The Role of Weeds in Survival of *Pseudomonas syringae* pv. *phaseolicola* in Northern Tanzania

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ABSTRACT

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Of 16 weed species belonging to 10 families, only *Neonotonia wightii* served as a perennial reservoir of *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight. The weed survived dry periods along fences and hedgerows, by roadsides, on ditch banks, and in corners of farmers' fields. Only race 1 of *P. s. phaseolicola* was recovered from *N. wightii*. With artificial inoculation, however, all three races of the bacterium caused lesions in the weed. Because race 1 of *P. s. phaseolicola* was not seedborne in *N. wightii*, the weed cannot spread the pathogen into new areas by means of seed dispersal.

The role of the foliage of weeds and nonhost species as alternate sources of inoculum for disease epiphytotics has been documented for several plant-pathogenic bacteria, including *Xanthomonas campestris* pv. *campestris* (11, 17,19), *X. c. vesicatoria* (10), and *Pseudomonas syringae* pv. *syringae* (4). Saprophytic survival of plant-pathogenic bacteria on root surfaces was first shown by Valleau et al (26), who demonstrated that *P. angulata (P. syringae* pv. *tabaci)* and *X. c. vesicatoria* overwintered in close association with the roots of several crops and weeds. *X. c. malvacearum* was detected in roots and leaves of various weeds collected from blighted cotton fields (22), and growth of soft rot *Erwinia* sp. multiplied in the rhizospheres of weeds common in fields of Chinese cabbage (8).

In many parts of Tanzania, one crop season is well separated from the next by a dry season. Endemic plant pathogens therefore survive between seasons despite the discontinuity of host crop populations. Weeds and wild plants serve as alternative hosts of infection or epiphytic populations and thus contribute to outbreaks of plant diseases by bridging between seasons, crops, or locations. Weeds also constitute a reservoir of pathogens that may be exchanged with cultivated crops (2). Moreover, there is no way yet to predict when and how a minor pathogen of a wild plant or weed will spread into cultivated crops and become a major problem.

Epiphytic survival of *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al was first reported by Ercolani et al (4), who showed that the bacterium multiplied to some extent on hairy vetch (*Vicia villosa* Roth), a leguminous weed growing near bean (*Phaseolus vulgaris* L.) fields in Wisconsin. Recently, Taylor et al (25) isolated several strains of *P. s. phaseolicola* from *Neonotonia wightii* (Graham ex Arnott) Lackey in South America and in Africa, including Tanzania.

Efforts are being made to control halo blight in the bean crop, but the disease is not being controlled in weeds or wild plants. No studies have been conducted in Tanzania to determine the role of weeds and nonhost species in the survival of the halo blight bacterium and as sources of inoculum. Therefore, a study was conducted to investigate the role of weeds in halo blight epidemics in northern Tanzania.

MATERIALS AND METHODS

Sampling and isolation procedures. From November 1988 to January 1990, attempts were made to isolate *P. s. phaseolicola* from weed plants with and without leaf lesions. Weeds were collected from bean-growing areas of the Arusha and Kilimanjaro regions of northern Tanzania in locations where halo blight was reported to have been severe on the bean crop the previous growing season. In addition, samples were collected from roadsides and hedges around backyards of farmers' houses and along irrigation ditches. The samples were placed in plastic bags and sent to the laboratory, where they were kept at 5 C until processed within 24–48 hr.

The samples were separated into leaves, stems, and roots. Then, 10–15 g of each component was placed in 500-ml flasks containing 100–300 ml of 0.01 M phosphate buffer (pH 7.2) with 0.01% Tween 20. Samples were shaken for 30 min with a wrist-action shaker at 22–25 C. The supernatant liquids were serially diluted 10-fold four times in the same buffer, and 0.01-ml portions from each dilution were plated on King's medium B (KB) (9) supplemented with 100 μg of cycloheximide per milliliter to inhibit fungal growth. Inoculated plates were incubated at 24 C for up to 5 days. Plates were examined under short- (254-nm) and long-wave (366-nm) ultraviolet light to determine production of fluorescent pigments (9). Cultures associated with such pigments were transferred and further purified on KB with a series of single-colony transfers.

Storage of cultures. Purified cultures were maintained as very thick suspensions in glycerol and 0.1 M phosphate buffer (1:1, v/v) and on nutrient agar slants at 0 C. Cultures were also stored at 22 ± 2 C in dry bean leaf powder prepared from artificially inoculated bean plants; leaves were air-dried and ground to a fine powder with sterile mortars and pestles, and the powder containing each bacterial isolate was stored in sterile glass vials.

Identification of bacterial cultures. Identification procedures were based on those described in Schaad's manual (18) and by Hayward (7). Three tests were conducted for each bacterial strain, and two known strains of *P. s. phaseolicola* (1299A and 1375A) were included as positive controls. Cultures were tested for levan, oxidase, production of pectolytic enzymes, arginine dihydrolase, and hypersensitivity in tobacco (LOPAT) (12) and also for carbon source utilization (18), ice nucleation activity (14), and bacteriophage sensitivity and serology (24). The method of Lindow et al (13–15) at −5 C was used to determine ice nucleation activity in suspensions from cultures grown for 2–3 days, then suspended in 0.1 M phosphate buffer (pH 7.0) and adjusted with a spectrophotometer to an optical density of 0.1 at 620 nm. Suspensions were 10-fold serially diluted three times in the same buffer. Ice nucleation activity was determined by placing 30 10-μl droplets from each of the dilutions on paraffin-coated aluminum weighing boats floating on 50% ethanol maintained at −5 C. The freezing of each droplet was determined visually at 1-min intervals for up to 3 min. Droplets from blank phosphate buffer dilutions were included as negative controls. Stock solutions of mannitol, sorbitol, and inositol were prepared in glass-distilled water and added to Dye's medium at 2 C, according

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to the procedures given by Schaad (18). The medium was left to stand for 3 days, then log phase bacterial cultures grown on KB were streaked on the medium in triplicate and plates were incubated for up to 10 days. Growth was compared with that on plates containing no added carbon sources. Sensitivity to bacteriophage and reaction to *P. s. phaseolicola* antiserum were tested with the methods described by Taylor (24) and J. D. Taylor and D. M. Teverson (*personal communication*) and cited by Mabagala (16).

Pathogenicity tests. All strains were evaluated for pathogenicity on susceptible cv. Canadian Wonder plants. The abaxial leaf surfaces of screenhouse-grown bean plants were sprayed with a hand-operated atomizer to runoff without water-soaking when the first trifoliolate leaf was fully open (10–14 days). Each strain was tested on three bean plants. A known strain 1299A and sterile phosphate buffer were included as positive and negative controls, respectively. Inoculated bean plants were incubated in the screenhouse and observed daily for symptom development. Pathogenicity was evaluated 10–14 days after inoculation. Appearance of typical halo blight symptoms confirmed identification of *P. s. phaseolicola*.

Strains of *P. s. phaseolicola* obtained from weeds were identified as to race by inoculation of the differential cultivars Canadian Wonder (universal susceptible), Edmund (universal resistant), Red Mexican U.I.-3 (resistant to race 1), and Tendergreen (resistant to race 3). Both pod and plant leaf reactions were used for race determination (3,16).

Assay of *N. wightii* parts for presence of *P. s. phaseolicola*. Flower buds, open flowers, and young flat pods of plants with typical halo blight symptoms were collected from the Monduli district in the Arusha region and from the Hai district in the Kilimanjaro region of Tanzania during 1989 and 1990. Plants from each site were marked so that the same plants could be sampled periodically. Samples from each site were placed in separate plastic bags to allow correlation of any seed infection with weather conditions, which were recorded for each site. After collection, samples were sent to the laboratory and kept at 5 C until processed within 24–28 hr.

For each sampling, 10 flower buds, 10 open flowers, and 10 young flat pods were weighed separately, surface-sterilized with 2.6% NaOCl for 2–3 min, rinsed three times in sterile distilled water, and ground in 10 ml of sterile phosphate buffer (pH 7.0). Aliquots of 0.1 ml were plated on KB-cycloheximide and incubated as described above for 5 days. Plates were incubated and observations were made daily to detect the presence of fluorescing colonies.

Tests for transimission of *P. s. phaseolicola* in seed of *N. wightii*. Mature pods collected from *N. wightii* plants at selected sites were placed in plastic bags, sent to the laboratory, and air-dried. The dried pods were shelled by hand, and both seeds and shells were assayed for *P. s. phaseolicola* (20). The seeds were surface-sterilized as described above, rinsed three times, and blotted dry on sterile filter papers in a laminar flow chamber. Seed lots (500–900 seeds) from each site were transferred to sterile test tubes containing 10 ml of phosphate buffer with 0.01% Tween 20. The tubes were shaken for 30 min on a wrist-action shaker, then the seeds were removed aseptically and divided into two lots. One lot was dried aseptically on sterile filter papers in a laminar flow chamber, plated hilum down on KB-cycloheximide agar, and incubated for 5 days. Plates containing bacterial growth around seeds were observed under ultraviolet light to detect production of fluorescent pigments.

The seed wash water was either diluted and plated on the same medium or used to inoculate five 10- to 15-day-old seedlings of cv. Canadian Wonder by injection and leaf water-soaking methods. In the latter, the water was infiltrated with a syringe into four sites on primary and fully open first trifoliolate leaves. Each plant was considered a replicate. Inoculated plants were maintained in the screenhouse and observed for halo blight symptoms for up to 14 days. Control plants were inoculated with *P. s. phaseolicola* at 10^3 to 10^4 cfu/ml to simulate the low amount of inoculum that may occur in the soakings; negative control plants were similarly treated with sterile phosphate buffer.

Another portion of the sample from each test tube was planted in the screenhouse. Seedlings were examined for water-soaked lesions on stems and leaves. Also, starting from the primary leaf stage, leaves, stems, and roots of five plants were sampled at random at 4-day intervals for 3 wk. The separate plant components were weighed, surface-sterilized for 1 min in 2.6% NaOCl, rinsed, and ground in phosphate buffer. The suspensions were serially diluted and plated on KB-cycloheximide. Plates were observed for up to 5 days.

The mature pods shells of *N. wightii* also were assayed. For each site and sampling date, 3 g of shells was surface-sterilized, ground, and mixed with phosphate buffer. Dilutions of suspensions were plated on KB-cycloheximide and observed for colonies resembling those of *P. s. phaseolicola* for up to 5 days.

Reaction of *N. wightii* to races 1, 2, and 3 of *P. s. phaseolicola*. Noninfected plants were identified from three locations: Lyamungu, Lambo, and Monduli. Mature pods were harvested and air-dried at 22–27 C. Dry pods were then shelled by hand, and seeds from each site were kept separate. Seeds were examined to ensure absence of internal populations of *P. s. phaseolicola*. Seeds from each location were surface-sterilized and rinsed as described above, mixed with 10 ml of phosphate buffer containing 0.01% Tween 20, and shaken for 30 min. Seeds were removed from test tubes, and the resulting wash water was diluted and plated as described above. Observations to detect *P. s. phaseolicola* were made daily for 5 days.

Surface-sterilized, pathogen-free seeds, 100 from each site, were planted in 16-cm-diameter sterile plastic pots, 20 seeds per pot. Three locations (Lyamungu, Lambo, and Monduli), two plant ages (9 and 15 days), and three isolates for each race were included. Two of the three race 1 strains were isolated from *P. vulgaris*; the third (1281A) was obtained from *P. coccineus* and supplied by J. D. Taylor. All of the race 2 and race 3 strains were obtained from *P. vulgaris*; one of the race 2 strains produced a brown, diffusible pigment.

Inoculum for each strain was produced as described above. A hand-operated atomizer was used to spray 9- to 15-day-old *N. wightii* plants to runoff, without water-soaking. Control plants were spray-inoculated with race 1 isolated from *N. wightii* and with sterile phosphate buffer. Inoculated plants were kept in the screenhouse and observed daily for symptom development for 15 days. The experiment was done twice.

RESULTS

Sixteen weed species belonging to 10 families were assayed for *P. s. phaseolicola* (Table 1). Only one weed species, *N. wightii* of the family Leguminosae, was found to be a natural host of the halo blight bacterium. Symptoms were restricted to leaves. Initially, small, water-soaked spots appeared on the undersides of young leaves. Later, a greenish yellow halo developed around the water-soaked area (Fig. 1). Symptoms were more evident on young leaves a few weeks after onset of the short rains (October–December). During the dry season, halo development around necrotic areas was restricted and sometimes did not occur.

P. s. phaseolicola was isolated from samples of *N. wightii* collected in the Kilimanjaro and Arusha regions throughout the year because the weed survives dry seasons in the field. In some fields, halo blight symptoms occurred only on the foliage and pods of bean plants growing near *N. wightii*. Disease occurred especially on the leeward side of hedges, suggesting that *P. s. phaseolicola* was disseminated from infected weeds to the bean crop.

Identification of bacterial strains. Of 27 strains of fluorescent pseudomonads characterized, 22 were from *N. wightii* and five from *Bothriocline laxa* N.E. Br. The 22 strains from *N. wightii* were positive for levan sucrase and negative for

oxidase, arginine dihydrolase, ice nucleation activity, and pectolytic activity and produced a rapid hypersensitive reaction on tobacco leaves. These strains also did not utilize mannitol, sorbitol, or inositol as carbon sources. They were sensitive to bacteriophages 11P, 12P, and 48P, as evidenced by plaque formation, and agglutinated with *P. s. phaseolicola* antiserum. Bacterial strains from *B. laxa* were positive for ice nucleation activity, oxidase, and arginine dihydrolase, and two of the five isolates utilized the three carbon sources. All five isolates were negative for levan sucrase production and pectolytic activity, did not produce a hypersensitive reaction on tobacco, and were insensitive to the three bacteriophages used. Thus, the five isolates from *B. laxa* were determined to be *P. fluorescens*.

Identity of the 27 bacterial strains was confirmed in pathogenicity tests. All strains from *N. wightii* were pathogenic on the susceptible bean cultivar Canadian Wonder, whereas those from *B. laxa* were not. Water-soaked lesions developed within 3–4 days of inoculation, and typical halo blight symptoms developed 7–10 days after inoculation. Systemic chlorosis was also evident on young leaves.

Race identification. On the basis of leaf and pod reactions of bean differential cultivars, all 22 strains isolated from *N. wightii* were typical of race 1, as shown by a resistant reaction on cv. Red Mexican U.I.-3. All compatible reactions involved the development of large water-soaked lesions, especially on cv. Tendergreen, and the production of bacterial ooze at the point of inoculation on the stem. Spray-inoculation of leaves and pod inoculation produced similar results. Rapid hypersensitive reactions were indicated on leaves and pods of cv. Edmund by tissue browning at the inoculation point. The 10 race 1 strains from *N. wightii* showed no differences in incubation period and symptom development, as compared with 10 race 1 strains from bean sources on the differential cultivars.

Populations of *P. s. phaseolicola* in parts of *N. wightii*. Throughout 7 mo (August–December 1989 and January–February 1990) of testing, no flower bud, open flower, or flat pod samples were found infected with *P. s. phaseolicola*, despite severe infection of the foliage of sampled plants. Results were similar for all three locations studied. Even random samples of reproductive parts collected within 5–10 cm of highly diseased shoots were free of detectable pathogen. Moreover, symptoms were not seen on any reproductive part examined. Pods on *N. wightii* plants were very hairy, which may have prevented inoculum from reaching the pod surface, but this was not true for flower buds or parts of open flowers. The restriction of symptoms to foliage and the absence of the halo blight bacterium in flower buds, open flowers, and flat pods suggest that *P. s. phaseolicola* was not able to move systemically in the vascular tissue to these reproductive tissues under the different weather conditions that existed in these locations.

***N. wightii* seed infection and transmission assay.** All mature pods on severely diseased *N. wightii* plants were symptomless, as were the seeds from these pods. Results of direct plating of 2,733 seeds and of plating of seed washes and pod shell suspensions were negative for the pathogen. Canadian Wonder plants inoculated with seed soakings did not show symptoms of halo blight, whereas control plants showed typical symptoms

Fig. 1. Typical symptoms of halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola*, on leaves of *Neonotonia wightii*.

Table 1. Weed families and species assayed for presence of *Pseudomonas syringae* pv. *phaseolicola* in northern Tanzania

Families Species	Location	Symptoms
Asteraceae (Compositae)		
Bidens pilosa L.	Kilacha, Lyamungu, Monduli	Necrosis and chlorosis of leaves
Bothriocline laxa N.E. Br. (= *Erlangea laxa* N.E. Br.) S. Moore)	Lyamungu	Marginal necrosis of stems and leaves
Conyza sumatrensis (Retz) E. H. Walker	Miwaleni, Lyamungu	None
Galinsoga parviflora Cav.	Lyamungu, Monduli, Sanya Juu	None
Boraginaceae		
Trichodesma zeylanicum (Burm.f.) R. Br.	Lambo, Monduli, Kirua, Tengeru	Necrotic lesions on leaves
Commelinaceae		
Commelina benghalensis L.	Lambo, Monduli	None
Cyperaceae		
Cyperus rotundus L.	Lyamungu	Necrosis, water-soaking, and chlorosis of stems and leaves
Leguminosae		
Neonotonia wightii (Graham ex Arnott) Lackey (= *Glycine wightii*)	Lambo, Monduli, Sanya Juu, Lyamungu, Tengeru	Necrotic lesions, water-soaking, and halos on leaves
Pueraria sp.	Lyamungu	Necrotic lesions on leaves
Malvaceae		
Sida alba L.	Lambo	Necrosis, water-soaking, and chlorosis of stems and leaves
Nyctaginaceae		
Boerhavia erecta L.	Lambo, Miwaleni	None
Poaceae		
Panicum sp.	Lambo, Selian	None
Setaria sp.	Kibosho, Lyamungu	Necrotic lesions on leaves
Polygonaceae		
Oxygonum sinuatum (Meissn.) Dammer	Lambo, Monduli	None
Solanaceae		
Nicandra physalodes (L.) Gaertn.	Lambo, Narumu, Sanya Juu, Monduli, Tengeru	Necrosis and halos on leaves
Solanum incanum L.	Sanya Juu, Lyamungu	Necrotic lesions and chlorosis on leaves

12 days after inoculation. However, seeds from all three locations contained other endophytic bacteria. The proportion of seed containing unidentified endophytic bacteria for each sampling period at the different locations ranged from 20 to 85%. Most of the colonies of these bacteria were creamy-white and a few were orange; none fluoresced under ultraviolet light. These bacteria were not tested for pathogenicity.

In the screenhouse experiments, 2,860 seedlings were assayed for infection by *P. s. phaseolicola*. None of the *N. wightii* seedlings from seeds collected from plants naturally infected with the halo blight bacterium showed symptoms on stems or leaves. The bacterium also was never recovered from root, stem, and leaf samples from such seedlings.

Reaction of *N. wightii* to races 1, 2, and 3 of *P. s. phaseolicola*. All three races produced susceptible reactions in plants inoculated at 9 or 15 days of age. The incubation period and the type of symptoms produced on these seedlings did not differ for all three races. Typical halo blight symptoms appeared 8 days after inoculation at 19–27 C; results were consistent for both experiments. Stunting of plants, chlorosis of young leaves, and presence of yellowish green halos around water-soaked and necrotic lesions were evidence of toxin production by isolates of all three races.

DISCUSSION

Infected seed is generally considered to be the most important source of *P. s. phaseolicola* for primary inoculum in bean crops, especially because pathogen-free seed programs are lacking in Tanzania and because farmers retain portions of their harvest for the next planting (5). However, we have shown that at least one weed species, *N. wightii*, can serve as a perennial reservoir of *P. s. phaseolicola* in northern Tanzania. The halo blight bacterium was detected in this weed throughout the year, even during dry periods when beans were not growing in the field, evidence that *N. wightii* provides a suitable habitat for long-term survival and multiplication of *P. s. phaseolicola*.

The pathogen was readily disseminated from the weed to neighboring bean plants under different weather conditions existing in the two regions of Tanzania studied. These findings may account for the occasional outbreaks of bean halo blight that have been reported in the Kilimanjaro region, even when pathogen-free seed was used. Because some of the farmers' bean crops were free of halo blight disease, infected seed may not always be the source of initial inoculum in the region. Furthermore, halo blight was observed only at the edges of the fields in some areas in the region, additional evidence that *N. wightii* may be an important source of supplementary initial inoculum for this pathogen. Gondwe (6) speculated that occasional outbreaks of halo blight disease in the Kilimanjaro region were due to the occurrence of a new virulent race, probably race 3. However, in an extensive survey of the prevailing races in northern Tanzania, race 3 was not recovered (16).

Because *N. wightii* is a common weed on fences and hedges around farmers' backyards and because most farmers thresh their bean crop in backyards, infected bean debris and diseased volunteer plants may be sources of primary inoculum for the *N. wightii* found in such areas. In addition, the bacterium is probably spread from prunings from hedges containing diseased weeds. *N. wightii* is also used as a feed for animals kept under the zero-grazing system common in the Kilimanjaro region, which may be another factor in dissemination of the bacterium. The halo blight bacterium has been reported to survive for 9 mo after passage through sheep under Australian conditions (23). Such may also be the case in the Kilimanjaro region, especially in dry areas.

The finding of only race 1 of *P. s. phaseolicola* in *N. wightii* throughout the period of this study is surprising but agrees with other reports (1,25). Screenhouse experiments revealed that races 2 and 3 could cause disease in artificially inoculated *N. wightii*. However, there are only two reports of race 2 isolates from the same weed under natural conditions, one from Rwanda and another from Tanzania (25). Thus, under natural conditions, susceptibility of *N. wightii* seems to be restricted to race 1. We do not know why race 2 was not recovered from *N. wightii* in this study, despite its high frequency on bean in northern Tanzania (16). One reason may be that the minimum bacterial population required for disease occurrence in the weed was not attained. Infectivity titrations and genetic studies of the host-pathogen relationship are needed to understand the relationship between races of *P. s. phaseolicola* and *N. wightii*.

Because we found no infected seeds of this weed, we can hypothesize that under field conditions, a nearby infected bean crop acts as a source of initial inoculum for *N. wightii* plants. A diseased weed remains so until the next season, when it may serve as a source of inoculum for a pathogen-free bean crop grown nearby. The restriction of symptoms of halo blight disease to foliage and the failure to detect any internal infection in flower buds, blossoms, young pods, mature dry seeds, and pod shells indicate that the pathogen does not move systemically in the vascular tissue of this weed to the seed. The mechanism by which *P. s. phaseolicola* is excluded from vascular transmission to the seed of this weed is unknown. Also not known is why other bacteria occurred in *N. wightii* seed at very high numbers or how the bacteria gained access to the seeds. The fact that *P. s. phaseolicola* is not seedborne in *N. wightii* has epidemiological significance in that spread of the halo blight pathogen into new areas through infected weed seed is precluded. Although such spread may occur by way of infected bean seed, procedures for producing bean seed relatively free from the halo blight bacterium are feasible (21).

Attempts to control halo blight disease in northern Tanzania and in other areas must take into consideration infected *N. wightii* plants as reservoirs of the pathogen. Control measures should aim at disengaging this source of inoculum from the new bean crop by use of pathogen-free seed and sanitation measures. Because *N. wightii* plants survive primarily outside cultivated fields, destruction of such plants around bean fields would protect a disease-free bean crop from infection by inoculum from weeds. These disease control measures could be augmented by the introduction of bean cultivars resistant to halo blight.

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