Black Rot of Crucifers in Thailand

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ABSTRACT

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Twenty-five crucifer fields in eight provinces in Thailand were surveyed for black rot between 7 July and 15 August 1982. *Xanthomonas campestris* pv. *campestris* was isolated from plants with symptoms of black rot and pseudomonads and erwiniae, from plants with soft rot. Twenty-eight strains of *X. campestris* were recovered from 21 fields. Three strains of *Pseudomonas* sp. and four strains of *Erwinia* sp. were recovered. *X. campestris* was isolated from one of five locally obtained commercial crucifer seed lots. No cruciferous weeds were observed in or around crucifer fields. Isolation of *X. campestris* from seed together with failure to observe cruciferous weeds plus the knowledge that *X. campestris* does not survive in soil in the humid tropics of Thailand suggest use of black-rot-free seeds and a 1-yr crop rotation for control of black rot of crucifers in Thailand.

Black rot of crucifers, caused by *Xanthomonas campestris* pv. *campestris* (*X. campestris*), is known in most major agricultural countries in the world (2,3,16,17), including Thailand (5). Several cruciferous crops, including cabbage (*Brassica oleracea* L. var. *capitata* L.), cauliflower (*B. oleracea* L. var. *botrytis* L.), broccoli (*B. oleracea* L. var. *italica* Plenck), and Chinese cabbage (*B. pekinensis* Rupr.), are important in Thailand.

Thailand has a humid tropical climate with little variation in temperature. The summer season extends from March through June and has a mean temperature of 30 ± 2 C. The period from July through October is referred to as the rainy season and has a mean temperature of 27 ± 2 C. The period from November through February is dry and cooler with a mean temperature of 25 ± 2 C. In the somewhat cooler mountains of the north and northeast, the mean temperature is generally 2–5 C cooler than in the rest of Thailand. Because of these moderate temperatures, crucifers are grown year-round. Most are hybrid cultivars developed especially for the humid tropics and are imported from Japan or Taiwan. Cultivars developed for temperate climates are sometimes grown in the mountains of the north and northeast. Although black rot causes heavy losses in crucifers throughout Thailand (N. Thaveechai, *unpublished*), little is done to control the disease.

The purpose of our investigation was to determine the distribution and severity of black rot in Thailand and to collect representative strains for future serological studies on identification of *X. campestris* in Thailand.

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MATERIALS AND METHODS

Survey of black rot. Twenty-five fields in eight provinces in the major crucifer-growing areas of north, northeast, and central Thailand were surveyed for black rot between 7 July and 15 August 1982 (Fig. 1). Two districts approximately 100 km apart were included in each province except Kanchana Buri and Nonta Buri (one each). Samples of plants showing black rot or black rot-like symptoms (10) were collected. Samples with symptoms of bacterial soft rot were also collected, because symptoms of black rot and soft rot are sometimes difficult to distinguish. All samples were placed in polyethylene bags on ice, and isolations of bacterial pathogens were made on the same day. An extensive search by four or five persons was made for cruciferous weeds and/or wild cruciferous plants in and around each crucifer field surveyed. Samples of crucifer seed of several commonly grown typical cultivars were obtained from a local seed distributor in Bangkok. The samples were brought to the United States to determine the presence of *X. campestris* in seeds.

Isolation of bacteria. Leaf samples were washed under running tap water for 5 min and blotted on paper towels. Small tissue segments (approximately 1–2 × 3 mm) were excised from margins of lesions, surface-sterilized with a fresh preparation of 0.5% NaOCl (Clorox) in water (1:9, v/v) for 2–3 min, and washed

in sterile distilled water. The tissue was comminuted in a drop of sterile distilled water on a sterile microscope slide and a loopful of the suspension streaked onto yeast extract-dextrose-calcium carbonate (YDC) agar (18) and also on nutrient-starch-cycloheximide agar (NSCA) (12). Plates were incubated at 27–30 C for 48–72 hr. Colonies tentatively identified as *X. campestris* on YDC (4,13) or NSCA (11), using 23 g of nutrient agar, were cloned by streaking onto YDC. Erwiniae colonies were tentatively identified on YDC by acid production that caused a clearing around the colony. King's medium B (7) was used to isolate pseudomonads from soft rot tissues. A pure culture of each strain was transferred to a YDC slant and stored at 4 C.

Pathogenicity tests. Inocula were prepared by inoculating a loopful of a 24-hr culture from a YDC plate into liquid medium 523 (6) and incubating for 18–24 hr on a rotary shaker at room temperature (27–30 C). Suspensions were adjusted to an optical density of 0.08–0.1 at 640 nm and diluted to 10^{-4}. Cauliflower plants at the four-leaf stage were inoculated with approximately 0.1 ml of bacterial suspension using a 1-ml syringe and 25-gauge needle (14).

Seed assay. Crucifer seed samples were assayed for black rot by standard NSCA seed washing assay (11). In addition, triplicate plates of two semiselective media (8) were used for 10^{-1} dilution. Indirect immunofluorescence (9) and pathogenicity tests (14) were used to confirm the identity of suspected colonies. The following samples of tropical hybrid seed were tested: cauliflower cultivar 45 Days, cabbage cultivar 60 Days, and Chinese cabbage cultivars Thianchin and Thianchin 23. A sample of broccoli cultivar Green Comet originating from Japan and obtained from Khon Kaen University, Khon Kaen, Thailand, was also tested. All seed tested originated from Taiwan or Japan. Because Thailand has no uniform labeling laws, seeds are often sold under local names without reference to the original seed lot or cultivar.

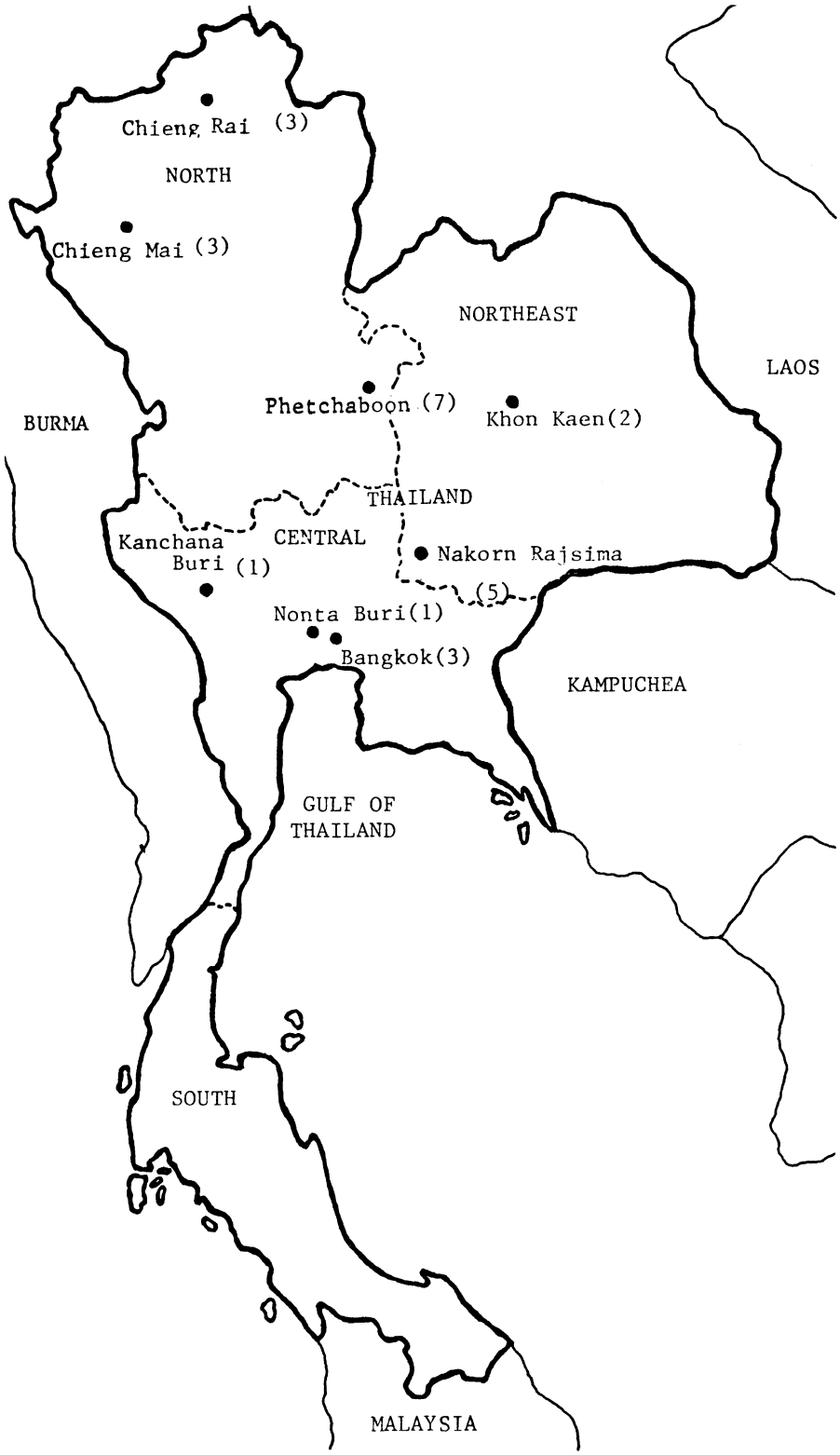

Fig. 1. Broken lines divide Thailand into north, northeast, central, and south. Black dots are locations of the eight provinces (states) surveyed for black rot of crucifers, with number of fields surveyed in parentheses.

RESULTS

Black rot symptoms were observed in 21 of the 25 fields surveyed. A total of 28 strains of *X. campestris* were isolated from cabbage, cauliflower, broccoli, Chinese cabbage, and kale (*B. oleracea* L. var. *acephala* D.C.) (Table 1). Black rot symptoms were not observed in one field in the districts of Pasee Charoen, Lom Sak, Pak Chong, and Muang. Strains BT-1, BT-2, BT-3, BT-5 through 18, BT-21, BT-24, BT-25, BT-27, and BT-28 were isolated from plants with typical yellow leaves with black veins, whereas strains BT-4, BT-19, BT-20, BT-22, BT-23, and BT-26 were isolated from plants with chlorotic spots, wilting, and black veins without yellow leaves. Three strains of *Pseudomonas* sp. and four strains of *Erwinia* sp. were isolated from cabbage and cauliflower with symptoms of soft rot (Table 1).

X. campestris was not isolated from plants with symptoms of soft rot. All tentatively identified strains of *X. campestris* were pathogenic on cauliflower. *X. campestris* was isolated from one of the five seed lots sampled, the cabbage cultivar 60 Days. The pathogen was recovered at 10^{-3} dilution on all three media but not at 10^{-1} dilution owing to overgrowth by saprophytic bacteria. This seed strain, designated BT-29, was immunofluorescent-positive and pathogenic on the cabbage cultivar Market Victor.

No cruciferous weeds were observed in any of the surveyed fields or in areas adjacent to the cruciferous fields.

Table 1. Origin of strains of *Xanthomonas campestris* pv. *campestris* and other bacterial pathogens isolated from cabbage (*Brassica oleracea* L. var. *capitata* L.) and other crucifers in Thailand in July 1982

Growing area	Province and district	Total fields observed	Disease	Host	Pathogen	Strains
Central	Bangkok, Pasee Charoen	3	Black rot	Cauliflower (*B. oleracea* L. var. *botrytis* L.)	*X. campestris*	BT-1,2
			Black rot	Kale (*B. oleracea* L. var. *acephala* D.C.)	*X. campestris*	BT-3,4
			Soft rot	Cauliflower	*Pseudomonas* sp.	CT-1
	Nonta Buri, Bang Bua Thong	1	Black rot	Cauliflower	*X. campestris*	BT-28
	Kanchana Buri, Tha Mung	1	Black rot	Cabbage	*X. campestris*	BT-27
North	Phetchaboon, Lom Sak	7	Black rot	Cabbage	*X. campestris*	BT-15 through 18
			Soft rot	Cabbage	*Erwinia* sp.	AT-2
			Soft rot	Cauliflower	*Erwinia* sp.	AT-1
	Chieng Mai, Chom Thong	3	Black rot	Cabbage	*X. campestris*	BT-19
			Black rot	Cauliflower	*X. campestris*	BT-20
			Black rot	Broccoli (*B. oleracea* L. var. *italica* Plenck)	*X. campestris*	BT-21,22
			Soft rot	Cabbage	*Erwinia* sp.	AT-3
	Chieng Rai, Mae Sruay	3	Black rot	Cabbage	*X. campestris*	BT-23,24
			Black rot	Cauliflower	*X. campestris*	BT-25,26
			Soft rot	Cabbage	*Erwinia* sp.	AT-4
Northeast	Nakorn Rajsima, Pak Chong	5	Black rot	Cabbage	*X. campestris*	BT-5 through 11
			Soft rot	Cabbage	*Pseudomonas* sp.	CT-2,3
	Khon Kaen, Muang	2	Black rot	Broccoli	*X. campestris*	BT-12,13
			Black rot	Chinese cabbage (*B. pekinensis* Rupr.)	*X. campestris*	BT-14

DISCUSSION

The presence of *X. campestris* in 21 of 25 (85%) fields surveyed and on five different cruciferous hosts indicates that black rot of crucifers is widely distributed throughout Thailand. The occurrence of *X. campestris* in one of five seed lots assayed establishes the presence of infested seeds in commercial seed lots in Thailand but does not determine the role or importance of seed as a primary source of inoculum.

Our failure to observe any cruciferous weeds in July or August in Thailand is not unexpected, since most crucifers are cool-season plants. Our observations are, however, in sharp contrast to those in Georgia where cruciferous weeds are very common in and around transplanted beds most of the year (10). Unlike in Georgia, crucifers are grown in Thailand with intensive hand cultivation in small 0.4–2 ha family-owned fields. Because of the abundance of hand labor, one seldom observes weeds in vegetable crops in Thailand (N. Thaveechai, *unpublished*). Still, only by a month-to-month survey can the absence of cruciferous weeds be confirmed. Whereas the apparent absence of cruciferous weeds is in contrast to the situation in Georgia and California (10), the failure of *X. campestris* to survive longer than a year in debris in soil in plots of Kasetsart University in Bangkok (15) is in agreement with results in southern Georgia (14).

Finding several fields with both black rot and soft rot in Thailand was not surprising because of the humid tropical conditions. In India (1) it has been suggested that *X. campestris* predisposes host plants to infection by soft rot organisms. Various black rot symptoms were observed in Thailand, but the presence of dark veins in yellow areas or in green leaves was still diagnostic. Chlorotic spots, wilting, and black veins without yellowing were unexpected observations.

Black rot will most likely become more widespread and a more serious disease of cruciferous plants in Thailand if proper control methods are not initiated. Because *X. campestris* does not survive longer than a year in debris in soil in Thailand (15) and because cruciferous weeds apparently are absent, crop rotation and use of black-rot-free seeds are perhaps the most beneficial recommendations for controlling black rot in Thailand. However, most vegetable growers grow crucifers continuously because cruciferous crops have a high market value and because they do not want to grow different crops. Another common practice that increases the chances of black rot is the sowing of seeds for plant production for the next crop (often the same year) in one corner of the current production field. This was observed in most of the fields we visited.

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LITERATURE CITED

1. Chakvavarti, B. P., and Hegde, S. V. 1972. Occurrence of bacterial soft rot in black rot affected cabbage plants in Udaipur Rajathan. Indian Phytopathol. 25:118-122.
2. Chupp, C., and Sherf, A. F. 1960. Vegetable Diseases and Their Control. Ronald Press, New York. 693 pp.
3. Dawson, W. J. 1957. Plant Diseases Due to Bacteria. Cambridge University Press, London. 232 pp.
4. Dye, D. W. 1980. *Xanthomonas*. Pages 45-49 in: Laboratory Guide for Identification of Plant Pathogenic Bacteria. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. 72 pp.
5. Giatgong, P. 1980. Host Index of Plant Diseases in Thailand. 2nd ed. Mycology Branch, Plant Pathology and Microbiology Divisions, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. 118 pp.
6. Kado, C. I., and Heskett, M. C. 1970. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*. Phytopathology 60:969-976.
7. King, E. O., Ward, M. K., and Raney, D. R. 1954. Two simple media for the demonstration of pyrocanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
8. Randhawa, P. S., and Schaad, N. W. 1982. Improved assay media for isolating *Xanthomonas campestris* pv. *campestris* from crucifer seeds. (Abstr.) Phytopathology 72:944-945.
9. Schaad, N. W. 1978. Use of direct and indirect immunofluorescence tests for identification of *Xanthomonas campestris*. Phytopathology 68:249-252.
10. Schaad, N. W., and Dianese, J. C. 1981. Black rot of crucifers: Cruciferous weeds as sources of inoculum of *Xanthomonas campestris*. Phytopathology 71:1215-1220.
11. Schaad, N. W., and Donalson, R. C. 1980. Comparison of two methods for detection of *Xanthomonas campestris* in infected crucifer seeds. Seed Sci. Technol. 8:383-391.
12. Schaad, N. W., and Kendrick, R. 1975. A

qualitative method of detecting *Xanthomonas campestris* in crucifer seed. Phytopathology 65:1034-1036.
13. Schaad, N. W., and White, W. C. 1974. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.
14. Schaad, N. W., and White, W. C. 1974. Survival of *Xanthomonas campestris* in soil. Phytopathology 64:1518-1520.
15. Soontarasing, S., and Kositratana, W. 1981. Studies on survival and pathogenicity of *Xanthomonas campestris* from infested soil in different range of time. Research Reports, Kasetsart University, Annual Reports 1981–1982. 11 pp. (English summary)
16. Walker, J. C. 1952. Diseases of Vegetable Crops. McGraw-Hill, New York. 592 pp.
17. Williams, P. H. 1980. Black rot: A continuing threat to world crucifers. Plant Dis. 64:736-742.
18. Wilson, E. E., Zeitoun, F. M., and Frederickson, D. L. 1967. Bacterial phloem canker, a new disease of Persian Walnut trees. Phytopathology 57:618-621.