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Identification of fungal disease in a *Vanilla planifolia* Jacks plantation in the central zone of the state of Veracruz, Mexico

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ABSTRACT

The state of Veracruz is the main producer of vanilla in Mexico. In recent years, this crop has been seriously affected by severe phytosanitary problems caused mainly by fungal pathogens. For this reason, it was proposed to develop this study with the objective of determining the causal agents of the disease, incidence, and severity observed in a vanilla plantation located in the locality "El Palmar," Municipality Emiliano Zapata, Veracruz. A census sampling was carried out on one hectare of the plantation to determine the incidence of the disease. Disease severity per plant was determined using a 4-grade scale. Leaves and stems with disease symptoms were collected for morphological description of the pathogenic agents by scanning electron microscopy. The results showed the presence of two species of phytopathogenic fungi, *Fusarium oxysporum* f. sp. vanillae and *Puccinia sinanoemea*, known to cause root rot and rust diseases in vanilla. The infection resulted in the death of most individuals on the plantation. The incidence was classified as severe (a grade of 4), with 80% infestation by both pathogens. This study contributes to understanding the phytosanitary problems this crop faces due to mixed infections with fungal pathogens.

Keywords: phytopathogens, incidence, rust, *Fusarium*.

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INTRODUCTION

Vanilla (*Vanilla planifolia* Jacks.) is an orchid native to Mexico of great economic importance because vanillin, the second most expensive aromatic spice in the food industry after saffron, is extracted from pods of this plant (Anilkumar, 2004). Because of its great commercial value, both in the confectionery industry and in cosmetology, it is grown commercially in the "Totonacapan region" of the state of Veracruz, México, which accounts for 70% of national vanilla production (Hernández, 2011).

In recent years, this crop has suffered serious phytopathogenic effects. Among the main diseases that reduce production in this crop, there are the root rot disease caused by *Fusarium oxysporum* f. sp. vanilla and the basal rot caused by *Phytophtora* spp. Basal rot of stems and roots. These can affect vanilla production per unit area and even cause premature death of the plants (Santa-Cardona *et al*., 2018).

Other fungal pathogens that attack vanilla are anthracnose (*Colletotrichum* sp.) and rust (*Uromyces joffrini*) (Hernández, 2011). All these pathogens, especially those caused by *Fusarium* spp., are causing serious losses worldwide (FAOSTAT, 2017; Ramírez-Mosqueda *et al*., 2019). Although, in the state of Veracruz, Mexico, the greatest affectations caused mainly by *Fusarium* spp. have been reported, as the causal agent of basal rot (Santa-Cardona *et al*., 2018), affectations caused by other phytopathogenic fungi such as rust have recently been detected in vanilla-growing areas. However, there is not enough information on the effects of this disease on this valuable crop. For this reason, it was proposed to determine the causal agents of the disease, its incidence, and its severity on a commercial vanilla plantation located in the central zone of the state of Veracruz.

MATERIALS AND METHODS

A census-type sampling was carried out on one hectare of vanilla (*Vanilla planifolia* Jacks.) plantation established in the "Palmar region", Municipality of Emiliano Zapata, Veracruz. The studio area has average annual temperatures of 23.4 °C and average annual precipitation of 122.2 mm; the warmest months are from April to September with average maximum temperatures between 26-28 °C. The plantation was established for approximately 8 years under shade canopy conditions. Disease incidence was determined. For this purpose, a diseased plant showed symptoms of necrotic lesions on leaves and stems, typical of fungal diseases.

In a plantation established in the "Palmar region", Municipality of Emiliano Zapata, Veracruz, the incidence of the disease was determined. For this purpose, a plant was considered infected when it showed symptoms of necrotic lesions on leaves and stems, which are typical of fungal diseases.

Ten leaves with pustules were collected from each diseased plant and taken to the laboratory for fungal isolation, determination of the causal organism, and morphological description of the pathogen using the keys of the *Fusarium* Laboratory Manual (Leslie and Summerell, 2006). For Puccinia, the Keys from Uredinales (rusts) from Mexico: Puccinia were used (Gallegos and Baker 2018, Sandoval-Sánchez *et al*., 2020). Subsequently, using the scale proposed by Vidal-Martínez *et al*. (2011), the percentage of disease was determined for both phytopathogens based on the damage caused in each plant according to the census carried out; so, the degree of infection was determined for each plant. The infection was measured according to the following grades: grade 0: no presence of the disease; grade 1: 10% incidence; grade 3: 26-50% incidence; grade 4: >50% incidence (Figure 1). Using the methodology of Ortega-Centeno (2009), leaves with yellow-orange pustules were visually detected and collected.

Subsequently, infected leaves were selected and cut from the base; they were placed on absorbent paper in zip-lock bags to be transferred to the laboratory. Once the plants were collected, they were taken to the Institute of Biotechnology and Applied Ecology of the Universidad Veracruzana (INBIOTECA) laboratory.

To carry out the study of infections, the following methodology was used: with a sterilized toothpick, the leaves were infected by placing 1×10^6 spores as inoculum for infection of the fungi *Puccinea sinamonea* and *Fusarium oxysporum*; infected leaves were checked every day for five days. Young leaf cuts were placed on filter paper, sealed, and sprayed with distilled water to form a humid chamber. Four Petri dishes were placed at room temperature (22-24 °C), and other 4 Petri dishes were placed in an oven at 28 °C, the infected leaves were checked every day for five days. The small leaf cuts were made and placed on filter paper, sealed, and sprayed with distilled water to form a humid chamber.

To carry out microscopic studies of rust, samples of infected leaves were placed in a humid chamber with absorbent paper in an airtight bag. The samples were transferred to the laboratory to be processed the next day, and fragments of 1 cm^2 were cut from the areas with the presence of uredospores and processed with the conventional technique for scanning electron microscopy. The material was observed with a scanning electron microscope (FEI QUANTA 250 FEG) at the Advanced Microscopy Unit of the Institute of Ecology, A.C. In the case of *F. oxysporum*, samples were taken from the infected leaves stored in the humid chambers in a similar way as in the case of rust, and the samples were prepared for observation under the scanning electron microscope. The samples obtained were photographed with a JEOL model JSMIT300 scanning electron microscope.

RESULTS AND DISCUSSION

Two pathogens, *Fusarium oxysporum* f sp. vanillae and *Puccinea sinamonea*, were found as the almost destruction of the vanilla plantation in the locality of "El Palmar". The *Fusarium* observed symptoms were chlorotic rings on the stems and basal rot. (Figure 1A); in the case of rust, there were pustules on the leaves that were yellowish to reddish brown (Figure 1C). Of the total number of plants tested, 80% were found to be severely deteriorated with necrotic stems and leaves (Figure 1A), and 15% showed low levels of infection by both fungi with isolated spots or pustules without reaching necrosis on stems and leaves (Figure 1C). Five percent of the plants were healthy.

Regarding the incubation of the humid chambers, those at ambient temperature (22-24 °C) and those exposed to a temperature of 28 °C, both conditions resulted in the development of fungal infection; we found a more significant (about 10%) infection in the leaf segments. It was observed that the severity of these fungi increased exponentially due to the predominant environmental conditions of the site, characterized by abundant humidity and predominant temperatures between 28-30 °C. It has been proposed that the increase in temperatures is one of the factors that have most influenced the spread of diseases in plants since it impacts more significant quantities of fungal spores that increase the inoculum and give rise to more substantial infection, increasing the probable rate of genetic changes and thus a more rapid evolution of these pathogens (Hamada and Ghini 2011; SAGARPA 2012).

On the other hand, some predictions consider that there could be an increase of up to 1.5 °C by the middle of this century, increasing the probability of imbalance in all ecosystems, especially for Mexico; which, due to its geographical location and diversity of climatic and orographic conditions, could have severe problems for the cultivation of different species in our agricultural fields (Zamora-Martínez, 2015). The results of rust incidence and damage sampling revealed a 100% incidence, with disease incidence ranging from grade 2 to grade 4.

In the initial stages, yellowish-green leaves with slight disease scores on the underside (grade 2) were observed. Then the leaves turned yellowish, with an increase in the presence of yellow-orange pustules to brown pustules that later became necrotic (grade 4). Similar symptomatology was also observed by Álvarez-Morales and Salazar-Yepes (2014) in lemon grass (*Cymbopogon citratus* DC.) affected by rust. Samples placed in Petri dishes showed that no rust was present because no mycelium developed. In the case of *Fusarium oxysporum*., the symptoms were a yellowish-brown halo with cottony mycelium (Figure 1A).

F. oxysporum is a pathogenic fungus whose growth is characterized by growing by forming colonies of various colors (white, pale pink, red, orange, purple, light blue, and olive green) in a moderate to rapid manner (Jiménez-Quesada *et al.*, 2015). The results of microscopic observation showed that *Fusarium* has many septate hyphae, and *Fusarium conidia* were observed, as reported by Robles Yerena *et al.* (2017). In this study, *Fusarium hyphae* could be seen intermingled with rust hyphae (Figure 1B).

According to our microscopic observations, it was found that rust infection begins on the underside of the leaf, penetrating through the stomata. Then, infection spreads through the mesophyll until it colonizes the entire leaf tissue with hyphae, and subsequently hyphae exit through one or more stomata on the upper side of the leaf. Thus, the pathogen manages to completely expand uredospores to the outside and spread spores to infect other leaves (Figure 1D).

Microscopic studies showed that rust spores are strawberry-shaped with many regularly arranged dorsal and lateral spines. The results found demonstrate the presence of two pathogens that invaded the vanilla plantation under study. These were *F. oxysporum*, of

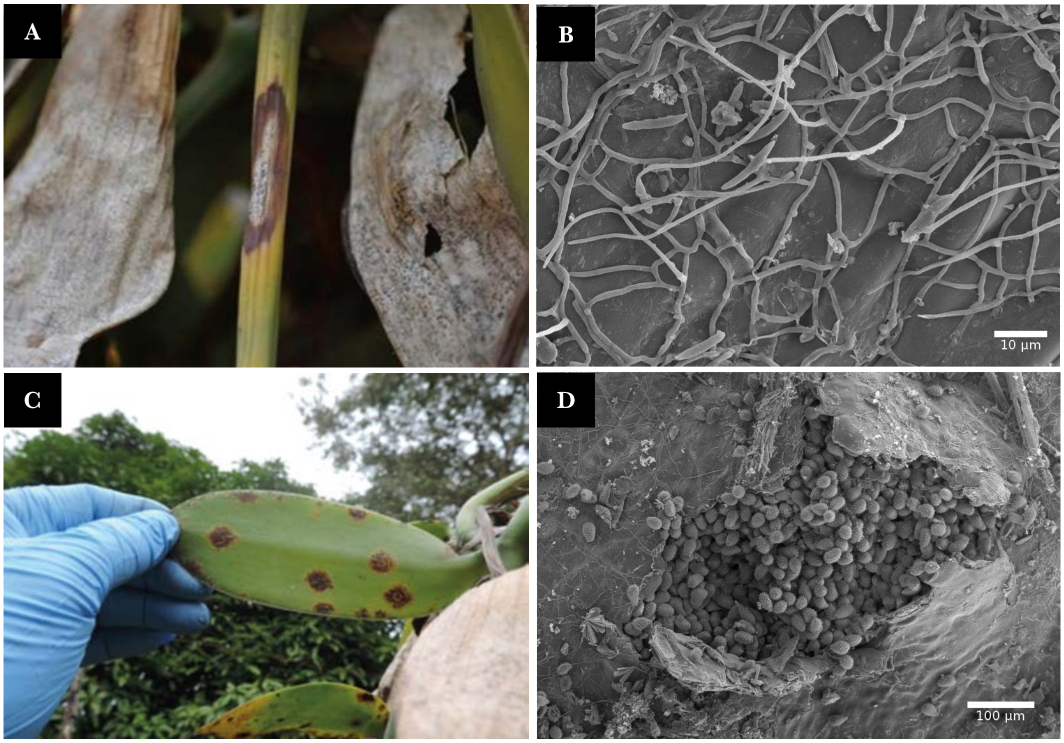

Figure 1. *Fusarium oxysporum* leaf and stem damage (A). Micrograph of hyphae and conidia of *Fusarium oxysporum* (B). Presence of rust pustules on the leaves of *Vanilla planifolia* (C). Micrograph showing the invasion of rust in the mesophyll and epidermis of vanilla leaves (D).

which the main symptom observed was the necrosis of the stems until separated from the roots. This fungus is the devastating causal agent causing stem and root rot in vanilla.

The symptoms of rust (*Puccinea sinamonea*) are yellowing of the leaves and the presence of orange pustules, mainly on the underside of the leaves. The presence of rust has also been observed on the new leaves of lemongrass (*Cymbopogon citratus* (DC.) (Alarcón, 2011). The results obtained in this study contribute to the phytopathological study of *Vanilla* as a crop, considering that to date there has been no adequate diagnosis and control of these pathogens in the *Vanilla* growing areas of Veracruz (Borbolla-Pérez *et al.*, 2016). It is expected to continue with the characterization of the rust strains observed in the field and to carry out the corresponding molecular studies.

CONCLUSIONS

The presence of rust and fusarium wilt in the vanilla area of the locality of Emiliano Zapata was confirmed. These pathogens were identified as *Puccinea sinamonea* and *Fusarium oxysporum*. These organisms are reported as devastating agents of the vanilla crop. Fungi incubated in humid chambers at 28 °C showed significant proliferation and greater infection damage in young vanilla leaves, coinciding with the predominant high humidity and temperature conditions in the fields from May to September. The highest infestation (80%) grade 4 observed in the field corresponds to plants with both fungal infestations.

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