RES. BULL. PL. PROT. JAPAN No. 41: 65～70 (2005)

Short Communication

Transmission of *Erwinia amylovora* from Blighted Mature Apple Fruit to Host Plants via Flies

Takanori TSUKAMOTO, Hiroshi UEMATSU*, Akifumi MIZUNO* and Shigeru KIMURA*

Yokohama Plant Protection Station
1-16-10, Shinyamashita, Nakaku, Yokohama 231-0801, Japan
*Research Division, Yokohama Plant Protection Station

Abstract: The possibility of transmission of *Erwinia amylovora* from infected mature apple fruit to host plants via flies was investigated. *E. amylovora* was detected from the bodies of 13 of 23 greenbottle flies that contacted blighted mature apple fruit under an experimental condition. An average of 2.6×10^2 cfu of *E. amylovora* was isolated from the bodies of the flies. The flies, contaminated with large numbers of *E. amylovora*, carried the bacteria to wounded young fruit and shoots of European pear, and consequently caused fire blight.

Key words: *Erwinia amylovora*, Fire blight, Apple fruit, Fly

Introduction

Fire blight, caused by *Erwinia amylovora* (Burrill 1882) Winslow *et al.* 1920, is an extremely destructive bacterial disease of apple, pear and other plants of the family Rosaceae. Many reports have speculated that long-range dissemination of *E. amylovora* was caused by movement of the nursery stock, budding or grafting material from infected plants (BONN, 1979; KIMURA *et al.*, 2005; VAN DER ZWET and WALTER, 1996), and that short-range dissemination was caused by rain, wind, birds, insects or spiders (SCHROTH *et al.*, 1974; VAN DER ZWET and KEIL, 1979). Some researchers considered water to be the most important agent for fire blight dissemination (BROOKS, 1926; GOSSARD and WALTON, 1922), whereas others considered insects to be of equal or greater importance (SCHROTH *et al.*, 1974; VAN DER ZWET and BEER, 1992). VAN DER ZWET and BEER (1992) described that *E. amylovora* may be carried by wind, rain, and insects, from overwintering cankers to blossoms or young shoots, where infection may start. THOMAS and ARK (1934) showed that ants and flies transmitted *E. amylovora* from bacterial ooze on pear-blight cankers to flowers.

Recently, TSUKAMOTO *et al.* (2005) and KIMURA *et al.* (2005) suggested the possibility that mature apple fruit infected with *E. amylovora* could be a mode of long-range dissemination. As such, we investigated the possibility of short-range dissemination of *E. amylovora* from infected mature apple fruit to host plants via flies. The objectives of this study were to determine if flies would visit blighted apple fruit infected with *E. amylovora*, and thus, if they could attach the bacterium to their bodies, such that, when visiting the fruit, they can cause fire blight symptoms host plants.

Materials and Methods

Bacterial strain and flies used in this study

E. amylovora strain ICMP 1499, isolated from an apple tree in New Zealand, was imported under permission MAFF 5Y2501 and used. The strain was cultured at 26℃ for 72 hours in bacto-Nutrient Broth medium supplemented with 5% sucrose and 0.5% bacto-Yeast Extract.

Four-day-old greenbottle flies, *Protophormia terraenovae*, were used. Also, this study was implemented in a strictly confined laboratory.


1

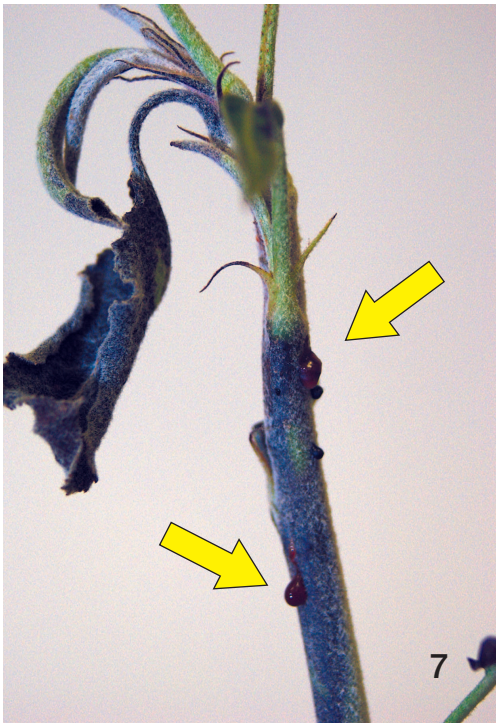

2


3


4


5


6


7


8


9

Detection of *E. amylovora* from flies that contacted blighted apple fruit

A commercially produced mature apple fruit (cv. Sun-Fuji) was inoculated with 1 ml of *E. amylovora* suspension (ca. 10^8 cfu/ml), by means of a hypodermic syringe, followed by incubation at 26℃ for 38 days. It caused fruit blight with exuding liquid on the base of pedicel (Fig. 1). The fruit was cut horizontally along an equatorial plane and was used as inoculum. The lower part of the apple was put into a beaker (volume, 1 litter), and then 23 flies were released into the beaker. The beaker was covered with flannel, to prevent escape of flies. After six hours, each fly was separately put on modified-MS medium (M-MS) (MIZUNO *et al.*, 2002) plates, and the plates were incubated at 26℃ for 14 days. The colonies of *E. amylovora* that formed on the plates were confirmed by each of examining colony morphology on M-MS plates, serological characteristics by slide agglutination test using antiserum against *E. amylovora* (SUETSUGU *et al.*, 1981), and pathogenicity to young Asian pear fruit (cv. Kousui) in accordance with the method of SHOLBERG *et al.* (1988).

Epiphytic population of *E. amylovora* on the flies

A commercially produced mature apple fruit (cv. Fuji) was inoculated with strain ICMP 1499, the same as above, and kept at 26℃ for 44 days. Ten flies were put into the flannel-covered beaker with a lower part of horizontally cut fruit. After six hours, each fly was collected separately and killed in a freezer, and washed with sterilized water. The serially diluted washing waters were spread on M-MS plates, and the plates were incubated at 26℃ for 7 days. The formed colonies of *E. amylovora* were counted. Identification of these colonies was conducted using the methods described above.

Infection of host by flies contaminated with *E. amylovora*

Forty-eight flies were anesthetized with carbon dioxide spray and soaked in bacterial suspension, prepared to a concentration of ca. 10^9cfu/ml, in a beaker. After 10 minutes, a piece of sponge was put in the beaker, and the beaker was placed in an acryl box (W, 41cm; D, 61cm; H, 70cm) with host plants of 5 young fruit and 3 vegetative shoots of European pear (cv. Nouveau Poiteau), and 3 young fruit and 3 vegetative shoots of apple (cv. Fuji). Regarding the young fruit, they were used while attached to branches. The tips of the shoots and the surfaces of the fruit were pricked by sterilized needles. Infection with *E. amylovora* was confirmed by fire blight symptoms that developed on host plants and isolation of the bacterium.

Infection frequency of young pear fruit with *E. amylovora* transmitted by flies

Thirty-four flies were soaked in bacterial suspension (ca. 10^9 cfu/ml) as mentioned above. These flies were put into the acryl box with 60 young pear fruit (cv. Brandywine), which had part of the skin peeled out roundly (ca. 1cm in diameter) with a sterilized knife. Infection with *E. amylovora* was confirmed by fire blight symptoms that developed on the fruit and isolation of the bacterium.

Figure legends

Fig.1. Blighted Sun-Fuji mature apple fruit inoculated with *E. amylovora*. The fruit was kept at 26℃ for 38 days after inoculation. Exuding liquid on the base of pedicel was observed.

Fig.2. Flies in a beaker visited the flesh section of the lower part of blighted fruit. Flies fed on liquid on the flesh.

Fig.3. Flies that contacted the flesh were put on M-MS plates. Numerous bacterial colonies were formed on the plates.

Fig.4. The results of pathogenicity test for isolated colonies using Kousui Asian pear fruit. The right fruit section was inoculated with water, as a negative control.

Fig.5. A fly on Nouveau Poiteau European pear fruit. The fruit were used while attached to branches.

Fig.6. Lesion on Nouveau Poiteau pear fruit after 5 days of contact with flies. The area around the pricked points turned black.

Fig.7. Blight symptoms on a shoot of Nouveau Poiteau pear were observed at 7 days after contact with flies. Bacterial ooze exuded from pricked points (as indicated by arrows).

Fig.8. A contaminated fly on Brandywine young pear fruit.

Fig.9. Three days after the contact with the flies, blackening of the peeled points of Brandywine pear fruit were observed.

Results

Detection of *E. amylovora* from flies that contacted with blighted apple fruit

Flies in a beaker visited the flesh section of the lower part of blighted apple and fed on it (Fig. 2). Collected flies from the beaker formed numerous bacterial colonies on M-MS plates (Fig. 3). The results of slide agglutination test and pathogenicity test (Fig. 4) for bacterial colonies on the plate, showed that *E. amylovora* was detected in 13 of 23 flies (56.5%).

Epiphytic population of *E. amylovora* on the flies

Seven days after the incubation of M-MS plates, epiphytic *E. amylovora* was recovered from 7 of 10 flies. The populations of bacteria were 5.0×10^{1} to 5.5×10^{2} cfu per fly (2.6×10^{2} cfu, on average).

Infection of host by the flies contaminated with *E. amylovora*

The wings of contaminated flies became dry after they climbed onto the sponge, and the flies visited the young fruit and shoots (Fig. 5). Four days after the visiting, bacterial ooze exuded from pricked points on Nouveau Poiteau pear fruit. These points and their peripheral areas turned black with oozing in 5 days after the contact by flies (Fig. 6), and the fruit became black all over in 7 days. Four of five pear fruit were observed to turn black. Slide agglutination test clarified that bacterial colonies on M-MS plates isolated from the ooze on these lesions were *E. amylovora*. After 7 days, the area around the pricked point of a shoot of pear turned black, and brown- to black-colored bacterial ooze exuded (Fig. 7). *E. amylovora* was also isolated from the ooze. No fire blight symptoms were observed on young apple fruit and shoots of Fuji apples in the same box.

Infection frequency of young pear fruit with *E. amylovora* transmitted by flies

Some flies visited the peeled Brandywine pear fruit (Fig. 8). Three days after the contact with the flies, blackening of the peeled points was observed (Fig. 9). After seven days, 13 of 60 fruit (21.7%) were blighted, and some fruit exuded bacterial ooze from the peeled part of fruit. *E. amylovora* was isolated from all blighted fruit.

Discussion

VAN DER ZWET and BEER (1992) stated that flies, wasps, ants and other crawling insects often contact or feed on ooze and become contaminated with *E. amylovora*, and contaminated insects may then carry the pathogen to natural infection courts. In this study, we showed that flies that contacted blighted mature apple fruit attached *E. amylovora* to their bodies. MILLER and SCHROTH (1972) examined the presence of *E. amylovora* on insects that collected from blighted pear orchard, and showed that 56% of the insects, *Pegamya* sp., *Minettia* sp., and syrphid flies, carried *E. amylovora*, ranging from ca. 10^{1} to 10^{5} cells per insect.

Flower blight symptoms by blossom infection occur with a small inoculum. THOMSON (1986) reported that 62% of flowers showed fire blight symptoms, under outdoor condition, when the stigmas of pear were inoculated with 5.6×10^{3} cfu of *E. amylovora*. NORELLI and BEER (1984) stated that flower blight was observed with as few as about 7×10^{2} cfu of *E. amylovora* per apple blossom. Tsukamoto *et al.* (unpublished data) performed inoculation studies of apple flowers, and found that ca. 10^{2} cfu of *E. amylovora* per stigma caused blossom blight with oozing from pedicel. Further, HILDEBRAND (1937) reported that inoculation of hypanthium of apple flowers with single cells of *E. amylovora* resulted in infection. There is a record that greenbottle flies used in this study visited apple flowers (Iwate Horticultural Experiment Station, 1979), and under laboratory condition, we showed that the flies attached, on average, 2.6×10^{2} cfu of *E. amylovora* to their bodies. Therefore,

the probability of short-range dissemination of *E. amylovora* from infected mature apple fruit to host plants in an orchard, garden, park, etc., by flies, would be not so low. Although it was an extreme example, we confirmed that flies contaminated with large numbers of *E. amylovora*, by soaking them in bacterial suspension, caused fire blight in young fruit and shoots of European pear.

Acknowledgements

We thank Drs. Kouji Yoshida (National Institute of Fruit Tree Science, Tsukuba), Kazuyuki Abe (National Institute of Fruit Tree Science, Morioka), and Yuji Ito (National Agricultural Research Center for Hokkaido Region, Sapporo) for provision of young pear fruit, and Tetsuo Imamura (Yokohama Plant Protection Station, Yokohama) for rearing of flies, and Ren Iwaizumi (Yokohama Plant Protection Station, Narita Sub-station) for identification of flies.

References Cited

BONN, W.G. (1979) Fire blight bacteria in symptomless dormant apple and pear buds. *Can. J. Plant Pathol.* 1: 61-62.

BROOKS, A.N. (1926) Studies of the epidemiology and control of fireblight of apple. *Phytopathology* 16: 665-696.

GOSSARD, H.A. and R.C. WALTON (1922) Dissemination of fire blight. *Ohio Agr. Exp. Sta. Bull.* 357: 81-126.

HILDEBRAND, E.M. (1937) Infectivity of the fire-blight organism. *Phytopathology* 27: 850-852.

Iwate Horticultural Experiment Station (ed.) (1979) Chapter 1: Current state and problem of using flower visiting insect. *Bull. of the Iwate Hort. Exp. Sta.* special issue No.1: 5-45.

KIMURA, S., H. KATSUMATA, S. SAITO, T. TSUKAMOTO, A. MIZUNO, K. YOSHIDA, K. SUYAMA and M. GOTO (2005) The probability of long-distance dissemination of bacterial disease via fruit. *J. Gen. Plant Pathol.* 71: (in press)

MILLER, T.D. and M.N. SCHROTH (1972) Monitoring the epiphytic population of *Erwinia amylovora* on pear with a selective medium. *Phytopathology* 62: 1175-1182.

MIZUNO, A., T. TSUKAMOTO and A. KAWAI (2002) Detecting method of *Erwinia amylovora* from internal tissues of mature apple fruits. *Res. Bull. Pl. Prot. Japan* Supplement 38: 9-12.

NORELLI, J.L. and S.V. BEER (1984) Factors affecting the development of fire blight blossom infections. *Acta. Hort.* 151: 37-39.

SCHROTH, M.N., S.V. THOMSON and D.C. HILDEBRAND (1974) Epidemiology and control of fire blight. *Annu. Rev. Plant Pathol.* 12: 389-412.

SHOLBERG, P.L., A.P. GAUNCE and G.R. OWEN (1988) Occurrence of *Erwinia amylovora* of pome fruit in British Columbia in 1985 and its elimination from the apple surface. *Can. J. Plant Pathol.* 10: 178-182.

SUETSUGU, T., S. SATOH, M. TAKAYAMA and A. YAMAUCHI (1981) Studies on the diagnosis of foreign bacterial plant diseases of major plant quarantine. Ⅱ. Detection of *Erwinia amylovora*. *Res. Bull. Pl. Prot. Japan* 17: 77-85.

THOMAS, H.E. and P.A. ARK (1934) Fire blight of pears and related plants. Univ. of California Agr. Exp. Sta. Bull. 586, Berkeley, California.

THOMSON, S.V. (1986) The role of the stigma in fire blight infections. *Phytopathology* 76: 476-482.

TSUKAMOTO, T., K. AZEGAMI, T. MATSUURA, T. OHARA, Y. INOUE, A. MIZUNO, K. YOSHIDA, H. BESSHO, S. KIMURA and M. GOTO (2005) Infection frequency of mature apple fruit with *Erwinia amylovora* deposited on pedicel and its survival in the fruit stored at low temperature. *J. Gen. Plant Pathol.* 71: (in press)

VAN DER ZWET, T. and H.L. KEIL (1979) Fire blight -a bacterial disease of rosaceous plants. USDA Agriculture Handbook No.510, Science and education administration, Washington DC.

VAN DER ZWET, T. and S.V. BEER (1992) Fire blight -its nature, prevention, and control: A practical guide to integrated disease management. USDA Agriculture Information Bulletin No.631, United States Department of Agriculture, Washington DC.

VAN DER ZWET, T. and J. WALTER (1996) Presence of *Erwinia amylovora* in apparently healthy nursery propagating material. *Acta. Hort.* 411: 127-130.

和 文 摘 要

火傷病感染リンゴ成熟果実から宿主植物へのハエを介した伝搬

塚本貴敬・上松　寛*・水野明文*・木村　茂*
横浜植物防疫所・*横浜植物防疫所調査研究部

火傷病感染リンゴ成熟果実から宿主植物へのハエを介した伝搬の可能性について調査を行った。果実腐敗を生じた成熟リンゴ果実に接触したルリキンバエ23頭中13頭から火傷病菌が検出された。ルリキンバエの虫体からは平均で2.6×10^{2}cfuの火傷病菌が分離された。多量の火傷病菌に汚染したルリキンバエは付傷した洋ナシ幼果及び新梢に火傷病菌を伝搬し、火傷病症状を引き起こした。