



Original Research Paper

Lesion spreading of ceratocystis canker on 'Masui Dauphine' fig trees

Accepted January 3rd 2014

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Characteristics of lesion-spreading of ceratocystis canker diseases caused by *Ceratocystis fimbriata* were investigated with shoot-cuttings and nursery stocks of fig (*Ficus carica* L.) tree. Wound inoculation of *C. fimbriata* produced lesions on shoots and trunks of 'Masui Dauphine' fig. The lesion length in the longitudinal direction was greater than in the tangential direction. In shoot-cuttings and intact nursery stocks, no difference was found in longitudinal lesion length between apical and basal parts. No posture of shoot-cutting (upright, inversion, and horizontal) affected the lesion size or shape. Lesser lesion spreading was observed near buds. Deep wound inoculation produced lesions spreading on the branch cross-section. Greater lesion spreading was observed in the cambium and pith. Results showed that lengthwise spreading is a fundamental characteristic for ceratocystis canker lesion on fig trees. The spreading was unaffected by gravity or the physiological polarity of fig trees. However, the difference in cell shape and wood component tissues affected the lesion spreading rate of ceratocystis canker lesions in fig wood.

Key words: *Ficus carica*, ceratocystis canker, Lesion

INTRODUCTION

The pathogen of ceratocystis canker (CC), a severe soil disease affecting fig (*Ficus carica* L.) culture, had been identified as *Ceratocystis fimbriata* Ellis et Halsted (Kato et al., 1982; Valarini and Tokeshi, 1980). But this fungus has identified as *C. fimbriata* sp. nov by Kajitani and Masuya (2011) recently. The fungus *C. fimbriata* belongs to ascomycete. *C. fimbriata* causes serious wilt disease in fig, but the dispersal process of *C. fimbriata* has not been fully clarified. The disease invades fig tree roots and trunks, eventually killing the tree. 'Masui Dauphine' ('San Piero' sensu Condit, 1955), a major fig cultivar in Japan, is sensitive to the disease. The disease has spread nationwide, affecting all productive areas in Japan. Infection by CC begins with inhibited shoot growth and leaf yellowing and wilting, eventually causing defoliation and tree death (Kato et al., 1982; Shimizu and Miyoshi, 1999; Togawa et al., 1999). Lesion spreading reflects the progress of illness in the tree. Generally, greater spreading of inoculated lesions on the trees indicates higher pathogenicity of the pathogen (Yamaoka, 2002). A comparison of lesion spreading on plant

tissues is used as a simple test for disease resistance. Inoculated lesions spreading on the branches or leaves were used to screen fig varieties for resistance to CC (Hosomi and Kwaradani, 2004; Shimizu and Miyoshi, 1999). Available resistant rootstocks such as 'Negronne' (Hosomi et al., 2012) and 'Kibaru' (Nogata et al., 2012) have been selected through such simple tests and field selection. However, the resistance levels of these rootstocks are imperfect, and *C. fimbriata* inoculation causes slight lesions on the roots and some growth inhibition of shoots, even on resistant rootstocks (Hosomi et al., 2012). Characteristics of lesion spreading on the rootstock or scion will be important to ascertain the recommended rootstock length to avoid lesion spreading effectively and to estimate how long the resistant rootstock prevents fig tree death from CC. Miwa et al. (2010) reported that soil conditions affect the mortalities of fig trees infected with CC. The lesion spreading information of characteristics on the woody parts including underground is also expected to be important to assess the relation between soil conditions and disease infection.

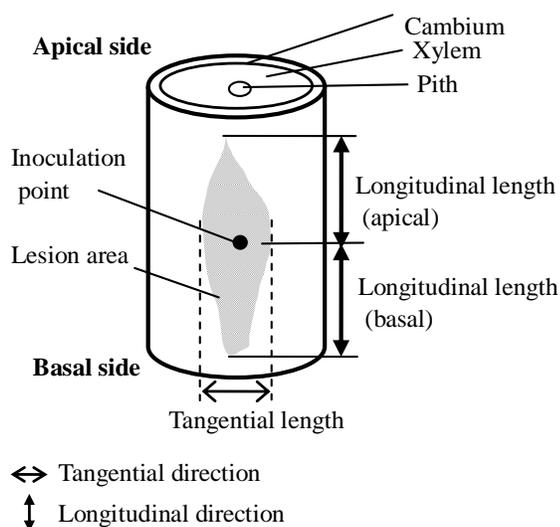


Figure 1: Components of fig wood and measurements of ceratocystis canker lesions.

The lesion spreading characteristics on woody plants were reported for some tree species and fungi combinations such as *Larix kaempferi* infected with *Ceratocystis laricicola* (Morimune et al., 2000), *Quercus crispula* infected with *Raffaelea sp.* (Kuroda, 2001), and *Picea jezoensis* infected with *Ceratocystis polonica* (Kuroda, 2005). The CC symptom is often observed on the basal part of the infected trunk of fig trees as an irregular circular brown lesion spreading lengthwise from the ground (Kajitani, 1995; Kato et al., 1982; Shimizu and Miyoshi, 1999). However, reports of CC lesions on fig trees are scarce (Kajii et al., 2013; Kajitani, 1995), and its spreading characteristics remain unknown. To ascertain their fundamental characteristics, we investigated whether gravity, the physiological polarity, and anatomical difference of the branches affect the inoculated CC lesion spreading.

MATERIALS AND METHODS

The pathogenic inoculum was *C. ficicola*, which was isolated from diseased fig trees cultivated in Kanan-cho, Osaka, Japan and which was subcultured on potato dextrose agar (PDA) at 25°C in the dark. The host plants were prepared as shoot-cuttings or potted nursery stocks of fig 'Masui Dauphine' trees at the Research Institute of Environment Agriculture and Fisheries Osaka Prefecture.

Effects of physiological polarity and gravity on lesion spreading in fig shoot-cuttings (Experiment 1)

In October 2008, fig shoots were cut into 30 cm and were

used for shoot-cuttings as host plants. A pinhole on the surface of each shoot-cutting was made using a needle. The cutting was inoculated with one spore of *C. ficicola* and was covered with parafilm. Each of 4–5 inoculated shoot-cuttings was set with a different posture (upright, inversion, and horizontal) and incubated at 25 °C. Seven days after inoculation, a lesion, a discoloured area around the inoculation point, was exposed by bark chipping. The lesion lengths in longitudinal and tangential directions were measured using a digital calliper. The length in the longitudinal direction was divided into apical and basal parts from the inoculation point (Figure 1).

Effects of the physiological polarity on the lesion spreading in fig nursery stocks (Experiment 2)

In March 2009, the rooted cuttings of fig 'Masui Dauphine' were transplanted to 750-ml plastic pots (11.5 cm diameter, 10 cm depth) filled with vermiculite, and were used as host plants for nursery stocks. In May 2009, one *C. ficicola* spore was inoculated into the internode of trunks of three nursery stocks using the same method as that described for Experiment 1. The nursery stocks were maintained at 25 °C. At 1, 5, and 14 days after inoculation, lesion spreading around the inoculated point was investigated for each of three nursery stocks using the same method as that described for Experiment 1.

Effects of wood anatomical characteristics on lesion spreading in fig shoot-cuttings (Experiment 3)

In September 2008, the fig shoots were cut into 30 cm, and were used for shoot-cuttings as host plants. One spore of *C. ficicola* was inoculated into each shoot-cutting and incubated using the same method as that described for Experiment 1. The inoculated points of shoot-cutting were the internode and immediately below the bud in each of three shoot-cuttings. Seven days after inoculation, the lesion spreading around the inoculated point was measured using same method as that described for Experiment 1.

In August 2010, the shoots were sampled and cut into 30 cm. They were used for shoot-cuttings as host plants. Using a drill, a 3-mm-diameter pit hole was made from the epidermis to the pith of each shoot-cutting. Each pit hole was plunged with the inoculum (mycelial tufts including PDA medium) and was covered with parafilm. Seven days after inoculation, the shoot-cuttings were cut in round slices with 1 mm thickness around the inoculated point. The lesion was observed on the cross-sections of the slices. Its spread in the shoot-cutting was estimated.

RESULTS

Experiment 1

Figure 2 shows the length of lesion on the shoot-cuttings in

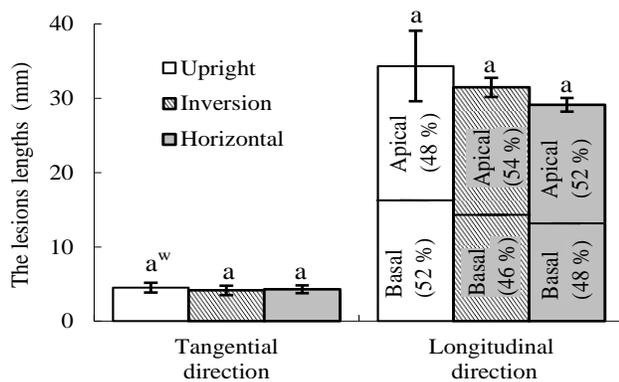


Figure 2: Effects of growth physiological polarity and gravity on lesion lengths in fig shoot-cuttings.

Shoot-cuttings inoculated with *C. ficicola* were set with differing postures: upright, inverted, and horizontal. The lesion lengths were measured in longitudinal and tangential directions. The length in the longitudinal direction was divided into apical and basal parts from inoculation points.

^w Different letters denote significance of lesion length at the 5% level found using a Tukey test among the postures of shoot-cutting ($n=4-5$). No significance was found in the ratio of the apical part for basal parts of the longitudinal length (markers are not shown).

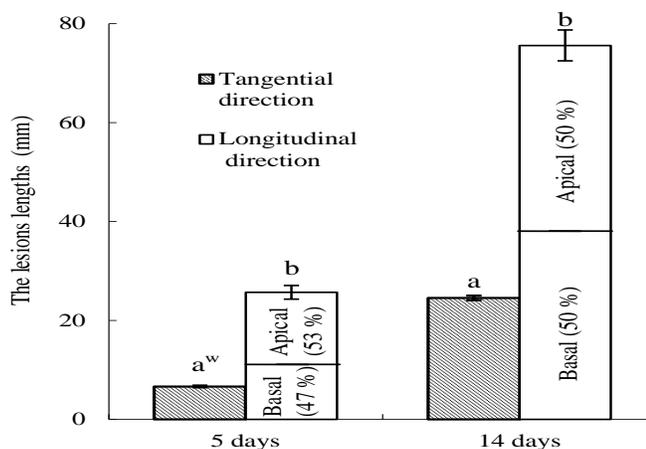


Figure 3: Effects of physiological polarity on lesion lengths in fig nursery stocks.

Lesion lengths were measured 1, 5, and 14 days after inoculation in the same manner as that shown for Figure 1.

^w Different letters denote significance between tangential and longitudinal lesion length at 5% level found using a *t*-test ($n=3$). No significance was found in the ratio of apical part for the basal part of longitudinal length (markers are not shown).

each direction. The lesion length in the longitudinal direction was apparently greater than in the tangential one in any posture of shoot-cutting. The ratios of the apical to basal parts of longitudinal length were 48:52% (upright), 54:46% (inversion), and 52:48% (horizontal). The parts were almost equal. No significant difference was found in

the ratios among the postures of shoot-cutting.

Experiment 2

Figure 3 shows the lesion spreading on the trunk in each direction. The lesion lengths were less than a detectable level at 1 day after inoculation (no data), and gradually spread over time (measured at 5 and 14 days) in both the longitudinal and tangential direction. The tendency of lesion spreading was similar with the results obtained for Experiment 1. The lesion lengths in the longitudinal direction were greater than in the tangential direction. The apical and basal parts of longitudinal lengths were almost equal: they were 53:47% (5 days after inoculation) and 50:50% (14 days after inoculation).

Experiment 3

Figure 4 shows lesion spreading on the shoot-cuttings in each direction of both inoculated portions. The tendency of greater longitudinal length than the tangential length was common in both inoculations. However, the longitudinal lesion length for the inoculation immediately below the bud was smaller than for internode inoculation. The ratio between apical and basal parts of longitudinal length was 53:47% (internode) and 47:53% (just below the bud). The ratios of both parts were almost half. No significant difference was found in the ratios among inoculations. Figure 5 shows the process of lesion spreading in the cross-section of cutting-shoots. The lesions spread longitudinally around the pit hole. The average of the maximum longitudinal length was 37 mm. The longitudinal lengths differed among shoot tissues. Larger lesion spreading was observed on the cambium and pith. For the sample of Figure 4, the longitudinal lesion length of the apical part was 30 mm on the xylem, but 50 mm on the surface structure and pith.

DISCUSSION

The polarity of fungal invasion affects the pattern of the lesion on the wood (Shain, 1967). In the case of *Ceratocystis*, e.g., the *C. laricicola* lesion did not spread in the tangential direction, but spread in longitudinal and radial directions in the Japanese larch *Larix leptolepis* Gord. (Morimune et al., 2000). The result obtained in the present study was similar: a lengthwise spreading was also a fundamental characteristic of CC in fig wood. Boddy and Rayner (1983) described that the longitudinally long shape of wood cells and the cell wall acts as mechanical barrier caused lengthwise spreading of the lesion. The shape of the observed lesion of CC is probably reflected in the wood cell morphology because the fig wood also has such long cells. The wood cells near the buds were arranged around them. The cells immediately below the bud exhibited a flat shape.

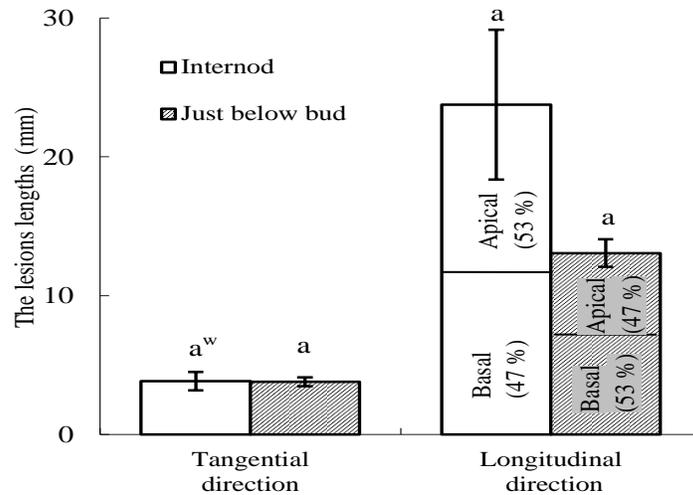


Figure 4: Effects of wood anatomical characteristics on the lesion lengths in fig shoot-cuttings. Shoot-cuttings were inoculated at the point of internode and just below the bud. The lesion lengths were measured in the same manner as that shown for Figure 1. ^w Different letters denote lesion length significance at the 5% level found using a *t*-test between both inoculation points (*n*=3). No significance was found in the ratio of apical part for basal part of longitudinal length (markers are not shown).

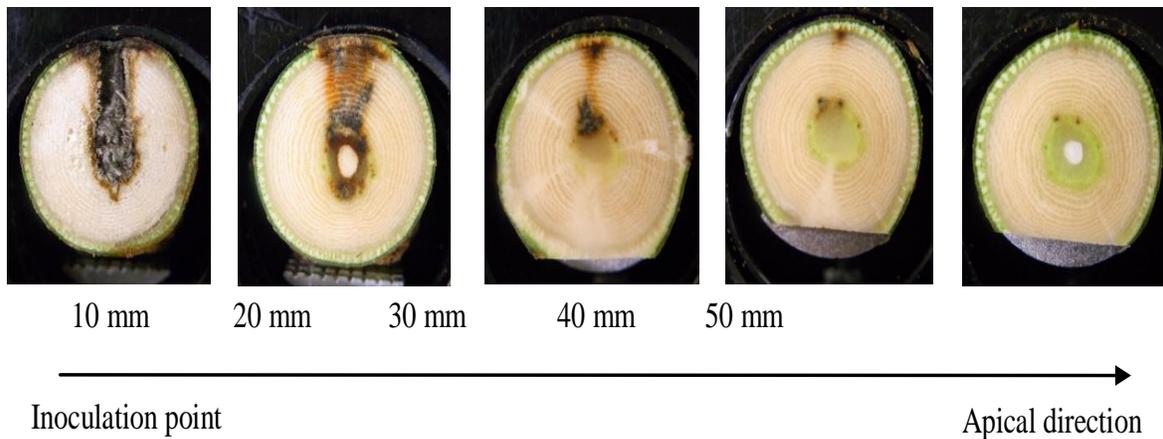


Figure 5: Lesion spreading on the cross-section of the branch. Images show cross-sections (10, 20, 30, 40, and 50 mm in the apical direction from the inoculation point) of an example of shoot-cutting.

Inhibitory spreading of the lesion below the bud in Experiment 3 suggests that the lesion spreading is affected by morphological characteristics of the wood cells.

In Experiment 1, the lesions on the shoot-cuttings spread equally from the inoculation point to apical and basal parts. This result was common in all postures of the shoot-cutting set. Such equal lesions spreading from the inoculation point were also observed on the internode of the trunk of intact nursery stocks in Experiment 2. Shimizu and Miyoshi

(2008) reported that the gene of *C. ficicola* was detected in a high branch site distant from the ground. In our study, however, no sign of elevatory spreading along the vessel was observed even when the lesions had penetrated deeply into the wood. Also in wilting *Quercus* spp., no evidence of a discoloration of vessel and no sign of a fungal transportation via sap flow was observed (Kuroda and Yamada, 1996), which suggests that the lesion spreading of CC is unaffected by gravity or the physiological polarity of fig trees,

although the possibility exists of transportation of a few fungal mycelia by sap flow. Lengthwise spreading is a fundamental characteristic for CC lesions on fig wood. A CC lesion with sharp triangle shape is often observed on the basal part of trunk (Kajitani, 1995; Kato et al., 1982; Shimizu and Miyoshi, 1999) reflects such a fundamental characteristic. The visible lesion is probably its top part. The other part of the lesion remains hidden under the ground with a more complicated shape reflecting the variable shape of roots.

The component of the *Ficus* wood consists of a vessel element, tree fibre, an axial parenchyma cell, and the radial parenchyma cell. The vessel pore distribution is diffuse. Larger pith is also a *Ficus* spp. characteristic. Experiment 3 demonstrated that the speed of lesion spreading differed between such components: the lesions tended to expand easily near the cambium and the pith. Yamaoka (2002) reported that the wood disease fungi were classified as a group invading the inner bark and cambium causing necrosis, and another group invading sapwood, causing an embolism. Discoloration of heartwood is often observed in the trunks of fig trees infected with *C. ficicola* in the field. Experiment 3 showed greater lesion spreading, observed on the cambium and pith of branch, in which the inoculation point was a deep wound (pit hole reaching to the pith). A core wood infection would induce the conventional lesion of CC on the heartwood of field fig trees. Penetration of ambrosia beetles as a carrier (Kajitani, 1996; Morita et al., 2012; Nitta et al., 2005; Valarini and Tokeshi, 1980) is a possible reason for core wood infection. However, heartwood lesion spreading alone did not induce tree death directly. Lesion spreading in sapwood would be rather severe for tree death because rapid tree wilting, as is characteristic of this disease, is probably induced by a blockage of vessels (Shimizu and Miyoshi, 1999). Slow spreading of the lesion in the sapwood was found in experiment 3. In addition, the characteristic of lengthwise spreading of a lesion is also useful in delaying tree death because a partly active vessel can maintain tree survival. Otherwise, rapid spreading in the cambium was observed in Experiment 3. The tree probably died when the reconstruction of a vessel in the cambium was inhibited by disease invasion. Subsequently, the damage spread throughout the vessels in either cross section of the trunk. Defining the evidence of embolism by lesion spreading must be done in future studies to elucidate fig tree death processes that occur because of CC.

ACKNOWLEDGMENTS

Financial support was received from funds for the Development of Practical Technologies to Implement New Agricultural, Forestry and Fishery Policies. Other assistance from Mr. Kiyotsug Okada and Mr. Mitsuo Kawaradani, Research Institute of Environment, Agriculture and

Fisheries, Osaka Prefecture, is gratefully acknowledged. We thank Ms. Akemi Nanno, Ms. Akemi Matsukiyo, and Ms. Emiko Sakakura for their kind help.

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Cite this article as: Miwa Y, Hosomi A, Ishii T (2014). Lesion spreading of ceratocystis canker on 'Masui Dauphine' fig trees. Int. J. Agric. Pol. Res. 2(2):055-060.