# STUDIES ON SEED TRANSMISSION OF XANTHOMONAS CAMPESTRIS PV PHASEOLI IN COMMON BEANS IN UGANDA

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## ABSTRACT

Studies on the seed transmission of Xanthomonas campestris pv phaseoli (XCP) that cause common and fuscous blight of beans were conducted in Uganda. Bean seed was the main source of primary inoculum. The population of XCP in farmers', commercial and research seeds averaged 10<sup>5</sup>–10<sup>9</sup> colony forming units (cfu) per 100 seeds; the levels of seed infection were 0.3–16.1%. There was a positive correlation between seed symptoms and population of XCP per seed. However, where seed was either slightly diseased or symptomless, XCP did not decrease bean germination irrespective of population density. The minimum population of XCP required to initiate infection in the field was 10<sup>2</sup> cfu per seed while 0.2% seed infection level resulted in serious disease incidence.

Key Words: Bacterial blight, Xanthomonas campestris beans, seed infection.

# INTRODUCTION

Common bacterial blight (CBB) and fuscous blight, caused by Xanthomonas campestris pv phaseoli (XCP), are among the most serious seed borne diseases affecting beans (Phaseolus vulgaris L.) in eastern Africa (5; 6; 11). Internally infected seeds form the main source of primary

inoculum (2; 19). Worldwide distribution of these diseases is partly associated with the ability of the pathogen to infect seeds of both resistant and susceptible genotypes (1). In Canada, 0.5% seed infection level has been shown to lead to disease epidemics (21). The causes of epidemics of blight in the tropics, that occur sometimes even in newly opened areas, needs further investigation.

This study was, therefore, initiated to investigate and provide quantitative data on both common and fuscous blight transmission in Uganda.

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#### MATERIALS AND METHODS

The study was conducted at Kawanda Research Station, Uganda, between June 1991 and July 1992, and covered laboratory, screenhouse and field experiments.

XCP infection in farmers' and non-farm seed. Seeds used to determine levels of seed infection were obtained from the National Seed Project at Kawanda, and from 25 farmers, seven markets and eight varietal trial centers in eleven districts. A total of 62 seedlots were tested. The seeds were clean, with no obvious symptoms of disease infection, which for farmers formed part of seed for planting in the following season.

Two media; MXP (a semi selective medium for XCP) and yeast dextrose carbonate agar (YDCA) were used in all isolations and cultures of XCP. MXP was prepared following the procedure described by Claflin et al. (3) while YDCA was prepared as described by Vidaver (19).

The most probable number method (MPN) (4) was used to estimate the level of CBB infection in the seed. Eleven samples were drawn from each lot, one of 500 seeds, five of 100 seeds and five of 10 seeds.

The seeds were plated, hilum downwards, on both media and records taken after incubating at 28 ± 2°C for 5 days. Seeds from which XCP was recovered were recorded as positive and those from which XCP was not recovered as negative. Colonies that hydrolyzed starch on MXP were assumed to be XCP (3). The level of infection was determined using the most probable number tables (13).

From each sample, 5 or 6 colonies with typical characteristics of XCP were selected for pathogenicity testing. After a further 48 hours growth, the increased inoculum was tested for pathogenicity by inoculating the second trifoliolate leaves of 18-day old seedling of the cultivar Kanyebwa grown in the screenhouse. The plants were grown in 20 pots of 25 cm-diameter, and filled with heat sterilized soil mixed with sand in the ratio of 2:1. Five bean seeds were sown per pot, NPK fertilizer (25-5-5) was applied at the rate of 1 g/7 kg of soil. The razor blade inoculation method (7) was used,

with XCP inoculum concentration of about 107 cfu/ml.

Relationship between seed symptoms, bacterial cell numbers per seed and seedling infection. Three bean cultivars: Kanyebwa, K 20, and XAN 112 were used, the first two being susceptible to XCP while the latter is resistant. A 2 kg sample of severely infected seed of each of the three cultivars was graded on the basis of visible symptoms in three categories:

Category 1: Symptomless, no lesion or discoloration on seeds.

Category 2: Slight to moderate symptoms — seeds with less than 10% butter-yellow discoloration or with discoloration in the hilum region.

Category 3: Severe symptoms — seeds with >10% discoloration with partial shrivelling.

Evaluation of the relationship between seed symptom, bacterial cell count/seed, and seedling infection was based on the method described by Taylor *et al.* (16).

The bacterial numbers in seed were determined using six groups of 100 seeds for each symptom category. Single seeds were ground in a sterile mortar containing 1 ml of 0.01M phosphate buffer at pH 7.2. The suspension obtained was shaken for 30 minutes and serially diluted ten-fold before plating. Aliquots of 0.1 ml (10<sup>-2</sup>, 10<sup>-4</sup>, 10<sup>-6</sup> and 10<sup>-8</sup>) were spread on media in triplicates and plates incubated for 5 days. From each set of seeds, a random sample of ten bacterial colonies were selected and tested for pathogenicity.

Disease transmission to seedlings was tested in the field and in the screenhouse. One hundred seeds from each category and each genotype were sown in 6 metre long two row plots, with three replications and a 1 m spacing between the plots. The design was a split plot with bean genotypes in the main plots and symptom categories in subplots. Maize was sown between the plots and in guard rows, two weeks earlier, to reduce interplot interference (16).

The same number of seeds and replications for each infection category were used in the screenhouse and in the field. Soil preparation and sowing was as described above for the

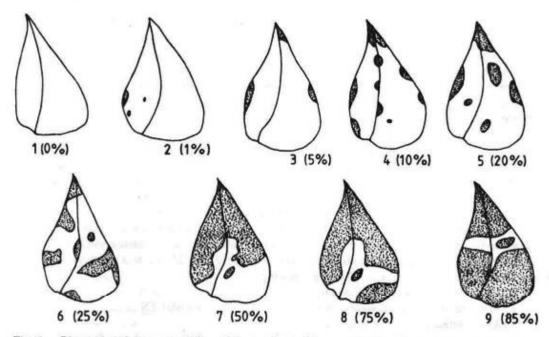


Fig. 1. Diagrammatic representation of the scale used to rate common bacterial blight of common beans on leaves.

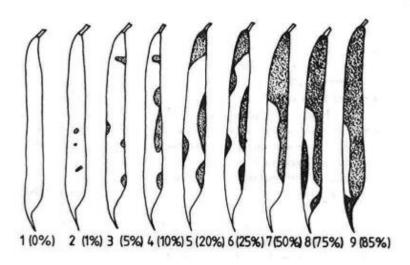


Fig. 2. Diagrammatic representation of the scale used to rate common bacterial blight of common beans on pods.

screenhouse. A total of 20 pots were used for each category for every replication.

Incidence and severity of the disease on cotyledons was recorded at seedling emergence. Thereafter, records were taken at 10, 18, 35, 42

and 56 days after sowing. Incidence was assessed by counting the number of infected plants and severity was scored using a modified CIAT 1-9 scale (Figs. 1 and 2).

Three bean genotypes: Kanyebwa, K 20 and

White haricot were used. The different levels of seed infection used were 0, 0.2, 0.4, 0.5, 1, 2, 3, 5, 10 and 20%. The clean seed used was multiplied in the off-season in the screenhouse at Kawanda. The infected seed was collected from severely diseased fields of the preceding season. Random samples of diseased and clean seed were assessed using the most probable number sampling method (14) to establish XCP inoculum levels before mixing the seeds in the proportions required. The samples were tested by plating seeds on MXP and YDCA and incubating as above. Where there was growth of XCP from presumably clean seed, such seed was discarded.

The experimental design used was a split plot, with genotypes in main plots and percentage infection in subplots, with three replications. The plot sizes were 6 x 6 m in 1991 and 12 x 12 m in 1992. Main plots and subplots were separated by 2 m space. Two rows of maize were sown between the plots to reduce interplot interference. The seedlings from infected seeds were tagged as soon as they were recognizable at two leaf stage. The presence or absence of bacterial blight symptoms on these plants was noted at tagging.

Disease incidence was determined as number of plants affected per plot, and severity was assessed using the modified CIAT 1–9 scale (Figs. 1 and 2). Incidence was determined by checking primary leaves of each plant at 10 and 18 days after sowing. Thereafter, four 1 m square quadrants were used per experimental unit and records taken weekly. Severity was assessed on 10 randomly selected plants, and a general score for the experimental unit taken. This was done at 18, 35, 42 and 56 days after sowing.

Relationship between level of visible seed infection and incidence of CBB in seedlings. Two experiments were conducted in the field and two in the screenhouse, to determine the minimum population of XCP necessary for successful seedling infection.

Seeds of three bean genotypes: Kanyebwa, K 20 and XAN 112 were inoculated with XCP at nine concentrations ranging from 0 to 5x108 cfu/ml. The experimental design was a split plot with varieties in main plots and concentrations in subplots. One hundred seeds were sown in the screenhouse as described above. Two 5 m row

plots separated by 1 m wide space were used in the field. Maize was sown between plots and in guard rows to reduce interplot interference.

A 48-hour old culture of XCP isolate 1010, obtained from a field at Kawanda and grown on YDCA, was used as the inoculum. The bacterial culture was washed into a sterile flask with sterile phosphate buffer (0.01m; pH 7.2), and the suspension adjusted to a concentration of 5 x 108 cfu/ml at a wavelength of 620nm, using a Milton Roy 20<sup>TM</sup> spectrophotometer. Seven serial dilutions were made in the same buffer. The seeds were inoculated by soaking in the different bacterial concentrations for four hours before sowing. The control seeds were soaked in the phosphate buffer. Disease incidence and severity were scored 10, 18, 35, 42 and 56 days after sowing.

#### RESULTS

XCP infection in farmers and non-farm seeds. The incidence of XCP infection in seed samples

from farmers, varietal testing centres and markets varied with the genotype and location (Table 1). Kabale had the lowest level of seed infection (0.3%) while Masindi had the highest (16.1%). The seed of White haricot was more infected compared to other genotypes from the same location. Kanyebwa and K 20, the most common genotypes encountered, had infection levels ranging from 0.8 to 3.5%. MCM 5001 had the lowest seed infection among seed obtained from varietal testing centers.

The XCP population was high for all seed lots. The number recovered ranged between 10<sup>5</sup> and 10<sup>9</sup> cfu/100 seeds. All the XCP isolates recovered from seed induced typical bacterial blight symptoms when inoculated onto the susceptible genotype Kanyebwa.

Relationship between common bacterial blight symptoms on seed, bacterial numbers per seed and production of infected seedlings. Seeds with severe symptoms (Category 3) had the highest population of XCP cells. Large number of bacterial cells (1.7 x 10<sup>6</sup> – 5.2 x 10<sup>6</sup>) were also recovered from apparently clean seeds (Table 2). There were no significant differences in number of XCP cells<sub>1</sub> recovered per seed between

TABLE 1. Incidence<sup>a</sup> and population<sup>b</sup> of Xanthomonas campestris pv phaseoli in bean seed collected from different parts of Uganda

District			Incidence (%	)	Population of XCP			
	Genotype	VTC	Farmers	Markets	VTC	Farmers	Markets	
Arus	K 20	=	-	1.8	-	-	4.8 x 10 <sup>9</sup>	
	White haricot	-	-	2.8	-	-	1.7 x 109	
	Black haricot	-	-	1.1	_	-	$8.4 \times 10^9$	
	K 20	-	3.5	1.8	-	8.4 x 10 <sup>8</sup>	$4.8 \times 10^{9}$	
Tororo	Kanyebwa	-	1.7	1.4	-	5.8 x 10 <sup>1</sup>	$3.8 \times 10^{7}$	
Mpigi	K 20	2.8	-	ш.	1.3 x 108	4	-	
(Bukalasa)	CAL 96	1.4	-	-	5.2 x 10 <sup>8</sup>	-	-	
Kabale	K 20	-	1.1	0.8	-	5.3 x 10 <sup>8</sup>	5.4 x 10 <sup>9</sup>	
	Mixture	-	0.3	1.1	-	$4.0 \times 10^{7}$	$5.3 \times 10^{5}$	
	G 5435	-	8.0	-	-	6.2 x 10 <sup>8</sup>	-	
Kampala	K 20	-	2.2	5.4	-	4.4 x 108	5.3 x 10 <sup>8</sup>	
	White haricot	-	-	9.2	-	-	$7.7 \times 10^9$	
	Kanyembwa	-	1.4	1.7	-	$6.3 \times 10^7$	$4.5 \times 10^7$	
	Kahura	-	-	1.4	-	-	$3.4 \times 10^7$	
	Kanyinja	-	-	1.3	-	-	$4.5 \times 10^7$	
	Kulyembarukye	-	-	2.2	-	-	$3.4 \times 10^7$	
Masindi	White haricot	-	3.5	16.1	-	3.6 x 10 <sup>8</sup>	8.4 x 10 <sup>8</sup>	
	Kanyebwa	-	1.8	2.8	-	5.2 x 10 <sup>6</sup>	1.8 x 10 <sup>8</sup>	
	K 20	2.8	3.5	3.5	$2.8 \times 10^{8}$	$3.5 \times 10^7$	$4.7 \times 10^{8}$	
	Mutike	-	1.7	-	-	-	2.4 x 10 <sup>6</sup>	
	CAL 96	1.1	=	-	$7.5 \times 10^6$	7	7	
Jinja	Kanyebwa	-	-	1.7	-	-	4.8 x 10 <sup>6</sup>	
	K 20	1.1	-	2.8	$4.8 \times 10^{8}$	-	2.3 x 10 <sup>8</sup>	
Mpigi	Trailer !	7427427						
(Kawanda RS)		2.8	-	_	-	_	3.4 x 10 <sup>9</sup>	
	Kanyebwa	0.5	-	-	-	-	4.4 x 10 <sup>7</sup>	
	White haricot	9.2	-	_	-	-	7.8 x 10 <sup>7</sup>	
	CAL 96 RWR 36	1.1	_	_	_	_	5.4 x 10 <sup>7</sup>	
		1.0				3.3 x 10 <sup>7</sup>		
Lowero	Mutike	-	1.4	-	-	5.2 x 10 <sup>7</sup>	-	
	CAL 96	-	2.2	-	_	1.9 x 10 <sup>7</sup>	_	
	CAL 98 K 20	_	1.8	-	-	4.7 x 10 <sup>9</sup>	-	
Manaka	MCM	0.7	-		3.8 x 10 <sup>9</sup>			
Masaka	CAL 96	1.1	-	_	7.8 x 10 <sup>9</sup>	_	_	
	CAL 98	1.0	-	-	6.2 x 10 <sup>9</sup>	-	-	
Mbale	K 20			2.8	_	_	_	
widale	Kanyebwa	_	_	5.4	_	_	-	
	MCM 5001	0.5	-	5.4	4.4 x 10 <sup>7</sup>	-	7.4 x 10 <sup>9</sup>	
	CAL 96	2.2	_	_	5.4 x 10 <sup>7</sup>	-	3.9 x 10 <sup>9</sup>	
	K 20	3.5	-	-	6.8 x 10 <sup>8</sup>	-	-	
					0.5 400			
Kasese	K 20	2.4	-		$2.5 \times 10^{8}$	-	-	

<sup>&</sup>lt;sup>a</sup>Incidence is the percentage of seeds infected with XCP as determined by the most probable number method and interpreted using tables of Taylor and Phelps (14).

<sup>b</sup>The population of XCP was determined as colony forming units (cfu) per 100 seeds.

VTC: varietal testing centres.

symptom Categories 1 and 2. Seed infection were highest for K 20, and lowest for XAN 112, their infection level being similar for all symptom categories (Table 2). For both Kanyebwa and XAN 112, infection for categories I and 2 were low, and statistically similar, but were significantly (P < 0.05) higher than infection for Category 3.

Seed germination failure ranged from 47.7% for symptom category 3 seeds of Kanyebwa and XAN 112, to only 0.3% for Category 1 of XAN 112 (Table 2). Higher seed infection significantly reduced germination. There were also significant ( $P \le 0.05$ ) differences among categories for seedling infection at 10 days after sowing (Table 2). Symptom category 3 had the highest incidence while Category 1 had the lowest incidence. Differences in number of infected plants between slightly diseased and symptomless seeds were not significant ( $P \le 0.05$ ).

Symptomless seeds contributed between 24.5-27.3% to the CBB transmission for Kanyebwa and 27-35% for K 20 (Table 2). Corresponding figures for slightly diseased seed

were 30–34% for Kanyebwa and 34% for K 20. No seed transmission occurred for these categories of seed for XAN 112. There was a positive and significant ( $P \le 0.05$ ) correlation between the number of infected seeds and the percentage of infected seedlings at 10 days after sowing (r = 0.72). Correlations between the number of XCP cells per seed with the number of infected seedlings were not significant. The ratio of seed infection to primary infection ranged from zero to 19:10.

Relationship between level of visual seed infection and incidence of CBB in seedlings. The 20% seed infection gave the highest number of infected seedlings at 10 days after sowing (Table 3). There were no significant (≤ 0.05) differences in number of infected seedlings produced at 10 days. The control remained free from common bacterial blight. At pod formation, and pod filling stages (42 and 56 days) 20% seed infection gave the highest incidence (97.1%) while 0.2% gave the lowest incidence (47.1%) at

TABLE 2. Relationship<sup>a</sup> between common bacterial blight seed symptoms, *Xanthomonas* campestris pv phaseoli (XCP) population per seed and seedling infection in 1991 in the field (F) and screenhouse (S)

Var.	Seed symptoms category	(%) infected seeds	Mean XCP seed	Seedling emergence (%)		Seedling infection at 10 days (%)		Contribution to primary transmission (%)		Seed infection to primary infection ratio	
				F	s	F	s	F	s	F	s
Kany	ebwa		50000 00000								
D. Ministra	1	4.3	2.0 x 10 <sup>6</sup>	90.3	92.5	2.8	3.5	24.5	27.3	1.9:1	1.5:1
	2	7.3	$4.7 \times 10^7$	79.7	81.5	4.2	4.2	32.5	30.4	1.91	1.3:1
	3	16.0	$9.0 \times 10^{8}$	48.0	47.7	5.3	5.5	43.0	42.3	1:1	1:1:1
K 20											
	1	15.7	5.2 x 106	83.7	92.7	3.6	3.5	34.9	34.1	1.5:1	1.5:1
	1 2 3	16.7	7.7 x 10 <sup>6</sup>	70.3	80.3	4.4	4.0	35.7	37.2	1.5:1	1.4:1
	3	16.0	1.3 x 10 <sup>8</sup>	56.3	54.5	4.5	5.0	29.4	28.7	1.2:1	1.1:1
XAN	112		*								
	1	0.7	1.7 x 10 <sup>6</sup>	98.3	99.7	0.0	0.0	0.0	0.0	1.2:1	0
	2	1.7	1.3 x 10 <sup>6</sup>	78.7	80.0	0.0	0.0	0.0	0.0	0	0
	3	6.0	2.3 x 10 <sup>6</sup>	47.7	52.5	0.7	0.6	100.0	100.0	1:1	1:2:1

<sup>&</sup>lt;sup>a</sup>Estimated level of infection by MPN method and interpreted with tables by Taylor and Phelps (14). Kanyebwa = 5.4; K 20 = 5.4; XAN 112 = 0.7.

TABLE 3. The effect of number of infected seeds in a seed lot on incidence of common bacterial blight in the subsequent bean crop at 10, 42 and 56 days after sowing

Percentage infected seed	Incidence of CBB (%)									
	10 days		42 0	lays	56 days					
	1991	1992	1991	1992	1991	1992				
20	14.6a	12.0a	72.8a	65.2a	97.1a	93.6a				
10	10.0b	8.1b	68.8b	61.0b	92.3b	89.7b				
5	6.5c	4.7c	49.4c	45.9c	88.8c	81.2c				
3	3.4d	2.0d	40.5d	31.7d	66.4d	58.4d				
2	1.6e	0.8e	26.3e	20.4e	64.0e	56.9e				
1	1.0ef	0.4ef	12.6f	10.0f	54.0h	56.1e				
0.5	0.4efg	0.3ef	13.1f	9.3f	54.6h	48.9f				
0.4	0.8efg	0.2ef	9.3f	7.1g	57.0g	48.1fg				
0.2	0.2g	0.2ef	13.2g	7.0g	59.7f	47.1g				
0	0.0h	O.Of	0.0h	0.0h	9.8i	7.0h				

Means in a column followed by the same letter are not significantly different at 5% Duncan's Multiple Range Test.

TABLE 4. The effect of population of Xanthomonas campestris pv phaseoli on seedling infection at 10 days (d) after sowing and adult (XCP) plant infection at 56 days after sowing

Population of XCP		1	991	1992				
	% infection		severitya		% plants infected		severity	
	10 d	56 d	10 d	56 d	10 d	56 d	10 d	56 d
108	8.2a	80.7a	2.1a	5.4ab	15.8ab	84.7a	2.3a	6.3a
107	8.6a	79.0a	2.2a	5.8a	17.4a	84.5a	2.7ab	6.4a
106	8.3a	78.4a	1.9ab	5.2ab	15.5ab	82.4ab	2.2ab	6.3a
105	8.0ab	76.1ab	2.0ab	5.0b	11.3ab	82.1ab	1.7b	6.1a
104	7.2ab	77.1ab	2.0ab	4.9bc	12.0ab	81.1ab	2.1b	6.3a
103	6.8abc	76.4ab	1.4bc	4.9bc	9.5ab	77.0b	2.1b	6.1a
102	4.6c	75.5b	1.2c	4.4c	7.6b	768b	1.5b	6.1a
101	0.0d	55.1d	1.0c	1.8c	0.0c	61.1c	1.0b	1.6b
Control	0.0d	31.2d	1.0c	1.2d	0.0c	54.4d	1.0b	1.2b

<sup>&</sup>lt;sup>a</sup>Severity assessed on a scale of 1-9.

Means in a column followed by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

56 days after sowing. This indicated that a seed infection level as low as one seed in 500 could lead to a high disease incidence provided conditions are favorable for disease development.

Minimum population of XCP necessary for successful seedling infection. The lowest XCP population on seed that produced diseased seedlings at 10 days after sowing was  $10^2$  cfu/ml (Table 4). An XCP population of  $10^7$  cfu/ml in seed gave the highest number of diseased seedlings in all experiments. The control and  $10^1$  cfu/ml did not result in seedling infection at 10 days after sowing in both years.

The severity and incidence of bacterial blight was highest in treatments with XCP populations of 10<sup>7</sup> cfu/ml 56 days after sowing (Table 4). Bacterial blight symptoms were observed on plants grown from seeds inoculated with 10<sup>1</sup> cfu/ml and control plots (buffer inoculated) at 56 days but not at other earlier stages. The appearance of blight symptoms in these plots at this stage was a result of secondary infection. The severity was, however, very low as compared to other treatments.

# DISCUSSION

This study demonstrated that the bean seed used by farmers in Uganda is heavily infected by XCP. The lowest level of seed infection was 0.3% and the bacterial population per seed ranged between 10<sup>5</sup> and 10<sup>9</sup> cfu/100 seeds. The minimum bacterial population per seed necessary to incite infection in the field was found to be 10<sup>2</sup> cfu/seed.

There was significant correlation between symptoms on seed and the population of XCP per seed. Symptomless and slightly diseased seeds gave rise to severely infected seedlings. This demonstrated that the normal cleaning, by removing colored and shrivelled seeds, would have limited effect on reducing the XCP infection of a seed stock if it was obtained from an infected field. Similar observations were made by Saettler (8) on common bacterial blight, and by Taylor (16) on halo blight of beans.

A level of seed infection of 0.2% was found to initiate and cause severe infection in the field, similar to those reported for halo blight of beans (10: 16) and for Xanthomonas campestris pv campestris for crucifers (14). In Canada 0.5% seed infection was capable of causing common blight epidemics (12). Trigalet and Bidaud (17) established a tolerance level of one infected seed per 20,000 for controlling halo blight of bean in France. A seed transmission ratio of Pseudomonas syringe pv phaseolicola of 2:1 found by Taylor (15; 16) compares well with the ratic found in this study.

The minimum population of XCP . initiate common bacterial blight infection was und to be 10<sup>2</sup> cfu/seed which was lower than 10<sup>3</sup>-10<sup>4</sup> cfu/seed found by Weller and Saettler (20) in

USA. This may be attributed to differences in inoculum and bean types used, and environmental conditions. The higher temperatures involved in the present study may have enhanced development of disease symptoms from the lower populations of XCP in seed. Santana (9) reported that development of common bacterial blight was much faster on plants at higher temperatures (29.4°C) than lower temperatures (23.8°C). It is recommended that research on the XCP tolerance level needs to be carried out using more bean genotypes and locations.

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