Occurrence of Bacterial Canker of Kiwifruit in Japan: Description of Symptoms, Isolation of the Pathogen and Screening of Bactericides

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Abstract

A bacterial canker disease of kiwifruit (Actinidia chinensis) has been observed in Shizuoka Prefecture, Japan. Symptoms appear on trunks, leaders and over-wintering canes from late winter to early spring as cankers and cracks, when red-rusty brown bacterial ooze exudes from these lesions or from apparently healthy buds, leaf scars, lenticels and joints of trunks, leaders and canes. In late spring, brown water-soaked lesions with halos appear on leaves, and wilt or blight of vigorous canes and flower buds is also observed. A characteristic white bacterium is consistently isolated from the affected tissues, isolates of which reproduce typical symptoms on kiwifruit and Actinidia arguta when inoculated either with or without wounding in spring and winter. Kiwifruit leaves are most susceptible to the pathogen just before maturation and are less susceptible at younger and older stages. Climatic conditions such as low temperatures, strong winds, and heavy rainfall appear to promote the disease. Applications of streptomycin, kasugamycin or inorganic copper formulations reduced disease development on leaves.

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Key words: kiwifruit, bacterial canker, symptoms, control.

INTRODUCTION

A canker disease of kiwifruit (Actinidia chinensis) has been observed in Shizuoka Prefecture, Japan. The disease was first recognized as a serious disorder when it was observed widespread on trunks and branches in mature orchards in early spring in 1984. From late spring to early summer, leaves and shoots which had just emerged on affected branches were also diseased, and the outbreak increased rapidly after heavy rainfall with strong winds. Affected vines were killed or suffered serious loss of production. Some growers had occasionally noticed a similar condition in mature orchards in the years preceding 1984. In 1984, the disease affected 56 ha in 162 ha of cultivated area in Shizuoka Prefecture. Some orchards were destroyed because of severe damage by the disease. The disease was also observed in Kanagawa, Aichi, Tottori, Fukuoka, Tokushima and Miyazaki Prefectures.

Because of the severity of the outbreak, we initiated investigation of the etiology, epidemiology and control of the disease. This paper deals with description of the symptoms, isola-
tion of the pathogen, pathogenicity tests and screening of bactericides for the control of the disease.

SYMPTOMS

Trunks and leaders. Although not readily discernible in January, a careful inspection of trunks and leaders will reveal the exudation of small white droplets of bacterial ooze. As winter progresses, the ooze increases in quantity and viscosity and becomes red-brown in color, and the bark from which the ooze exudes becomes discolored.

The symptoms are readily apparent from late winter to early spring months when the vines break dormancy. Red-rusty brown droplets appear on buds, joints of branches, forks of boughs, leaf scars and pruning scars (Plate I-1-6). Bark tissue in these areas is red-rusty brown and wet. Removal of the bark reveals a browning of the underlying vascular tissues which extends beyond the brown region of the bark. Affected bark becomes deeply shriveled and dried. Cracks of 1 to 2 mm in width are often formed on the affected branches, and wound-healing callus tissues are formed around them. Severely affected leaders died without buds sprouting or developing new shoots. Prolific suckering occurs from the healthy part of the trunk close to crown (Plate I-7).

Leaves. Symptoms appear from April. First, small water-soaked spots are formed on expanding leaves. They become brown to dark brown and angular in shape, 2 to 3 mm in diameter, delineated by small veins. Bright yellow halos, 3 to 5 mm in width, are formed around the spots (Plate II-1, 2). The halos become narrow and indistinct as leaves mature (Plate II-3). In high humidity and cool conditions, the spots remain water-soaked, and expand and coalesce to form large lesions without halos. Then, the whole leaf may become blighted and shriveled. On the lower surface of the leaf, white gruel-like bacterial exudate is produced from lesions in a way similar to the angular leaf spot of cucumber caused by *Pseudomonas syringae* pv. *lachrymans* (Plate II-4). The ooze becomes dry and shows scale-like appearance in dry conditions.

Canes. Vigorous canes are also infected in spring. The infected part of the cane becomes dark-green and water-soaked. Longitudinal cracks of 1 to 3 mm in length are often formed (Plate II-6). In high humidity condition, bacterial ooze is exuded from the cracks and from the lenticels on apparently healthy parts of the canes neighboring the lesions (Plate II-5, 6, 7). The lesions elongate and whole shoots become wilted and blighted. When canes are infected late in the season, lesions become surrounded by wound-healing callus tissues as on trunks and they form typical cankers.

Flowers. Flower buds also show symptoms. Most infected flowers turn brown and wither without opening. Infected flowers which open may have petals not fully developed (Plate III-3). Sepals are also affected and necrotic lesions are formed on them (Plate III-1, 2). These flower symptoms resembled to that of blossom rot disease, though, the former is characterized by necrotic sepals and apparently healthy petals at early stage of infection, in contrast to the latter which is characterized by browning of petals and healthy sepals (Plate III-4).

MATERIALS AND METHODS

Isolation of the pathogen. Plate culture isolations were made from the affected parts of kiwifruit cv. Hayward, from May to June in 1984. Samples were collected from 16 mature orchards in Shizuoka Prefecture. Small pieces of tissue were cut away from the edge of leaf spot lesions, canker lesions of canes and necrotic lesions of flower buds. Each piece was surface-sterilized with 70% ethanol and comminuted in 3 ml of sterile distilled water (SDW). For isolations from trunks and canes, bark was cut away and small chips of vascular tissue were removed from the margin of discolored parts. They were similarly comminuted in SDW. The suspensions were streaked on plates of peptone-sucrose-agar (PSA: peptone 10 g, sucrose 10
g, agar 14 g, distilled water 1 liter, pH 6.8), and incubated for 24~48 hr at 27 C. Fresh ooze on canes was collected and suspended in 10 ml SDW. These suspensions were also streaked on plates and incubated as described.

Colonies appeared on the PSA plates were picked up and subcultured on PSA slants. These isolates were stored at 4 C.

Pathogenicity tests. Isolates from kiwifruit to be tested for pathogenicity were spread onto PSA plates and incubated for 48 hr at 27 C. A suspension was prepared by scraping the growth from the plates, suspending it in SDW, and diluting it to $2 \times 10^8$ colony-forming-units/ml (cfu/ml). The suspensions were inoculated with or without wounding on potted 3-year-old female plants grown from seedlings of kiwifruit (cv. Hayward) in May 1984, and on these mature vines in the nursery in April 1986. For each isolate, 4 to 5 seedlings and 4 to 5 canes of the mature vine were inoculated.

For wound-inoculations, leaves of various ages, canes and fruits were punctured with a sterile needle through a 10 µl drop of the suspension. For inoculations without wounding, inoculum was sprayed over the plants with a glass sprayer. After inoculation, the plants were covered with plastic bags overnight to be maintained under moist conditions and then put out into field conditions. The reisolation of inoculated bacteria was performed 20 and 40 days after inoculation, as described above. Susceptibility of leaves at different ages was assessed by counting lesions on every leaf of each of four unwounded pot-grown seedlings 30 days after inoculation.

Dormant canes were also inoculated in December 1984. Bark tissue at the base of a bud on the canes was lightly shaved with a sterile knife. Pieces of absorbent cotton dipped in bacterial suspension were applied to the each wound site. Masking tape was used to cover each wound site to prevent the inoculum from drying out. Five canes were inoculated for each isolate. Symptom development was observed regularly until late April 1985.

Five- to 6-year-old plant of Actinidia arguta (Japanese common name is "sarunashi") was also inoculated in the same ways as described for kiwifruit.

Screening tests of bactericides. Control effects of some bactericides against the disease were investigated in a naturally infected field in 1985. Treatments consisted of streptomycin 200 ppm ("Agrepto": stm 20% WP. 1 g/liter), kasugamycin 50 ppm ("Kasumin": ksm 2% SL. 2.5 g/liter), inorganic copper formulation 270 ppm ("Kocido": copper hydroxide 54% 5 g/liter), and control (no treatments). The copper formulation was applied with calcium carbonate (CaCO$_3$ 95% 0.5 g/liter) to reduce phytotoxicity. For each treatment, four 7-year-old vines of kiwifruit (cv. Hayward) were first sprayed on 16 April and again at 7-day intervals for a total of 3 applications. Assessments of efficacy were made on 14 May. Disease levels were determined by counting the number of affected leaves and the number of lesions formed on them. Results of treatments and a disease severity index, calculated from the data, are described in Table 1.

RESULTS

Isolation of the pathogen

From all fresh plant tissues, a characteristic bacterium which formed round white glistening colonies was regularly isolated. When isolations were attempted from the bark tissues of severely affected trunks in late June, many other bacteria were obtained, and it became difficult to isolate the white bacterium. Most of the other bacteria formed large, yellow umbonate colonies which are characteristic of Erwinia herbicola.

Pathogenicity test

All isolates of the white bacterium described above, but none of the yellow isolates, produced pathogenic reactions on kiwifruit. Leaf spots on seedlings and nursery trees appeared five days after inoculation in April and May with or without wounding. Their appearance was
Fig. 1. Susceptibility of kiwifruit leaves to bacterial canker at various stages of development. Four seedlings of kiwifruit were inoculated without wounding. For each leaf, disease severity was assigned to one of five categories based on the numbers of lesions which had appeared 30 days after inoculation.

similar to that observed in the field. Ooze exudation and longitudinal cracks of ca. 1 cm in length were also produced on canes with wound inoculations. When the bark tissues of the inoculated canes were removed, brown stripes were seen at vascular tissues. The characteristic bacterium was consistently reisolated from leaf spots, cane lesions and ooze. Those reisolates were pathogenic on kiwifruit by reinoculation. The most severe infection occurred on leaves which had been inoculated just before maturation. Numbers of lesions decreased on younger and older leaves (Fig. 1).

Dormant canes of kiwifruit were also susceptible. Symptoms appeared in February and March (i.e. 2 to 3 months after inoculation), when the plant resumed growth. White to red-brown bacterial exudates were produced, and canker lesions were formed as observed in natural infections.

Fruits of kiwifruit proved to be not susceptible to the pathogen. Slight browning was observed at wound sites but progressive lesions were not produced.

The inoculation of Actinidia arguta in April and December resulted in the appearance of symptoms similar to those which developed in kiwifruit. For instance, leaf spots and tip withering with ooze exudation were observed on young shoots, and red-rusty brown exudates appeared on over-wintering canes (Plate III-5, 6).

Screening of bactericides
All of the three formulations applied were effective in preventing leaf infection, streptomycin being most effective (Table 1).

Phytotoxic reactions were sometimes observed on the leaves treated with streptomycin and inorganic copper. Streptomycin treatments produced chlorosis of leaf margins of immature leaves and in severe cases produced 'cupping' of leaves. The chlorosis disappeared as leaves matured, but the lamina of severely affected leaves were permanently cupped downwards. Inorganic copper treatments caused discoloration, and sometimes cracking of caprophores. In addition, on the lower surfaces of the leaves, small spots appeared similar to star-
a) Disease severity of each leaf was assigned to one of 5 categories based on the numbers of spots which appeared; A: more than 20, B: 11 to 20, C: 4 to 10, D: 1 to 3, and E: 0. Disease severity was calculated as follows: 
\[
\frac{(7n_A + 5n_B + 3n_C + n_D)}{7(n_A + n_B + n_C + n_D + n_E)} \times 100
\]
\(n_A\) to \(n_E\) represents the number of the leaves of category A to E, respectively. Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test (\(P=0.05\)).

melanosis of citrus and grapevine also caused by copper injury, and sometimes whole leaf became silver-brown.

**DISCUSSION**

A bacterial pathogen not previously isolated in Japan has been shown to be responsible for causing death and dieback of kiwifruit vines, for killing buds and flowers, and for causing a leaf spot.

Kiwifruit was introduced as a commercial crop into Shizuoka Prefecture in 1970, and the cultivated area has increased rapidly since 1980. The pathogen may have been introduced with the host plant but was not noticed until environmental conditions, such as a typhoon in fall (1983), the low temperatures in winter (1983-1984), and heavy rainfall and wind storms in spring (1984) combined to initiate the epidemic of 1984.

A consideration of disease development and temperature indicated in association between severity and cold weather. From December 1983 to March 1984, the temperature was unusually low. Temperature fell below 0°C on 42 days in the period in which it ranges from 10 to 20 in most years. In addition, disease severity in the prefecture was greater in areas where there were prevailing low temperatures than in areas where temperatures were high. In California, *Pseudomonas syringae*, the pathogen responsible for a similar canker disease, was reported to have ice nucleation activity (INA), and frost injuries were considered to promote the disease\(^4\). However, our unpublished data showed that the causal bacterium in Japan has no INA. Furthermore, the bacterium was able to infect the plants and caused typical symptoms when inoculated in late winter, despite relatively high temperatures. These facts suggest that frost injuries might be associated with infection processes but might not be necessary for disease development. Alternatively low temperatures may promote the disease by depressing defense activities of the trees.

Frost injury to kiwifruit has been reported in New Zealand to produce damage on trunks and branches similar to the canker disease\(^5\). We have also compared frost injury in Japan with the canker disease and we could easily differentiate them because the frost injury produced neither leaf spot symptoms nor bacterial ooze exudation.

Strong winds and heavy rainfall might also promote the disease, as recorded for many other bacterial plant diseases. Inoculation experiments showed that the pathogen can infect the plants through either stomata, hydathodes and wounds. Abundant exudation of bacterial ooze is characteristic of this disease. Strong winds during rain may both injure the plants and disperse the bacterial exudate to the infection sites. In fact, the severely affected orchards are concentrated in western and eastern parts of the prefecture where strong winds blow frequently,

<table>
<thead>
<tr>
<th>Treatment and concentration</th>
<th>Number of leaves investigated</th>
<th>Number of affected leaves</th>
<th>Disease severity a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin (200 ppm)</td>
<td>673</td>
<td>26 (3.9%)</td>
<td>0.5 a</td>
</tr>
<tr>
<td>Kasugamycin (50 ppm)</td>
<td>690</td>
<td>48 (7.0%)</td>
<td>1.3 a</td>
</tr>
<tr>
<td>Copper hydroxide (270 ppm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Calcium carbonate (475 ppm)</td>
<td>669</td>
<td>100 (14.9%)</td>
<td>3.2 a</td>
</tr>
<tr>
<td>Control (no bactericides)</td>
<td>769</td>
<td>339 (44.1%)</td>
<td>14.8 b</td>
</tr>
</tbody>
</table>

\(n_A\) to \(n_E\) represents the number of the leaves of category A to E, respectively. Values within a column followed by the same letter are not significantly different according to Duncan’s multiple range test (\(P=0.05\)).
but the disease has not been observed in the orchards which are well protected with wind-hedges despite the fact that they were located adjacent to severely affected orchards.

Damage associated with the canker disease of kiwifruit occurs in two phases. The first phase occurs in winter and involves damage to the main vine structure and over-wintering canes. The second phase occurs in spring and involves the new season's growth; the leaves, flowers and canes. The first phase has direct effects on yield by reducing the size of the productive vine. By contrast, the second phase has less direct effect on yield but is relevant to disease dispersal. For example, in some orchards, it was observed that only the leaf spot symptoms occurred in spring, with small yield losses, but that severe damage to trunks and branches occurred the following winter, causing serious yield losses. Because the two phases are closely correlated with each other, it may be possible to forecast the damage to trunks and branches from disease development in the previous growing season. Our field observations suggest that the severity of leaf damage in the previous spring, and temperature in winter may be good indicators of the damage to be expected in the following growing season. It is also probable that the application of bactericides in spring and fall may reduce damage to trunks, leaders and canes.

A similar diphasic disease progression was reported for bacterial canker of stone fruit caused by Pseudomonas syringae pv. syringae and pv. morsprunorum1,2). The symptoms, pathogen, and disease cycle of bacterial canker of kiwifruit have many similarities with bacterial canker of stone fruit. A detail comparison of these diseases may illuminate factors affecting bacterial canker of kiwifruit.

The pathogenicity of the kiwifruit pathogen was also confirmed on Actinidia arguta, a wild plant commonly found in Japan. Recently, a bacterial disease of A. arguta, similar to the bacterial canker of kiwifruit, was reported6). It is not clear whether the disease of kiwifruit preceded that of A. arguta or not, however, A. arguta may play as an alternative host in the transmission of the disease.

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Literature cited

和文摘要
芹沢伸夫・市川健・瀧川雄一・露無憲二・後藤正夫：わが国におけるキウイフルーツかいよう病の発生

1984年ころより、静岡県においてキウイフルーツ（Actinidia chinensis）に新しい細菌病の発生が認められた。本病の病徵は大々く二つの相に分けられた。一つは冬季から春先にかけて発生するもので、樹幹や枝に亀裂を生じ、赤褐色の溢出物が認められる。同時に、外観は健全な葉芽や葉痕、剪定痕、枝の分岐点などに白色ないし赤褐色の細菌痕の溢出も認められる。第二は春末から初夏にかけてで、新たに展開した葉にまず水浸症状を形成し、やがて膨大して大きさ2〜3 mmの赤褐色の角巻となり黄色のヘロイを伴う。同時に、新梢には亀裂を生じて潰瘍状を呈し、やがて先端は萎縮枯死する。花芽にも感染が認められ、枯死あるいは花腐れ状を呈する。葉も新梢、花蕾、花果の病変上にも白色の菌斑が認められる。これらの病組織および菌斑より分離を行ったところ、つねに一定の白色細菌が得られた。分離細菌は有傷接種、無傷接種ともにキウイフルーツおよびサルサン（A. arguta）に対して強い病原性を有しており、自然感染の病徵を再現した。葉位別にキウイフルーツ葉の感受性を調べたところ、成熟直前のものが最も感受性が高く、より若いものや完全
Explanations of plates

Plate I. Symptoms of bacterial canker on trunks, leaders and canes of kiwifruit occurring from winter to early spring.
1. Whole view of an affected tree with abundant red-rusty brown exudates on trunk (March).
7. Prolific suckers arising from around the base of a vine (July).

Plate II. Symptoms on newly developed shoots of kiwifruit from mid spring to summer.
1. Leaf spots on mature vine surrounded by small halos (May).
2. Leaf spots on seedling surrounded by large halos (May).
3. Small necrotic spots formed in summer without halos (July).
4. Water-soaked appearance of lesions on the lower side of a leaf with numerous ooze droplets, as seen during wet weather (May).
5. Bacterial ooze exudation from the lenticels of an affected shoot (May).
6. Cracks and cankers formed on an affected shoot with ooze exudation (May).
7. Bacterial ooze exudation at the base of a newly developed shoot (April).

Plate III. Symptoms on flower buds of kiwifruit.
1. Affected flower buds with necrotic sepals (right) and healthy bud (left) (May).
2. Bacterial ooze droplet on a sepal (May).
3. Affected sepals and petals not fully developed (June).
4. Flower buds affected by canker disease (upper two), and by blossom rot disease (lower five) (June).
The symptoms of canker disease are characterized by necrotic sepals and apparently healthy petals at early stage of infection. In contrast, the symptoms of blossom rot disease are characterized by browning of petals and healthy sepals.

Inoculations on Actinidia arguta with the canker pathogen.
5. Water-soaked spots formed on developing leaves (April).
Plate I
Plate II

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Plate III