A Comparison of Inoculation Methods of *Erwinia chrysanthemi* in Greenhouse Ornamentals

R. A. HAYGOOD, Graduate Research Assistant, and D. L. STRIDER, Professor, Department of Plant Pathology, North Carolina State University, Raleigh 27650

ABSTRACT

Haygood, R. A., and Strider, D. L. 1982. A comparison of inoculation methods of *Erwinia chrysanthemi* in greenhouse ornamentals. Plant Disease 66:461-463.

Concentrations of 2×10^6 and 2×10^8 colony-forming units per milliliter and undiluted inoculum of *Erwinia chrysanthemi* were inoculated to wounded and unwounded, attached and detached leaves of 29 greenhouse ornamentals. *Asplenium nidus, Begonia semperflorens, Begonia* × *hiemalis, Brassaia actinophylla, Crassula argentea, Kalanchoe blossfeldiana, Pelargonium* × *hortorum*, and *Peperomia argyreia* were new hosts of *E. chrysanthemi*. Unwounded attached and detached leaves of *A. nidus, C. argentea, K. blossfeldiana*, and *Philodendron selloum* were susceptible to *E. chrysanthemi* when inoculated with high inoculum concentrations, but wounding was usually required for infection of other cultivars. Stab-inoculation of stems and inoculation of wounded attached leaves with undiluted inoculum resulted in similar responses except for *Caladium* × *hortulanum, Chrysanthemum morifolium, Dracena marginata, Dracena sanderiana, Euphorbia pulcherrima, Pelargonium* × *hortorum*, and *Peperomia argyreia*. Wounded detached leaves in sand of *Caladium* × *hortulanum, Coleus blumei, E. pulcherrima*, and *Pelargonium* × *hortorum* became infected with undiluted inoculum, but attached leaves did not. Wounded leaves of several species developed extensive soft rot in petri plates but did not become infected in sand.

Additional key words: bacterial blight, floral crops, foliage plants

Erwinia chrysanthemi Burkholder, McFadden and Dimock (*Ec*) is a threat to the production of many plants. A list of plant species from which *Ec* has been isolated and an extensive literature review were recently published (2).

A recent study (Haygood and Strider, *unpublished*) indicated that *Ec* would survive in association with the attached leaves of several greenhouse ornamentals for 5 mo. Leaves of several plants considered to be nonhosts were found to be susceptible to *Ec* when bacterial cells were placed on wounded leaves in petri plates. Because a variety of inoculation methods has been used to determine responses of plant species to strains of *Ec* (3,4), we believed that a study was needed to determine the response of several plant species to one strain of *Ec* using different artificial inoculation methods.

Paper 6856 of the Journal Series of the North Carolina Agricultural Research Service.

Use of trade names implies neither endorsement of the products by the North Carolina Agricultural Research Service nor criticism of similar ones not mentioned.

Present address of senior author: Extension Plant Pathologist, Mississippi Cooperative Extension Service, Mississippi State 39762.

Accepted for publication 24 July 1981.

0191-2917/82/06046103/$03.00/0

Objectives of this study were to determine i) the responses of wounded and unwounded, attached and detached leaves of 29 greenhouse ornamentals to *Ec* using undiluted inoculum (UI) and inoculum concentrations of 2×10^6 and 2×10^8 colony-forming units (CFU) per milliliter and ii) responses of plants to stab-inoculation of stems with UI.

MATERIALS AND METHODS

Plants used in this study (Table 1) were either supplied by Mikkelsens, Inc. (Ashtabula, OH 44004), Speedling, Inc. (Sun City, FL 33586), or Yoder Brothers, Inc. (Barberton, OH 44203), or they were purchased locally. They were planted in Metro Mix 220 (W. R. Grace and Co., Cambridge, MA 02138) in 10-cm-diameter pots, fertilized with Peters 20-20-20 (2.6 g/L of water) weekly, and overhead irrigated twice a day for 8–12 wk before inoculation.

Ec strain Cu 242, isolated from *Philodendron selloum* Koch leaf tissue in Florida by J. F. Knauss (obtained from R. S. Dickey, Cornell University), was used in this study. The UI consisted of a loopful (0.2 cm diameter) of cells from 48-hr lima bean agar cultures. Inoculum concentration of 2×10^6 and 2×10^8 CFU/ml were determined turbidimetrically.

Four methods were used to inoculate 29 plant species. Sterile distilled water controls were used with each inoculation technique, and at least two replications were made by each method.

Method 1: Sterile wooden toothpicks were infested with cells from 48-hr lima bean agar cultures and then stabbed into stems of two plants of each species listed in Table 1.

Method 2: Twelve leaves of each species were wounded at the center of the blade by a multineedle device (6). Each inoculum concentration was applied to three wounded and three unwounded leaves. The UI was gently applied with a loop to wounds or to an area approximately 1.5 cm in diameter in the center of both surfaces of unwounded leaves. Bacterial suspensions were atomized onto leaves to runoff.

Method 3: Twenty-four leaves of each plant species (12 of which were wounded with the multineedle device) were detached and placed in sand in wood flats measuring 8 × 38 × 55 cm such that they had approximately the same orientation as attached leaves. Each inoculum concentration was then applied to three wounded and three unwounded leaves of each species as in the second method.

Method 4: Sixteen leaves of each plant species were detached, trimmed, and placed on moistened filter paper in 8-cm petri plates. Eight of the leaves were wounded with the multineedle device and each inoculum concentration was applied to two wounded and two unwounded leaves of each species as in the second method.

Stab-inoculated plants, plants with inoculated attached leaves, inoculated detached leaves in sand in flats, and detached inoculated leaves in petri plates were placed in a chamber that was maintained at 20–30 C and at 100% RH by intermittent mist. After 72 hr they were placed on a bench in a greenhouse at 20–30 C and 60–100% RH.

The disease rating scale for wounded leaves was −, +, or ++, where − = water-soaked lesions absent or restricted to the wounded area, + = water-soaked lesions developed within and extended 0.1 to 1 cm outside the wounded area, and ++ = water-soaked lesions developed and extended more than 1 cm outside the wounded area. Unwounded leaves were rated positive only if water-soaked lesions developed from the interior portions of the leaves or from the uncut leaf margins. Stab-inoculated plants were rated positive when water-soaked lesions developed and spread beyond the point of inoculation. Disease was rated 3 and 5 days after inoculation.

RESULTS

Water-soaked lesions appeared within 3 days after inoculation with all inoculum concentrations on unwounded attached and detached leaves of bird's nest fern and split-leaved philodendron and with the two higher inoculum concentrations on jade plant and kalanchoe. Symptoms appeared within 3 days on the unwounded detached leaves of geranium in petri plates inoculated with the UI and 2×10^8 CFU/ml, but symptoms appeared on the unwounded detached leaves in sand only when inoculated with UI. Negative ratings were recorded for unwounded leaves of remaining plants (Table 1) regardless of inoculum concentration or inoculation method. Disease reaction of wounded, detached leaves incubated in sand was similar to that recorded for wounded, attached leaves for all species with a few exceptions. Disease reaction was positive for wounded attached leaves of caladium, coleus, geranium, and poinsettia incubated in petri plates but negative when incubated in sand. A positive reaction (+) was observed in kalanchoe and wax begonia in sand, and the reaction was more pronounced (++) in leaves incubated in petri plates.

Results of stab-inoculation of stems with UI and inoculation of wounded attached and detached leaves with the three inoculum concentrations are summarized in Table 1. Disease ratings were made 3 days after inoculation of wounded attached and detached leaves. The same ratings were usually recorded 5 days after inoculation but are not presented.

The lower inoculum level (2×10^6 CFU/ml) produced positive ratings in wounded, detached leaves of many species in petri plates but only for detached leaves in sand and attached leaves of bird's nest fern, jade plant, and split-leaved philodendron. Disease reaction was positive for wounded, detached leaves of carnation, dumb cane, wax plant, emerald-ripple peperomia, and arrowhead plant leaves in petri plates inoculated with 2×10^8 CFU/ml but negative when inoculated with 2×10^6 CFU/ml. For several plants such as silver evergreen, wax begonia, and coleus, a negative response was recorded for attached and detached leaves in sand inoculated with 2×10^6 and 2×10^8 CFU/ml, although a positive response was recorded for corresponding detached leaves in petri plates.

The same response usually occurred on attached leaves and detached leaves in sand regardless of inoculum concentration. This was not the case, however, for caladium, coleus, poinsettia, and geranium leaves; wounded detached leaves in sand developed symptoms with the high inoculum concentrations but attached leaves did not.

Symptoms of inoculated detached leaves in petri plates were similar to those that developed in attached leaves in nature. However, disease ratings of detached leaves in petri plates were equal to or greater than those of attached leaves or detached leaves in sand. Leaves of the arrowhead plant and emerald-ripple peperomia were rated positive only in petri plates with the two higher inoculum concentrations.

Similar responses resulted from stab-

Table 1. Response of greenhouse ornamentals to *Erwinia chrysanthemi* inoculated by different methods

Species	Inoculum (CFU/ml)[a]	Wounded leaves[b]		Stems[c] (stab inoculated)
		Attached	Detached in petri plates	
Aglaonema commutatum Schott.	2×10^8	−	++	
(silver evergreen)	UI	+	++	+
Aglaonema commutatum Schott.	2×10^8	−	++	
'Treubii' (Treub's aglaonema)	UI	+	++	+
Aglaonema modestum Schott.	2×10^8	−	−	
(Chinese evergreen)	UI	−	+	−
Asplenium nidus L.	2×10^8	++	++	
(bird's nest fern)	UI	++	++	+
Begonia semperflorens Link &	2×10^8	−	++	
Otto 'Charm' (wax begonia)	UI	+	++	+
Begonia × *hiemalis* Fotsch.	2×10^8	−	++	
'Schwabenland red' (Reiger begonia)	UI	+	++	+
Brassaia actiniphylla Endl.	2×10^8	+	++	
(schefflera)	UI	++	++	+
Caladium × *hortulanum* Birsey	2×10^8	−	++	
(caladium)	UI	−	++	+
Chrysanthemum morifolium Ramut.	2×10^8	−	−	
'Bright Golden Anne' (chrysanthemum)	UI	−	−	+
Coleus blumei Benth.	2×10^8	−	++	
(coleus)	UI	−	++	−
Crassula argentea Thunb.	2×10^8	++	++	
(jade plant)	UI	++	++	+
Cyclamen persicum Mill.	2×10^8	−	−	
(cyclamen)	UI	+	+	+
Dianthus caryophyllus L.	2×10^8	−	+	
'Scania' (carnation)	UI	+	++	+
Dieffenbachia sp. 'Exotica'	2×10^8	−	+	
(dumb cane)	UI	+	++	+
Dracena marginata Lam.	2×10^8	+	++	
(red-margined dracena)	UI	++	++	−
Dracena sanderiana Sander.	2×10^8	−	−	
(ribbon plant)	UI	+	+	−
Epipremnum aureum (Linden and	2×10^8	+	++	
Andre) Bunt. (Pothos)	UI	++	++	+
Euphorbia pulcherrima Willd.	2×10^8	−	++	
'Rochford' (poinsettia)	UI	−	++	+
Hoya carnosa R. Br.	2×10^8	+	++	
(wax plant)	UI	++	++	+
Kalanchoe blossfeldiana Poellnitz	2×10^8	+	++	
'Serenade' (kalanchoe)	UI	++	++	+
Maranta leuconeura E. Morr.	2×10^8	−	−	
(prayer plant)	UI	−	−	−
Pelargonium × *hortorum* L. H. Bailey	2×10^8	−	++	
'Cherry Glow' (geranium)	UI	−	++	+
Peperomia argyreia E. Morr.	2×10^8	+	++	
(watermelon peperomia)	UI	++	++	−
Peperomia caperata Yurck.	2×10^8	−	+	
(emerald-ripple peperomia)	UI	−	++	−
Philodendron scandens C. Koch &	2×10^8	−	−	
Sello subsp. *oxycardium* (Schott)	UI	−	−	−
Bunt (heart-leaf philodendron)				
Philodendron selloum Koch.	2×10^8	++	++	
(split-leaved philodendron)	UI	++	++	+
Pilea cadierei Gagnep & Guill.	2×10^8	−	−	
(aluminum plant)	UI	−	−	−
Saintpaulia ionantha H. Wendl.	2×10^8	+	++	
'Helga' (African violet)	UI	++	++	+
Syngonium podophyllum Schott.	2×10^8	−	+	
(arrowhead plant)	UI	−	++	−

[a] Undiluted inoculum (UI) consisted of bacterial cell masses.

[b] Wounded leaves were rated as positive (+) or negative (−) where − = water-soaked lesions absent or restricted to the wounded area, + = water-soaked lesions developed 0.1 to 1 cm outside the wounded area, and ++ = water-soaked lesions developed more than 1 cm outside the wounded area.

[c] Stems were stab-inoculated by using toothpicks infested with UI and were rated positive when water-soaked lesions developed and spread beyond the point of inoculation.

inoculation of stems and inoculation of attached leaves with UI. However, stems of caladium, chrysanthemum, poinsettia, and geranium were susceptible, but attached leaves were not. Also, attached leaves of red-margined dracena, ribbon plant, and watermelon peperomia were susceptible, but no symptoms developed on stems.

DISCUSSION

Schefflera, watermelon, peperomia, bird's nest fern, kalanchoe, wax begonia, Reiger begonia, and jade plant were susceptible to *Ec* strain Cu 242. *Erwinia* spp. have been isolated from the latter three plants in Florida (*personal communication*, J. W. Miller, Division of Plant Industry, Gainesville, FL 32602), but we are not aware of any published reports on the susceptibility of any of these plants to *Ec*.

Miller and McFadden (8) obtained no response when they inoculated wounded schefflera leaves with *Ec*. Coleus, ribbon plant, prayer plant, emerald-ripple peperomia, Chinese evergreen, heart-leaf philodendron, aluminum plant, and arrowhead plant were not susceptible to the *Ec* strain used in this study. Miller and McFadden (8) also found that the latter four plants were not susceptible to an *Ec* strain obtained from split-leaved philodendron. However, in a later study, McFadden (7) found Chinese evergreen to be susceptible to an *Ec* strain obtained from *Aglaonema pictum*. Strains of *Ec* have been isolated from emerald-ripple peperomia and Chinese evergreen in Florida (J. W. Miller, *personal communication*). Dickey (3) has demonstrated that strains of *Ec* obtained from various sources often differ in host ranges, which probably explains variation in the susceptibility of these two plants to *Ec*.

Wounding enhanced or was required for infection of leaves of most plants at all inoculum concentrations. However, the nonwounded leaves of kalanchoe, jade plant, bird's nest fern, and split-leaved philodendron were very susceptible to *Ec* at the higher inoculum concentrations under the conditions of this study. The latter three plants were also the only plants to show a positive response when the wounded attached and wounded detached leaves in sand were inoculated with the lower inoculum concentration. The stems of chrysanthemum, caladium, poinsettia, and geranium were susceptible when inoculated with UI, but the wounded attached leaves were not. However, leaves of the latter three species were rotted by *Ec* when they were detached, wounded, and placed in sand or petri plates. Because the wounded attached leaves and wounded detached leaves in sand were inoculated and maintained alike, it is probable that a biochemical phenomenon is responsible for the variations in host reaction.

In most species, disease development was increased when the wounded leaves were placed in petri plates. The leaves of arrowhead plant and emerald-ripple peperomia were rotted by *Ec* only after they were detached, wounded, and placed in petri plates. Because this phenomenon was not observed among the wounded attached and wounded detached leaves in sand, it is possible that the build-up of some volatile compound, such as ethylene, inside petri plates was responsible for the increased tissue disintegration of leaves wounded by *Ec*.

We do not contend that new hosts can be determined by the reaction of wounded, detached leaves alone. However, similar methods have been used (1,5,9,10) and can provide a quick and convenient source of preliminary and substantiative information.

Because of limitations in time, space, and materials, we used only one *Ec* strain, which was isolated from philodendron in Apopka, FL, by J. D. Knauss and is believed to be representative of the population of the pathogen. However, we would not expect to obtain the same results with each *Ec* strain since *Ec* strains obtained from different species as well as from the same species often vary in host ranges (3).

In this study we have identified several new hosts of *Ec* and demonstrated that the response of many species to *Ec* depends on the inoculation method used. Because detached leaves are sometimes used to study efficacy of fungicides (1,5), resistance (10), and disease development (9), researchers should be cognizant that responses of attached and detached leaves to pathogens may vary.

ACKNOWLEDGMENT

We thank Christie Williford for technical assistance.

LITERATURE CITED

1. Benson, D. M. 1980. Chemical control of rhododendron dieback caused by *Phytophthora heveae*. Plant Disease 64:684-686.
2. Dickey, R. S. 1979. *Erwinia chrysanthemi*: A comparative study of phenotypic properties of strains from several hosts and other *Erwinia* species. Phytopathology 69:324-329.
3. Dickey, R. S. 1981. *Erwinia chrysanthemi*: Reaction of eight plant species to strains from several hosts and to strains of other *Erwinia* species. Phytopathology 71:23-29.
4. Haygood, R. A., and Strider, D. L. 1981. Influence of moisture and inoculum concentration on infection of *Philodendron selloum* by *Erwinia chrysanthemi*. Plant Disease 65:727-728.
5. Horn, D. L. 1964. A detached cucumber leaf method of screening fungicides for the control of anthracnose. Plant Dis. Rep. 48:331-332.
6. Huisingh, D., and Durbin, R. D. 1965. A device for rapidly and uniformly inoculating plants with *Agrobacterium rhizogenes* and other bacteria that require wounds. Plant Dis. Rep. 49:878-879.
7. McFadden, L. A. 1969. *Aglaonema pictum*, a new host of *Erwinia chrysanthemi*. Plant Dis. Rep. 53:253-254.
8. Miller, H. N., and McFadden, L. A. 1961. A bacterial disease of philodendron. Phytopathology 51:826-831.
9. Morton, D. J. 1966. Bacterial spot development in excised pepper and tomato leaves at several temperatures. Phytopathology 56:1194-1195.
10. Ward, C. H. 1959. The detached-leaf technique for testing alfalfa clones for resistance to black stem. Phytopathology 49:690-696.