Research Note

Growth of Bioluminescent *Xanthomonas campestris* pv. *campestris* in Susceptible and Resistant Host Plants

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Transposon Tn*4431* was used to introduce *luxCDABE* the operon into a highly pathogenic strain of *Xanthomonas campestris* pv. *campestris* (*X. c.* pv. *campestris*), causal agent of black rot of crucifers. The bioluminescent strain was indistinguishable from the wild-type strain in pathogenicity tests and *in planta* growth characteristics. Movement and growth of the bioluminescent strain of *X. c.* pv. *campestris* in susceptible and resistant cabbage seedlings after wound or mist inoculation was followed over time with a liquid nitrogen-cooled, charge-coupled-device camera. Wound-inoculation resulted in significantly higher bioluminescence levels in the susceptible plant than in the resistant plant. More leaves became infected in the susceptible host, and peak levels were reached after 5 days compared with 10–12 days in the resistant host. Peak bioluminescence levels were followed after 2–3 days by symptom development. Mist inoculation resulted in high bacterial population levels in mature susceptible leaves only. Hydathodes or wounds served as sites of infection in susceptible leaves, whereas entry was gained mainly through wounds on resistant leaves. Infection remained confined in and around damaged leaf areas in resistant leaves but spread rapidly through the vascular system of susceptible leaves.

Additional keywords: black rot, cabbage, genetically engineered microbe.

Xanthomonas campestris pv. *campestris* (*X. c.* pv. *campestris*) is the causal agent of the economically important disease known as black rot of crucifers (Williams 1980). Bacteria gain entry through hydathodes or wounds and colonize the vascular system (Cook *et al.* 1952). As bacteria advance into the vascular tissue, the veins become occluded, restricting water flow and resulting in characteristic V-shaped lesions and vein darkening. In resistant plants, the growth of the pathogen is restricted to the hydathode region (Staub and Williams 1972), and the plant response is often macroscopically visible as a localized area of necrosis and microscopically as tissue deformation (Brettschneider *et al.* 1989). Wound inoculation, insect injury, or hail damage are known to result in black rot lesions in resistant plants (Staub and Williams 1972). Resistance was found to be temperature, light (Staub and Williams 1972), and age dependent (Hunter *et al.* 1987). Generation times of *X. c.* pv. *campestris* in resistant versus susceptible genotypes were found to be similar (Staub and Williams 1972).

Transgenic incorporation of the *luxCDABE* operon in phytopathogenic bacteria provides a sensitive and nondisruptive technique for studying host-pathogen interactions *in planta* (Shaw and Kado 1986) and can provide valuable insights in compatible (susceptible) as well as incompatible (resistant) host-pathogen interactions. A bioluminescent phenotype of a highly pathogenic strain of *X. c.* pv. *campestris* (90-BMC, selected after field pathogenicity tests on black rot susceptible cabbage plants) was constructed using the lux-transposon, Tn*4431*. The transposon was introduced into a spontaneous

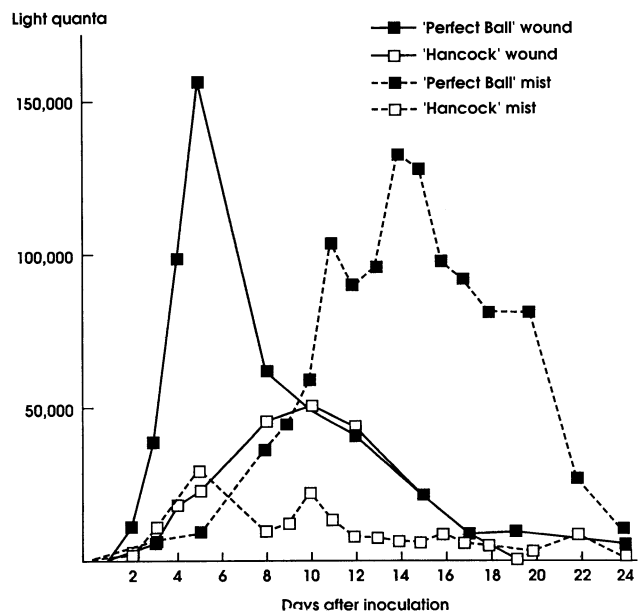

Fig. 1. Bacterial bioluminescence in black-rot-susceptible Perfect Ball or resistant Hancock cabbage seedlings followed over time from the day of wound- or mist-inoculatioin with FD91L. Bioluminescence is expressed as the number of pixels produced per plant.

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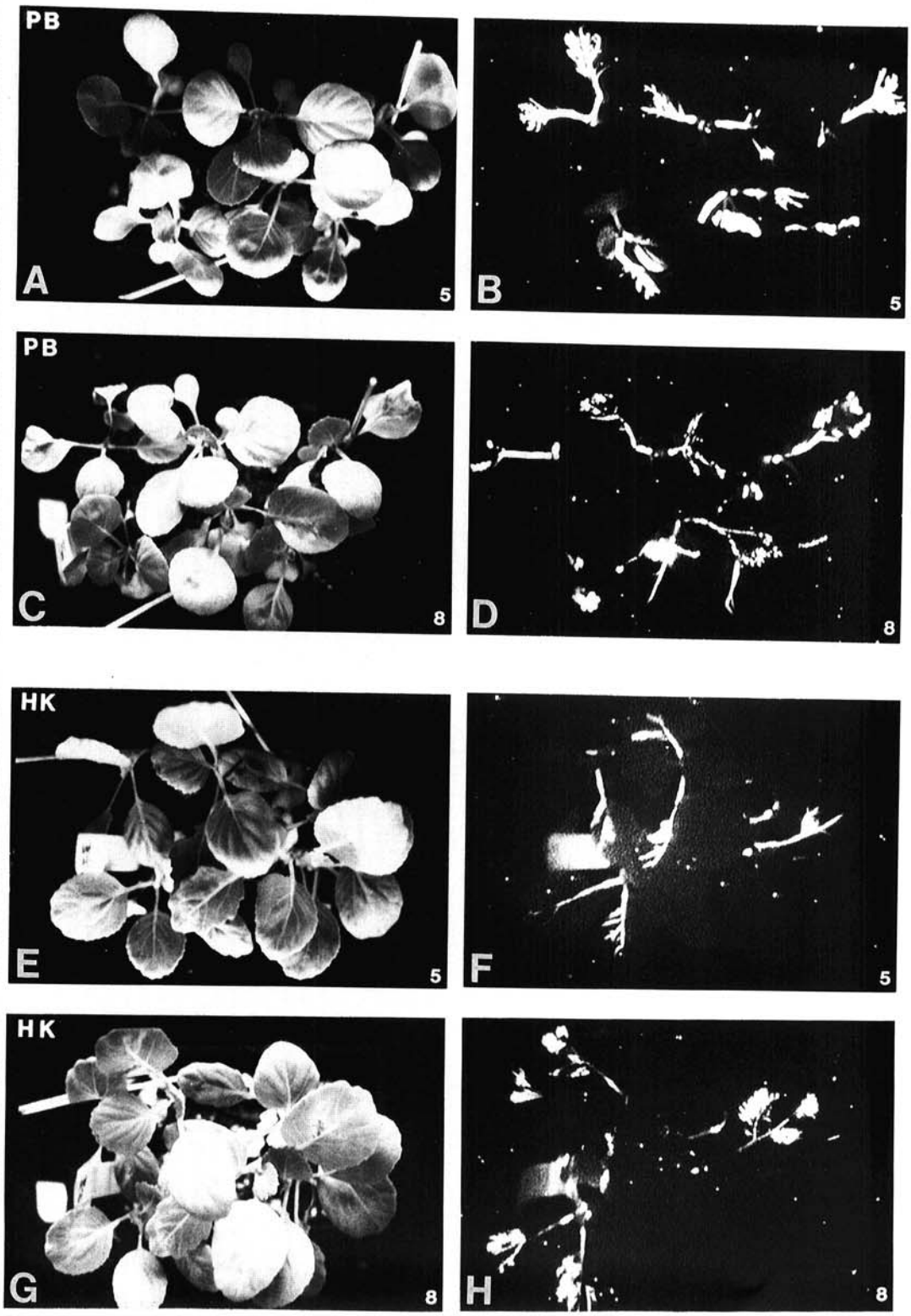

Fig. 2. Bacterial bioluminescence *in planta*. Six 4-wk-old, black rot susceptible Perfect Ball (PB) and resistant Hancock (HK) plants illuminated by bacterial bioluminescence 5 (**B, F**) and 8 (**D, H**) days or by incident light 5 (**A, E**) and 8 (**C, G**) days after wound inoculation with bioluminescent strain FD91L of *Xanthomonas campestris* pv. *campestris*. FD91L inoculum was injected into the petiole of two leaves per plant with a syringe. Inoculum was prepared by growing bioluminescent bacteria for 48 hr in a suspension culture of medium 210 at 29° C (Shaw *et al.* 1992). Images were obtained by focusing the charge-coupled-device camera on the seedlings and performing a 10-min exposure with the camera cooled to –110° C. Firmware was used to extract positional and quantitative data from images. The bacterial bioluminescence observed is a direct function of the colony-forming units per leaf.

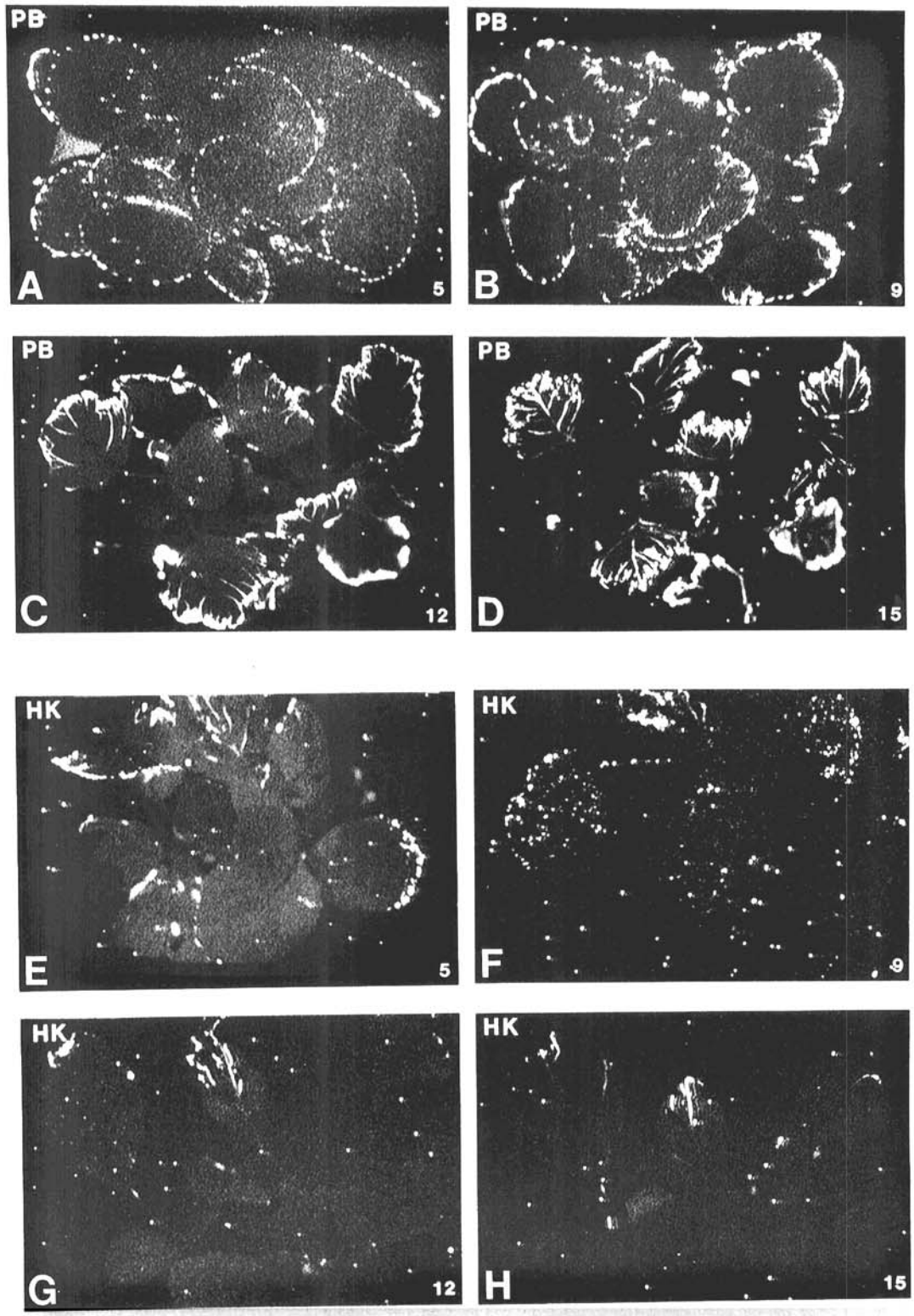

Fig. 3. Bioluminescence *in planta*. Six 4-wk-old, black rot susceptible (PB) or resistant Hancock (HK) plants illuminated by bacterial bioluminescence 5 (**A, E**), 9 (**B, F**), 12 (**C, G**), and 15 (**D, H**) days after mist inoculation with FD91L. Before mist-inoculation, Silwet (0.15%, Zidack *et al.* 1992) was added to the inoculum as a surfactant. A hand-held mister sprayer was used to deliver inoculum to the plants.

rifampicin-resistant derivative of 90-BMC by electroporation (Shaw and Kahn 1993). A strongly bioluminescent transposon recipient (FD91L) was selected and found to be indistinguishable from the wild-type strain in pathogenicity tests and *in planta* growth characteristics. Movement and growth of FD91L in susceptible (cv. Perfect Ball) and resistant (cv. Hancock) cabbage seedlings after wound or mist inoculation was followed with a charge-coupled-device (CCD) camera (Shaw *et al.* 1992). The CCD camera allows the visualization of bacterial population levels without disrupting the ongoing pathogen-host interaction.

Wound inoculation of 4-wk-old cabbage seedlings with FD91L resulted in significantly higher bioluminescent bacterial population levels in the susceptible plants. Successful infections were established more frequently in the susceptible host, and peak bioluminescence levels were reached 4–8 days after inoculation. In the resistant host, peak bioluminescence levels were reached 10–12 days after inoculation (Figs. 1 and 2), and several inoculated leaves failed to develop black rot symptoms or detectable levels of bioluminescence. Once a leaf of the resistant plant became infected, however, bioluminescence levels comparable to those in many of the susceptible leaves were reached. Peak bioluminescence levels were reached 2–3 days before symptom development. Black rot lesion development was slower in resistant than in susceptible tissue. In resistant plants, the first symptoms developed about 8 days after wound inoculation as interveinal chlorosis and necrosis, whereas whole leaf necrosis and drying were apparent in susceptible leaves by that time. Eventually infected leaves shriveled up and abscised. Systemic spread to uninoculated susceptible or resistant leaves was not observed, consistent with studies of Alvarez *et al.* 1987.

More drastic differences between susceptible and resistant plants could be observed after mist inoculation. Hydathode infections were clearly evident in the susceptible plants. Five days after inoculation, bacterial colonization was evident at the hydathodes of susceptible but not resistant hosts. The bacteria advanced rapidly through the veins of susceptible hosts until all mature leaves emanated bioluminescence. Symptoms were clearly visible in most Perfect Ball plants 10–12 days after inoculation. While bacterial growth was mainly intraveinal in the susceptible host, on the resistant plants bioluminescence was observed on and around lesions and insect-caused wounds (Figs. 1 and 3). High levels of bioluminescence were only observed in susceptible plant tissues, which is consistent with observations by Staub and Williams (1972). Wound inoculation techniques bypass the early host defense to *X. c.* pv. *campestris* infections in the incompatible interaction between pathogen and host (Daniels *et al.* 1988, 1991). Only through hydathode infection does the host fully respond to the pathogen, restricting its endophytic growth. The host response is developmentally dependent, since young leaves in the growing tip of the susceptible host do not show hydathode infection after mist inoculation. A vascular hypersensitive response, described by Kamoun *et al.* (1992) after wound inoculation of turnip cultivars with pathogenic *X. c.* pv. *campestris* strains, was not detectable in resistant Hancock cabbage or other hosts. Bioluminescent FD91L was observed to colonize the vascular system of radish (cv. Early Scarlet), turnip (cv. Yellow Globe), and rapid-cycling *Brassica* plants after either wound or mist inoculation. Nonhost plants (cucumber, tomato, and muskmelon seedlings) showed high bioluminescent bacterial population levels only after drought stress and the onset of senescence (unpublished), apparently overcoming the nonhost defense mechanism.

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