INTERACTION BETWEEN *XYLELLA FASTIDIOSA* AND *CURTOBACTERIUM FLACCUMFACIENS*, AN ENDOPHYTIC BACTERIUM

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ABSTRACT

Xylella fastidiosa (*Xf*) is a fastidious gram-negative, xylem-limited bacterium that causes diseases in many crops of economic importance, such as grape (Pierce's disease), almond, peach, coffee, plum and citrus. *Xf* can infect all known *Citrus sinensis* cultivars and causes Citrus variegated chlorosis (CVC). One major endophyte isolated from CVC-asymptomatic plants is the bacterium *Curtobacterium flaccumfaciens* (*Cf*). *Catharanthus roseus* (*Cr*) plants were inoculated together with *Cf* and *Xf* and in a similar experiment, plants were inoculated alone with *Xf* and *Cf*. Three phytopathology parameters, including number of flowers, height of plants, and disease symptoms were evaluated. Primers for *Cf* were designed to detect this endophytic bacterium in plant tissue when inoculated with *Xf*. These primers were able to detect *Cf* in the presence of *Xf* after inoculation in *Cr*. *In planta* interaction studies where *Cf* was inoculated together with *Xf* showed that there was an inhibition of disease symptoms caused by *Xf*.

INTRODUCTION

Endophytes are microorganisms that do not visibly harm the host plant but can be isolated from surface-disinfected plant tissue or the inner parts of plants (Hallmann et al. 1997). Since they colonize an ecological niche similar to that of phytopathogens, endophtytes are candidates for biocontrol agents (Hallmann et al. 1997). Members of the genus *Curtobacterium* have been isolated as endophytic bacteria from many plants, including red clover (Sturz & Christie, 1998), rice (Elbeltagy et al. 2000), potato (Sturz & Matheson, 1996), yam (Tor et al. 1992), citrus (Araújo et al. 2002; Lacava et al. 2004), and are associated with control of plant diseases in tobacco (Park & Kloepper, 2000), cucumber (Raupach & Kloepper, 2000) and potato (Sturz & Matheson, 1996); and plant growth-promotion of red clover (Sturz et al. 1997) or interacting with other bacteria in plant growth-promotion (Bent & Chanway, 1998).

CVC is a disease of sweet orange trees (*Citrus sinensis* L.) caused by one strain of the xylem-limited bacterium *Xf* (Hartung et al. 1994). *Xf* is transmitted by xylem-feeding sharpshooter leafhoppers (*Homoptera: Cicadellidae, Cicadellinae*; Roberto et al., 1996; Brlansky et al., 2002) or through seeds (Li et al., 2003). In Brazil, CVC is responsible for losses to the citrus industry of US $ 100 million per year (Coletta-Filho et al., 2001). In spite of the fact that *Xf* was the first plant pathogen to have its genome completely sequenced (Simpson et al. 2000), much remains to be learned about its pathogenesis, biology and ecology.

Araújo et al. (2002) and Lacava et al. (2004) demonstrated that *Cf* is isolated more frequently from CVC-asymptomatic than CVC-symptomatic orange and tangerine plants. Also, Lacava et al. (2004) found, through the use of *in vitro* interaction experiments that the growth of *Xf* could be inhibited by the presence of endophytic *Cf*.

OBJECTIVES

1. Evaluate, *in planta*, the interaction between *Xf* and *Cf* and the potential use of this endophytic bacterium in biological control

RESULTS

Sixty days after the inoculation of *Cr* seedlings, *Cf* was detected by PCR using primers CFC1 and CFC2. *Xf* was also specifically detected using primers 271-int and 272-int on extracts of *Cr* inoculated with *Xf*. In seedlings simultaneously inoculated (doubly-inoculated), *Cf* was detected by PCR with primers CFC1 and CFC2 and *Xf* with primers 271-int and 272-int respectively.

The first parameter analyzed to check the effects of inoculation of *Xf* and *Cf* was the number of flowers. Plants inoculated with sterile PW medium (negative control) and plants inoculated with *Cf* did not demonstrate a statistically significant difference (< P 0.05) in the number of flowers. Plants inoculated with *Xf* alone had a reduced number of flowers (< P 0.05)

during the same period (Figure 1). However, plants inoculated with both *Xf* and *Cf* demonstrated a number of flowers similar (< P 0.05) to those inoculated with PW media, *Cf* and *Xf* (Figure 1).

In the second parameter analyzed, the height of plants were statistically similar in plants inoculated with PW medium and plants inoculated with *Xf* and *Cf* (< P 0.05) (Figure 2), but plants inoculated with *Xf* demonstrated reduced height after 60 days (Figure 2).

After sixty days, plants inoculated with sterile PW medium (negative control) did not demonstrate symptoms of disease and neither did plants inoculated with C*f* or with both *Xf* and *Cf* (double-inoculation). However, plants inoculated with just *Xf* demonstrated characteristic symptoms of disease (Figures 3 and 4).

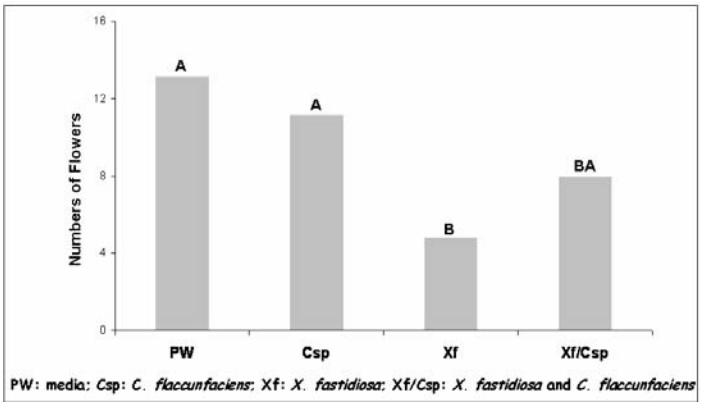

Figure 1. Interaction between *Cf* and *Xf* inside of host plant, *Cr*, after two months. The phytopathology parameter used to evaluate the interaction was number of flowers. Different letters on bars show statistical difference by Tukey's test at 5% of significance.

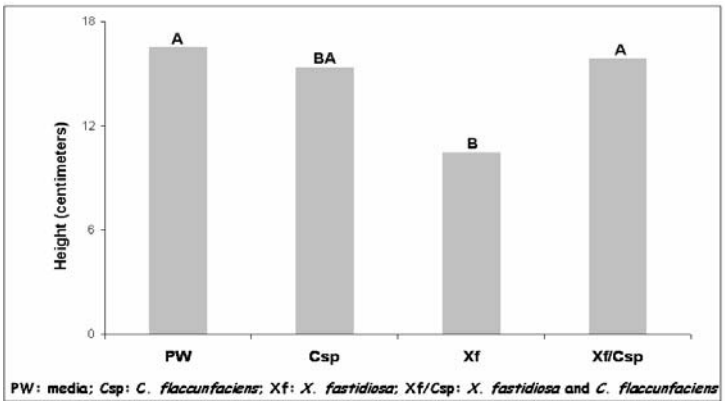

Figure 2. Interaction between *Cf* and *Xf* inside of host plant, *Cr*, after two months. The phytopathology parameter used to evaluate the interaction was height. Different letters on bars show statistical difference by Tukey's test at 5% of significance

Figure 3. *Cr* plants two months after inoculation with *Xf* (right) in comparison with *Xf* and *Cf* inoculated together doubly-inoculated (left).

Figure 4. *Cr* leaves two months after inoculation with *Xf* (left) and doubly-inoculated with *Xf* and *Cf* (right) in inoculated in plant.

CONCLUSIONS

The endophytic bacterium *Cf* was detected in extracts of *Cr* 60 days after inoculation using the primer pair CFC1/CFC2 in a PCR assay. In a similar experiment, where both *Cf* and *Xf* were inoculated into *Cr*, it was possible to detect both the endophyte and the pathogen using PCR. These data demonstrate that *Cf* has the ability to colonize plant tissue in presence of *Xf*. This is an important point to consider when evaluating this endophyte as a potential biocontrol agent for CVC.

The parameters measured to check the potential use of *Cf* against *Xf* include number of flowers, height, and disease symptoms. This study suggested that this endophyte was able to reduce the effect of the colonization of *Xf*. In plants inoculated with *Xf* and *Cf,* symptom remission probably occurred compared with plants inoculated just with *Xf*.

Recently, an interaction between *Cf* and *Xf* was strongly indicated (Araújo et al., 2002; Lacava et al., 2004). These authors suggested this interaction based in the frequency of isolation of *Cf* and in interaction experiments *in vitro* using both *Xf* and *Cf*. This article describes how *Cf* can reduce symptoms caused by *Xf in planta* when both the phytopathogen and the endophytic bacterium colonize the same plant.

This work described the effect of a possible interaction of *Cf* and *Xf in planta* under controlled conditions and the results reinforce the idea that endophytic bacteria, that colonize a similar niche as does *Xf*, could contribute to the reduction of the symptoms in the field.

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