ユズ「山根系」及び「要2号」果実におけるカンキツかいよう病感受性

誌名	農研機構研究報告. 果樹茶業研究部門 = Bulletin of the NARO. Fruit Tree and Tea Science
ISSN	24326631
著者名	今井,健司 篠崎,毅 足立,嘉彦 塩谷,浩
発行元	農研機構果樹茶業研究部門
巻/号	2号
掲載ページ	p. 1-7
発行年月	2018年3月

原著論文

Susceptibility of *Citrus junos* 'Yamane' and 'Kaname No. 2' fruits to *Xanthomonas citri* pv. *citri*

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Summary

To evaluate the susceptibility of fruits of *Citrus junos* 'Yamane' and 'Kaname No. 2' to *Xanthomonas citri* pv. *citri*, we inoculated attached fruits of *C. junos* and *Citrus sinensis* with the rifampicin-resistant strain KC20RR in June and July 2017. The bacterial populations in fruits of both species increased until 1 month after inoculation and then decreased. Symptoms developed by 14 days. The populations in lesions on 'Yamane' fruits were apparently smaller than those in *C. sinensis* 'Seike' fruits, possibly owing to physiological resistance of *C. junos*. There was no difference in the susceptibility of fruits or leaves between 'Yamane' and 'Kaname No. 2' by inoculation test. Populations in lesions on immature *C. junos* fruits were much larger than those on leaves at 2 months.

Key words: Yuzu, citrus canker

Received 26 September 2017, Accepted 17 November 2017

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Introduction

Citrus canker caused by *Xanthomonas citri* pv. *citri* (Constantin et al., 2016) is a major problem in citrus production worldwide. Although it has a broad host range among members of the Rutaceae, *Citrus junos* (called *yuzu* in Japan) is thought to be highly resistant. In Japanese cuisine, *yuzu* rind is used to garnish some dishes, and the juice is commonly used as a seasoning. There is demand for *yuzu* in Southeast Asia and Europe; Japan began exporting to France in 2012, but the quantity was limited owing to plant protection and quarantine requirements. The susceptibility of *C. junos* to *X. citri* pv. *citri* has been evaluated in investigations of foliage (Deng et al., 2010; Hyun et al., 2003; Koizumi and Kuhara, 1982; Lee et al., 2009; Myung et al., 2003). However, there is no information about the occurrence of the disease on fruits, which might allow *X. citri* pv. *citri* to enter uninfected citrus production areas. Thus, we investigated the susceptibility of *C. junos* fruits to *X. citri* pv. *citri* by spray inoculation test.

Materials and methods

Inoculum

Xanthomonas citri pv. *citri* strain KC20RR, which is resistant to rifampicin, was used as inoculum. This strain is a stable, spontaneous mutant of strain KC20 lacking the gene *hssB3.0*, which is related to host specificity (Shiotani et al., 2007), and is as pathogenic as other strains. Inoculum was prepared from cultures incubated at 27 °C for 16 h on potato semisynthetic agar medium (Wakimoto, 1967) supplemented with 100 mg/mL rifampicin.

Inoculation test

To evaluate the susceptibility of *C. junos* fruits to *X. citri* pv. *citri*, we inoculated young attached fruits of 'Yamane' 10 to 40 mm in diameter by spraying fruit-bearing trees with KC20RR at 10^6 cells/mL with a power atomizer in June 2017 in an orchard of the Tokushima Agriculture, Forestry and Fisheries Technology Support Center, Katsuura, Tokushima, and an orchard of the former Ehime Fruit Tree Experiment Station in Kihoku town, Ehime. For comparison with *C. sinensis*, we also inoculated 'Seike' in Katsuura and 'Shirayanagi' in an orchard of the Ehime Research Institute of Agriculture, Forestry and Fisheries, Shimoidai, Ehime.

In June 2017, natural infection of *C. junos* by citrus canker was observed on 'Kaname No. 2' in an orchard of the Tokushima Center, Ishii, Tokushima. Thus, to compare the susceptibility between the *C. junos* cultivars, we spray-inoculated attached fruits as above and pin-prick-inoculated attached mature leaves in the orchard as described previously (Shiotani et al., 2000). For comparison, we also inoculated leaves of *Citrus sudachi* 'Honda', as *C. sudachi* is susceptible to citrus canker (Koizumi and Kuhara, 1982).

Quantitation of bacterial cells

To investigate the size of the populations of bacteria that oozed from the fruit surface, we randomly collected 10 inoculated fruits (5 of *C. junos* 'Yamane' in Katsuura) every 1 to 2 weeks and sonicated them in 5 to 50 mL of deionized water for 2 min in a US-4 ultrasonic cleaner (SND Co., Ltd, Suwa, Japan). Appropriately diluted aliquots of the sonicates were plated on modified XCSM medium (Graham and Gottwald, 1990; Shiotani et al., 2000: starch 10 g/L, polypeptone 2 g/L, monopotassium phosphate 1 g/L, magnesium chloride 0.2 g/L, calcium nitrate 0.5 g/L, disodium hydrogen phosphate dibasic 1 g/L, agar 15 g/L, rifampicin 100 mg/L, kasugamycin 16 mg/L, cephalexin 16 mg/L, chlorothalonil 12 mg/L, methyl green 12 mg/L). The plates were incubated at 27 °C for 3 days and observed for the presence of colonies typical of the bacteria.

We quantitated the populations on rinds and leaves the same way by macerating an excised lesion in 0.85% sodium chloride and plating on the medium.

Results and discussion

Susceptibility of *C. junos* fruits to *X. citri* pv. *citri*

The populations on fruits of both *C. junos* 'Yamane' and *C. sinensis* 'Shirayanagi' and 'Seike' increased until 1 month after inoculation and then decreased, but populations in Ehime were apparently smaller than those in Katsuura (Fig. 1). Symptoms developed by 14 days (Fig. 2a). The greater populations in Katsuura might have been due to the more suitable conditions for infection there.

Populations in lesions on *C. junos* 'Yamane' fruits were also apparently smaller than those on *C. sinensis* fruits (Fig. 3). This result corresponds with observations that *C. junos* is highly resistant to citrus canker and can physiologically inhibit bacterial growth (Koizumi, 1979; Lee et al., 2009). The smaller populations on *C. junos* in both orchards (Fig. 1) might therefore be due to physiological resistance.

Same susceptibility of both *C. junos* cultivars

Natural infection of *C. junos* by citrus canker has been reported only twice. The first time was an experiment using potted trees placed around a heavily diseased citrus tree in the field (Hyun et al., 2003). The second time, in June 2017, we observed canker lesions on the leaves of 5-year-old *C. junos* 'Kaname No. 2' trees growing in the Ishii orchard (Fig. 2d) next to diseased *C. sudachi* trees. PCR assay of representative lesions excised and macerated in 0.85% sodium chloride (Miyoshi et al., 1998) identified the causal agent as *X. citri* pv. *citri*. These observations suggest natural infection in the orchard.

Despite the infection of 'Kaname No. 2' trees, neighboring 5-year-old *C. junos* 'Yamane' trees had no lesions. This observation suggests that 'Yamane' is more resistant to citrus canker than 'Kaname No. 2', as the susceptibility of *C. junos* accessions may depend on cultivar (Deng et al., 2010). Thus, we investigated the susceptibility of each by pin-prick inoculation of the leaves (Shiotani et al., 2000) as well as by spray inoculation of the fruits. Strain KC20RR could infect leaves of both *C. junos* cultivars and similarly increased in number to 10^4 CFU per lesion at 28 days after inoculation, but then decreased to near extinction at 60 days (Fig. 4a). The population was apparently larger on the leaves of *C. sudachi* 'Honda' than on those of either *C. junos* cultivar and remained large at 60 days. These results suggest that leaves of both *C. junos* cultivars are highly resistant to the disease, and the limited occurrence of the disease in 'Kaname No. 2' might be due not to its susceptibility but to close proximity to the source of infection.

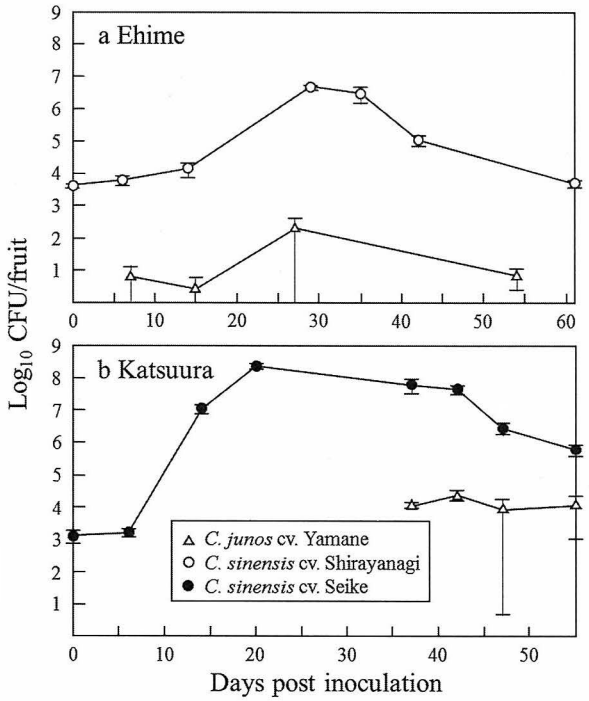

Fig. 1 Time course of *Xanthomonas citri* pv. *citri* populations on the fruit surface of *Citrus junos* 'Yamane' and *C. sinensis* 'Sirayanagi' and 'Seike' in orchards in (a) Ehime ('Yamane' in Kihoku and 'Shirayanagi' in Shimoidai, respectively) and (b) Katsuura. Data are means of 10 fruits (5 of *C. junos* 'Yamane' in Katsuura); vertical bars are standard error of the mean.

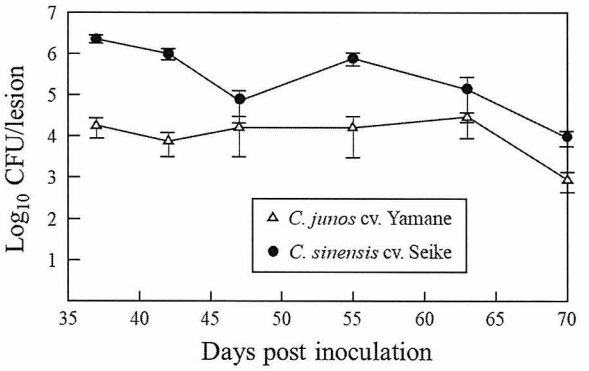

Fig. 3 Time course of *Xanthomonas citri* pv. *citri* growth in lesions on fruits of *Citrus* species in Katsuura. Data are means of 5 lesions; vertical bars are standard error of the mean.

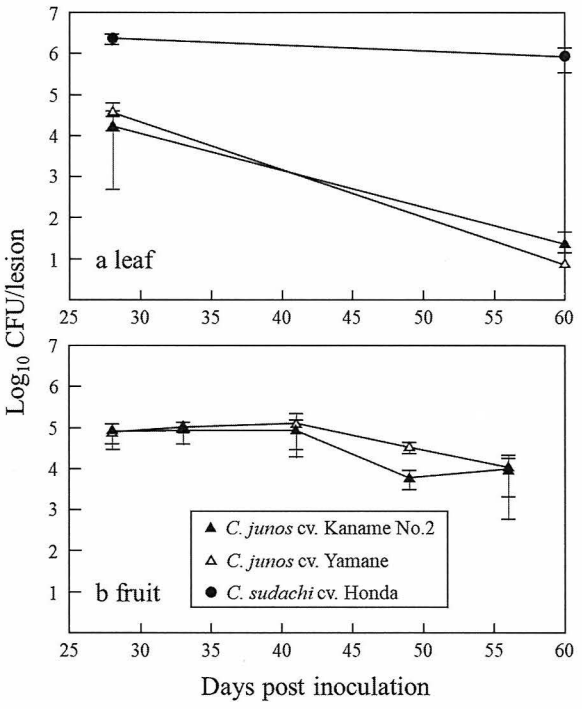

Fig. 4 Time course of *Xanthomonas citri* pv. *citri* growth in lesions on a (a) leaf and (b) fruit of *Citrus* species in Ishii. Data are means of 3 lesions; vertical bars are standard error of the mean.

Bacterial populations remain longer in lesions on fruits than on leaves

On fruits of both 'Yamane' and 'Kaname No. 2' in Ishii, typical canker symptoms, with a raised and corky appearance, developed by 1 month after inoculation (Fig. 2b, c). Bacterial populations in lesions of both cultivars were equivalent throughout the experiment (Fig. 4b). However, populations in lesions on fruits were three orders of magnitude larger than those on leaves of each cultivar at 2 months (Fig. 4). This result indicates that the immature rind of highly resistant species is susceptible for a longer time than leaves, as previously reported (Stall and Seymour, 1983).

Acknowledgements

This work was supported in part by a grant-in-aid for scientific research on plant protection from the Ministry of Agriculture, Forestry and Fisheries of Japan.

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ユズ「山根系」及び「要２号」果実におけるカンキツかいよう病感受性

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摘　要

樹上に着生したユズ*Citrus junos*の未成熟果実におけるカンキツかいよう病感受性を評価した。平成29年6月、徳島県立農林水産研究技術支援センター圃場（勝浦町）ならびに愛媛県南予地方局産業振興課地域農業室鬼北農業指導班圃場に植栽されたユズ「山根系」樹にリファンピシン耐性カンキツかいよう病菌KC20RR株を10^6 cells/mlの濃度で噴霧接種した。対照のネーブル着生果の場合と同様，いずれの圃場においても接種から14日後までにユズ「山根系」着生果で発病が確認されるとともに，ネーブルに比べて明らかに少ないものの病斑内での細菌増殖ならびに病斑からの細菌溢出が確認された。また，平成29年7月、徳島県農林水産研究技術支援センター圃場（石井町）に植栽されたユズ（「山根系」および「要２号」）樹にKC20RR株を噴霧接種し，ユズ品種間での着生果のかいよう病感受性を比較した。その結果，両品種とも発病するとともに果実に形成された病斑中での細菌増殖も同等で，これら品種間では感受性に違いがないと判明した。未熟なユズ果実上の病斑では葉上のものに比べてより多くの細菌が長く生存した。

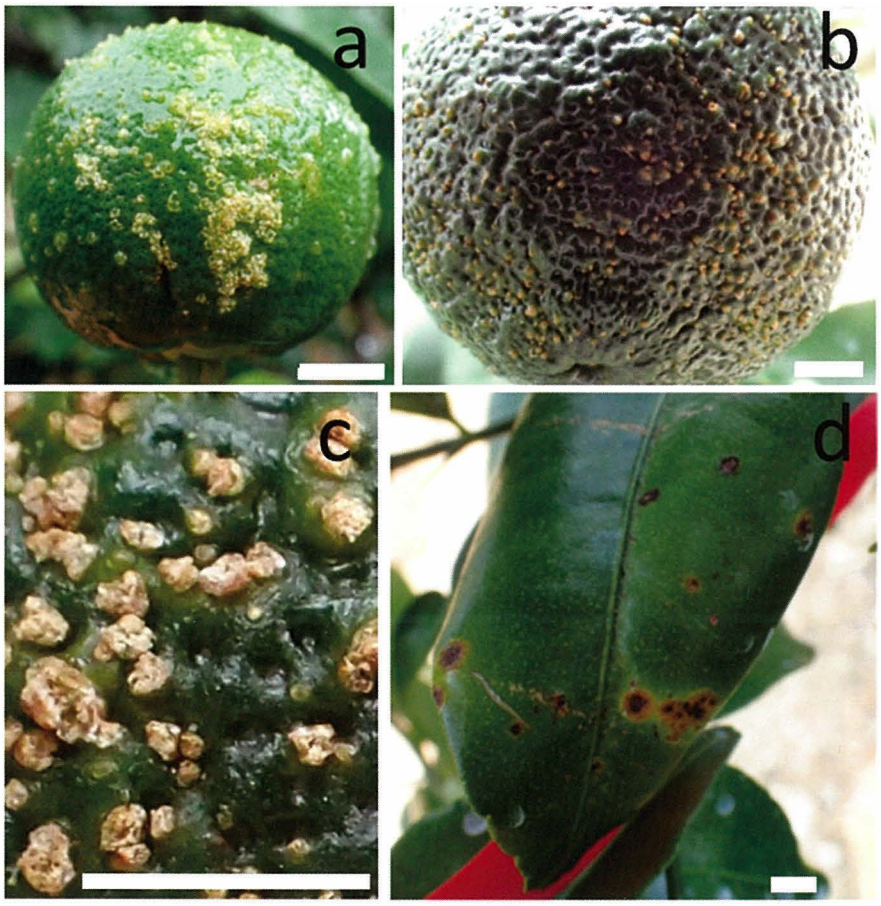

Fig. 2 Canker symptoms on fruits of (a) *Citrus sinensis* 'Seike', (b) *C. junos* 'Yamane', and (c) *C. junos* 'Kaname No. 2', and on leaves of (d) 'Kaname No. 2'. Symptoms developed at (a) 14 days in Katsuura and (b, c) 28 days in Ishii after spray inoculation with *Xanthomonas citri* pv. *citri*. (d) Symptoms naturally elicited on leaves of 'Kaname No. 2' in Ishii. Bars = 0.5 cm.