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Bacterial Blight of Geranium: A History of Diagnostic Challenges

Geraniums (*Pelargonium* spp.) have been an important part of greenhouse potted plant and bedding plant production for almost a century. Cultivars produced by vegetative propagation and by true seeds are grown and sold worldwide. In 1996, the wholesale value of geraniums exceeded \$205 million in the United States alone (2). *Pelargonium* species are affected by a number of fungal diseases, including rust (*Puccinia pelargonii-zonalis*), gray mold (*Botrytis cinerea*), and root rot (*Pythium* spp.). Several bacteria and numerous viruses also cause diseases on geranium. Indeed, the most destructive disease of geraniums is bacterial blight (*Xanthomonas campestris* pv. *pelargonii*). Although hybrid geraniums grown from seed are susceptible to bacterial blight, it is the vegetatively propagated cultivars of florists' geranium (*P. × hortorum*) and ivy geranium (*P. peltatum*) that are most commonly affected, because the causal organism inhabits the vascular tissue of infected plants and is carried in the cuttings. Munnecke (22) and Nichols (24) estimated 10 to 15% annual losses due to bacterial blight in the 1950s and 1960s, and such losses continue today (22,24). These loss figures are somewhat misleading because they are based on the industry-wide production of geraniums. In individual greenhouse operations, entire geranium crops have been destroyed by this disease. The reputations of several specialty propagators of *Pelargonium* have been ruined or severely damaged when these growers unknowingly sold infected cuttings and distributed them throughout the bedding plant industry.

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Despite efforts to eliminate this disease from geranium production systems, it continues to occur annually.

Symptoms of Bacterial Blight

Leaves. Spots may initially appear as small translucent or watery pustules, much like those characteristic of edema (Fig. 1). More frequently, small yellow spots (1 to 2 mm diameter) on the leaves gradually increase in size (2 to 3 mm), become irregular in shape, and become necrotic. The margins of the dead, dark brown, dry spots are well defined and seldom coalesce. Often, the individual dead spots are surrounded by yellow halos, or a large section



Fig. 1. Water-soaked spots on the underside of a *Pelargonium* leaf associated with the early-stage infection with *Xanthomonas campestris* pv. *pelargonii*.



Fig. 2. V-shaped leaf lesions characteristic of *Xanthomonas campestris* pv. *pelargonii*-infected *Pelargonium*.

of yellowed leaf may contain several necrotic spots. V-shaped yellow areas (Fig. 2) that later become necrotic are thought to develop when the bacteria enter via hydathodes. When infection of the vascular tissue has occurred, leaves on individual branches wilt and lack other symptoms (Fig. 3). Typically in *P. peltatum* (ivy geranium), entire leaves yellow, die, and fall but have no other symptoms. These symptoms mimic those caused by edema.

Stems. The vascular tissue of stems or branches with wilted leaves usually has brown discoloration. This can be indistinct. As the invasion continues, infected stems become brown or black but usually remain stiff as they desiccate (Fig. 4).

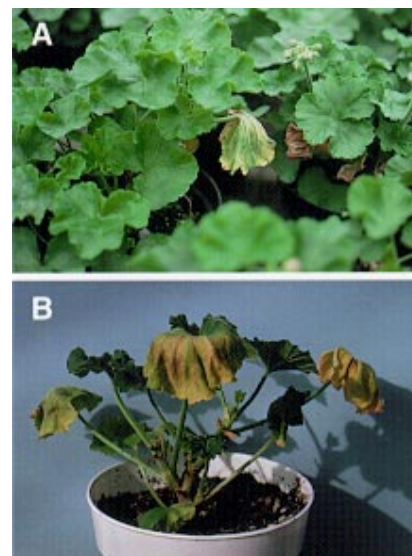


Fig. 3. Characteristic wilting in a single leaf (A) of a geranium in the greenhouse and in the whole plant (B), associated with *Xanthomonas campestris* pv. *pelargonii* infection. In early stages, affected plants may recover from wilting in the cool of the evening.

Signs of Bacterial Blight

When discolored vascular tissue, leaf spots, or the narrow end of V-shaped yellowed leaf lesions are excised and placed in water on a microscope slide, cloudy masses of bacteria ooze from the tissue (Fig. 5).

History of Bacterial Blight

Pelargonium species are believed to have been brought to Europe from South Africa in 1632 and were in use in gardens by the early 1700s (18). They played a significant role in ornamental landscapes and were sought by those involved in the increasingly popular glasshouse cultivation of exotic plants and the plant hybridization craze of the 1800s. Up to that time, there were no reports of diseases in Europe or the United States similar to bacterial blight on *Pelargonium*. Perhaps the extensive hybridization of *Pelargonium* resulted in new genotypes with increased susceptibility to bacterial blight. Lequet in France and Galloway in the United States described a stem disease that was later correlated with the leaf spot phase of the disease (12,19) and noted the presence of bacteria in the rotted stems. Stone and Smith (34) described the leaf spot phase, noting, "The leaves began to turn yellow in small spots, which gradually increased in size, the leaf tissue dying away at those points; thus the leaves soon became covered with dead spots of considerable size and lost their vitality completely." Although they found bacteria in abundance, they concluded, "We do not consider this a genuine disease of the geranium nor do we expect to find it occurring in the future." In 1907, however, Stone and Monahan wrote, "In a previous publication of this station a short note was made of a bacterial disease of geraniums occurring in the State (Massachusetts). It has appeared every year since it was first noted here in 1898 and has also been reported from other sections of the United States within recent years." That report (33) concluded, "...should it become more troublesome some remedy for it will have to be discovered."

Thus began a battle that continues today, waged by specialty propagators, greenhouse operators, government plant inspectors, university researchers, and consumers. It must be kept in mind that in the late 1800s and early 1900s, many people doubted that bacteria were truly plant pathogens. However, work on bacterial blight continued. Apparently, the leaf spot phase was most commonly observed and therefore most extensively studied. In 1914, Lewis reported a leaf spot disease on *Erodium* (wild crane's bill) and *Pelargonium* in Texas (20). Culture characteristics of the isolates and inoculation of plants indicated that the bacteria from *Erodium* and *Pelargonium* were identical and that the bacteria (named *Bacterium erodii*) attacked only *Erodium* and *Pelargonium*.

Brown noted that the circular to irregularly shaped spots form either at the leaf margin or on the blade of young or old leaves and that bacteria are easily isolated. She was able to produce typical spots within 4 days of inoculation by spraying bacteria onto the leaves of healthy geraniums when day temperatures were about 27°C. When similar inoculations were done in greenhouses, where temperatures ranged between 12 and 15°C, only a trace of infection was observed. Brown raised the question of whether her bacteria were the same as those of Lewis. Although she was not able to compare cultures, she concluded from an examination of Lewis' illustrations and descriptions that they were not the same and named her organism *Bacterium pelargonii*.

While Munnecke (22) provided a comprehensive description of symptom development, including the formation of V-shaped yellowed areas on the leaves, it was Hellmers who first described and illustrated the small watery "blisters" on *Pelargonium peltatum*, very much like edema, but which continue to develop into necrotic spots with "small yellowish-grey excrescence of dried-up bacterial slime" protruding at their centers (13). Bugbee and Anderson observed that at low temperatures (18 to 21°C), the leaf blisters remain present for 4 months but develop further when plants are moved to a higher temperature (5).

Further symptom descriptions were provided by Dodge and Swift, who noted black stem rot on the hybrid zonal geraniums at the New York Botanical Garden that appeared to begin along the middle or upper parts of the stem (10) (Fig. 4). Although they found bacteria, they made no attempt to ascertain morphological or cultural characteristics. Hellmers' detailed studies showed that the organism capable of causing the typical leaf spot can also cause stem rots like those described by Lequet, Galloway, Dodge, and Swift, and as described to him by Brown in a personal letter (13). Kivilaan and Scheffer (16)



Fig. 4. Black stem rot associated with the advanced stages of *Xanthomonas campestris* pv. *pelargonii*.

found that the disease is favored by high temperatures (21 to 27°C) and slowed by low temperature (10°C). They noted that external symptoms on stems may not become evident until the bacteria begin to invade the parenchyma. They also found that invasion could occur via roots.

Munnecke noted that the bacteria can survive in moist field soil for 3 months in California (22). He demonstrated that the bacteria are harbored in the vascular tissue of infected plants, can be spread on knives used to remove cuttings, and can spread from plant to plant in propagation beds. The most important means of survival, however, is the carryover from season to season in symptomless plants. The possibility of whiteflies (*Trialeurodes* spp.) vectoring the bacteria is quite high (5), and *Geranium* spp., commonly grown for use in herbaceous perennial gardens, may be a source of inoculum for greenhouse-grown *Pelargonium* crops (9).

As the taxonomy of bacteria in general changed, so did the name of the bacterial blight organism. In 1923, Brown proposed the name *Bacterium pelargonii* (4). Five years later, Stapp changed the name to *Pseudomonas pelargonii* (Brown) Stapp (31). This was changed to *Xanthomonas pelargonii* (Brown) Starr & Burkholder in 1942 (32). In the current taxonomy, the name of the pathogen is *Xanthomonas campestris* pv. *pelargonii* (Brown) Dye (11).

Throughout the history of this disease, one of the greatest challenges to professional phytopathologists has been the ability to diagnose bacterial blight of geranium with a high degree of accuracy, timeliness, and confidence. Because of this, many avenues of disease diagnosis and pathogen identification have been explored. All methods have certain advantages but at the same time present diagnostic professionals with some degree of uncertainty.

Methods of Disease Detection

Culture indexing. Specialty propagators of geraniums require a system for presymptomatic detection of dangerous pathogens. For many years, this has been accomplished largely through the use of a process called "culture indexing." Culture indexing is the procedure whereby plant



Fig. 5. Bacterial streaming from the cross section of a leaf spot from a bacterial blight symptomatic *Pelargonium*.

material is incubated in artificial media in order to detect the presence of particular target fungi and bacteria that will grow in those media (Fig. 6). This is one of the simplest ways to detect *X. campestris* pv. *pelargonii*, because the bacterium inhabits the vascular tissue of the plant and readily grows on common culture media. When indexing was first developed, specialty propagators selected a plant that was apparently healthy and had all the desirable culture characteristics of the cultivar. Branches were removed either by breaking them off or by cutting them off with a disinfested knife. Three or four 1- to 2-mm-thick cross sections were removed from the base of each sample, placed in tubes of sterile broth, and incubated for several days. The parent plant was discarded. If the broth became cloudy with bacterial or fungal growth, all cuttings from the parent plant were discarded. No attempt was made to identify the bacteria or fungi. If the broth remained clear, the cuttings were used to produce nuclear stock plants (23,25,39).

In modern production systems, culture indexing is usually part of a larger program of tissue or meristem tip culturing and virus testing. The goal is to target for elimination from the production system the viral diseases of *Pelargonium* as well as pathogenic bacteria and fungi. In the United States, plants are grown at very high temperatures in order to inhibit virus replication while the meristem tip slowly elongates, thereby increasing the volume of tissue that may be free of viruses. Culture indexing is conducted as meristem tip cultures are being established. Culture indexed plantlets found free of bacteria are then indexed for viruses. When no viruses are detected, these plants become the elite nuclear stock plants. Some specialty propagators, particularly those in Europe, have done the virus indexing and meristem tip culturing first and assumed that these

steps eliminate bacteria and fungi that reside in the vascular tissue (27). These propagators have used techniques other than culture indexing to determine whether *X. campestris* pv. *pelargonii* is present, such as immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) methods (40). However, it is recognized that bacteria can go undetected during the meristem tip culturing, only to create problems later (6,26).

The nuclear stock plants are individually tested and kept under a rigorous regime of insect, mite, and disease management in a greenhouse accessible only to trusted employees aware of the importance of protecting these plants from pathogens and other pests. These plants are isolated from all other plants. They are indexed two more times at later dates. This redundancy in testing is extremely important because (i) *X. campestris* pv. *pelargonii* can be harbored in symptomless plants, especially if grown at cool temperatures, and (ii) only a small portion of the plant is tested, leaving the possibility that the pathogen will be missed during sampling. In the United States, it is common practice to renew the nuclear stock annually by starting the process over again for each new candidate plant. In Europe, it has been suggested that nuclear stock can be maintained as long as it is kept under strict pest management conditions and indexed regularly (40). Regardless of the methods used to establish nuclear stock plants, it is acknowledged that the plants are not totally disease-free or pathogen-free. It is possible that these plants harbor one or more organisms for which they are not indexed and that they will be infected by ubiquitous pathogens such as *Botrytis* while being grown in a production greenhouse.

Cuttings harvested from the nuclear stock are used to establish the propagation stock or increase stock. Many thousands of production plants must be grown under less rigorous insect, mite, and disease control measures than the nuclear stock and are tested only randomly for the organisms targeted in the indexing program. At this point in the production system, regular and skilled scouting for disease symptoms becomes very important because of the large quantities of plants being grown. Cuttings taken from these plants are ulti-

mately sold to the consumer. Often, production and increase blocks are grown at facilities remote from the nuclear stock and managed by growers other than the specialty propagator in charge of the nuclear stock. These growers are usually under a licensing agreement with the specialty propagator and are required to follow specific guidelines to help ensure that the plants will remain free of the target organisms. These secondary propagators may sell plants directly to the consumer or to other greenhouse operators or florists who sell to the consumer. There are known cases where *X. campestris* pv. *pelargonii* has infected plants during this secondary propagation step. Because it is usually difficult to trace the source of the *X. campestris* pv. *pelargonii* when contamination occurs during the increase phase of production, it has been suggested that the bacteria are always present at a low level on the plants, even on indexed plants, and that it is not possible to totally eliminate the bacteria. However, the past success of specialty propagators in establishing and maintaining blight-free plants in areas where conditions are highly conducive to disease development is a strong argument that a zero tolerance threshold is possible to demand and meet (17).

A potential weakness in the geranium production system is that purchasers of plants from the secondary, licensed propagators will at times take one or more unauthorized cuttings from these plants and root them under conditions totally uncontrolled by the specialty propagator or his licensed cooperators. These tertiary propagators usually maintain their own collection of geranium cultivars all year, year after year, under less than sanitary conditions with no indexing or routine testing of their health. It is well known that *X. campestris* pv. *pelargonii* often is reintroduced during tertiary propagation from plants grown outside the indexed stream of production.

Other diagnostic methods. If the specialty propagators' system for clean stock production through culture indexing and other associated techniques is not successful, growers will be faced with symptoms of bacterial blight in their crops. The symptoms, however, are not unique to bacterial blight; therefore, laboratory diagnosis is essential for accurate disease identification. Bacterial blight has traditionally been diagnosed by isolation of the pathogen from plants with typical symptoms. A diagnostician working with a geranium sample should look for round, water-soaked or brown leaf spots, 2 to 3 mm (1/16 to 1/8 in) diameter (Fig. 1). These represent localized infections and are commonly seen on geraniums with overhead irrigation. In some instances, wedge-shaped necrotic areas are seen (Fig. 2). On subirrigated plants (plants watered from the bottom), leaf spots may be absent. As

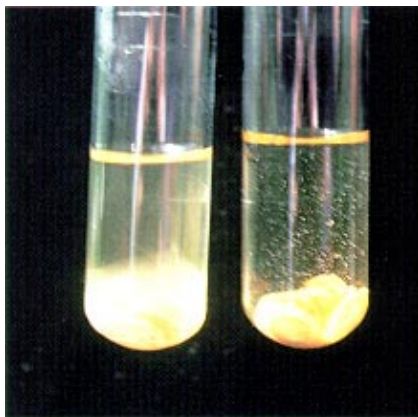


Fig. 6. Culture indexing of geraniums in nutrient broth plus 10% glucose. Left tube, *Xanthomonas campestris* pv. *pelargonii*-infected plant, and right tube, healthy plant. (R. Wick, University of Massachusetts)

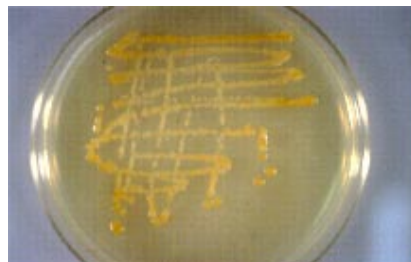


Fig. 7. Pure culture of *Xanthomonas campestris* pv. *pelargonii* grown on yeast-dextrose-calcium carbonate agar.

infections develop within symptomless plants, leaves wilt, turning yellow and finally brown (Fig. 3). The presence of both small leaf spots and wilting is strong field evidence for bacterial blight, as no other geranium pathogen produces both of these symptoms. Wilting of geraniums is often due to *Pythium* root rot, less frequently to *Fusarium oxysporum*, *Verticillium* spp., or *Ralstonia (Pseudomonas) solanacearum*. Common causes of leaf spots on geraniums include three other bacteria (*Pseudomonas syringae*, *P. cichorii*, and *Acidovorax* spp.), as well as *Botrytis cinerea*.

The petioles of wilting leaves are the best tissues to use when isolating *X. campestris* pv. *pelargonii*, in contrast to stems, which are often contaminated by saprophytes. Petioles may be plated directly, or vortexed in test tubes containing approximately 5 ml of distilled water, a sample of which is then streaked onto agar. Morphological characteristics are most distinctive on yeast extract-dextrose-CaCO₃ (YDC) or potato dextrose agar (PDA). *X. campestris* pv. *pelargonii* typically has a light yellow color and a highly mucoid, dome-shaped colony (Fig. 7). Other nonpathogenic *Xanthomonas* species that may be present in the tissue usually produce a deeper yellow pigmentation than *X. campestris* pv. *pelargonii*.

Observations for bacterial streaming will help to determine whether leaf spots are due to a bacterial pathogen, including possibly *X. campestris* pv. *pelargonii* (Fig. 5). Plants with severe systemic infections by *X. campestris* pv. *pelargonii* may show internal stem discoloration, but this is not always evident. Squeezing petioles or stems of systemically infected plants will sometimes allow droplets of thick, bacteria-filled sap to ooze out of the xylem (normal geranium sap is watery and clear).

Because of the difficulty of visually verifying the isolation of *X. campestris* pv. *pelargonii*, reinoculation of healthy geraniums and the reproduction of typical symp-

toms is often used to confirm that bacterial isolates are *X. campestris* pv. *pelargonii* (24). Nichols (1966) developed a system of wound inoculating stems of healthy cuttings with candidate bacteria using a toothpick, wrapping the wounds in Parafilm, incubating these in the laboratory for 5 to 7 days, and then checking for characteristic brown lesions at the point of inoculation (24) (Fig. 8). This system is slow, but it allows the diagnostician to check for morphological traits appropriate for a xanthomonad and to confirm the pathogenicity of the isolate. The presence of yellow pigments (xanthomonadins) typical of the genus *Xanthomonas* is not a completely reliable trait; strains that were mucoid but cream-colored have caused disease outbreaks in the past (M. L. Daughtrey, unpublished observation).

More rarely, physiological traits have been used to confirm the identity of a presumptive *X. campestris* pv. *pelargonii* isolate. Unlike *Pseudomonas* spp., *X. campestris* pv. *pelargonii* is unable to utilize asparagine as a sole source of carbon and nitrogen. Starch is utilized, but slowly. Slow growth on starch and beef extract (SX) agar medium and paler yellow color help to distinguish *X. campestris* pv. *pelargonii* from other xanthomonads that might be found in geranium tissue as saprophytes (30).

Fatty acid analysis is another option for identifying *X. campestris* pv. *pelargonii*. Xanthomonads biosynthesize 20 to 25 fatty acids (28). Most *X. campestris* pv. *pelargonii* isolates cluster tightly when analyzed using this method, allowing confirmation of a bacterial blight diagnosis (14). Bacterial isolates must generally be sent to a commercial laboratory for fatty acid analysis. Only a few university laboratories have access to this procedure.

The BIOLOG GN Microplate (Biolog, Inc., Hayward, CA) allows identification of bacteria by delineating their carbon-source utilization abilities, using 96-well microtiter plates. Twenty-four hours after

inoculation with a pure culture of the organism to be tested, a pattern of colored wells is analyzed by a computer program to provide bacterial identification, and the likelihood of a correct diagnosis is given for each set of data analyzed. This system is an excellent supplement to symptom observation and examination of isolate morphology, and requires 4 to 5 days less time than the bioassay method. Both BIOLOG and fatty acid systems can fail to recognize "unusual" isolates of *X. campestris* pv. *pelargonii* (with physiological traits unlike those encountered in the previously submitted isolates that form the databases for these systems).

Because culture-based methodologies are time-consuming and require special facilities and expertise, in the past decade other technologies have been brought into use for bacterial blight diagnosis. Serological methods have been employed in the detection of plant pathogenic bacteria for many years (29). In the past, most serological tests were confined to the identification of plant pathogenic bacteria grown in pure culture. Based on the nature of the tests and the quality of the antibodies used, these early tests were not always reliable and lacked the sensitivity to identify bacteria in vivo (29). A variety of tests have been developed for detection of *X. campestris*; however, most assays focused on pathovars other than *pelargonii*. Not until the introduction of ELISA in 1977 were researchers capable of using serological assays to detect the presence of *X. campestris* pv. *pelargonii* in infected plant tissue (7). Variations on ELISA using different solid-phase substrates and nonenzymatic conjugated antibodies allowed for greater sensitivity and ease of use (3,15). In 1990, Anderson and Nameth reported the development of a polyclonal-based antibody diagnostic assay in which antibodies made to *X. campestris* pv. *pelargonii* were conjugated with gold and used to detect the presence of *X. campestris* pv. *pelargonii* in sap samples from infected

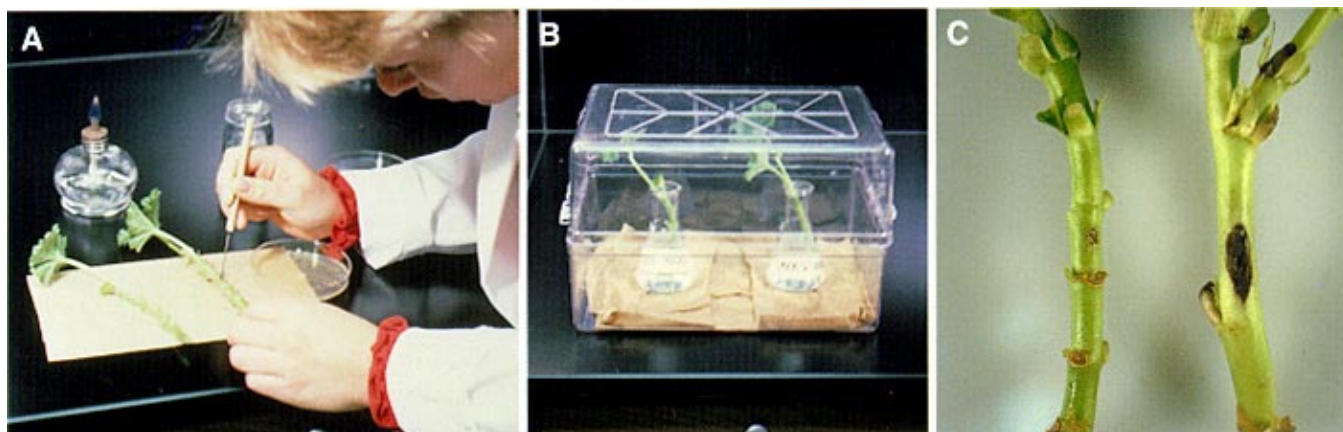


Fig. 8. Modification of the bioassay used to identify *Xanthomonas campestris* pv. *pelargonii* in infected *Pelargonium* developed by Nichols in 1966. (A) Stab inoculation of geranium cutting with pure culture of *X. campestris* pv. *pelargonii*, (B) incubation of cutting in a warm and moist environment, and (C) black lesion of *X. campestris* pv. *pelargonii* positive compared with the water control.

geraniums (1). Horseradish peroxidase-conjugated antibodies were also used to develop an indirect ELISA employing polystyrene microtiter plates. Both assays were capable of detecting the presence of *X. campestris* pv. *pelargonii* in asymptomatic geraniums. In some cases, positive ELISA results were obtained 3 days after inoculation. This was 6 days before the onset of the first symptoms (Fig. 9).

Today, ELISA is routinely used in private laboratories and university diagnostic clinics throughout the United States and Europe. However, it does have limitations, and because of the economic ramifications to the producer associated with a positive *X. campestris* pv. *pelargonii* result, ELISA-based diagnosis should be used only in conjunction with other diagnostic methods (8). The drawback of ELISA is that they may result in occasional false positives (when xanthomonads other than *X. campestris* pv. *pelargonii* are found in the tissue—some tests may not be pathovar-specific) and occasional false negatives (when *X. campestris* pv. *pelargonii* strains serologically distinct from those used to develop the antisera used in the test are encountered).

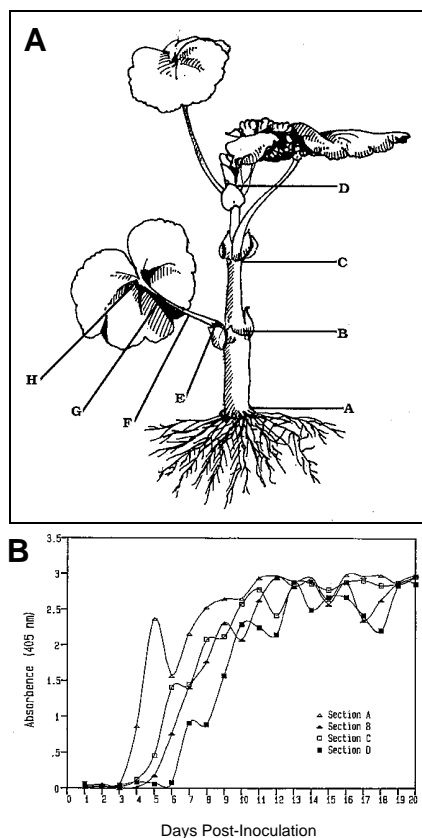


Fig. 9. Diagram of a geranium plant given a root-dip inoculation (A) showing location of various enzyme-linked immunosorbent assay (ELISA) sampling points. (A to D, stem sections), and (B) graph showing corresponding stem section ELISA values versus days post-root inoculation. Infected plants did not express bacterial blight symptoms until day 9.

Specialty propagators are interested in monitoring for disease in healthy-appearing stock. Detection of *X. campestris* pv. *pelargonii* in asymptomatic geraniums demands extreme analytical sensitivity, because the pathogen is often present in low population numbers in latently infected plants. Such sensitivity demands could be appropriately satisfied by application of polymerase chain reaction (PCR) amplification. Requirements for PCR detection of *X. campestris* pv. *pelargonii*-infected plants include design of pathovar-specific amplification primers and appropriate techniques for sampling and rapid extraction of plant tissues. Different approaches were used in two separate laboratories in an effort to design pathovar-specific PCR assays to detect *X. campestris* pv. *pelargonii*. Both approaches began with the same strategy of attempting to identify pathovar-specific regions of the bacterial genome, followed by the design and synthesis of pathovar-specific PCR primers.

Manulis et al. used the random amplified polymorphic DNA (RAPD) technique to identify DNA fragments that provided specificity for the pathovar *pelargonii* (21). Using the RAPD technique, a single 1.2-kb region of DNA was identified as pathovar specific. The region was cloned and partially sequenced, and a PCR primer pair was designed to amplify this target. When used in PCR amplification, the primer pair specifically amplified the same DNA product from all 53 pathovars of *pelargonii* strains tested but did not amplify DNA from 46 other *Xanthomonas* strains tested. Using pure laboratory cultures as the target, assay sensitivity was estimated to be between 10 and 50 cells of *X. campestris* pv. *pelargonii*.

Sulzinski et al. used two sets of highly conserved bacterial DNA sequences to

generate agarose gel fingerprints after PCR amplification of DNA (<10 to 200 ng) extracted from pure laboratory isolates of bacteria (35). With these fingerprints, they were able to identify all 19 pure isolates of *X. campestris* pv. *pelargonii* examined and distinguish them from 18 additional isolates representing 10 other pathovars of *X. campestris*.

In 1996, Sulzinski et al. described a set of pathovar-specific PCR primers (different from those of Manulis et al.) identified from one of the consensus sequence amplicons (36). The primers were used in amplification to detect *X. campestris* pv. *pelargonii* in infected symptomatic geraniums. In this study, it became evident that geranium extracts contained one or more substances inhibitory to PCR amplification, and removal of these inhibitors during extraction was clearly one of the most critical issues for developing an *X. campestris* pv. *pelargonii*-PCR assay (or for that matter, any enzyme-based diagnostic test). To remove amplification inhibitors, Sulzinski et al. performed a standard extraction procedure that involved multiple phenol-chloroform extractions and ethanol precipitations. While the phenol-chloroform extractions and ethanol precipitations rendered the extracts "amplifiable," this procedure was both laborious and time-consuming (several hours long), and therefore was not suitable for large-scale screening of geraniums for the presence of *X. campestris* pv. *pelargonii*.

In an effort to address the extraction challenges in geranium, several different extraction procedures were examined, and a rapid extraction protocol was developed (37). The procedure involved a quick grinding and treatment of the macerate with a commercially available extraction matrix, GeneReleaser, followed by a 6-min

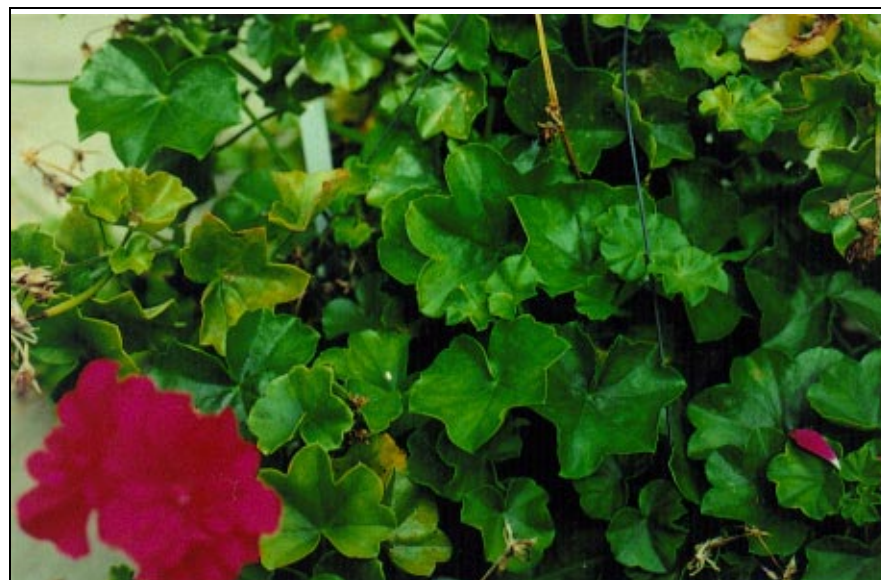


Fig. 10. Ivy geranium infected with *Xanthomonas campestris* pv. *pelargonii* showing nondescript leaf yellowing and leaf margin necrosis. Note the lack of the classical wilt symptom often seen in florists' geraniums infected with *X. campestris* pv. *pelargonii*.

heat treatment in a microwave oven and brief (30 s) microcentrifugation. The entire procedure involved less than 10 min, with minimal sample handling, and made possible the detection of *X. campestris* pv. *pelargonii* in the leaves, petioles, and stems of symptomatic geraniums (37). Using the same extraction and amplification procedure, no amplification products were detected in geraniums infected with *Rhodococcus* (*Corynebacterium*) *fascians* or *Pseudomonas cichorii*, two other common geranium pathogens. This study established both the applicability of the rapid extraction technique and pathogen-specific detection of *X. campestris* pv. *pelargonii* in symptomatic geraniums.

For applications to bacterial blight certification programs, PCR amplification must be able to detect latent infections of asymptomatic geraniums. Sulzinski et al. reported that latent infections could be detected in geranium petioles and stems after petiole inoculation with a minimal (known) inoculum load. Immediately after inoculation, sections of the petioles and stems were harvested and incubated for a 24- or 48-h biological enrichment in nutrient broth. Bacterial cells were collected by centrifugation, followed by rapid Gene-Releaser extraction and PCR amplification. With this biological enrichment/PCR amplification protocol, it was possible to detect infection induced by a single CFU of *X. campestris* pv. *pelargonii* in inoculated geranium tissue (38). Moreover, using the same biological enrichment/PCR amplification protocol, it was possible to detect systemic movement of *X. campestris* pv. *pelargonii* in tissues of latently infected geranium plants sampled 24 h postinoculation after inoculation with a minimal inoculum load of 4 CFU (38).

Refinements in extraction techniques have established the feasibility of detecting the pathogen in low population numbers in asymptomatic, latently infected plants. The ability to accurately and reliably detect the pathogen in asymptomatic tissue is an important step in establishing "pathogen free" certification programs for bacterial blight of geraniums.

Where Do We Go From Here?

It is clear that despite all the technological advances in the detection and identification of *X. campestris* pv. *pelargonii* in geraniums, more work is needed before we can use any one technique with a high degree of diagnostic confidence.

Perhaps the best diagnostic system for new *X. campestris* pv. *pelargonii* strains entering the production system from wild sources is the original, cumbersome, and time-consuming method of using geranium plants to confirm pathogenicity of presumptive *X. campestris* pv. *pelargonii* isolates. Because other bacterial pathogens (*Pseudomonas syringae*, *P. cichorii*, and

Management of Bacterial Blight of Geraniums (*Pelargonium* spp.)

Prevent introduction of the bacteria into geranium crop

- Purchase culture-indexed stock plants or cuttings taken from such stock plants.
- Properly indexed plants and cuttings taken from these plants should be relatively free of the bacteria.
- Insist that workers not handle *Pelargonium* or *Geranium* spp. in other locations prior to working in the greenhouse. If they do, insist that the workers thoroughly wash their hands.
- Do not grow *Geranium* spp. in or around a facility where *Pelargonium* spp. are being produced. Hardy, perennial *Geranium* spp. are susceptible and can be nonsymptomatic carriers of the bacteria.

Establish practices at beginning of production cycle that will minimize crop losses should the disease occur

- Keep seed-grown geraniums separate from vegetatively propagated geraniums. The bacteria are not seedborne, but seed-grown cultivars are susceptible. Therefore, bacterial blight in seed-grown cultivars usually originates from bacteria harbored in vegetatively propagated cultivars.
- Do not grow ivy geraniums in hanging baskets over benches of potted geraniums. Ivy geraniums may exhibit few or no symptoms when infected by the bacteria and therefore may be nonsymptomatic carriers of the disease.
- Keep geraniums from different propagator sources separate. If there is an outbreak of bacterial blight, it may thus be limited to plants from a single propagator.
- Insist that geranium workers thoroughly wash their hands and disinfect tools, such as knives used for taking cuttings, frequently. This will reduce the potential for spreading the bacteria from a single infected plant to a large number of plants.
- Irrigate plants in a manner that prevents or minimizes splashing. Bacteria are dispersed by splashing water.
- Individual stock plants should be raised slightly above the surface of solid-topped benches so that adjacent plants do not rest in a common puddle of water. It is known that the bacteria can infect via roots in water.

Scout for disease and be prepared to act quickly should it develop

- Know what symptoms should be sought at each stage of plant growth for each type of geranium grown. Symptoms differ on ivy versus florists' geraniums and on seedlings versus stock plants (Fig. 10).
- Work with diagnostic facilities that can accurately confirm the presence of the bacteria blight in a timely fashion. Some clinics do not routinely deal with this disease. False positive and false negative tests can occur, resulting in long delays in sample processing. Deal with a clinic or diagnostic service that routinely handles geranium diseases. Geranium suppliers can guide you to reliable diagnostic facilities.
- Once a positive diagnosis is confirmed, immediately discard symptomatic plants and those immediately adjacent to symptomatic plants. Plants purchased or produced from the same source as the positive plants should be tested.
- Consider spraying the remainder of the crop with a legally registered copper-containing pesticide. Certain copper-containing pesticides will sometimes induce rapid yellowing of bacterial blight-infected plants. Such plants are then more easily identified and discarded.

If bacterial blight occurs, begin preparation for next production season immediately

- Discard all geraniums remaining at the end of the production cycle.
- Remove all geranium plant debris from the greenhouse. Do not attempt to compost geranium debris with the idea of using the compost for later geranium production.
- Keep the greenhouse free of geranium for as long as possible before resuming the geranium production cycle.

Acidovorax spp.) may be found causing leaf spots, and *Ralstonia (P.) solanacearum* may cause vascular wilt of florists' geranium, a cursory diagnosis (based on symptoms, ooze, etc.) may be incorrect. Although time-consuming, the bioassay does offer the greatest degree of confidence.

Serologically based assays are relatively quick (a few hours); yet they are not 100% reliable. Great care must be taken when sampling suspect plants. Multiple samples must be taken when testing individual plants. Because *X. campestris* pv. *pelargonii* is not evenly distributed in the plant, samples taken from tissue with low *X. campestris* pv. *pelargonii* concentrations could result in false negatives. Samples taken from florists' geraniums that were root inoculated with *X. campestris* pv. *pelargonii* resulted in stem sections that tested negative by ELISA even though the stem sections above and below the *X. campestris* pv. *pelargonii*-negative section tested positive for the bacteria (1). Work needs to continue on producing serological tests with greater sensitivity, along with addressing the issue of bacterial distribution in the host.

The specificity offered by PCR and other molecular techniques has the potential to eliminate the necessity of a bioassay and serological tests to confirm diagnosis. The most useful PCR assay will employ sampling procedures targeted for the greatest likelihood of detecting the minimal population numbers of *X. campestris* pv. *pelargonii* expected with latent infections. To optimize the sampling process, several questions need to be addressed concerning the interaction of *X. campestris* pv. *pelargonii* with its host: How is *X. campestris* pv. *pelargonii* distributed in geranium tissues during latent infections? How, and under what environmental conditions, does *X. campestris* pv. *pelargonii* multiply and move on and in asymptomatic geraniums? What types of tissues are most likely to contain *X. campestris* pv. *pelargonii* in a latently infected plant? If *X. campestris* pv. *pelargonii* is present as a foliar epiphyte, how and when does it become a pathogen, and under what conditions are symptoms induced or repressed? To what extent do geranium species or varieties resistant to bacterial blight (e.g., Martha Washington's geraniums, *P. domesticum*) support multiplication of *X. campestris* pv. *pelargonii*, and how important are such varieties as potential reservoirs of inoculum? These issues are the subject of current study (M. A. Sulzinski, unpublished).

Thus, there remain significant gaps in basic knowledge of the biology of *X. campestris* pv. *pelargonii* that affect our ability to design efficient, accurate, practical detection systems. In order to maximally exploit the increased sensitivity offered by PCR amplification, these knowledge gaps



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affecting the sampling strategy must be resolved. Another, more technical challenge still unresolved is to adapt the PCR amplification technology (or any other appropriate DNA amplification alternatives) to inexpensive and user-friendly formats for large-scale grower level testing. The specificity offered by PCR and other molecular techniques has the potential to eliminate the need for a bioassay to confirm bacterial blight and to make it possible to detect *X. campestris* pv. *pelargonii* in asymptomatic latent infections of geranium.

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