

## Detection of *Phytophthora parasitica* from Soil and Host Tissue with a Species-Specific DNA Probe

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### ABSTRACT

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*Phytophthora parasitica* was detected with a species-specific DNA probe by employing several common techniques for isolating *P. parasitica* from soil, and then extracting DNA from the increased biomass. DNA of *P. parasitica* was detected with the probe following dilution plating of infested soil onto semiselective medium covered with a membrane, crushing portions of fungal colonies on a membrane, crushing tomato leaf disk baits on a membrane, and isolating DNA from colonized leaf disks. These methods were sufficiently sensitive to detect propagule concentrations in soil below those associated with yield loss in tomatoes. *P. parasitica* was also detected in infected tomato roots by modifying the DNA isolation

method developed for leaf disk baits. To determine the feasibility of quantifying *P. parasitica* in soil with a DNA hybridization assay, the amount of *P. parasitica* DNA detected in leaf disk baits was compared to the number of propagules estimated by the most probable number method for the same soil samples. The relationship between the two sets of data, however, varied with sampling date. Although this assay method did not reliably quantify populations of *P. parasitica*, the results demonstrate that a DNA probe can detect *P. parasitica* in soil at population levels comparable to those detected by baiting methods.

DNA probes are increasingly being used to identify and detect plant pathogens (2,4,7,10,13,20,21). Several random, cloned DNA fragments have been identified that are specific to *Phytophthora parasitica* Dastur, an important pathogen of tomato and several other crops (8). These DNA probes hybridize to repetitive DNA of *P. parasitica*, but do not hybridize to DNA of tomato plants, several *Pythium* species, or several other *Phytophthora* species. There are a number of advantages to using a DNA hybridization assay to identify *P. parasitica*. In particular, a species-specific DNA probe does not require the use of pure cultures or the observation of taxonomically important fungal structures, which may be highly variable or difficult to produce in culture.

Considerable potential exists for the use of DNA probes in epidemiological and ecological studies of plant pathogens. However, to be effective in these applications, a hybridization assay must be able to detect an organism in its natural environment at population levels below those causing yield loss. Propagules of *P. parasitica* normally survive in soil and infect roots of several crops (19). For tomatoes, early-season inoculum levels of 1-4,000 propagules of *P. parasitica* per kilogram of soil result in little root rot and no loss in yield, whereas considerable root rot and yield loss occurs at  $25 \times 10^3$  propagules per kilogram of soil (D. A. Neher and J. M. Duniway, unpublished). Inoculum of *P. parasitica* consists primarily of chlamydospores that contain only one or a few nuclei. Therefore, direct detection of *P. parasitica* DNA in soil at inoculum levels below those causing yield loss

would be difficult with a DNA probe because relatively little DNA would be present. However, pathogen detection would be greatly improved by permitting the inoculum to germinate and then probing the DNA extracted from the increased fungal biomass. For soilborne bacteria, a DNA probe detected as few as 10-100 cells per gram of soil when populations were first incubated with semiselective culture medium (6). In contrast, a hybridization assay only detected populations as low as  $4 \times 10^3$  cells per gram of soil when bacterial DNA was extracted from cells taken directly from soil (11).

Traditional procedures developed to isolate *P. parasitica* from soil also result in an increase in fungal biomass. The most common isolation procedures involve baiting flooded soil with susceptible plant parts or plating diluted soil suspensions on semiselective medium (23). The purpose of this research was to combine these common isolation procedures with the use of a pathogen-specific DNA probe to provide relatively simple, unambiguous detection of *P. parasitica* obtained from soil. The utility of a hybridization assay to quantify populations in soil and detect *P. parasitica* in infected tomato roots was also investigated. Differences among the alternative detection methods in their technical complexity, sensitivity, and ability to quantify *P. parasitica* are also discussed.

### MATERIALS AND METHODS

**Fungal isolates and inoculum.** Fungal isolates used in this study were: *P. parasitica* 5-3A and W-1 isolated from tomato in Yolo Co., CA; *P. parasitica* C-2CL from citrus in Arizona; *P. cactorum* (Leb. & Cohn) Schroet. W5475 from walnut in Yolo Co., CA;

*P. cinnamomi* Rands SB216-1 from avocado in CA; *P. citrophthora* (Smith & Smith) Leon. P1323 from citrus in CA; *P. cryptogea* Pethybr. & Lafferty RI from safflower in Arizona; and *Pythium ultimum* Trow 1786 from cotton in California. Cultures were stored on cornmeal agar at 25 C.

To inoculate soil, chlamydozoospores of isolates 5-3A or W-1 of *P. parasitica* were produced in liquid culture (22). Chlamydozoospores were freed of viable hyphal fragments and other spores to produce isolated chlamydozoospores (18), which were mixed with pasteurized Yolo fine sandy loam.

**DNA hybridization.** The plasmid pPP33, which was used as the DNA probe in this study, contains a DNA fragment specific to *P. parasitica* (8). Plasmid DNA was labeled with  $^{32}\text{P}$ -dATP to a specific activity of approximately  $10^8$  cpm/ $\mu\text{g}$  (5). All nylon (Nytran, Schleicher and Schuell, Keene, NH) or nitrocellulose membranes (BA45, Schleicher and Schuell) containing DNA samples were baked at 80 C for 2 hr under vacuum, prehybridized, and hybridized according to Kirkpatrick et al (13). After hybridization, the membranes were washed (30 min each) twice in  $2\times$  SSC ( $1\times$  SSC = 0.015 M sodium citrate, 0.15 M sodium chloride) and 0.1% SDS (sodium dodecyl sulfate) at 37 C, once in  $0.2\times$  SSC and 0.1% SDS at 60 C, and then once in  $0.2\times$  SSC and 0.1% SDS at 37 C. After washing, membranes were exposed to XAR X-ray film (Kodak) for 16 hr to 7 days at  $-70$  C with intensifying screens (Lightning Plus, Du Pont), unless otherwise specified.

All experiments describing the detection of *P. parasitica* in soil and host tissues with the DNA probe were replicated at least three times unless otherwise stated.

**Detection of *P. parasitica* on soil dilution plates.** Pasteurized soil was infested with 1–4,000 chlamydozoospores per kilogram of soil as previously described. For colony hybridization, infested soil was diluted in 0.25% water agar, and 1 ml was plated onto a nylon membrane placed over semiselective agar medium (12). After incubation for 24 hr at 22 C, the soil particles were washed gently from the membrane, which was subsequently removed from the agar, and placed on 3 MM Whatman filter paper moistened with 10% *N*-lauryl sarcosine. After 5 min of incubation, the membrane was transferred to a 3 MM Whatman filter paper moistened with 0.5 M NaOH and 1.5 M NaCl and incubated for 5 min. The membrane was transferred to 0.5 M Tris-HCl, pH 8, and 1.0 M NaCl, incubated for 5 min, and then moved to a 3 MM filter moistened with  $20\times$  SSC for 5 min. The membrane was finally placed in  $18\times$  SSC and 1.5% *N*-lauryl sarcosine for 10 min, and then air-dried.

**Detection of *P. parasitica* grown on agar.** Colonies were grown in pure culture on V-8 agar medium or grown from infected tomato leaf disk baits that were incubated on semiselective agar medium (12) for 3 days. Agar plugs (7 mm diameter) containing mycelium were excised and crushed on a nylon or nitrocellulose membrane that had been placed on 3 MM Whatman filter paper moistened with 10% *N*-lauryl sarcosine. Membranes containing mycelium were then processed as previously described for soil dilution plates.

**Detection of *P. parasitica* in tomato leaf disk baits and roots.** Pasteurized soil was infested with  $1 \times 10^3$  or  $25 \times 10^3$  chlamydozoospores per kilogram, and 50-g samples were placed in individual plastic cups and flooded with 25 ml of sterile  $\text{H}_2\text{O}$ . Ten 7-mm-diameter leaf disks of tomato (*Lycopersicon esculentum* Mill. 'VF6203') were floated on the water surface. After 48 hr at 22 C, leaf disks were blotted dry and assayed for colonization by *P. parasitica*. Half of each leaf disk was surface sterilized in 70% ethanol for 30 sec, rinsed in sterile water, and placed on semiselective medium (12). Plates were observed for colony development after incubation at 25 C in the dark. Negative controls consisted of leaf disks that were incubated on flooded pasteurized soil without addition of chlamydozoospores and leaf disks that were processed immediately after excision from tomato leaves.

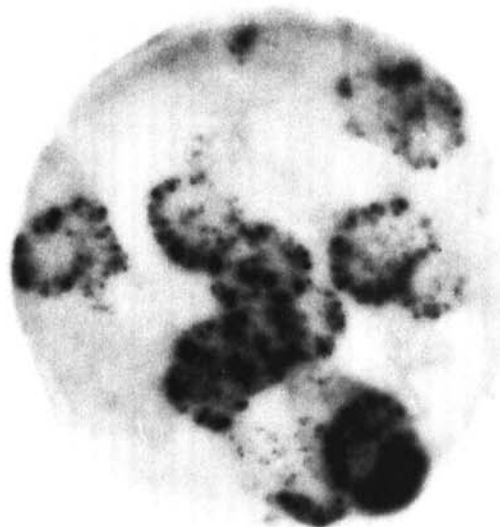
DNA was extracted from the remaining leaf disk halves by either a leaf crush or leaf DNA miniprep method. For the leaf crush method, leaf disk halves were crushed with a glass rod on a nylon or nitrocellulose membrane that had been placed on

3 MM Whatman filter paper moistened with 10% *N*-lauryl sarcosine, and then processed as described for the soil dilution plate method. Leaf disk debris was removed during incubation in  $18\times$  SSC and 1.5% *N*-lauryl sarcosine. For the leaf DNA miniprep method, leaf disk halves were frozen with liquid  $\text{N}_2$ , ground to a fine powder in a 1.5-ml microfuge tube, and 0.25 ml of extraction buffer (100 mM Tris, 40 mM EDTA, 0.2% SDS [w:v], and 0.2% 2-mercaptoethanol [v:v], pH 8) was added. After gentle shaking for 1 min, the lysate was incubated at 68 C for 20 min. The lysate was then extracted with an equal volume of phenol:chloroform (1:1), followed by an equal volume of chloroform:isoamyl alcohol (24:1). The aqueous supernatant solution was boiled for 5 min and applied to a nylon membrane, or adjusted to  $6\times$  SSC, boiled 5 min, and applied to a nitrocellulose membrane in a dot-blot manifold.

The leaf DNA miniprep method was modified to extract DNA from tomato roots. DNA was extracted from 2-wk-old seedlings that had been inoculated 7 days earlier with zoospore suspensions and 8-wk-old plants that had been inoculated 3 wk earlier with cultures of *P. parasitica* grown in sterile vermiculite saturated with V-8 broth. Three grams of root tissue was frozen with liquid  $\text{N}_2$  and ground to a fine powder with a mortar and pestle. Three milliliters of extraction buffer was added, and the samples were further processed by the leaf DNA miniprep method.

**Detection of *P. parasitica* in natural soil.** During 1988, 25-cm-deep soil cores were taken in a stratified random sampling pattern (14) from 32 plots of an experimental field of processing tomatoes. The field was located in Davis, CA, and had been infested with various populations of *P. parasitica* in June 1987. The top 5 cm of each soil core was discarded, and the cores from each plot were sieved (2-mm mesh) and mixed thoroughly. The soil was either assayed directly or serially diluted with sterilized Yolo fine sandy loam. Soil samples were maintained at 23 C with a water content at approximately field capacity, and processed within 30 hr of sampling. Each soil sample (55 ml) was placed onto 300 ml of sterile soil in a 400-ml plastic cup that was perforated on the bottom for drainage. The soil was saturated with water and allowed to drain for 96 hr. The perforation was then blocked, and the soil was flooded with distilled water. These soil moisture changes favor sporangial formation and zoospore release from *P. parasitica* (1).

Six leaf disks were placed on the water surface of each flooded, undiluted soil sample and incubated for 48 hr at 22 C. The disks then were divided into two groups of three disks and processed by the leaf DNA miniprep method previously described. The DNA extracted from each set of three leaf disks was applied to a nylon



**Fig. 1.** Hybridization of pPP33 with DNA from colonies of *Phytophthora parasitica*. Colonies developed from infested soil, which was diluted and plated onto a nylon membrane covering selective medium.

membrane. Dilutions of purified DNA of *P. parasitica* (isolate 5-3A) were also applied to the membrane. After hybridization and washes, the area of the nylon membrane containing either the purified DNA or the DNA obtained from the soil sample were excised, placed in 5 ml of liquid scintillation fluid (Scinti Verse II, Fisher), and the radioactivity of the samples determined in a liquid scintillation spectrometer. The relationship between the amount of radioactivity and the quantity of purified *P. parasitica* DNA between 0.05 and 1 ng was determined by linear regression analysis. The level of nonspecific hybridization of the probe to DNA of healthy tomato leaf disks was subtracted from the sample values.

To quantify populations of *P. parasitica* by the most probable number (MPN) baiting method (17), five leaf disks were placed on the surface of flooded, serially diluted soil samples. Each soil dilution was replicated 10 times. After incubation for 24–48 hr at 23–26 C in the greenhouse, the leaf disks were removed, surface-sterilized, rinsed in sterile water, and placed on semiselective medium (12) containing Hymexazol (Sankyo Co., Tokyo, Japan) at 50 µg/ml (16). Plates were observed for colony development originating from the leaf disks after 5 days of incubation in the dark. The frequency of replicate soil samples with infected leaf disks at each soil dilution was determined, and the MPN of propagules was calculated with an MPN computer program (17).

## RESULTS

**Detection of *P. parasitica* DNA from colonies grown on soil dilution plates.** When artificially infested soil was diluted and plated on nylon membrane-covered selective medium, colony development appeared to be normal, and equivalent numbers of colonies were observed on the nylon-covered medium and the selective medium alone. No growth of *P. parasitica* was detected on selective medium covered with a pure (i.e., not detergent-treated) nitrocellulose membrane (data not shown). On nylon membranes, colonies grew on the surface of the membrane and occasionally spread into the agar below. The hyaline mycelia of most colonies were not easily visible on the white nylon membranes, making it difficult to count the colonies before hybridization. Large colonies (>2.0 cm diameter), however, were more easily visualized because the mycelia tended to trap soil particles even after washing the membrane. While the hybridization pattern was mottled and more intense at the margins, most individual colonies could be recognized and unambiguously identified by the probe (Fig. 1). Similar

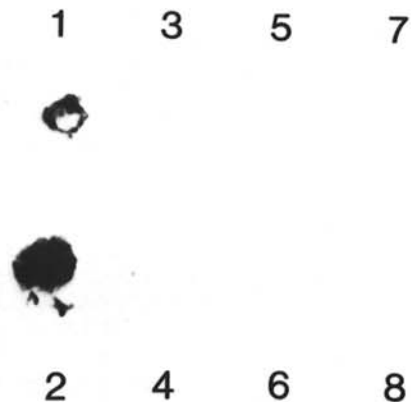


Fig. 2. Hybridization of pPP33 with DNA from fungal colonies grown in V-8 agar medium and crushed onto a nitrocellulose membrane. 1, *Phytophthora parasitica* C-2CL; 2, *P. parasitica* 5-3A; 3, *P. citrophthora* P1323; 4, *P. cryptogea* RI; 5, *P. cactorum* W5475; 6, *P. cinnamomi* SB216-1; 7, *Pythium ultimum* 1786 (7), and 8, uninoculated V-8 agar.

hybridization patterns were also observed with pure cultures grown on nylon-covered medium.

**Detection of *P. parasitica* DNA from colonies grown on agar media.** *P. parasitica* DNA was released and detected by the DNA probe when agar plugs containing mycelium were crushed on nylon or nitrocellulose membranes (Fig. 2). No significant hybridization was observed with crushed agar plugs containing mycelium of *Pythium ultimum* or other *Phytophthora* spp. (Fig. 2). This method also identified colonies of *P. parasitica* that were grown from infected leaf disks placed on semiselective medium, and was used to positively identify putative isolates of *P. parasitica* obtained from naturally infested soil (data not shown).

**Detection of *P. parasitica* DNA in leaf disks.** For all leaf disks in which colonies of *P. parasitica* grew on semiselective medium, *P. parasitica* DNA was also detected by the hybridization assay. With the leaf crush method, positive hybridization signals were observed for leaf disks incubated on soil infested with  $1 \times 10^3$  and  $25 \times 10^3$  chlamydospores per kilogram (Fig. 3). Some leaf disks at the lower infestation level showed a relatively weak hybridization signal, but these signals were still distinguishable from the healthy controls. Stronger detection signals were obtained in other experiments when whole rather than half leaf disk baits were used (data not shown). No significant hybridization was observed with crushed leaf disks excised from uninfected leaves or leaf disks that were incubated with flooded pasteurized soil. Detection of *P. parasitica* was similar whether leaf disks were crushed on nylon or nitrocellulose membranes, but nylon membranes are stronger and more tear resistant. The results shown in Figure 3 were for samples crushed on a nitrocellulose membrane, which gave a slightly lower background than a nylon membrane.

With the leaf disk miniprep method, *P. parasitica* DNA was detected on both nitrocellulose and nylon membranes (Fig. 4). Adjusting a sample to  $6\times$  SSC before its application to nylon or nitrocellulose membranes resulted in some plugging of the membranes. Although only half of the sample could pass through the nitrocellulose membrane before plugging, this amount was sufficient to detect colonized leaf disks (Fig. 4A). The effect of adding SSC to samples was the same for both control and infected leaf disks. The addition of salts, such as SSC, to DNA samples is required for efficient binding of nucleic acids to pure nitrocellulose, but is not required for binding to positively charged nylon. When SSC was not added to the sample DNA before application on a nylon membrane, the entire sample readily passed through the membrane, resulting in a stronger detection signal (Fig. 4B). Three half leaf disks could be combined, and the extracted DNA blotted completely onto a nylon membrane (Fig. 4C). This resulted in a greater hybridization signal than DNA

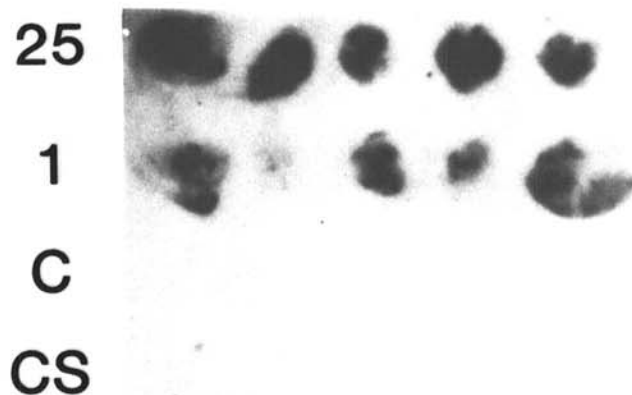
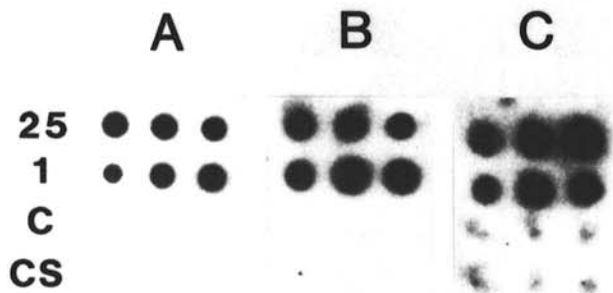


Fig. 3. Hybridization of pPP33 with DNA released from crushed leaf disks. Leaf disks were crushed following excision from uninfected leaves (C), incubation over flooded pasteurized soil (CS), or incubation over flooded soil containing  $1 \times 10^3$  (1) or  $25 \times 10^3$  (25) chlamydospores per kilogram of soil. Five replicate leaf disks were placed in each horizontal row.

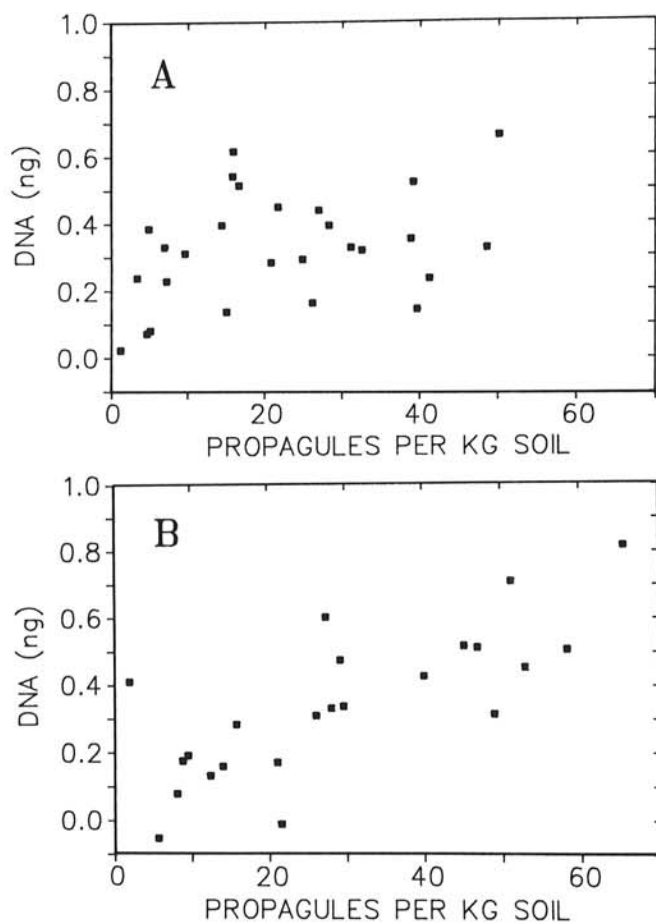
samples extracted from a single half leaf disk, but there was also an increase in nonspecific binding of the probe with DNA extracted from control leaf disks (Fig. 4B and C).

With a modified DNA miniprep method, positive hybridization signals were obtained for roots of seedling and mature tomato plants inoculated with *P. parasitica* in the greenhouse. No hybridization was observed with DNA extracted from uninoculated seedlings and plants (data not shown).

**Detection and quantification of *P. parasitica* in natural soil.** To obtain a range of pathogen population levels in natural soil, soil samples were taken preplanting (April), midseason (July),



**Fig. 4.** Dot hybridization of pPP33 with DNA extracted from leaf disks. DNA was extracted from leaf disks after they were cut from uninfected leaves (C), incubated over flooded pasteurized soil (CS), or incubated over flooded soil containing  $1 \times 10^3$  (1) or  $25 \times 10^3$  (25) chlamydospores per kilogram of soil. DNA samples were applied to a nitrocellulose membrane (A), a nylon membrane with one leaf disk half per sample (B), or a nylon membrane with three leaf disk halves per sample (C). Three replicate samples were applied in each horizontal row.



**Fig. 5.** Relationship between the quantity of DNA detected in leaf disk baits as determined by hybridization to probe pPP33 and the number of propagules of *P. parasitica* estimated for the same soil sample by the most probable number baiting method. Soil samples were taken from a tomato field **A**, before planting and, **B**, at midseason.

and preharvest (August) from experimental plots infested the previous year with *P. parasitica*. Sources of inoculum, therefore, should be relatively similar to those naturally occurring in tomato fields. The pathogen was detected in all of the soil samples by baiting with leaf disks, and incubating the disks on semiselective culture medium. Population densities, as determined by the most probable number baiting method, ranged from 1 to 1,500 propagules per kilogram of soil. MPN values at  $P > 0.05$  and/or with a uniformly high incidence of leaf disk infection in diluted soil (up to 64-fold dilution) were excluded from the analysis (17). The higher populations observed for preharvest samples (up to 1,500 propagules per kilogram of soil) appeared to be too large to be accurately quantified with the soil dilutions chosen for the MPN assay, and therefore much of the data was excluded.

To quantify propagules of *P. parasitica* with the hybridization assay, it was first necessary to determine with a liquid scintillation counter the amount of radiolabeled probe that hybridized to DNA extracted from leaf disk baits. A dilution series of purified *P. parasitica* DNA was included as standards. Linear regression equations of the standards (e.g.,  $Y = 55.1 + 187X$ , where  $Y$  = disintegrations per minute and  $X$  = ng of DNA) that had high coefficients of determination ( $r^2 = 0.997-0.998$ ) were used to calculate the quantity of *P. parasitica* DNA detected by the probe.

To determine whether the quantity of *P. parasitica* DNA in leaf disk baits was related to the number of propagules in soil, correlations were made between the two sets of data obtained from the same soil samples (Fig. 5A and B). A significant correlation ( $P < 0.05$ ) was found for preplanting samples, ( $r = 0.36$ ;  $Y = 2.89 + 0.092X$ , where  $Y$  = ng of DNA and  $X$  = propagules per kilogram of soil), midseason samples ( $r = 0.88$ ;  $Y = -6.36 + 0.252X$ ), and preharvest samples ( $r = 0.44$ ;  $Y = 0.22 + 0.002X$ ; data not shown). However, preharvest results are less complete due to insufficient soil dilutions for the MPN assay in 15 of the 32 samples. The high degree of variation of the correlation indicates that the assay was affected by unknown factor(s), which differed between sampling dates.

## DISCUSSION

DNA probes have been employed to detect plant pathogenic fungi (10,20), bacteria (4,7,21), mycoplasmalike organisms (13), and nematodes (2). To extract DNA, plant pathogens have been homogenized with the host tissue (13,20), or first recovered from the host tissue and then lysed directly on a membrane or homogenized (2,4,7,21). DNA of plant pathogens also has been detected by crushing insect vector or plant tissue onto a membrane and lysing the cells (3,7,13). To detect DNA of *Phoma tracheiphila*, lignified branch tissue was first incubated on culture medium to increase the amount of extractable DNA (20).

In the present study, relatively simple and direct methods were found that allowed a *P. parasitica*-specific DNA probe to detect the fungus in infected leaf disks and roots. Although these methods are sufficiently sensitive to detect *P. parasitica* in soil at populations below those causing yield loss, they differ in their sensitivity, simplicity, and potential to quantify *P. parasitica*.

Detection of *P. parasitica* DNA with a modified soil dilution plating method was simple and could permit direct quantification of propagules of *P. parasitica* in soil. Although detection and enumeration of fungal colonies on a membrane with the DNA probe is more difficult than counting colonies directly on plates, the use of the DNA probe offers the advantage of unambiguous species identification. Of course, the use of the DNA probe does not overcome the limitations of sensitivity and accuracy inherent in dilution plate methods at population levels much below  $1 \times 10^4$  propagules per kilogram of soil (23).

The use of the crushed agar colony method also permitted simple and rapid processing of samples. This method can be employed to identify any discrete fungal colonies grown in agar medium that originated from soil or elsewhere. This method, the colony hybridization, and the DNA miniprep methods have all been used to confirm the identification of isolates in culture collections (data not shown).

Two alternative methods were developed to detect *P. parasitica* DNA in leaf disk baits. The leaf crush method was quicker and simpler for processing samples; however, closely spaced samples could be cross-contaminated during the crushing process, and, therefore, more membrane area was required to keep the samples separated. The leaf DNA miniprep method required more processing of the bait tissues, but application of multiple samples was simplified because the membrane was immobilized in a dot-blot manifold. In addition, the discrete spots of DNA provide a more intense and uniform hybridization signal, and they were easily quantified with a liquid scintillation spectrometer. Difficulties encountered in applying the DNA solution to a nitrocellulose membrane were avoided by using a nylon membrane that binds DNA without the addition of SSC. The cause of the membrane plugging induced by SSC is unknown. With only slight modifications, the leaf DNA miniprep method was also used to extract DNA of *P. parasitica* from tomato roots.

In combination with leaf disk baits, the DNA probe could detect *P. parasitica* in field soil at populations below those causing considerable root rot and yield loss of tomatoes. At populations from 1 to 65 propagules per kilogram of soil, the quantity of *P. parasitica* DNA detected by the DNA probe increased significantly ( $P < 0.05$ ) with the level of inoculum. Examination of the results of each sampling date, however, revealed that the relationship was variable. For the DNA probe to accurately quantify populations of *P. parasitica* with the leaf disk method, the amount of DNA of *P. parasitica* in infected leaf disks must be quantitatively related to the number of propagules in the soil. Bait tissue is infected by zoospores that are released in relatively large numbers from sporangia. For a quantitative relationship to exist, the number of lesions should be directly related to the number of propagules. Also, each lesion must have similar amounts of DNA of *P. parasitica*, and, therefore, all the infections must occur during a limited period of time and develop at similar rates. If, for instance, the release of zoospores occurs over a prolonged period of time, then a quantitative relationship will not exist because lesions will differ in size and content of mycelium. A number of additional factors could be responsible for the variable results, such as slight temperature differences affecting the growth rate of the lesions. In addition, the MPN method of estimating soil populations has a number of limitations and uncertainties. One of the major limitations is that the efficiency of leaf disk infection was probably low, and therefore not all propagules in a given aliquot of soil are detectable, which contradicts a basic assumption of the MPN method (9,15,17). Also, it was not always possible to predict the appropriate dilution series of soil that would give the range of positive and negative leaf disk colonizations needed for the MPN baiting method (9,15,17).

Despite the limitations and uncertainties, a good correlation was observed for midseason samples between the quantity of detected DNA and the soil populations estimated by the MPN method. For these samples, there was also a good correlation between the fraction of leaf disks colonized by *P. parasitica* in undiluted soil samples and the DNA detected in leaf disks with the probe (data not shown). In addition, midseason samples had the best distribution of colonized and uncolonized leaf disks, thus best fulfilling the assumptions of the MPN method (15). Preplanting samples showed a much lower correlation between the DNA detected and both the MPN values and the fraction of leaf disks colonized, which illustrated the variability of these assays.

Although *P. parasitica* was not reliably quantified by the method described in this study, the use of the DNA probe with leaf disk baits and soil dilution plates did specifically and reliably detect *P. parasitica* in soil. Several alternative methods of increasing *P. parasitica* from soil samples and extracting its DNA are available by using relatively simple techniques. These methods should be useful in epidemiological and ecological studies, and to provide correct diagnosis for disease management.

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