Artículo Original

Probability of occurrence of the mixed infection *Canditatus* phytoplasma sp. - *Bean golden yellow mosaic virus* (BGYMV) in *Phaselous vulgaris* L.

Probabilidad de ocurrencia de infección mixta *Canditatus* phytoplasma sp. - *Bean golden yellow mosaic virus* (BGYMV) en *Phaselous vulgaris* L.

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ABSTRACT: The objective of this work was to determine the probable occurrence of the mixed infection phytoplasma - BGYMV in *Phaselous vulgaris* L. and the symptoms associated. This probability was calculated using classical probability and a smoothed adjustment of the series of not centered moving median was used to estimate the presence of a mixed infection in two close points. The conditional probabilities of a mixed infection knowing the *a priori* probabilities of phytoplasma and begomovirus were calculated using the classical formula of probability. The influence of present symptoms in the agricultural ecosystem on the probability of this mixed infection to increase and its association with the vector presence were evaluated using principal component analysis and canonical discriminate test. The occurence of a mixed infection was more probable when it was known *a priori* that the phytoplasma was present than when it was the begomovirus. Symptoms in the bean crop associated with the mixed infection were wrinkled leaves, mosaic, and reduced size of the leaves. Even in the presence of the vector, leaf chlorosis and deformation were associated with a low probability of mixed infection, which suggested the presence of a unique virus or another disease. We suggest that this association can be used in the prediction of mixed diseases as an initial prognosis leading to a rapid diagnosis with reduced costs due to a better selection of samples.

Key words: Bayes theorem, BGYMV, phytoplasma, canonical discriminate analysis, Phytopathogens, Phytoplasma.

RESUMEN: El objetivo del presente trabajo fue determinar la probabilidad de ocurrencia de infección mixta *Canditatus* phytoplasma sp. - BGYMV en *Phaselous vulgaris* L. y los síntomas asociados. La probabilidad se estimó utilizando un ajuste suavizado de la serie de la media móvil no centrada. Las probabilidades condicionales de presencia de infección mixta, conociendo *a priori* la presencia de fitoplasmas o begomovirus, se calcularon usando la fórmula clásica de probabilidad. Se evaluó, además, la influencia de síntomas presentes en el agroecosistema sobre la probabilidad de incremento de la infección mixta y su asociación con la concurrencia del vector, mediante un análisis de componentes principales y discriminante canónico. La existencia de infección mixta fue más probable cuando se conoce *a priori* que el fitoplasma está presente, que cuando se establece primero la presencia de begomovirus. Los síntomas en el cultivo de frijol que más se asociaron con la presencia de infección mixta fueron arrugamiento de las hojas, mosaico y reducción del tamaño de las hojas. Aun en presencia del vector, la clorosis de la hoja y la deformación se asociaron con una baja probabilidad de infección mixta, lo cual sugiere la presencia de un virus único u otra enfermedad. Este resultado puede ser utilizado en la predicción de enfermedades mixtas, como una prognosis inicial que conduzca a un diagnóstico rápido con costo reducido, al hacer una mejor selección de las muestras; asimismo, apoyará la toma de decisiones para el manejo integrado de plagas en el cultivo.

Palabras clave: BGYMV, discriminante canónico, fitopatógenos, fitoplasma, teorema de Bayes.

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INTRODUCTION

Phytopathology has historically dealt with those diseases caused only by a causal agent and its identification and pathogenic mechanisms in a particular crop. However, we frequently find complex agricultural ecosystems where biotic and abiotic relationships exist. The biotic interactions can cause complex diseases where the pathogen activity is strengthened by the effect of other pathogen (synergism) (1).

When several pathogens occur in a crop, mixed infections can be found and the symptoms may be confused, being the diagnosis only possible by using molecular techniques (2,3).

In particular, the common bean (*Phaselous vulgaris* L.), an important component of the animal and human diet, for its high protein value (4), is highly vulnerable to the attack of diverse insects with a negative influence on the crop (5). These insect behave like vectors of viral infections. Important viral species are the bean common mosaic virus (BCMV) and the bean common mosaic necrosis virus (BCMNV), both transmitted by seeds, mechanically and by aphids (6); the beet curly top virus (BCTV), transmitted by *Circulifer tenellus* Baker; the *bean golden mosaic virus* (BGMV); and the *bean golden yellow mosaic virus* (BGYMV), transmitted by white fly (*Bemisia tabaci* Gennadius) (7). Also, species of tysanopterans have been informed associated with the transmission of tospovirus in bean, like *Caliothrips phaseoli* Hood, *Frankliniella insularis* Franklin, *Frankliniella williamsi* Hood, *Frankliniella vespiformis* Crowford, *Thrips palmi* Karny, *Thrips tabaci* Lindeman (8). Other important insects are the Empoasca spp. vectors of phytoplasma and have been informed associated with begomovirus producing the symptom complex (9)

The existence of a mixed infection phytoplasma - virus has been demonstrated using molecular analysis by PCR of field samples (9,10). The probability of existence of mixed infection can be calculated using Bayes' theorem; it establishes the relationship between the prior and posterior probabilities, with the advantage that the use of verisimilitude permit quantifying the probability of infection or disease in one plant. However, this technique has not been used very much by psychopathologists (11). Besides, there are methods such as the canonical discriminant analysis that permit identifying variables that are correlated with the presence - absence of another variable (12), these can be useful to identify symptoms correlated with mixed infections.

Also, the good use of statistics techniques will permit answering the several questions: Can the symptoms appearing in the bean crop be attributed to the presence of a mixed infection? Which is the frequency percentage of mixed infection occurrence?

Then, the objective of this work was to determine the probability of the mixed infection phytoplasma - begomovirus in Cuban localities and the major symptoms correlated with the mixed infection.

MATERIALS AND METHODS

During the period 2011-2012, the commercial cultivar Bat-304 of *P. vulgaris* was sampled at two famer properties of the municipalities of Güines, four fields of San José de las Lajas (Mayabeque province located at 22°34' -23°12' N latitude and 82°28' - 81°40' W longitude), and two fields of Cotorro (Havana province located at 23°01'34 " N latitude and 82°14'51 " W longitude). A total of 120 samples were collected from each corner and the center of a rectangle in each field. After eliminating the damaged samples, between 7 and 14 samples for location, a total of 99 samples with different phenological ages was left: 11 with 35 days, 58 with 60 days and 30 with 70 days.

The presence or absence of begomovirus (13) or phytoplasma (14) characteristic symptoms appearing in each sample was recorded. The symptoms examined were mosaic, wrinkled leaves, and reduction of the leaf size, yellowing, dwarfism, chlorosis, violaceous stems, deformed leaves, dark-colored nerves, and spotted and thick stems. The presence of *Ca.* phytoplasma sp. and BGYMV in each sample was tested by specific PCR and nested PCR multiplex (15)

Prediction by smoothed adjustment

The not centered moving median was calculated by predicting the probability of a fourth sample to be positive to mixed infection if two of three samples taken consecutively were positive.

This series is constructed with the average of the value in one point and two foregoing items ($\overline{Y_t} = median\ \{Y_{t-2}; Y_{t-1}; Y_t\}$) (12)

Classical formula of probability

Taking the presence of mixed infection (success A) and the presence of phytoplasma of begomovirus(success B) as processes , the conditional probability of the mixed infection presence P (mixed/begomovirus) and P(mixed/phytoplasma) were calculated. For it, the classical formula of probability was applied (16,17):

P(A/B)=Number of success/Number of possible events

The conditional probabilities were compared using Wald with correction method (18).

Associations in the ecosystem

The most frequent symptoms that occurred in each locality were identified by a principal component analysis and a biplot graph. The typical symptoms of a mixed infection were selected using a canonical discriminate analysis and the chi square independence test. The statistical system InfoStat 2.0 was used (12).

RESULTS AND DISCUSSION

Prediction by smoothed adjustment

The prediction by a smoothed adjustment showed 85.8 % of coincidence (Fig. 1). This showed that a correct prediction would be possible if the samples were arranged by a geographic location order. In consequence, if two of three samples taken consecutively were positive to a mixed infection, the probability of a fourth sample to be positive was higher than 0.8.

Classical formula of probability

Of the total of the analyzed samples, 55.5 % presented phytoplasma and 61.6 % begomovirus. Both diseases were present in 44.4 % of the samples. The presence of mixed infection was more probable when it was known *a priori* that phytoplasma was present than when it was the begomovirus (*p<0,01*). It is important to know that when the phytoplasma or the begomovirus were present the probability of finding a mixed infection was above 70 % (Table 1).

P(mixed/begomovirus)=44/55=0.721

P(mixed/phytoplasma)=44/61=0.80

The mixed infection of phytoplasma - begomovirus was favored in areas with a maximum temperature between 28 and 30°C, a minimum of 18 to19°C, an annual average of 121 days with rains and a relative humidity of 77 to 85 %. In general, the mixed infection occurred more frequently than by pyhtoplasma or begomovirus alone. This effect could be observed in a farm in Güines, in three farms in San José, and in a special way, in a farm in Cotorro, where simple infections did not appear. Besides, the incidence of these diseases was markedly lower in both farms in Cotorro than in the other farms

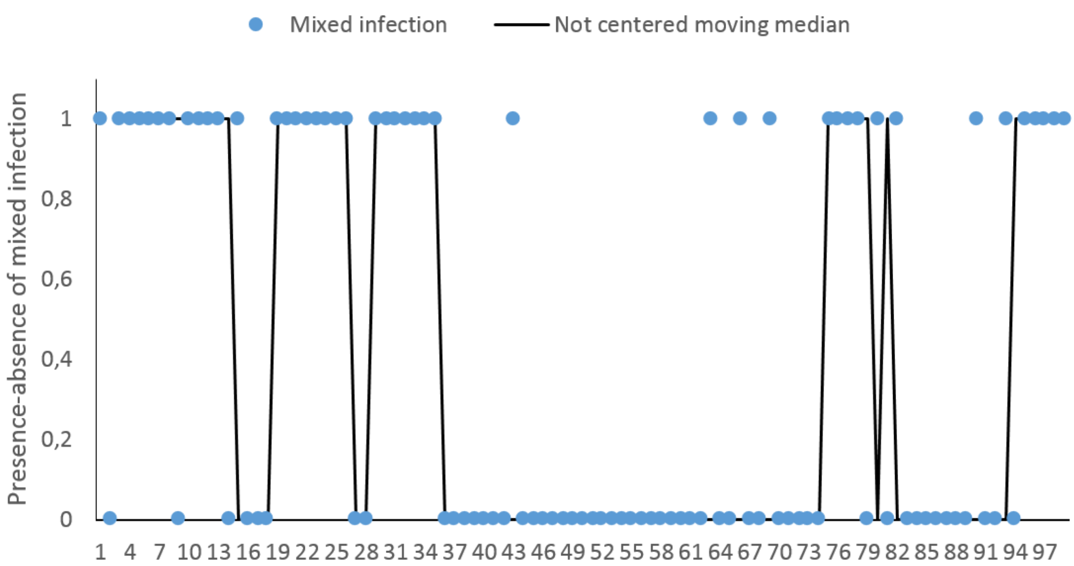

FIGURE 1. Mixed infection predicted using not centered moving median. / Infección mixta predicha mediante la serie media móvil no centrada

(Fig. 2). Mixed infections occurred less frequently in urban areas, indicating the importance of crop management and the control of those factors associated with the development of the diseases in smaller extensions of land. The crops in these areas are adjacent to arvensis plants, which may function as alternative vector hosts contributing to reduce the inoculum pressure in the field and, consequently, the occurrence of diseases (19).

This fact demonstrated that, in addition to the environmental factors, crop management is important to counteract the diseases caused by the presence of phytopathogens.

Different factors favor the proliferation of diseases in the crops, such as the existence of an active pathogen agent, an appropriate host and environmental conditions for the infection, colonization and reproduction of the pathogen agent. The environmental factors also affect the competitive capacity of the vector. The interaction host-pathogen - environment has allowed to use ecological principles to reduce the losses from diseases (19).

In contrast, the BGYMV has been considered devastating for bean production (20), with most of the farmers making indiscriminate use of chemicals (21) affecting the environment and the

TABLE 1. Simple and mixed infections in samples from Cuban localities. Comparison of conditional occurrence percentages. / Infección simple y mixta en muestras de localidades cubanas. Comparación de la probabilidad de ocurrencia condicional

Mixed infection	phytoplasma		begomovirus	
	+	-	+	-
+	44	0	44	0
-	11	44	17	38
Total	55	44	61	38
χ^2	63.36		16.42	
p	0.0001		0.0001	
Percentage of conditional occurrence	80 % a		72.13 % b	

Different letters indicate significant differences, according to Wald´s test with correction for continuity (*p≤0.05*)

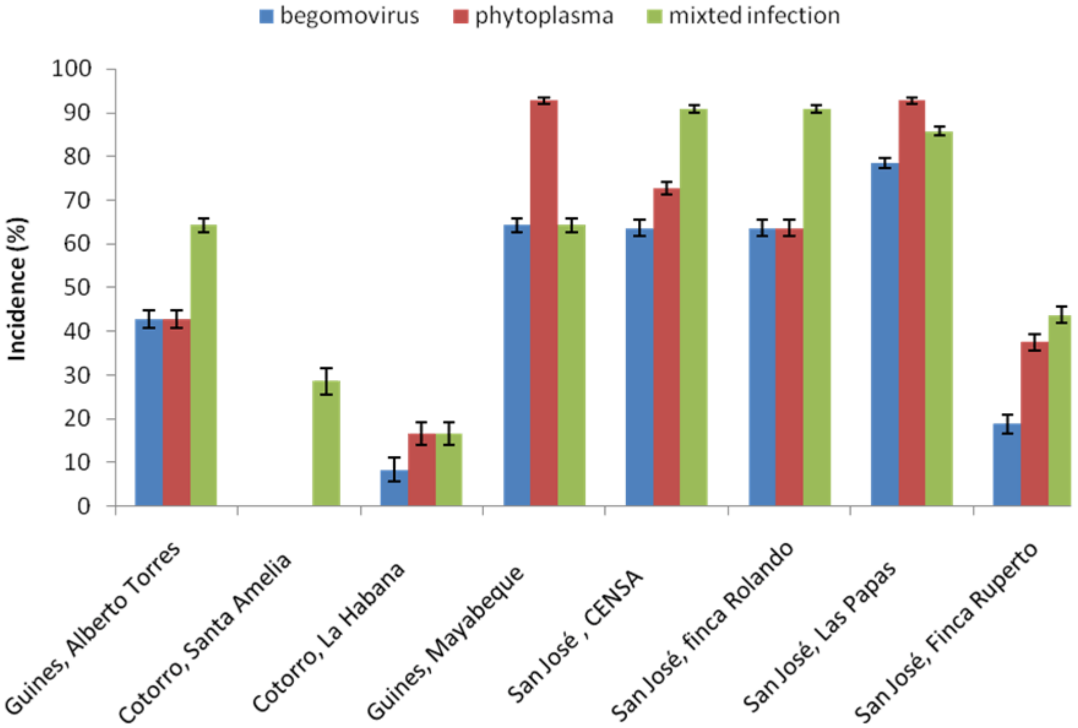

FIGURE 2. Single and mixed infection incidence by locality. / Por ciento de incidencia de infección simple y mixta para cada localidad

biodiversity among other elements of the ecosystem.

The principal factors that contribute to the proliferation of single and mixed diseases in crops are the excessive use of pesticides and of infested plant materials (9). In these affected localities, the biological control can lead to a reduced use of insecticides when they are included within the management strategy. This should be together with an appropriate farmer training (22).

Associations in the ecosystem

The most frequent symptoms in each locality were identified (Fig. 3) and informed to farmers in order to associate these symptoms with the possible presence of important diseases because some farmers could confuse the causes of these symptoms.

The symptoms most associated with the presence or absence of mixed infection were mosaic, wrinkled leaves, reduction of the leaf size, chlorosis, deformed leaves, and the presence of the vector. (Table 2)

The results of the present work allow introducing some important aspects into the disease management in the bean crop and into the comprehensive training of farmers. For example, these results allow implying a high probability of mixed infection occurrence when mosaic, wrinkled leaves, or the reduction of the leaves size are present, even when the presence of white fly or leaf hoppers are not observed. In addition, if a plant is positive to phytoplasma, it is estimated to have an 80 % of probability of being also positive to mixed infection.

Of course, it is advisable to take measures to control vectors, for their synergic or antagonistic biotic interactions with unpredictable pathological consequences (23). Mixed infections have to be analyzed with an epidemiological approach, keeping in mind the interaction of the vectors (24).

It is convenient to remark that the appropriate use of statistical methods makes possible an effective prognostic. The canonical discriminate analysis proved to be a useful method in the identification of symptoms that increased the probability of a mixed infection presence. This

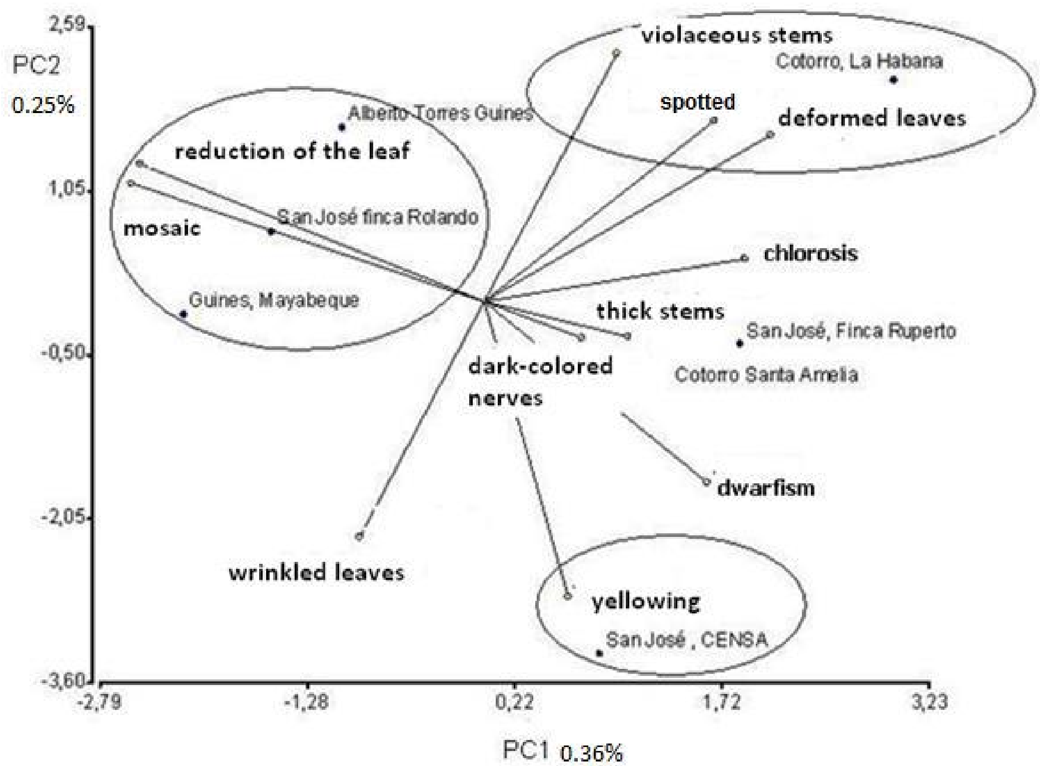

FIGURE 3. Characterization of the sampled localities according to the most frequent symptoms present in the crop. / Caracterización de las localidades muestreadas de acuerdo con la frecuencia de aparición de síntomas en el cultivo

TABLE 2. Symptoms associated with the presence of the mixed infection phytoplasma - begomovirus in common bean in localities of Cuba. / Síntomas asociados a la presencia de infección mixta fitoplasma-BGYMV en frijol común en localidades de Cuba

Symptoms	Wilk´s Lambda	F	p	χ^2	p
mosaic	0.823	20.812	0.001	15.83	0.001
wrinkled leaves	0.940	6.191	0.015	4.99	0.025
reduction of the leaf size	0.865	15.139	0.001	11.92	0.001
yellowing	0.982	1.767	0.187	1.06	0.302
dwarfism	0.994	.610	0.437	0.303	0.582
chlorosis	0.886	12.468	0.001	9.506	0.002
Violaceous stems	1,000	.025	0.875	0.026	0.873
Deformed leaves	0.957	4.369	0.039	3.08	0.079
Dark-colored nerves	0.992	.798	0.374	0.808	0.369
spots	0.992	.798	0.374	0.808	0.369
thick stems	0.980	1.996	0.161	0.978	0.323
presence of vector	0.936	6.632	0.012	6.34	0.012
plant age	0.962	3.862	0.052	4.52	0.104

$p \leq 0.05$ indicate symptom associate with mixed infection.

allows a reduction of costs of sample analysis, since the visual identification of more severe symptoms presupposes the existence of multiple infections in a crop (19).

Our work agrees with Martínez *et. al.* (25) in that the management actions so far adopted are not enough to strengthen the management program of the white fly - begomovirus complex. We add the importance of looking at the relationships with other vectors like the leaf hoppers to avoid the proliferation of mixed infection.

The coexistence of insect vectors in the ecosystems affects with the proliferation of phytoplasma, begomovirus and tospovirus (26) in correspondence with multiple factors (27), which must be approached by projects for the management of populations in consequence with the natural enemies conservation, the selection of more resistant varieties, the rational use of water, the identification of the best sowing time, among other factors.

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