Survival of *Xanthomonas campestris* pv. *citri* in Citrus Plant Debris and Soil in Florida and Argentina

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ABSTRACT

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Citrus nurseries infested with the Florida nursery strain of *Xanthomonas campestris* pv. *citri* (Xcc-FN) and dooryards and an orchard infested with the Asiatic strain (Xcc-A) were sampled to determine survival of the bacterium in soil and plant debris. Xanthomonads presumptively identified as *X. campestris* were recovered from nonsymptomatic plants and soil before and after eradication of citrus plants in nurseries but were not pathogenic to citrus. Pathogenic Xcc-A was detectable in leaf lesions of grapefruit and Pineapple sweet orange at least 90 days after defoliation under relatively dry conditions in the spring of 1986 in Florida. Under similar conditions in Argentina, Xcc-A was detected after 120 days in lesions of grapefruit leaves placed on the soil surface but only up to 85 days when leaves were buried. In air-dried sandy soil (−1,500 cb soil water potential), Xcc-FN was detected up to 105 days, but under slightly moist to saturated conditions (−70 to 0 cb), it survived for less than 24 days. Soil fumigants methyl bromide (MC2), methyl bromide-chloropicrin (66–33%) (MC33), metam-sodium, and dichloropropene-methyl isothiocyanate killed citrus roots that otherwise resprout to produce susceptible host tissue. Metam-sodium and dichloropropene-methyl isothiocyanate were more effective than MC2 in reducing populations of Xcc-FN in grapefruit leaves, whereas MC33 had no effect on populations. In field trials in Argentina, MC2 and methyl isothiocyanate did not eradicate Xcc-A in lesions of grapefruit leaves but reduced the survival time in leaves from 85 days or more to less than 45 days.

Additional key words: epiphytic survival

The current outbreaks of citrus canker in Florida were caused by a previously undescribed group of strains of *Xanthomonas campestris* pv. *citri* (Hasse) Dye isolated from nurseries (Xcc-FN) (18), whereas the Asiatic strain (Xcc-A) was isolated from dooryards and a nearby citrus orchard on the west coast. Questions have been raised about the survival of the bacterium through eradication efforts that attempt to destroy the aboveground portion of the plant. If *X. c.* pv. *citri* can survive in plant debris and soil until the area is replanted, then soil treatments must be applied to completely eradicate the bacterium. Otherwise, the area must be quarantined against replanting of citrus for some period of time.

Saprophytic survival of *X. c.* pv. *citri* in soil and plant residues has not been established conclusively. Generally, populations are thought to decline rapidly in soil, in lesions on detached leaves and fruit, and on infested host and nonhost roots in soil (1), but bacterial survival of up to 8 mo has been reported on weeds, in soil, and on the roots of citrus and other plants (5–7). The significance of survival of *X. c.* pv. *citri* in plant residues and soil is not completely known. However, Goto et al (7) suggested that as few as 10^2 cfu/g soil or plant material could result in successful infection of wounded *Citrus natsudaidai* leaves.

In this report, studies were conducted to confirm whether *X. c.* pv. *citri* resided in soil and plant debris in field nurseries and beneath mature trees under Florida conditions. Because xanthomonads are presumed to survive (5–7), cultural practices such as soil fumigation, irrigation, and burial of plant material in soil were investigated in Florida and Argentina with respect to the survival of host citrus roots and the longevity of *X. c.* pv. *citri* in detached leaves.

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MATERIALS AND. METHODS

Recovery of *X. c.* pv. *citri* from infested citrus nurseries, dooryards, and orchards. Soil and plant material were collected before and after eradication of citrus plants in nurseries determined to be infested with Xcc-FN by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) (18). In one greenhouse and one field nursery, samples of lesioned and asymptomatic leaves of Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. × *C. paradisi* Macf.) and soil were collected before eradication. In two field nurseries after eradication, soil and residual plant debris in the form of rootstock stems and sprouts of Swingle citrumelo, Carrizo citrange (*C. sinensis* (L.) Osb. × *P. trifoliata*), Cleopatra mandarin (*C. reticulata* Blanco), and sour orange (*C. aurantium* L.) and scion stems and leaves of sweet orange (*C. sinensis*) were collected. Sample types and sizes were: soil, 1 g; roots, 1–3 g; stems, 1–10 g; and leaves, 0.5–5 g. Depending on type and size of samples, material was shaken for 1 hr in 20–50 ml of sterile Na-K phosphate buffer (Na_2HPO_4, 5.8 g/L; KH_2PO_4, 3.5 g/L; pH 7.0) plus 0.1% peptone. Samples (0.1 ml) of the wash solution and 10-fold serial dilutions with sterile buffer were spread on duplicate plates of Tween medium C (14). Plating efficiency for *X. c.* pv. *citri* from leaves and soil on Tween medium C was 92–100% compared with that on both King's medium B and Difco nutrient agar (14). Populations were expressed as colony-forming units per gram fresh weight of sample material.

In Manatee County, Florida, one grapefruit tree in each of two dooryards and two Pineapple sweet orange trees in an orchard infested with Xcc-A were surveyed. Replicated sets of three leaves with lesions that were on the tree, recently fallen on the ground (0–30 days old), or decomposing in the leaf litter (30–90 days old) were collected in June 1986. Bacterial populations in lesions of leaves from each tree were estimated as follows: one lesion from each of three leaves was removed with a cork borer, and the leaf disks were pooled and ground in sterile buffer with a tissue grinder. Likewise, epiphytic populations were assessed by washing three lesioned leaves in buffer and measuring leaf area with a Li-Cor leaf area meter. Populations in lesions and leaves were expressed as colony-forming units per lesion and per square centimeter of leaf area, respectively.

Influence of soil moisture and burial on survival in leaves. Because of quarantine restrictions, laboratory studies on the survival of Xcc-FN were conducted under isolation at the DPI quarantine facility in Gainesville, FL, and field studies on Xcc-A were conducted under similar soil and climatic conditions to Florida at the INTA Citrus Experiment Station in Concordia, Entre Rios, Argentina.

In the laboratory, the influence of soil moisture content on the survival of one strain of Xcc-FN in leaves was evaluated

under controlled air temperature (25 ± 2 C) and relative humidity (70 ± 10%). Nine liters of nonsterile, nonfertilized Candler fine sand (sand 96.5%, silt 2%, clay 1.5%; pH 6.8) were placed in 10-L buckets and adjusted with tap water to −70 cb (slightly moist), −7 cb (moist), −3 cb (container capacity), and 0 (saturation) soil water potential (ψ soil) or left at −1,500 cb (air-dried). Leaves of grapefruit seedlings were infiltrated with a 0.1-ml suspension containing 10^8 cfu/ml of Xcc-FN (DPI 084-3048), which equates to about 10^6 cfu/cm^2 of leaf surface in the intercellular spaces. The infiltrated area was outlined with a marker. Leaves were air-dried under the above temperature and moisture conditions for 7 days at which time the populations had dropped to about 10^4 cfu/cm^2. The air-drying simulated leaf drop and leaf drying on the soil surface in the field. Leaves were put in nylon mesh bags and placed on the soil surface or buried 10 cm deep in three replicate buckets of soil at each moisture level. The buckets were then covered with a plastic bag to prevent drying of the soil at the surface. All treatments were replicated three times. Periodically, leaves were removed, air-dried for 2 hr to facilitate the removal of attached soil, and then the infiltrated area previously marked was removed with a cork borer. Populations of the bacterium in leaf disks were determined as described. Sampling was discontinued after Xcc-FN was not detected on two consecutive sampling dates or until leaves had decomposed beyond recognition.

In a field trial in Argentina in November 1985, diseased leaves of the current spring flush were collected from mature grapefruit trees and immediately placed in nylon mesh bags. To simulate the incorporation of leaf debris into soil by disking, bags were buried 10 cm deep or placed on the surface at three locations in a cleared field of unfertilized Yuqueri sand (sand 96.1%, silt 1.3%, clay 2.6%; pH 5.8). Leaves were periodically recovered from the bags during the spring and summer (November through February) over an 85-day period. Populations of Xcc-A in leaf lesions were determined as described for dooryard and orchard trees.

Influence of soil fumigants on survival of roots and bacteria in leaves. The effects of soil fumigants on *X. c.* pv. *citri* populations in leaves, soil, or roots were investigated both in Florida and in Argentina. Laboratory studies were conducted in buckets of soil as described before. Roots of rough lemon (*C. jambhiri* Lush.) about 20 cm long and 1–2 cm in diameter were collected from mature trees in February 1986. Two buckets of soil per treatment contained either three leaves or three roots at each of three soil depths (0, 10, and 20 cm). Soils at −7 cb ψ soil were treated with 98% methyl bromide-2% chloropicrin (MC2) or 66% methyl bromide-33% chloropicrin (MC33) at 0.23 kg/m^3 by injection and were maintained in a sealed chamber for 2 days at 18 C. Metam-sodium (Vapam) and 40% 1,3 dichloropropene-20% methyl isothiocyanate (Vorlex/Trapex) were drenched into soil at 110 ml/m soil surface with sufficient tap water to give a ψ soil of −3.5 cb. Buckets were covered to maintain this moisture level for 10 days at 16.5 ± 1.5 C. Leaves and roots were recovered after the fumigation period and populations of Xcc-FN in leaves were measured as described above for the soil moisture laboratory study. Root survival was evaluated by sticking roots in a peat-vermiculite mist bed. Roots were considered alive if any part of the cambium was living after 6 weeks.

The ability of MC2 and Trapex (Vorlex) (9.3% 1,3-dichloropropene-2.35% methyl isothiocyanate) to eradicate Xcc-A in diseased grapefruit leaves on the soil surface or buried in soil was evaluated in Argentina in the field location described before. Leaves were collected as before and buried 0, 10, and 20 cm deep in a single location. Soils were then treated with MC2 at 0.05 kg/m^2 under a 4-mil-thick polyethylene tarp, with Trapex (injected 10 cm deep at 120 ml/m^2), or left untreated. Leaves were periodically recovered from November through February and populations in lesions determined as described for the soil moisture field experiment.

RESULTS

Recovery of *X. c.* pv. *citri* from infested nurseries, dooryards, and orchards. In the greenhouse and field nurseries before eradication, asymptomatic leaves on diseased Swingle citrumelo seedlings contained Xcc-FN populations of 10^2–10^3 cfu/g. No xanthomonads were isolated from a peat-perlite medium in three pots containing symptomatic plants in the greenhouse, but a xanthomonadlike bacterium was recovered from beneath one of two symptomatic plants in the field nursery. The bacterium was identified as *Xanthomonas* by xanthomonadin pigment analysis (9) and *X. campestris* by conventional determinative tests (17). Infiltration of Rutgers tomato with 10^6 and 10^8 cfu/ml gave a positive hypersensitive reaction (HR+) comparable to that of *X. c.* pv. *campestris* (Pammel) Dowson. In repeated tests, however, this strain was not pathogenic on grapefruit after infiltration of leaves with 10^8 cfu/ml.

In two field nurseries after eradication, rootstocks with intact root systems that resprouted and whole plants of rootstocks and scion/rootstock trees remained. In one field nursery, isolations from 36 leaf, stem, and root samples were negative, except for one leaf sample that yielded *X. campestris*. This strain was HR+ on tomato but not pathogenic on grapefruit. In the other field nursery, *X. campestris* was recovered from two of 12 stem samples and two of 12 leaf samples but was not recovered from roots with attached soil. Populations ranged from 1.5×10^2 to 3.3×10^3/g fresh weight. All strains were HR+ on tomato and not pathogenic on grapefruit.

On mature Pineapple sweet orange trees in the orchard, populations of Xcc-A ranged from 10^5 to 10^6 cfu/cm^2 of leaf or per lesion, those in recently fallen leaves were between 10^4 and 10^5 cfu/cm^2, and populations in decomposing leaves ranged from nondetectable to 10^4 cfu/cm^2. Selected colonies were tested for pathogenicity by infiltrating Swingle citrumelo leaves with 10^5 cfu/ml. The lesions formed on leaves were erumpent, which is characteristic of Xcc-A but not Xcc-FN. For a grapefruit tree in a dooryard, populations of Xcc-A from leaves on the tree and recently fallen leaves were about 10^3 cfu/cm^2 of leaf or per lesion. In decomposing leaves, populations ranged from nondetectable to 10^3 cfu. In a second dooryard, where the canopy floor was wetted frequently, leaves on the tree and recently fallen leaves had about 10^5 cfu/cm^2 of leaf or per lesion, whereas decomposing leaves were skeletonized and Xcc-A was not detected.

Influence of soil moisture and burial on bacterial survival in leaves. In uniformly moistened soils (0 to −70 cb ψ soil) under controlled temperature and humidity conditions, there was no significant effect of leaf placement or ψ soil on bacterial survival. Therefore, data from the moistened soil treatments were combined to demonstrate the effect of moist vs. air-dry soil conditions. In moistened soil, Xcc-FN populations increased to 10^6 cfu/cm^2 of leaf within 5 days (Fig. 1). By 24 days, however, bacteria were not detected in any of the moistened soil treatments. Some leaf decomposition was observed under all but the slightly moist conditions (−70 cb). Buried leaves had decomposed beyond recognition in the container capacity (−3 cb) and saturated treatments (0 cb). In air-dried soil (−1,500 cb), populations were detected up to 105 days but not after 120 days (Fig. 1). Leaf placement did not affect survival of the bacterium.

Under field conditions in Argentina, ψ soil ranged from −1,000 to −15 cb from November through February (spring and summer). Populations of Xcc-A in leaf lesions declined from 10^6 to 10^2 cfu/lesion for leaves on the soil surface and declined to zero by 85 days for buried leaves (Fig. 2). The rate of decline in bacterial populations was significantly greater in buried leaves than in surface leaves. After 85 days, buried leaves were decomposed nearly beyond recognition,

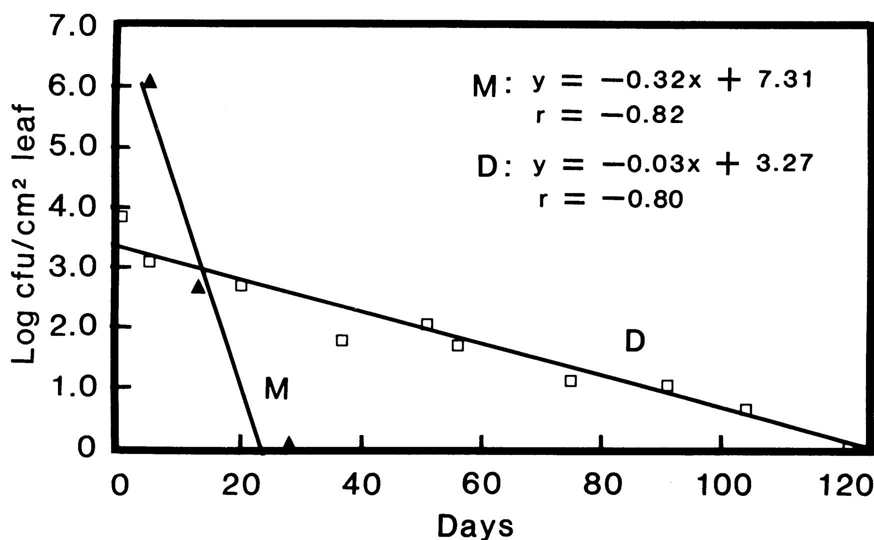

Fig. 1. Survival of *Xanthomonas campestris* pv. *citri* (Florida nursery strain) in grapefruit leaves in sandy soil under moist (M) (0 to −70 cb soil water potential) and air-dried conditions (D) (−1,500 cb). Correlations are significant at $P \leqslant 0.01$. Regression equations have significantly different slopes according to Student's *t* test ($P \leqslant 0.01$).

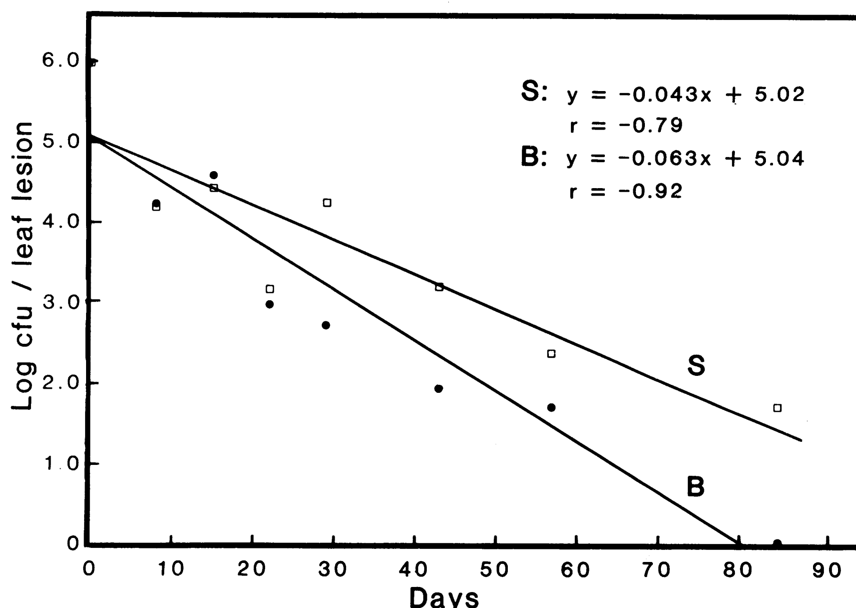

Fig. 2. Survival of *Xanthomonas campestris* pv. *citri* (Asiatic strain) in lesioned grapefruit leaves on the surface (S) or buried (B) in a sandy soil in Argentina. Correlations are significant at $P \leqslant 0.01$. Regression equations have significantly different slopes according to Student's *t* test ($P \leqslant 0.01$).

Table 1. Survival of the Florida strain of *Xanthomonas campestris* pv. *citri* (Xcc-FN) in grapefruit leaves and of rough lemon roots 2 days after soil fumigation with mixtures of methyl bromide and chloropicrin (98 and 2%, MC2; 66 and 33%, MC33)

Treatment	Soil depth (cm)	Xcc-FN[a] (log cfu/cm²)	Root survival (%)
Nonfumigated	0	4.64 ± 0.04	67
	10	5.44 ± 0.76	100
	20	5.51 ± 0.69	100
Methyl bromide (MC2)	0	2.81 ± 1.40	0
	10	0.99 ± 1.32	0
	20	ND[b]	0
Methyl bromide-chloropicrin (MC33)	0	3.82 ± 0.37	0
	10	5.70 ± 0.50	0
	20	5.70 ± 0.36	0

[a] Initial population was 6.72 ± 0.18 log cfu/cm² of leaf area.
[b] Nondetectable.

whereas surface leaves dried periodically and were still intact.

Influence of soil fumigation on survival of roots and bacteria in leaves. Under chamber conditions, MC2 fumigation treatment reduced populations of Xcc-FN in leaves buried in the soil to a greater extent than in leaves on the soil surface compared with the respective nonfumigated treatments (Table 1). Bacteria were not detected in leaves treated with MC2 at the 20-cm depth. In contrast, MC33 had little effect on populations in either surface or buried leaves. Both fumigants killed sections of rough lemon roots whether on the surface or buried in soil.

Drench treatments with the fumigants metam-sodium and Vorlex reduced populations to nondetectable levels in buried leaves and to about 10^1 cfu/cm² in surface leaves compared with 10^3 and 10^6 cfu/cm² in the respective nonfumigated treatments (Table 2). Roots did not survive either of the fumigation treatments.

In Argentina, populations of Xcc-A in nonfumigated leaves declined in surface and buried treatments in the same way as in the soil moisture experiment (Table 3, Fig. 2). After 85 days, populations in surface leaves were about 10^1 cfu/lesion and in buried leaves were not detected at either the 10- or 20-cm soil depth. After 8 days, MC2 applied under tarp had not affected bacterial survival in surface leaves but had reduced populations in buried leaves compared with the nonfumigated treatment. By 45 days, bacteria were not detected in surface and buried treatments. Trapex reduced populations to below 10 cfu/lesion in surface and buried leaves, and bacteria were not detected after 45 days.

DISCUSSION

The survey of citrus nurseries infested with Xcc-FN led to the recovery of xanthomonads from nonsymptomatic plant material and soil that were not pathogenic to citrus. These xanthomonads were identified as *X. campestris*. Their genomic DNA fingerprints (8) are different from those of Xcc-FN strains pathogenic to citrus in each location studied (J. S. Hartung, *unpublished*). Thus, these *X. campestris* strains are not identical to the strains of Xcc-FN previously isolated at each location and may not be pv. *citri*. Recently, *X. campestris* has been reported to occur in fruit with soft rots and in leaf spots mixed with other phytopathogenic bacteria on hosts including tomato and pepper (4,13). Common features of these strains are lack of virulence on tomato and pepper but strong pectolytic activity with the ability to cause soft rot of their fruits. Our putative *X. campestris* strains are also pectolytic and may share other characteristics in common with these opportunistic xanthomonads.

Pathogenic Xcc-A survived in diseased leaves left on the soil surface for at least 90 days after defoliation during relatively dry conditions in the spring of 1986 in Florida. In Argentina, under similar soil and climatic conditions, the maximum survival period of Xcc-A in leaf lesions was estimated to be 120 days in surface leaves. Burial of leaves in soil significantly increased the rate of leaf decomposition and shortened the survival period of Xcc-A to 85 days. Periodic wetting of the canopy floor in a dooryard in Florida accelerated leaf decomposition and killed Xcc-A in diseased leaves within 90 days.

In air-dry soil, Xcc-FN survived after 105 days in leaves, whether on the surface or buried in the soil, because humidity conditions were kept uniform at the soil surface. Moistening the soil decreased survival to less than 24 days. Soil water potential directly influenced decomposition of the leaf, which accelerated the decline of populations in the leaf. These observations confirm several earlier studies (3,6,12) in which Xcc-A was able to persist in dry or sterilized soil in the absence of microbial activity for several months; however, with the addition of moisture, populations declined rapidly. The ability of *X. c.* pv. *citri* to survive up to 120 days in decomposing citrus leaves compares with a survival time of 6 mo of *X. c.* pv. *vesicatoria* in tomato crop residue in Florida (10).

In the presence of living citrus not removed during the eradication process, *X. c.* pv. *citri* could survive in the rhizosphere and on leaves and stems that resprout from the rootstock. Goto et al (6) reported that 10^2 cfu/g of roots survived on *C. natsudaidai* after 10 mo. Xanthomonads were not detected on roots or in attached rhizosphere soil from field nurseries, but *X. campestris* was isolated from root sprouts, stems, and leaves. Thus, for eradication of *X. c.* pv. *citri*, residual roots in soil must be killed to ensure that the rootstock does not survive and resprout susceptible shoots. We found that fumigants applied at maximum recommended rates killed rough lemon roots under controlled conditions. In Florida orchards, these fumigants are used as a preplant treatment to prevent sprouting of residual rough lemon roots after tree removal (11).

The fumigants were not completely effective as eradicants of the bacterium in leaves. Metam-sodium, Vorlex, and MC2 significantly reduced populations in leaves under controlled conditions. In the field, treatment with MC2 and Trapex (Vorlex) accelerated the decline in populations. There was a decrease in survival time in leaves from 85 days or more to less than 45 days. The more rapid decline of *X. c.* pv. *citri* may have resulted from the reduction of the microflora in the soil and the leaf, which allowed for subsequent colonization and decomposition of leaves by primary colonizing fungi and bacteria. Ohr et al (16) found that activity of *Trichoderma* in soil increased after treatment of *Armillaria mellea* in citrus roots with sublethal levels of methyl bromide. Colonization of roots by *Trichoderma* eventually killed *A. mellea*.

Compared with methyl bromide alone, the mixture of methyl bromide and chloropicrin did not immediately reduce bacterial populations in leaves under fumigation chamber conditions. Chloropicrin was more effective than methyl bromide for full-season control of *Pseudomonas solanacearum* in tomato transplant beds where the inoculum consisted of infested tomato plants incorporated into the soil before treatment (2). However, methyl bromide penetrates plant tissue more effectively than chloropicrin (15). Citrus has a thick cuticle, which tomato lacks, that may have inhibited the penetration of chloropicrin more than methyl bromide. Methyl bromide and chloropicrin were equally effective in killing *P. solanacearum* alone in soil (2). The ability of fumigants to kill bacteria may depend on direct contact between the fumigant and the bacterium. The drench fumigants, metam-sodium and Vorlex, were more effective than the gaseous fumigants, especially when leaves were buried.

In summary, *X. campestris* can survive as an epiphyte on citrus stems and leaves. Killing of citrus plants with fumigants provides an alternative to mechanical removal of citrus roots during plant eradication. If all host material is killed, it is unlikely that *X. c.* pv. *citri* would survive in plant debris in soil more than 6 mo. The bacterium declines most rapidly under conditions that favor decomposition. The longevity of *X. c.* pv. *citri* can be reduced significantly by soil fumigation and irrigation treatments that accelerate leaf decomposition.

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Table 2. Survival of the Florida nursery strain of *Xanthomonas campestris* pv. *citri* (Xcc-FN) in grapefruit leaves and rough lemon roots 10 days after soil fumigation with metam-sodium and dichloropropene-methyl isothiocyanate (Vorlex)

Treatment	Soil depth (cm)	Xcc-FN[a] (log cfu/cm²)	Root survival (%)
Nonfumigated	0	3.71 ± 0.42	67
	10	6.11 ± 0.04	100
	20	6.15 ± 0.80	100
Metam-sodium	0	1.03 ± 0.89	0
	10	ND[b]	0
	20	ND	0
Dichloropropene-methyl isothiocyanate (Vorlex)	0	0.84 ± 1.11	0
	10	ND	0
	20	ND	0

[a] Initial population was 6.72 ± 0.18 log cfu/cm² of leaf area.
[b] Nondetectable.

Table 3. Survival of the Asiatic strain of *Xanthomonas campestris* pv. *citri* (Xcc-A) in grapefruit leaf lesions in Argentina after soil fumigation with methyl bromide and chloropicrin (98 and 2%, MC2) or dichloropropene-methyl isothiocyanate (Trapex)

Treatment	Soil depth (cm)	Xcc-A (log cfu/lesion)[a]		
		8[b]	45[b]	85[b]
Nonfumigated	0	3.32 ± 1.26	2.45 ± 0.92	1.70 ± 0.50
	10	5.03 ± 0.54	1.70 ± 0.50	ND[c]
	20	4.60 ± 1.68	1.70 ± 0.50	ND
Methyl bromide (MC2)	0	4.45 ± 0.92	ND	ND
	10	1.37 ± 0.71	ND	ND
	20	1.71 ± 0.88	ND	ND
Dichloropropene-methyl isothiocyanate (Trapex)	0	0.90 ± 0.35	ND	ND
	10	0.90 ± 0.09	ND	ND
	10	0.90 ± 0.35	ND	ND

[a] Initial population was 6.01 ± 0.92 log cfu/lesion.
[b] Days after fumigation.
[c] Nondetectable.

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