Influences of Cropping Practices on *Verticillium dahliae* Populations in Commercial Processing Tomato Fields in Ontario

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ABSTRACT

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The abundance of *Verticillium dahliae* in the soil and the incidence of *V. dahliae*-infected plants were determined for 12 commercial processing tomato fields in Kent County, Ontario. Comparison of the data with those from a previous survey of fields in adjacent Essex County showed that soil inoculum levels and incidence of infection were generally lower in Kent County fields and that race 2 *V. dahliae* was not common in Kent County. From the two counties, 128 isolates were characterized by restriction fragment length polymorphism (RFLP) analysis, using the sub-

species-specific repetitive DNA sequence E18. A subset of these isolates was also characterized by vegetative compatibility and DNA hybridization analysis with a second subspecies-specific DNA sequence. Isolates with E18 RFLP profiles highly similar to those of isolates previously collected from potato fields in North America were prevalent in Essex County tomato fields but not common in Kent County fields. The data are consistent with the hypothesis that the group I isolates were introduced into southwestern Ontario with potato and that the different cultural practices in Essex County and Kent County have contributed to the differences in the accumulation of these isolates in the two regions.

Additional keyword: Verticillium wilt.

Verticillium wilt, caused by the imperfect fungi *Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke & Berthold, has been reported on plants in as many as 45 families in both temperate and tropical regions. Several strategies are currently used to control this disease. In Ontario, fumigation is commonly used for preplant soil sterilization and greenhouse applications but is both costly and detrimental to the environment. The efficacy of crop rotation as a disease control strategy is limited by the longevity of the *V. dahliae* microsclerotia in soil (39) and the ability of *V. dahliae* to persist in the roots of nonsymptomatic hosts, including weeds and native plants (29). Long rotations that do not include crops susceptible to *V. dahliae* can reduce the level of the pathogen in the soil (11) but may not be economically practicable for growers. Lastly, disease control through the cultivation of *Verticillium*-resistant cultivars is often limited by a lack of resistance genes. *Verticillium*-resistant tomato cultivars that carry the *Ve* resistance gene (35) are widely used by commercial growers. However, within 10 years after the introduction of *Ve* cultivars, *V. dahliae* isolates virulent on these cultivars were recovered from Ohio fields (1). These strains, now designated race 2, have since been isolated in California, Canada, and Europe (4,6,15,21,27) and present a chronic problem for tomato production.

A 1993–1994 survey of commercial processing tomato fields in Essex County, a major tomato-growing region in southwestern Ontario, showed that race 2 strains of *V. dahliae* were predominant in fields where tomato had been cropped intensively (15). In this region, potato, which is also susceptible to Verticillium wilt,

may be included as a rotation crop and is frequently grown adjacent to tomato fields (K. Dobinson, *unpublished data*). In contrast to the situation in Essex County, Verticillium wilt is not currently a concern to commercial tomato growers in adjacent Kent County, where tomatoes are typically grown under long rotations and potatoes are not widely grown on a commercial scale (J. Dick, Nabisco Ltd., *personal communication*). We therefore hypothesized that the apparent differences in disease pressure were related to differences in these cropping practices between the two counties. However, wilt incidence had not been previously assessed in Kent County. The first objective of our study was, therefore, to determine if the levels of *V. dahliae* in the soil and the numbers of infected plants in Kent County tomato fields differed from those in Essex County fields.

The second objective of this study was to investigate genetic diversity among *V. dahliae* isolates from southwestern Ontario tomato fields. In recent years, molecular methods, particularly restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA analyses, have been used to characterize *V. dahliae* isolates from around the world, collected from various hosts (7,28,31,32,34). These studies demonstrated that within the global population of *V. dahliae* there is considerable genetic diversity, but limited information is available regarding genetic variation among *V. dahliae* isolates from restricted geographical locales, particularly from regions subject to different cultural practices. The results of a recent study suggested that Ontario race 2 isolates of *V. dahliae* arose from more than one genetic background (14). However, that study examined only a small number of isolates. We have, therefore, characterized a larger number of isolates from southwestern Ontario tomato fields using molecular and biological characters including RFLPs, race type, and vegetative compatibility.

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MATERIALS AND METHODS

***V. dahliae* isolates.** Vegetative compatibility group (VCG) tester strains T9 (VCG 1), PH (VCG 2A), 115 (VCG 2B), 70.21 (VCG 3), BB (VCG 4A), and CW (VCG 4A/B) were from R. Rowe (The Ohio State University, Wooster). VCG tester strains Dvd-T5 (VCG 2A; ATCC 201177), Dvd-T3 (VCG 2B), Dvd-E6 (VCG 4A), and Dvd-3 (VCG 4B) and all other isolates used in this study originate from southwestern Ontario. The Ontario isolates were cultured directly from field-grown plants, from soil (isolate numbers prefixed by S), or from eggplant, tomato, or potato plants grown in field soil under environmentally controlled conditions (isolate designations prefixed by E, T, and P, respectively). Monoconidial cultures of each isolate were maintained in long-term storage on filter paper disks or as silica gel stocks, as previously described (15). Unless otherwise indicated, isolates were grown on complete medium (CM) (5,13) agar or in liquid CM.

Determination of *V. dahliae* soil inoculum levels and incidence of *V. dahliae*-infected plants. The abundance of *V. dahliae* in the soil was determined for 12 commercial processing tomato fields in Kent County. Six fields were sampled in 1996 and six in 1997. Prior to planting, soil samples were collected from each commercial field in late May from a 100-m transect centered on a randomly selected tomato bed and positioned at least 20 m from the edge of the field. The transect was divided into five 20-m blocks, and soil samples, consisting of three cores (2.5 cm in diameter $\times$ 10 cm in depth), were collected from the center of each block. Between sampling in different fields, the soil sampler was cleaned with a solution of 1.5% sodium hypochlorite. All soil samples were processed and assayed by the Anderson sampler method, as described previously (15). For each sample, a total of 200 or 250 mg of soil was assayed (five replicate plates with 40 or 50 mg of soil per plate). The amount of *V. dahliae* in each transect was calculated as the mean number of colony forming units per gram of soil in the five samples.

The incidence of *V. dahliae*-infected tomato plants in Kent County commercial processing fields was determined essentially as described previously (15). Briefly, in mid-August leaf samples were collected from the lower portions of 8 to 10 randomly selected plants in each block along the transects from which the soil samples had been collected. A total of 48 to 50 plants was sampled from each field. The leaf petioles were surface-sterilized, cross sections were taken and incubated on soil pectate tergitol agar medium (15), and the presence of *V. dahliae* in the cross sections was determined microscopically.

Race type analysis. *V. dahliae* strains Dvd-T5 and Dvd-2 were used as race 1 and race 2 standards, respectively, and infection assays were carried out as previously described using tomato cvs. Bonny Best (*ve*) and H1350 (*Ve*) and a root dip inoculation method (15). Disease symptoms were assessed 28 days postinoculation using a five-point rating scale (0 to 4) similar to that of Ashworth (2). Race 2 isolates produced disease symptoms (scores between 2 and 4) on both cultivars, while race 1 isolates produced symptoms only on cv. Bonny Best. Isolates that were not pathogenic to tomato did not produce symptoms on either cultivar.

RFLP analysis. Genomic DNA was isolated from *V. dahliae* spores using a glass bead breakage method, as previously described (13). For DNA hybridization analysis, genomic DNA (200 to 300 ng) was digested overnight with *Eco*RI (New England BioLabs, Beverly, MA) under conditions suggested by the supplier. The digested DNA was fractionated by electrophoresis through 0.8% agarose gels, transferred by capillary blotting to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL), and fixed to the membranes by UV cross-linking. The two hybridization probes were E18, a 310-base pair dispersed, repetitive genomic DNA sequence from the *V. dahliae* race 2 tomato isolate Dvd-2, and BGC22-S, a low-copy-number DNA sequence from *V. dahliae* Dvd-T5 (33). The E18 probe was generated using the digoxigenin (DIG) DNA labeling kit, as described previously (14). The BGC22-S probe

was prepared by the random primed labeling method using the DIG DNA labeling procedure and conditions suggested by the manufacturer (Roche Diagnostics, Laval, Quebec, Canada). High stringency hybridizations, washes, and the chemiluminescent detection were carried out as previously described (13,14).

The similarities of E18 fingerprints were assessed by scoring for the presence or absence of 63 polymorphic bands detected by DNA hybridization. Strains Dvd-T5, Dvd-2, Dvd-3, and Dvd-S85 (14) were used as standards for the RFLP analysis. A distance matrix was calculated using a modified Jacquard coefficient ($1 - S_j$) (37), and a phenogram was generated from the data by cluster analysis using unweighted pair grouping by mathematical averaging (UPGMA). Parsimony analysis was done using PAUP 4.0b4a, with a heuristic search (terminated after 90,000 equally parsimonious trees) and default options.

Statistical tests. Linear regression analysis of soil inoculum level and percent infection data was done using the statistical program of SigmaPlot version 5.0 (Jandel Scientific Software, San Rafael, CA). Other statistical analyses used Minitab release 12 (Minitab Inc., State College, PA).

Vegetative compatibility analysis. Nitrate-nonutilizing (*nit*) mutants, which are unable to use nitrate as a sole nitrogen source (9), were selected as previously described (14) on potato dextrose agar amended with 2.5% KClO_3 or on 0.005% rose bengal medium (17) amended with 2.5% KClO_3 . Classification of mutants as *nit1*, *nit2*, or *nitM* was done as previously described (24), and monoconidial cultures of *nit* mutants that retained wild-type growth on CM agar were used for vegetative compatibility tests.

Vegetative compatibility was determined by pairing *nit* mutants of each Ontario isolate with complementary mutants of the VCG testers on minimal medium agar containing sodium nitrate as the sole nitrogen source (24). Whenever possible, *nit* mutants of two different phenotypes were tested. In cases in which only one mutant phenotype was obtained, at least two different mutants of that phenotype were paired with the tester strains. Compatibility was indicated by prototrophic growth and microsclerotia production at the site of contact between mycelia of paired isolates, as previously described by Joaquim and Rowe (24).

RESULTS

***V. dahliae* soil inoculum levels and incidence of *V. dahliae*-infected tomato plants in southwestern Ontario fields.** To determine if the amount of *V. dahliae* in the soil and the incidence of infected plants differed between Essex County and Kent County tomato fields, 12 Kent County fields were surveyed in 1996 and 1997 for *V. dahliae* and the data compared with those reported previously for 10 Essex County fields (15). Of the 12 fields, nine had not been cropped with tomato during the previous 10 years; the remaining three had been infrequently planted with solanaceous crops (J. Dick, Nabisco Ltd., *personal communication*). The Essex County fields, in contrast, had been intensively cropped with tomato; rotations were typically short (1 or 2 years without tomato) and included one or more of sweet corn, seed corn, cabbage, and potato (K. Dobinson, *unpublished data*).

The mean number of viable *V. dahliae* propagules in soil samples from the Kent County fields ranged from 0 to 15 CFU/g of soil, and disease incidence ranged from 0 to 31% (Fig. 1). In eight of the fields, soil inoculum levels were less than 5 CFU/g of soil; *V. dahliae*-infected plants were detected in only four fields (Fig. 1). Both the soil inoculum levels and the incidence of *V. dahliae*-infected plants in the Kent County fields were significantly lower than those reported previously for Essex County (Fig. 1).

Pathogenic variation among Ontario *V. dahliae* isolates. A small number of *V. dahliae* isolates (18 from soil and 13 from plants, including nine from plants in a single field) were obtained from the Kent County commercial fields during 1996 and 1997. Race type analysis was done on a representative selection of these iso-

lates and on isolates from soil samples from an experimental field in which tomatoes had been grown intensively for several years under various crop rotation regimes (22,25). In contrast to isolates collected from the Essex County tomato fields, the majority of which were race 2, most of the Kent County isolates were either race 1 or not pathogenic to tomato (Table 1).

E18 RFLP analysis. The genetic variation among 137 *V. dahliae* isolates from Essex (76 isolates), Kent (52 isolates), and three other Ontario counties (9 isolates) was assessed by RFLP analysis using the E18 DNA probe. Eighteen isolates did not contain E18 sequences and were classified as nonhybridizing. One hundred nineteen (87%) of the isolates contained E18 sequences; 62 RFLP patterns were identified. In the resulting UPGMA phenogram generated from a distance matrix of the RFLP data, several loose clusters were apparent (Fig. 2). For the purposes of further analysis, 14 E18 groups were defined, in which each group contained isolates with E18 profiles having at least 50% similarity (Fig. 2). In a parsimony analysis of the RFLP data, isolates in each of the three largest E18 groups (I, III, and VI) clustered together (bootstrap values of 85 to 100%) (data not shown), supporting the 50% cutoff value.

E18 sequences were detected in moderate copy number (14 to 22 hybridizing bands) in the group I isolates, and in many cases, the presence or absence of only one E18 band differentiated the isolates (Fig. 3, top panel; and data not shown). The majority of isolates (47 isolates) from Essex County were members of E18 group I, as were nine Kent County isolates (Fig. 4). Eight of the Kent County group I isolates were from plant tissue from three commercial fields; one was from soil in the experimental field. Six isolates from other counties in southwestern Ontario also belonged to this group (Fig. 2; and data not shown). As with group I, most of the *V. dahliae* isolates in groups III and VI were from Essex County (Fig. 4). A low E18 copy number characterized members of these groups (three to seven hybridizing bands).

In contrast to the Essex County *V. dahliae* isolates, which were assigned to only three E18 groups, Kent County isolates belonged to 13 different groups (Figs. 2 and 4). Isolates in groups II, V, X, XI,

and XII contained moderate numbers (12 to 22) of E18-hybridizing bands. Group XI included seven identical isolates collected from four different fields, while group XII included four identical isolates from three different fields. Kent County isolates that contained few (less than seven) E18-hybridizing bands were assigned to seven groups (III, IV, VI, VII, IX, XIII, and XIV).

Association of molecular markers and biological characters.

Fungal strains that are vegetatively compatible are presumed to be more genetically similar to each other than to isolates from different VCGs (26). To assess the validity of the genetic relationships defined by the E18 fingerprint data, we therefore selected for vegetative compatibility analysis 11 *V. dahliae* isolates that had been race-typed and were representative of eight E18 fingerprint groups. As previously reported for *V. dahliae* (14), VCG was not strictly associated with race type (Fig. 2). One of the isolates, Dvd-S207 (E18 group XIII), did not complement any of the tester strains and was self-incompatible. In the case of the other isolates, those in the same E18 fingerprint group belonged to the same VCG (Table 2). Isolates in E18 groups I and III were assigned to VCG 4B; those in E18 groups V and XI were in VCG 2B; and isolates in groups VI, VII, and XIV were in VCG 2A. VCGs were also determined for 14 Kent County isolates that did not contain E18 sequences. The majority of these isolates were included in VCG 2B; two were in VCG 1 (Table 2).

The genetic similarities of the isolates was further assessed by DNA hybridization analysis using the low-copy-number DNA sequence designated BGC22-S. Previous work in our laboratory suggested that this sequence, originally isolated from a race 1 strain of *V. dahliae*, was associated with VCG 2A (33). BGC22-S sequences were absent from isolates in E18 groups I through IV but present in isolates in E18 groups V through XII (Table 2; Fig. 3). Neither group XIV (VCG 2A) isolates nor VCG 2B isolates lacking E18 sequences contained BGC22-S (Table 2; Fig. 3).

DISCUSSION

Long crop rotations that exclude *V. dahliae*-susceptible crops have been reported to reduce both the level of the pathogen in the soil and the subsequent infection of susceptible crops (11,29). Consistent with these findings, the levels of *V. dahliae* in the Kent County commercial tomato fields were generally lower than those in neighboring Essex County (Fig. 1), where tomatoes are grown in short rotations. Moreover, in two experimental fields in these counties, both of which had been intensively planted with tomato for 8 years and subjected to similar crop rotations (25), *V. dahliae* soil inoculum levels were similar to each other and higher than those in the Kent County commercial fields (22). Taken together, these data suggest that the long rotation strategies used in Kent County have so far been successful in limiting the accumulation of *V. dahliae* in the soil.

As with the level of *V. dahliae* in the soil, the overall incidence of *V. dahliae*-infected tomato plants in the Essex County fields was higher than in the Kent County fields. However, in neither county was there a strong correlation between incidence of infection and

Fig. 1. Comparison of *Verticillium dahliae* soil inoculum levels and incidence of *V. dahliae*-infected tomato plants in Kent County and Essex County commercial processing tomato fields. Essex County fields were sampled in 1993 and 1994. Kent County fields were sampled in 1996 and 1997. In three of the Kent County fields, both soil inoculum level and percent infected plants were zero. According to a linear regression analysis of the data for each county, the proportion of infected plants in Essex County was not dependent on soil inoculum level ($r^2 < 0.001$) and in Kent County was only weakly correlated with soil inoculum level ($r^2 = 0.067$). According to a two-sample t test with unequal variance, the mean incidence of infected plants in Kent County fields (3.4%) was significantly different ($P = 0.000$) from that in Essex County fields (36.6%). By the same test, the mean soil inoculum levels in Kent County (4.2 CFU/g) and Essex County (31.2 CFU/g) fields were significantly different ($P = 0.016$).

TABLE 1. Pathotypes of *Verticillium dahliae* isolates from Essex County and Kent County tomato fields

Geographical location	Race 1	Race 2	NP ^a
Kent County (commercial) ^b	3	3	2
Kent County (experimental) ^c	10	1	1
Essex County (commercial) ^d	4	120	0
Essex County (experimental) ^c	0	2	0

^a Not pathogenic to tomato.

^b Three isolates from plants in two different fields; five isolates from soil in four fields.

^c Soil isolates (22).

^d Forty-four isolates from soil; eighty isolates from trap plants and field-grown tomato plants (15).

soil inoculum levels (Fig. 1). A similar lack of correlation has been observed for Verticillium wilt of cotton (3,12). A number of factors may influence disease potential, and it has been proposed that the nonuniform distribution of the pathogen in the soil

(23,36) and the limitations of current soil assay methods (38), such as the one used in the current study, contribute to the inability to use inoculum density to reliably predict Verticillium wilt incidence.

Fig. 2. Unweighted pair grouping by mathematical averaging phenogram generated from E18 restriction fragment length polymorphism (RFLP) profiles of 119 Ontario *V. dahliae* isolates. Terminal nodes marked with an asterisk indicate RFLP patterns represented by more than one isolate. For representative isolates, indicated in parentheses, vegetative compatibility (inc = self-incompatible) and race type were determined. Solid vertical lines and Roman numerals adjacent to the phenogram indicate the 14 E18 subgroups. Counties from which the isolates originate are indicated as follows: E (Essex), H (Hamilton-Wentworth), K (Kent), M (Middlesex), and P (Peel). Isolates Dvd-3 (E), Dvd-158 (H), and Vd-JM (M) are from potato, strawberry, and Japanese maple, respectively. All other isolates are from tomato plants or soil from tomato fields.

The overall level of genetic variation was quite high among the southwestern Ontario *V. dahliae* isolates, as assessed by the E18 RFLP profiles. Although several clonal groups were identified, in many cases the level of similarity between isolates was less than 50%, and 42 of the 62 different E18 profiles were represented by a single isolate (Fig. 2). The apparently high level of genetic variation could result if the E18 sequence was highly mutable. However, *V. dahliae* isolates with highly similar E18 RFLP profiles have been collected from a wide range of geographical locations over at least 5 years (14,30), suggesting that the E18 sequences are not highly mutable or unstable.

Isolates within a VCG are expected to be more genetically similar to each other than to isolates in different VCGs (26). In the soil-

borne fungus *Fusarium oxysporum*, which is characterized by a high level of genetic and pathogenic variation, VCG diversity is high and VCGs typically define genetically distinct, clonal lineages (18–20). In contrast to *F. oxysporum*, *V. dahliae* is characterized by a low level of VCG diversity. Only four major VCGs, two of which (VCGs 2 and 4) are further divided into subgroups, have been definitively identified (8,10,16,24). The data presented in the current study indicate that *V. dahliae* isolates that are genetically similar by RFLP analysis are members of the same VCG (Table 2). However, genetically distinct isolates may also be members of the same VCG. The Ontario VCG 2B isolates, for example, appear to comprise at least two genetically distinct groups, one of which contains BGC22-S and E18 sequences and one of which lacks both

Fig. 3. Southern blot analysis of *EcoRI*-digested *Verticillium dahliae* genomic DNA of representative isolates (Fig. 2). The blot was hybridized with the E18 probe (top panel) and then stripped of the probe and hybridized with BGC22-S (bottom panel). Vegetative compatibility and E18 fingerprint groups are indicated. Lane 1, Dvd-S100; lane 2, Dvd-S85; lane 3, Dvd-S132; lane 4, Dvd-S90; lane 5, Dvd-T5; lane 6, Dvd-S136; lane 7, Dvd-S185; lane 8, Dvd-S197; lane 9, Dvd-S216; lane 10, Dvd-S45; lane 11, Dvd-S156; lane 12, Dvd-3; lane 13, Dvd-2; lane 14, Dvd-31; lane 15, Dvd-198; lane 16, Dvd-T9; lane 17, Dvd-137; and lane 18, Dvd-S207. Positions of molecular weight standards (kilobases) are indicated to the right of each panel. Ethidium bromide staining of the gel (data not shown) indicated that equivalent amounts of DNA were loaded in each lane.

sequences (Table 2). Similarly, the VCG 2A isolates that we analyzed all contain E18 sequences, yet only one group of these isolates contains BGC22-S sequences. These results suggest that for *V. dahliae* the utility of VCGs to infer genetic similarity has some limitations. We also speculate that the parasexual cycle, which provides the opportunity for recombination between genetically distinct but vegetatively compatible isolates (26), is a potential source of molecular variation within VCGs of *V. dahliae*.

The distribution of E18 genotypes among the Essex County and Kent County tomato fields was significantly different, with group I/

Fig. 4. Distribution of isolates from Essex County ($n = 75$) and Kent County ($n = 52$) tomato fields among E18 restriction fragment length polymorphism groups. NH indicates isolates that do not contain E18 sequences. Group VIII (data not shown) did not contain isolates from either county. The distribution of isolates among the fingerprint groups was significantly different for the two counties according to a chi-square test ($\chi^2 = 70.79$, degree of freedom = 8, $P = 0.000$) in which groups were combined (II, IV, and V; VII and IX; and X, XIII, and XIV) so that expected frequencies for all classes were greater than one.

TABLE 2. Vegetative compatibility groups (VCGs) and presence/absence of BGC22-S DNA sequences among *Verticillium dahliae* isolates from Essex County and Kent County^a

E18 group	Number of isolates tested	VCG ^{b,c}	BGC22-S hybridization
I	5	4B	—
	21	n.d.	—
III	2	4B	—
	9	n.d.	—
IV	1	n.d.	—
V	1	2B	+
VI	4	2A	+
	8	n.d.	+
VII	1	2A	+
VIII	1	n.d.	+
IX	1	n.d.	+
X	2	n.d.	+
XI	1	2B	+
	6	n.d.	+
XII	4	n.d.	+
XIII	1	inc	—
XIV	1	2A	—
NH ^d	12	2B	—
	1	1	—
	1	1	n.d.

^a Vegetative compatibility tests and DNA hybridization analysis were done as described in Materials and Methods.

^b Includes VCG data for isolates Dvd-2 (E18 group I), Dvd-3 (E18 group III), and Dvd-T5 and Dvd-S85 (E18 group VI) (14).

^c n.d. = not done, and inc = self-incompatibility and incompatibility with tester strains of all VCGs.

^d NH = isolates with no E18 sequences.

race 2 isolates predominating in Essex County (Table 1; Figs. 2 and 4) and a number of E18 groups detected in Kent County but not in Essex County (Fig. 4). The group I isolates are of particular interest, since isolates of this type have also been collected from seed potato tubers from the northeastern United States (K. F. Dobinson, M. A. Harrington, M. Omer, and R. C. Rowe, *unpublished data*) and from potato fields in Simcoe (30), Hamilton-Wentworth, and Peel Counties (14) (Fig. 2; and data not shown). Since seed potato used in Ontario is not screened for *V. dahliae* and potato is frequently grown in Essex County, it is therefore possible that the *V. dahliae* group I isolates were introduced into Essex County by potato (15) and have since accumulated in the fields because of the subsequent, intensive cultivation of tomato.

In contrast to their prevalence in Essex County, few group I isolates were collected from the commercial Kent County fields (Fig. 2) and the majority of Kent County isolates that were race-typed were either race 1 or nonpathogenic to tomato (Table 1). Although only a small number of isolates were available for analysis, the data suggest that E18 group I/race 2 isolates are not yet widespread in Kent County. The data presented in this study are also consistent with the hypothesis that the different cultural practices in Essex County and Kent County have contributed to the differences in the accumulation of E18 group I isolates in the two regions.

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