

Maize bushy stunt phytoplasma transmission by *Dalbulus maidis* is affected by spiroplasma acquisition and environmental conditions

Elizabeth DE OLIVEIRA, Jaciara Cristina SANTOS, Paulo César MAGALHÃES, Ivan CRUZ
Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil

Abstract

Effects of acquisition-sequence of corn stunt spiroplasma and maize bushy phytoplasma on phytoplasma transmission by *Dalbulus maidis*, were studied under different environmental conditions. Acquisition of spiroplasma before phytoplasma and lower air temperatures reduced phytoplasma-detection frequency in leafhoppers and in plants. Results suggest that phytoplasma development could be reduced by spiroplasma competition and low air temperatures.

Key words: maize bushy stunt phytoplasma, corn stunt spiroplasma, *Dalbulus maidis*, maize.

Introduction

The leafhopper *Dalbulus maidis* (DeLong and Wolcott) is a vector of maize bushy stunt phytoplasma, and corn stunt spiroplasma (Nault, 1980). Infection of susceptible maize seedling by one of these *Mollicutes* result in severe damage on plant development, nutrition, and kernel production (Oliveira *et al.*, 2002b; Oliveira *et al.*, 2005). The maize bushy stunt phytoplasma transmission by *D. maidis* has been studied (Nault, 1980; Legrand and Power, 1994), however, the interactions between phytoplasma and spiroplasma transmission are not completely understood. In this study, we evaluated the effects of the order of spiroplasma and phytoplasma acquisition by *D. maidis* on phytoplasma transmission, under different environmental conditions.

Materials and methods

Two experiments, with same treatments, were carried out in greenhouse protected against insects, at Embrapa maize and sorghum experimental station, Brazil, during different months. The experiment 1 was carried out under high temperatures, during the months of October, November and December. The experiment 2 was carried out under low temperatures, during part of April, May, June and part of July. Temperatures and air relative humidity were collected at Embrapa experimental station. For each experiment, healthy young adults of *D. maidis* were first confined on spiroplasma-infected maize, and after that, on phytoplasma-infected maize (treatment 1), and others leafhoppers were first confined on phytoplasma-infected maize, and after that on spiroplasma-infected maize (treatment 2), for acquisition periods of four days. After that, leafhoppers were kept in insect cages feeding with healthy maize seedlings. Thirty days after acquisition (latent period), only one leafhopper was confined for four days on one healthy maize seedling, eight days old, growing on pots with 3 kg of fertilized soil, for mollicute transmission. Each treatment was repeated 30 times. From each treatment, 10 leaf-

hoppers used for mollicute-inoculation were randomly collected and tested by multiplex PCR for phytoplasma and spiroplasma detection (Oliveira *et al.*, 2005). Also, samples of plants in which these leafhoppers had feeding were tested by multiplex PCR for phytoplasma and spiroplasma detection, 45 days after inoculation. The experiments were carried out for three months since acquisition, and plant symptoms were evaluated at the end (60 days after sowing).

Results

Results are presented on table 1, for environmental conditions, and for mollicute detection. The frequency of phytoplasma detection in *D. maidis* was reduced when the sequence of acquisition was phytoplasma and, after that, spiroplasma, and this reduction was higher in experiment 2, under low air temperatures, than in experiment 1. For leafhoppers as well as plants, phytoplasma-detection frequency in experiment 2 was lower than in experiment 1, even when the order of acquisition was spiroplasma and, after that, phytoplasma. In some cases, phytoplasma or spiroplasma was detected by PCR test, only in leafhopper or in the corresponding plant. Diagnostic symptoms of corn stunting spiroplasma, characterized by chlorotic stripes that extend toward leaves tips (Nault, 1980) predominated in plants of both experiments.

Discussion

These results suggest that phytoplasma development could be reduced by spiroplasma competition, and by temperatures lower than 29 °C (T_{max}) and 17 °C (T_{min}). Nault (1980) comparing maize bushy stunt phytoplasma and corn stunt spiroplasma at 31/25 °C and 27/18 °C (day/night), observed that phytoplasma infected plants died before maturity, at high temperatures.

It is possible that phytoplasma needs higher temperatures for multiplication, than spiroplasma. In Brazil,

Table 1. Monthly averages of maximum and minimum temperatures (°C) and air relative humidity (RH) (%) at Embrapa maize and sorghum experimental station, during the three months of each experiment.

	Tmax	Tmin	RH	Tmax	Tmin	RH	Tmax	Tmin	RH												
Experiment 1	30.35	17.19	50.47	29.26	18.61	66.05	29.38	19.10	70.65												
Experiment 2	27.80	15.80	71.50	25.70	13.50	71.50	25.40	13.40	67.00												
Sequence of acquisition																					
Experiment 1	Spiroplasma/phytoplasma										Phytoplasma/spiroplasma										
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Leafhoppers	Phyto	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-
	Spiro	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Plants	Phyto	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
	Spiro	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sequence of acquisition																					
Experiment 2	Spiroplasma/phytoplasma										Phytoplasma/spiroplasma										
		1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
Leafhoppers	Phyto	+	+	-	-	-	+	-	-	+	+	-	-	-	-	+	-	-	-	-	-
	Spiro	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Plants	Phyto	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-	+	+	-	-
	Spiro	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-

(+) = detected; (-) = not detected.

corn stunting diseases are common in maize crop, however plants infected simultaneously by both mollicutes are seldom detected. Usually, one of them has predominance (Oliveira *et al.*, 2002a). It is possible that the air temperature variation could be the main epidemiological factor determining whether phytoplasma or spiroplasma predominates under field conditions.

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Corresponding author: Elizabeth DE OLIVEIRA (e-mail: beth@cnpm.embrapa.br), Embrapa Milho e Sorgo C.P. 151, CEP 35701-970, Sete Lagoas, MG, Brasil.