initially visible symptoms (LATUNDE-DADA *et al*. 1999). Assymptomatic stages in some infected plant tissues dificults the knowledge of the process of pathogenesis. This work pretends to contribute to the clarification of the latency phase of *C. acutatum* in the foliar tissue of the strawberry plants throughout the

study of the conidium development and ultrastructural in the relationship host-pathogen.

MATERIALS AND METHODS

Susceptible cv. Camarosa leaves, were inoculated with 100 μl drops of a conidial suspension (10^6 conidia per ml distilled steril water) of *C. acutatum* CECT 20240 (ARROYO *et al*. 2001). Plants were incubated at 25°C, 100% RH and 16 h light photoperiod in a growth chamber. For the study of the conidium development on the foliar surface using scaning electronic microscopy (SEM), 1 cm^2 pieces of leaf were sampled at 4, 8, 12, 16, 20, 24, 36, 48, 72, 96, and 120 hours after-inoculation (hpi), then they were fixed in glutaraldehyde, dehydrated and dried up by means of the critical point drying technique. The study of the infection process at ultrastructural level using transmission electronic microscopy (TEM) was carried out in strips of tissue obtained at 24, 48 and 120 hpi. Samples were fixed in glutaraldehyde and postfixed in OsO_4, dehydrated with acetone and embedded in Epon 812 resin.

RESULTS

Conidia of *C. acutatum* were left adhered on foliar surface through matrix extracellular secreted by themselves. Germination on both, upper and down foliar surfaces began within 4 hpi.

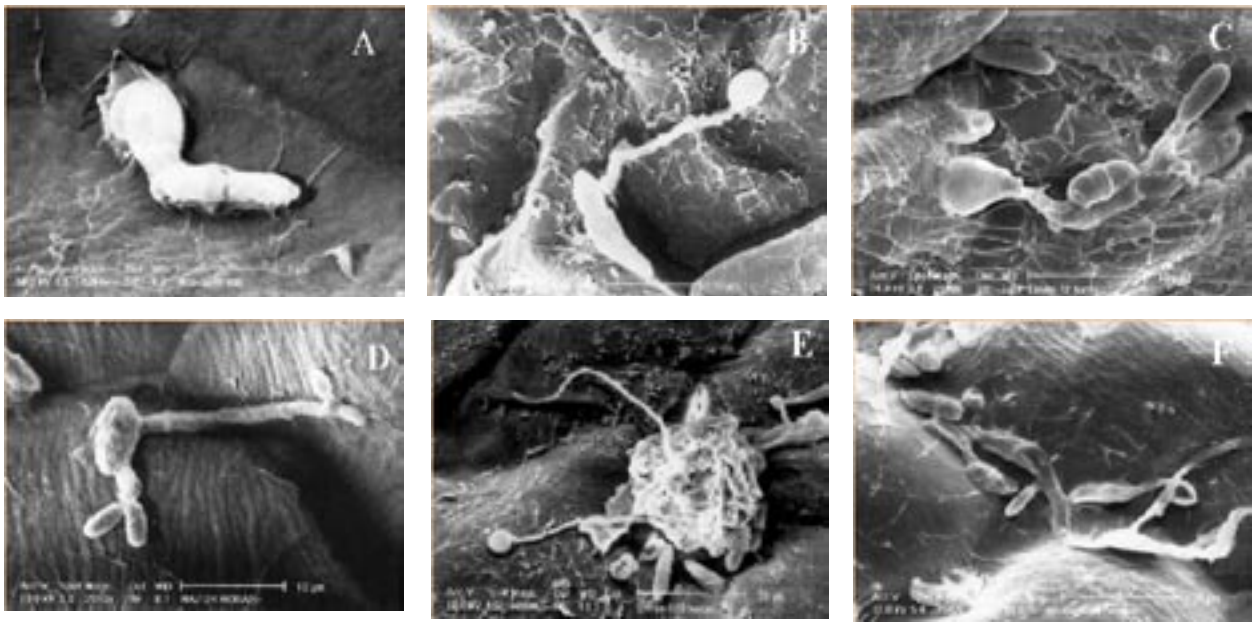
At 8 hpi some appressoria were observed in both sheets of the leaf surface that grew from germinative tubes, generally short tubes or sessile on the upper leaf surface and longer on the lower one (Figures 1A and 1B, respectively). At this time it was also appreciated formation of secondary conidia (microcyclic conidiation) originated from conidiogenes structures represented by hypha and conidia phialide (Figures 1C and 1D).

Anastomoses of both hypha and primary conidia were detected and they became to make accumulations until 100 μm. From this structures were also observed processes of conidiogenesis (Figure 1E).

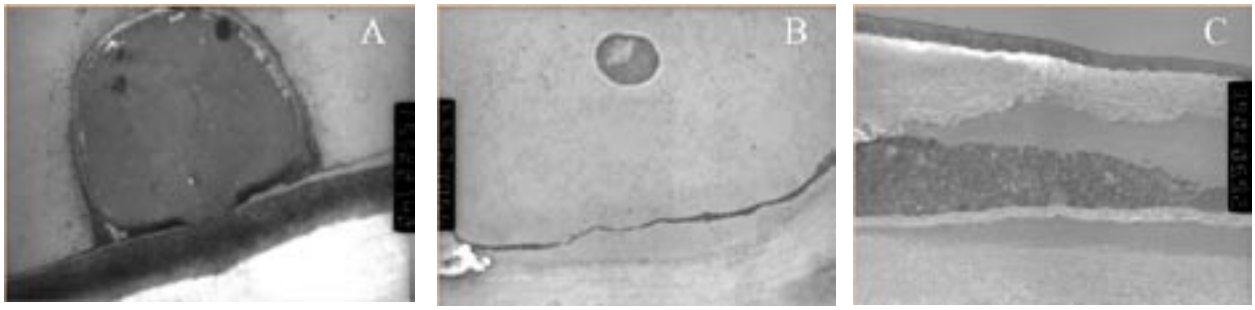
At 72 hpi was perceived on the upper leaf surface a collapse and lysis of fungi structures that it was increased at 120 hpi (Figure 1F).

Penetration of cuticle from appressorium was observed at 24 hpi, not being a synchronous process (Figure 2A). Infection intracellular was also manifested in the lower leaf surface at 24 hpi whereas in the upper one it could only be detected hypha inside cell wall (Figures 2B and 2C, respectively).

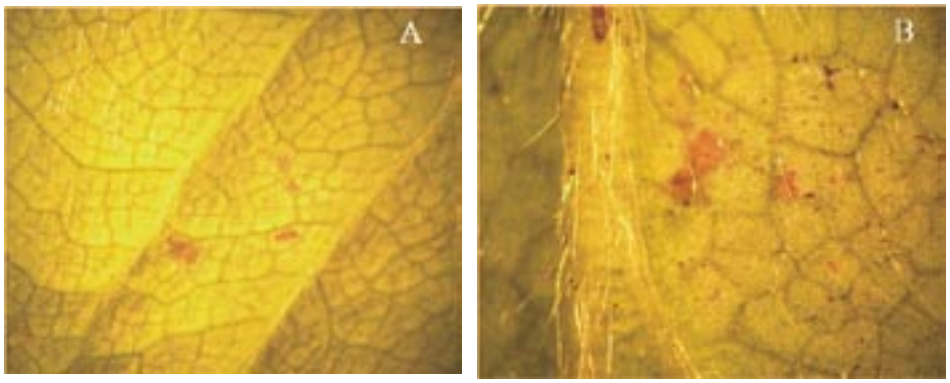
Macroscopics symptoms on leaves reveals a differential answer between both foliar surfaces, necrotic spots of several mm in the lower leaf surface and symptomless in the upper one. Observation of these small spots in the lower leaf surface seems to be cor-



Figures 1. Conidial development on the leaf surface. A and B, appressoria on the upper and lower leaf surfaces resp. at 8 hpi. C and D, hypha and conidia phialide at 72 hpi. E, anastomoses on the lower one at 120 hpi. F, fungi collapse on upper one at 120 hpi



Figures 2. Transmission electron micrographs of interaction between *C. acutaum* and strawberry leaves. A, appressorium on the upper leaf surface at 24 hpi, which is forming a penetration peg. B, intracellular hypha in the lower one at 24 hpi. C, fungi inside epidermic cell wall with shows a large amount of vesicles, it is observed degradation of cell wall



Figures 3. A and B, macroscopic symptoms on the lower leaf surface

related with a reduced number of cells died by hypha intracellular, mainly in veins but also in the sheet (Figures 3A and 3B). In the upper leaf surface this pathogen appears to survive inside epidermic cell wall where is considered an intense activity of enzymatic secretion, manifested by a large amount of endo- and exocytosis vesicles and the degradation of wall in this area, can exist.

The recovery of the pathogen is 100% in both leaf surfaces at 120 hpi.

Conclusions

The infection and development of pathogen in the foliar tissue of strawberry plants shows the capacity of it to be located and to remain asymptomatic, being able to contribute to the disease dispersion through crop fields.

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