USE OF A BIOTINYLATED DNA PROBE FOR DETECTION OF THE ASTER YELLOWS MYCOPLASMALIKE ORGANISM IN *DALBULUS MAIDIS* AND *MACROSTELES FASCIFRONS* (HOMOPTERA: CICADELLIDAE).

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ABSTRACT

A DNA probe was used to detect aster yellows mycoplasmalike organism acquisition by the corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott), an insect that does not transmit aster yellows mycoplasmalike organism, as well as by *Macrosteles fascifrons* (Stål), a vector insect. Results show the effectiveness of the probe for pathogen detection in both the non-vector and the vector insect.

RESUMEN

Se utilizó una prueba de DNA para detectar la adquisición del organismo de micoplasma de las estrias amarillas (AYMLO) por la chicharrita del maíz, *Dalbulus maidis* (DeLong & Wolcott), un insecto que no transmite AYMLO, así como de *Macrosteles fascifrons* (Stal) un insecto vector. Los resultados muestran la efectividad de la prueba para detectar el patógeno en el insecto no vector y en el insecto vector.

Positive and negative effects have been reported in vector and non-vector insects after feeding on diseased plants (Vega 1992). In at least one case, an expansion of host plant range occurred when a non-vector insect species fed upon a diseased host (Maramorosch 1958). This system involved the aster yellows mycoplasmalike organism - an aster leafhopper [*Macrosteles fascifrons* (Stål); Homoptera: Cicadellidae] (Sorensen & Sawyer 1989) transmitted a plant pathogen with host plants in over 40 different families (McCoy et al. 1989) - and the corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott), (Homoptera: Cicadellidae).

D. maidis is a *Zea* spp. specialist (Nault & DeLong 1980) that cannot normally survive on China asters [*Callistephus chinensis* (L.) Nees] (Maramorosch 1958) nor does it transmit the pathogen causing aster yellows (Maramorosch 1952). However, if *D. maidis* feeds on asters infected with the aster yellows mycoplasmalike organism, not only can it survive on the plant, but after a minimum of three days on the plant it can survive on healthy asters and other non-host plants, such as carrots and rye, on which

it typically would not survive (Maramorosch 1960; Purcell 1988). This phenomenon is called conditioning (Maramorosch 1958).

Recently, Vega et al. (1993) confirmed the presence of the aster yellows mycoplasmalike organism in *D. maidis* by using the polymerase chain reaction (PCR). The objective of this study was to determine whether a biotinylated DNA probe could be used to detect the mycoplasmalike organism in a vector and a non-vector insect.

Materials and Methods

Healthy and aster yellow-infected aster plants, as well as healthy and infected *M. fascifrons*, were provided by the fifth author. The non-vector insects (i.e., *Dalbulus maidis*) had been maintained in colonies at the University of Maryland greenhouse for 15-20 generations.

Pathogen Acquisition

Vector and non-vector insects were placed on asters infected with the 'Severe' (SAY) strain of the aster yellows mycoplasmalike organism for various acquisition access periods (AAP). The AAP was followed by various incubation periods, during which insects were held on uninfected asters in the case of *M. fascifrons*, or on uninfected corn (cv. Aristogold Bantam Evergreen) in the case of *D. maidis*. When *D. maidis* was allowed to feed on healthy aster plants, the insect died within 4 days (Maramorosch 1958). To reduce mortality of the non-vector insect after the AAP on SAY-infected asters, we held *D. maidis* on corn for the incubation period. *D. maidis* was given an AAP of 7 days followed by incubation periods of 0, 7 or 14 days and an AAP of 14 days followed by incubation periods of 0 or 7 days. *M. fascifrons* was given an AAP of 7 days followed by incubation periods of 0, 7, or 14 days and an AAP of 14 days followed by an incubation period of 0, 7, or 14 days. Negative controls consisted of insects that fed on corn (*D. maidis*) or on aster (*M. fascifrons*). *M. fascifrons* AAP and incubation periods were conducted at the University of California, Berkeley. *D. maidis* AAP and incubation periods were conducted at the University of Maryland greenhouse. Insects were frozen (-86 °C) after each treatment combination until DNA analysis.

DNA Extraction

Batches of five insects were separately placed in 12 ml glass tissue homogenizers (Bellco Biotechnology, Vineland, N.J.) and macerated several times in 400 µl of extraction buffer (100 mM Tris, 50 mM EDTA, 500 mM NaCl, pH 8.0), 2 µl of ß-mercaptoethanol, and 20 µl of 20% sodium dodecyl sulfate (SDS). The solution was transferred to a 1.5 ml Eppendorf tube and re-macerated with a mini-pestle. After clarifying three times by centrifugation, nucleic acids were extracted using chloroform-isoamyl alcohol and TE-saturated phenol. To precipitate nucleic acids, 2.5 volumes of cold absolute ethanol was added to samples followed by 30 min at -86 °C, and 20 min of centrifugation at 14,000 rpm. The DNA pellet was resuspended in 100 µl 6x saline sodium citrate (SSC; 1x= 0.9 M NaCl, 0.09 M sodium citrate, pH 7.0).

Filter Preparation

Resuspended DNA was denatured by adding to each 50 µl SSC, 3 µl of 2N NaOH, boiling for 10 min, cooling in ice and neutralizing with 3 µl of 2M TRIS and 1 µl 1.5

M NaAc. Three microliters of undiluted and serial dilutions were spotted on nitrocellulose paper and baked at 80 °C for 2 h under vacuum.

Filter Pre-hybridization

Filters were pre-hybridized with gentle rotation at 42°C for 2-4 h in presence of 50% formamide, 5x SSC, 5X Denhardt's solution (0.5 g Ficoll, 0.5 g polyvinylpyrrolidone, 0.5 g bovine serum albumin), 25 mM sodium phosphate and 0.5 mg/ml of denatured salmon sperm DNA.

Probe Hybridization and Visualization

Hybridization was conducted at 42 °C using 5% Dextran sulfate, 45% formamide, 5X SSC, 1X Denhardt's solution, 20 mM sodium phosphate (pH 6.5), 0.2 mg µl of denatured salmon sperm DNA, and approximately 0.3 mg of biotinylated DNA probe per ml. The probe was prepared by cloning aster yellows mycoplasmalike organism DNA fragments into plasmid vectors pSP64 or pSP65 which were then amplified in *Escherichia coli* strain JM83 (for a full description see Lee & Davis 1988). After 12-16 h incubation, filters were rinsed twice for 3 min in 2X SSC with 0.1% SDS, 2 rinses of 0.2X SSC with 0.1% SDS (both rinses at room temperature), and 2 rinses at 50 °C for 15 min using 0.16X SSC with 0.1% SDS. For signal detection, filters were rinsed for 1 min in buffer 1 (0.1 M Tris-HCl, 0.15 M NaCl, pH 7.5), and blocked for 1 h at 64 °C in buffer 2 (3% bovine serum albumin in buffer 1). Filters were incubated with gentle rotation for 10 min using streptavidin-alkaline phosphatase diluted in buffer 1, followed by two 15 min rinses in buffer 1 and one 10 min rinse in buffer 3 (0.1 M Tris-HCl, 0.1 M NaCl, 0.05 M $MgCl_2$, pH 9.5). To visualize reactions, filters were incubated for about 30 min in the dark using nitroblue tetrazolium and 5-bromo-4-chloro-indolylphosphate diluted in buffer 3. To stop reactions, filters were washed in termination buffer (20 mM Tris, 0.5 mM EDTA, pH 7.5). A bluish color indicated a positive reaction, i.e., presence of the pathogen's DNA.

Results and Discussion

For *D. maidis* given a 7-day AAP, higher amounts of DNA were detected as the incubation period increased from 0 to 7, and then to 14 day (Table 1). There was a reduction in the SAY DNA detected as the incubation period increased from 0 to 7 days in insects given a 14-day AAP (Table 1). Similar reductions in aster yellows concentration have been reported in the alimentary canal of *M. fascifrons* by Sinha & Chiykowski (1967). SAY DNA was detected in *M. fascifrons* at all dilutions and in all treatments except for the control and for insects given a 7 day AAP and no incubation period (Table 1).

Our results (Table 1) indicate that a biotinylated DNA probe can be used for the detection of SAY MLO in vector and non-vector insects. Even though sensitive techniques such as PCR are now routinely used for pathogen detection, biotinylated DNA probes are a sound alternative in cases where PCR technology is not available. In this study, the use of a biotinylated DNA probe yielded information that suggests an increase in pathogen titer as the incubation period increased for non-vector insects with a 7 days AAP.

If widespread, the conditioning phenomenon could play an important role in the expansion of insects host plant range. The availability of different pathogen detection

TABLE 1. SAY MLO DETECTION IN *D. MAIDIS* AND *M. FASCIFRONS* USING A BIOTINYLATED DNA PROBE.

		Dalbulus maidis			
		Nucleic acid dilutions			
AAP[1]	IP[2]	1X[3]	1/2X	1/4X	1/8X
0	0	–[4]	–	–	–
7	0	+[5]	+	–	–
7	7	+	+	+	–
7	14	+	+	+	+
14	0	+	+	+	+
14	7	+	+	–	–
		Macrosteles fascifrons			
		Nucleic acid dilutions			
AAP	IP	1X	1/2X	1/4X	1/8X
0	0	–	–	–	–
7	0	–	–	–	–
7	7	+	+	+	+
7	14	+	+	+	+
14	0	+	+	+	+
14	7	+	+	+	+
14	14	+	+	+	+

[1]AAP, acquisition access period, in days.
[2]IP, incubation period following the AAP, in days.
[3]1x = undiluted DNA; 1/2X = DNA sample diluted in half, etc.
[4]Negative reaction, i. e. no hybridization signal.
[5]Positive hybridization reaction.

techniques will allow research aimed at understanding the mechanism(s) responsible for conditioning.

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