

First aeromycological study in an avocado agroecosystem in Mexico

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Abstract Avocados (*Persea americana* Mill.) are economically and nutritionally valuable despite their susceptibility to several fungal diseases. This study was conducted in an agroecosystem of avocado trees in México. The air samples were collected every week by gravimetric methods at a height of 2 m from the ground level. Fungal colonies were isolated and transferred onto PDA and identified using morphological methods. During air sampling, different meteorological variables were measured. The symptoms of avocado diseases were determined by visual observations. To the best of our knowledge, this is the first study on aeromycological characterization in an avocado agroecosystem. Thirty-two airborne fungal genera were identified; *Fusarium* (97.2 %) and *Colletotrichum* (94.4 %) were the most common fungal pathogens present in the atmosphere of avocado. In addition, seven genera of important phytopathogenic

fungi of other crops (*Alternaria*, *Capnodium*, *Pestalotia*, *Stemphylium*, *Rhizopus*, *Curvularia*, and *Phyllachora*) were isolated. The maximum concentration of total fungi was observed in June (358 CFU m⁻³) and the minimum in September (83 CFU m⁻³). The total fungal concentrations were significantly negatively correlated with the temperature. The symptoms of five diseases of avocado, viz. anthracnose, scab, spot, canker trunk, and vascular wilt, were identified in the area study. It was observed that the symptoms of all the above-mentioned diseases were present in June; and in the same month, the fungal concentrations were highest.

Keywords Aerobiology · Avocado · *Colletotrichum* · *Fusarium* · Airborne fungi · Fungal diseases

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1 Introduction

Avocado (*Persea americana* Mill.) belongs to the family, Lauraceae. It is grown in the tropics and subtropics. Recently, the consumption of avocado fruit has increased significantly in the developed as well the developing world (Menzel and Le Lagadec 2014). These fruits carry important economical and nutritional values. Avocado is predominantly consumed as fresh fruits; however, different industrial products can be obtained from the avocado pulp. The worldwide

total avocado production is more than 3.5 million metric ton. Hass and Fuerte are two of the most important cultivars that have been cultured in different countries; Mexico is the largest producer of avocado (Bost et al. 2013; SIAP 2015).

The trees and fruits of avocado are susceptible to several fungal diseases. Avocado scab is caused by *Sphaceloma perseae* Jenkins. The symptoms present as lesions on the upper and lower surface of young leaves or irregular brown spots on fruits (Jenkins 1934; Ávila-Quezada et al. 2003). *Verticillium* species cause vascular wilt; recently, these species have been identified by phylogenetics and taxonomic studies (Inderbitzin et al. 2011). In affected trees, the leaves and branches appear brown for several weeks (Pérez-Jiménez 2008). In some cases, *Cercospora purpurea* Cooke or *Pseudocercospora purpurea* Cooke (Deighton) produces small angular spots on leaf margins, initially of brown color and later purple, and the lesions on fruit appear as spots surrounded by yellow halos (Deighton 1976; Pérez-Jiménez 2008). The most important diseases in avocado are caused by *Fusarium* spp. and *Colletotrichum* spp. *Fusarium* sp. (vector *Euwallacea fornicates*) has also been found to cause dieback in avocado in California (Eskalen et al. 2012). Earlier, in Mexico, *Fusarium oxysporum* Schltdl. and *Fusarium solani* (Mart.) Sacc. were isolated from avocado orchards in Michoacán State, and both species produced small dark spots with white powder on inoculated avocado seedlings (Ceja-Torres et al. 2000). On the other hand, the anthracnose disease, which is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc., *Colletotrichum acutatum* J.H. Simmonds, and *Colletotrichum boninense* Moriwaki, Toy. Sato and Tsukib., severely affects avocado production (Silva-Rojas and Ávila-Quezada 2011). Recently, a new causal agent, *Colletotricum godetiae* Neerg., has been identified, which affects the avocado fruits (Hernández-Lauzardo et al. 2015). The symptoms observed on the avocado fruits include brown-black spots in the exocarp and soft rot in the mesocarp, regardless of the *Colletotrichum* species isolated.

In general, propagules of several species of pathogenic fungi can spread through air and can be deposited on the surface of plants causing new infections (Vásquez-López et al. 2012; Cannon et al. 2012). Aerobiology explores the abundance, diversity, survival, and transport of microorganism in the

atmosphere; recently, an interest in their active roles has emerged (Morris et al. 2011). The knowledge of the scale of atmospheric dispersal of plant pathogens can assist growers in making vital decisions on plant disease management (Schmale III and Ross 2015). Several aeromycological studies have provided valuable information on crop protection such as rice (Almaguer et al. 2012), vineyard (Fernández-González et al. 2013), and wheat (Prussin et al. 2014), among other important crops that cause economic losses in agriculture worldwide. In this regard, it is important to understand the airborne fungi present in the avocado agroecosystems; however, to the best of our knowledge, there are no available reports on the characterization of airborne fungi in avocado. The aims of this study were: (1) to characterize the airborne mycobiota of an avocado agroecosystem; (2) to evaluate the influence of meteorological variables on fungal concentrations; and (3) to estimate the incidence of diseases in avocado orchards.

2 Materials and methods

2.1 Study area

The study was conducted in an avocado agroecosystem located in Ocuilco, Morelos, Mexico (18°52'16.10"N, 98°46'9.70"O) during the period from March (floration stage) to November 2014 (harvest stage). The Ocuilco climate is humid subtropical with a dry period (from November to May) and a rainy season (from June to October). The orchard under study has been growing avocado trees cv. Hass for the past 30 years (Fig. 1). The sampling site (1 ha with 240 trees) was selected taking into account five points (one point in the interior area and four at the edge of the field).

2.2 Air sampling and calculation of fungal concentrations

The samples were collected every week at 3:00 p.m. by gravimetric methods at a height of 2 m from the ground. To collect fungal propagules, four Petri plates containing potato dextrose agar (PDA) were exposed to air for 60 min at each sample point. The Petri plates were incubated at 30 ± 1 °C for 7 days, and fungal colonies growing on each Petri plates were counted. The colony-forming unit per cubic meter of air

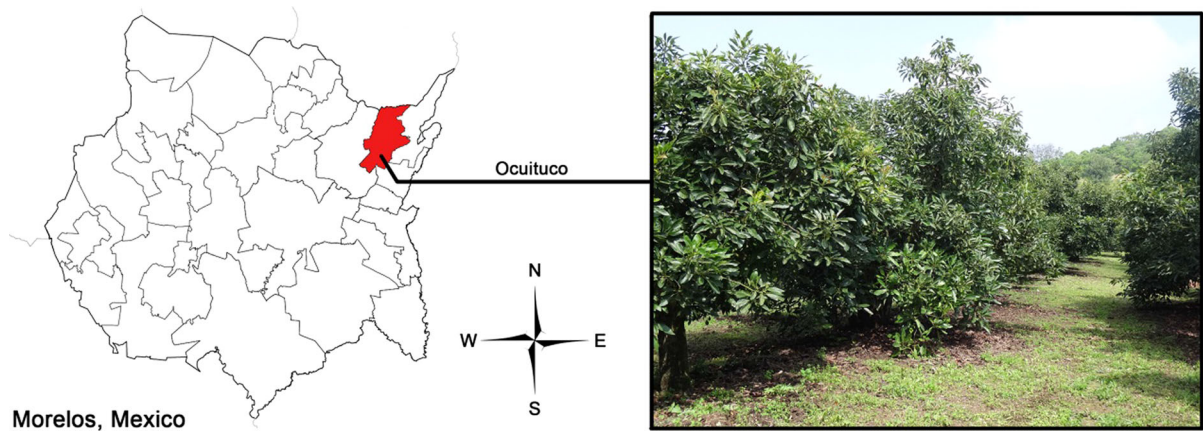


Fig. 1 Localization of the sampling area in Morelos, Mexico

(CFU m⁻³) was calculated using the following Omelyansky formula, according to Grbić et al. (2013):

$$N = 5a \times 10^4 (bt)^{-1}$$

where N is the fungal concentration (CFU m⁻³); a is the number of fungal colonies per Petri plate; b is the area of dish (cm²); and t is the period of exposure (min).

2.3 Identification and classification of isolates

The individual fungal colonies were transferred onto PDA. To identify the fungal genus, morphological characterization was carried out by taxonomic keys using the criteria of Barnett and Hunter (1998), Leslie and Summerell (2006), and Seifert et al. (2011). Fungal genera were classified according to the degree of pathogenicity as: avocado phytopathogenic fungi, important phytopathogenic fungi of other crops, and other fungi. Subsequently, relative frequency and relative density were calculated and expressed as percentages.

2.4 Meteorological measurements

During the air sampling day, the following meteorological variables were recorded by INIFAP Meteorological Station located in Ocuituco, Morelos (500 m from the study area): rainfall (mm), mean and maximum temperature (°C), solar radiation (W/m²), wind speed (km/h), and daily minimum and maximum relative humidity (%). Average values of the sampling

day were used to evaluate the correlations between fungal concentrations and meteorological variables.

2.5 Disease incidence

Any symptoms of avocado diseases such as anthracnose, scab, spot, canker trunk, and vascular wilt were determined by visual observations of the trees in the area of study according to Puente Raya (2011). Ten plants were randomly selected per week using a random sampling design. The incidence of disease was calculated as the percentage of symptomatic plants out of the total number of sampled plants (Kawube et al. 2015).

2.6 Statistical analysis

Fungal concentrations were analyzed by ANOVA (SigmaPlot 12.0, USA). The influence of meteorological variables on fungal concentrations was determined using Spearman's correlation test (IBM SPSS Statistics 23.0, USA).

3 Results

3.1 Aeromycological characterization

In this study, 32 airborne fungal genera were identified in the studied avocado agroecosystem. *Fusarium*, *Colletotrichum*, *Verticillium*, *Sphaceloma*, and *Cercospora* are the causal agents of important diseases of

avocado. In addition, seven genera of important phytopathogenic fungi of other crops (*Alternaria*, *Capnodium*, *Pestalotia*, *Stemphylium*, *Rhizopus*, *Curvularia*, and *Phyllachora*) and 20 other genera fungi (*Nigrospora*, *Penicillium*, *Ulocladium*, *Epicoccum*, *Aspergillus*, *Beauveria*, *Trichoderma*, *Drechslera*, *Paecilomyces*, *Monodictys*, *Chaetomium*, *Torula*, *Chrysosporium*, *Gilmaniella*, *Cladosporium*, *Trichocladium*, *Thysanophora*, *Periconia*, *Humicola*,

and *Oidiodendron*) were isolated. Figure 2 presents the relative frequency and relative density of the incidence of the fungi. *Fusarium* (97.2 %) and *Colletotrichum* (94.4 %) were the most common fungal pathogens present in the atmosphere of avocado; however, their density was found to be 7.9 and 5.4 %, respectively. On the other hand, *Alternaria* was found in all sampling (100 %), and it showed the highest values of relative density (40.7 %).

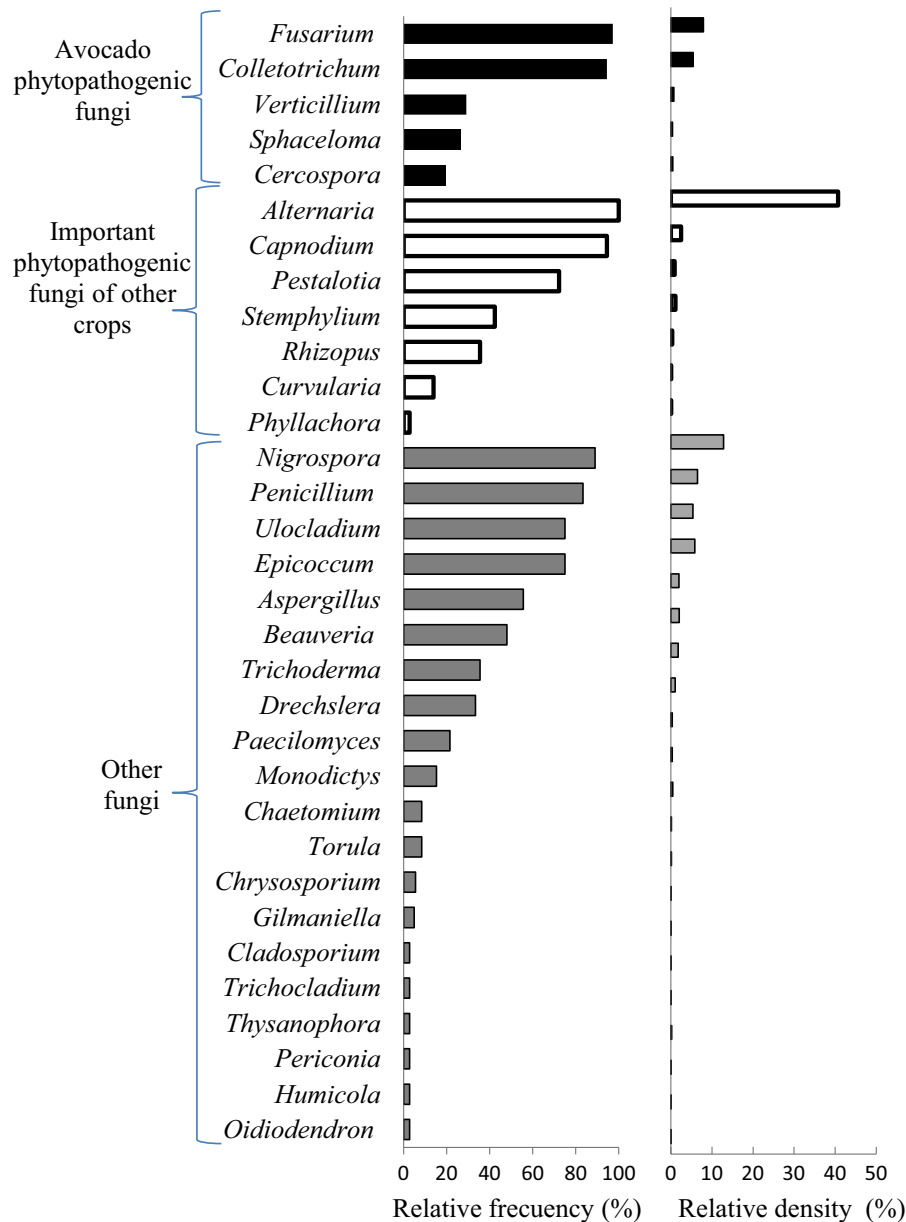


Fig. 2 Relative frequency and relative density of the fungi genera obtained at 2 m height of level ground from an avocado orchard in Ocuituco, Morelos

3.2 Concentration of airborne fungi

Figure 3 presents the variations in the concentration of total fungi, *Colletotrichum* spp. and *Fusarium* spp. obtained during the period of study. The maximum concentration of total fungi was observed in June (358 CFU m^{-3}) and the minimum in September (83 CFU m^{-3}). There was no statistically significant difference between the values obtained in May, July, August, October, and November. Similarly, there was no statistically significant difference in the concentration of total fungi in March, April, and September. *Colletotrichum* spp. and *Fusarium* spp. exhibited a similar behavior. In June, the above two fungal genera presented the highest level of concentrations, 18 and 23 CFU m^{-3} , respectively. In the case of *Colletotrichum* spp., there was no statistically significant difference in the values with respect to the other months, except for November. Similar results were obtained for *Fusarium* spp., except for October. In general, the highest count of fungal concentrations of *Colletotrichum* spp. and *Fusarium* spp. with respect to the total fungi was detected in June and the lowest in September. Other pathogenic fungi of avocado such as *Verticillium*, *Sphaceloma*, and *Cercospora* were found with a relative density $<1\%$, and there was no observed statistically significant difference in their concentrations between different periods of time.

3.3 Influence of the meteorological variables on fungal concentrations

The correlation between fungal concentrations and meteorological variables calculated using Spearman's correlation test is given in Table 1. This test revealed that the meteorological variables exert an influence on the concentration of the total fungi and in the genera studied except for *Cercospora* spp. Rainfall induced a variable effect on the concentration of total fungi (positive) and *Verticillium* (negative) propagules. Temperature was the most influential variable on the concentrations of airborne fungi. A significant negative correlation was observed between temperature and the fungal concentrations of *Colletotrichum*, *Fusarium*, *Sphaceloma*. On the contrary, a positive effect was observed on the concentration of propagules of *Verticillium*. In this study, solar radiation and wind speed showed a similar influence on fungal concentrations. Both the meteorological variables

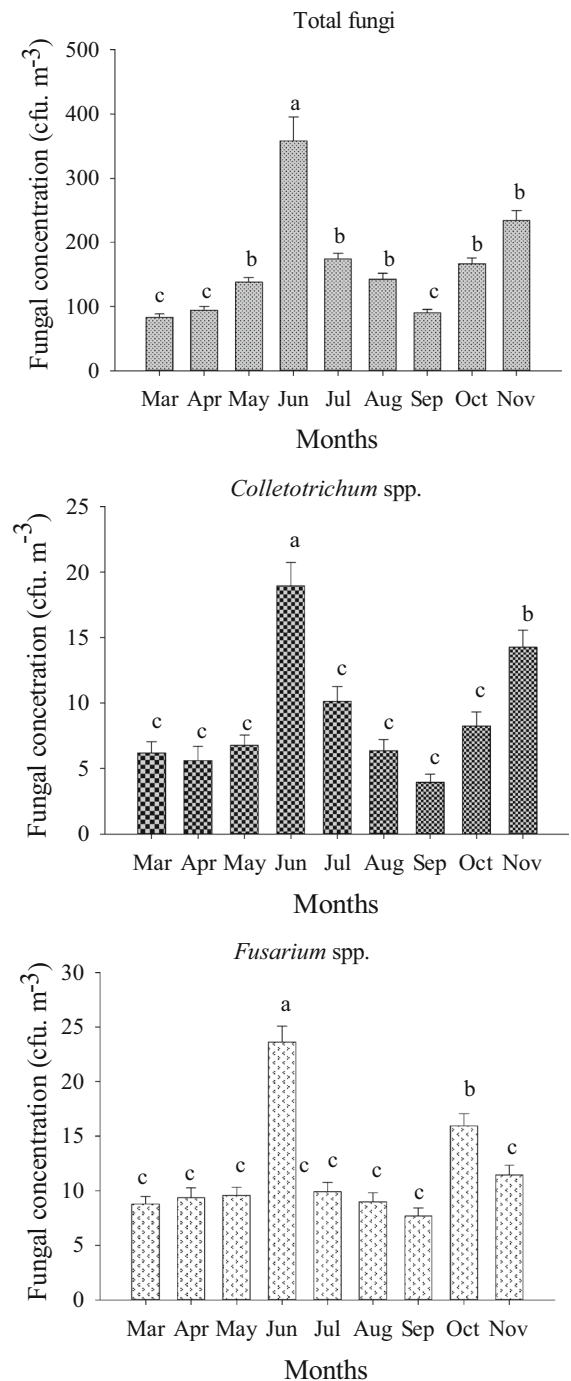


Fig. 3 Variation of the concentration of total fungi, *Colletotrichum* spp. and *Fusarium* spp. obtained at 2 m height during the study period

induced a significant negative effect on the concentrations of the total fungi and *Colletotrichum* and a positive influence on the concentration of airborne

Verticillium. A different pattern was observed for the effect of relative humidity. It did not have any effect on the fungal propagules of *Sphaceloma* and *Cercospora*, but showed a negative influence on *Verticillium*. However, the presence of fungal propagules of total fungi, *Colletotrichum*, and *Fusarium* was increased by the effect of high relative humidity.

3.4 Estimation of incidence of disease in avocado orchards

The symptomatic characterization of avocado diseases such as anthracnose, scab, spot, canker trunk, and vascular wilt was determined by visual observations in the study area (Fig. 4). The most important disease affecting avocado worldwide is the anthracnose. The symptoms of anthracnose disease were observed in fruits at the beginning as brown-black small spots on the fruit surface; later, the lesion increased and extended to other fruits (Fig. 4a–c). Another important disease of avocado is scab. The symptoms of scab were observed on the avocado leaves and fruits. The leaves showed small discrete lesions, and the fruits exhibited irregular-shaped brown spots that enlarged and coalesced to form rough surfaces (Fig. 4d–f). On the other hand, damage by leaf spot was observed in the avocado orchard as brown circular spots with a reddish border (Fig. 4g–i).

Canker trunk is an important disease that can cause death of trees. Initial symptoms of canker were observed in the trees and appeared as circular lesion with exudates and typical mycelium of *Fusarium*, while in other cases it was observed as tissue necrosis (Fig. 4j–l). In addition, symptoms of vascular wilt were observed in the study area. The affected trees

Fig. 4 Symptoms of avocado diseases in the studied orchard located in Ocuilco, Morelos, Mexico. **a–c** Anthracnose; **d–f** scab; **g–i** spot; **j–l** canker trunk; **m–o** vascular wilt

showed wilt on branches or on the whole tree (Fig. 4m–o). By taking into account the overall symptoms of the diseases described above, the incidence of the diseases was estimated, and the results are shown in Fig. 5. In March, a low incidence of the diseases was observed, as only spot leaf was found. In April, an increase in spot leaf was observed (30 %), while anthracnose (5 %) and scab (5 %) started to appear in the avocado orchard. In May, anthracnose and scab started to spread (32.5 %), and leaf spot was detected. June was the only month that recorded all the types of diseases studied: anthracnose (65 %), scab (40 %), leaf spot (12.5 %), canker trunk (5 %), and vascular wilt (5 %). In July, an increase in the anthracnose disease was observed (72.5 %) followed by scab, leaf spot, and canker trunk. The month of August showed a similar behavior to that in July. In September, the symptoms of only three diseases were observed: scab, anthracnose, and vascular wilt. October and November showed a similar pattern wherein only scab and anthracnose were detected. In October, the highest incidence was of scab (75 %); November recorded higher incidence of anthracnose (30 %) than does scab.

4 Discussion

Aerobiological research is important to understand the pattern of spread of fungal genera by air, their spatial

Table 1 Correlation between the fungal concentrations and meteorological variables using Spearman's correlation test with two levels statistical significance ($p \leq 0.01^{**}$ and $p \leq 0.05^{*}$)

Meteorological variables	Total fungi Fungal concentrations (ufc m ⁻³)	<i>Colletotrichum</i>	<i>Fusarium</i>	<i>Sphaceloma</i>	<i>Cercospora</i>	<i>Verticillium</i>
Rainfall (mm)	0.075*	0.033	0.025	−0.030	−0.025	−0.078*
Mean temperature (°C)	−0.266**	−0.168**	−0.095*	−0.086*	−0.034	0.088*
Maximum temperature (°C)	−0.271**	−0.157**	−0.099**	−0.086*	−0.026	0.098**
Solar radiation (W/m ²)	−0.217**	−0.094*	−0.027	−0.062	0.007	0.114**
Wind speed (km/h)	−0.140**	−0.096**	−0.056	−0.011	−0.005	0.082*
Mean RH (%)	0.195**	0.085*	0.071	0.027	0.007	−0.098**
Minimum RH (%)	0.207**	0.106**	0.090*	0.035	0.007	−0.099**
Maximum RH (%)	0.192**	0.070	0.044	0.000	0.001	−0.120**

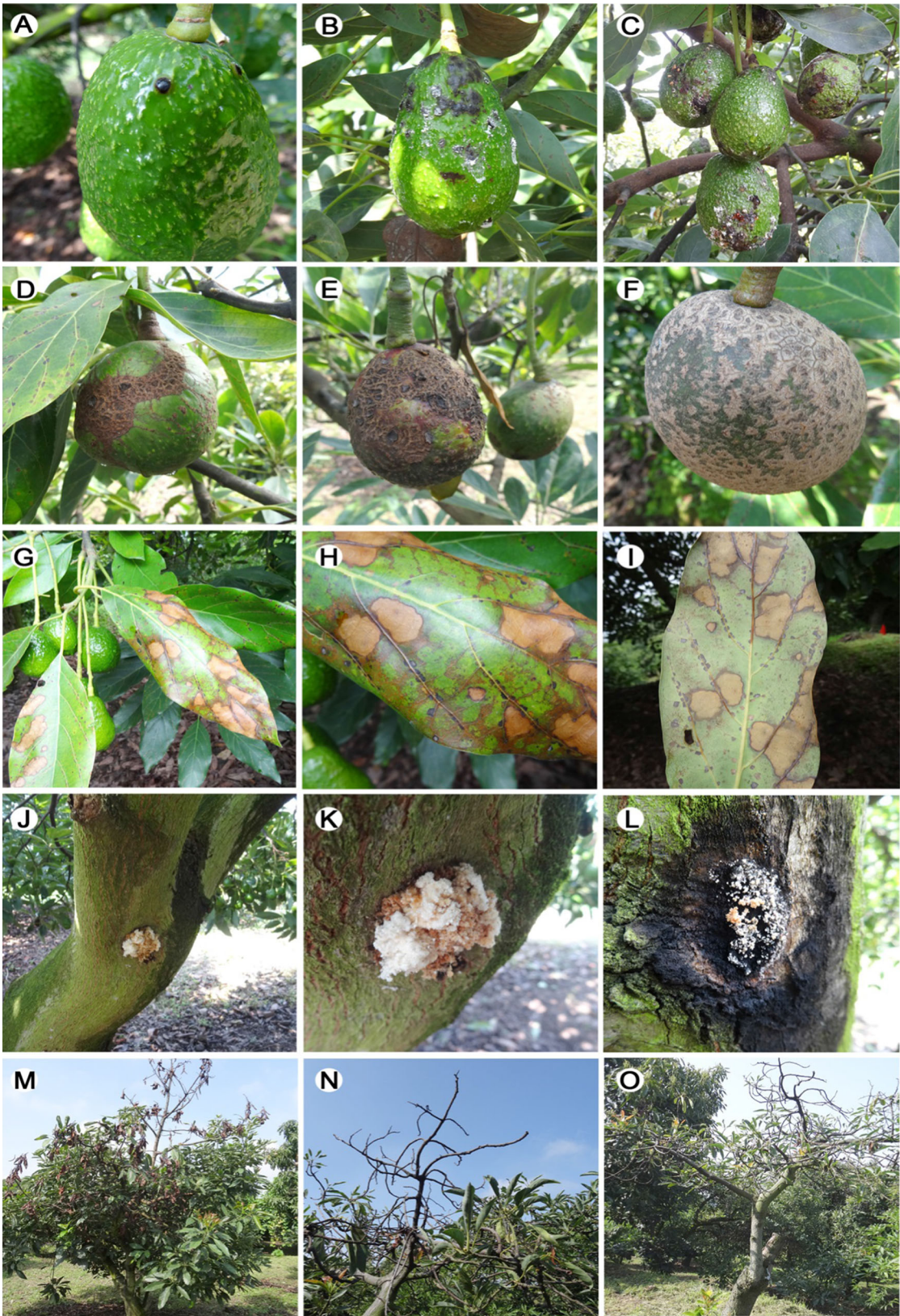
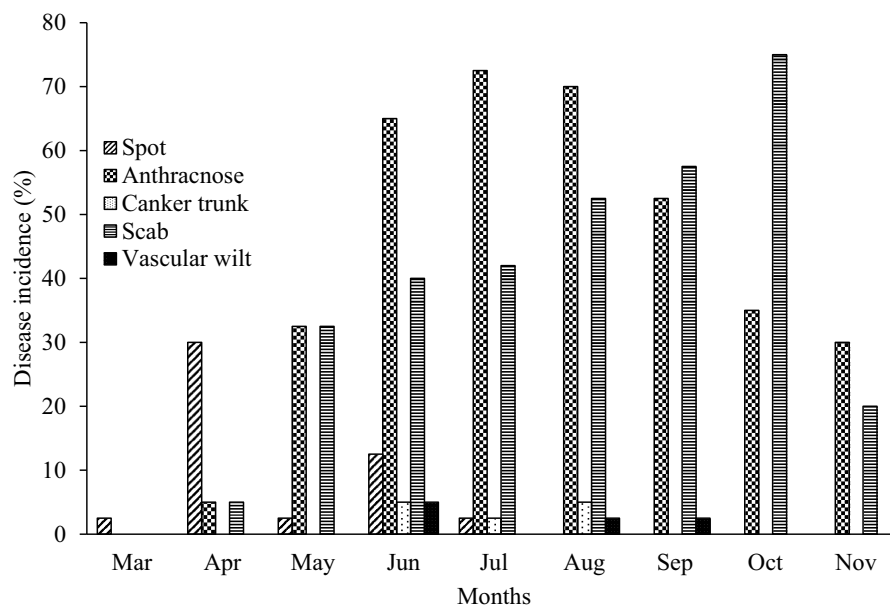


Fig. 5 Disease incidence: spot, anthracnose, canker trunk, scab, and vascular wilt in the avocado orchard studied. Plants were randomly selected weekly using a diagonal sampling design



and temporal distribution, and to derive strategies for integrated pest management (IPM) to control fungal diseases. To the best of our knowledge, this is the first study on aeromycological characterization in an avocado agroecosystem. In this work, we identified 32 airborne fungal genera in the sampling area. Five of them are causal agents of important diseases of avocado, and seven belong to important pathogenic fungi of other crops. Similar results were reported in a rice field in Cuba wherein the authors found 39 fungal genera including six important pathogenic fungi of rice (Almaguer et al. 2012). *Fusarium* spp. cause diseases in several crops, and their spores could be transported through the atmosphere over long distances (1–100 m) above ground level (Binbin et al. 2014). Therefore, it is important to identify this genus in the avocado atmosphere to predict the damage in this crop. In this study, *Fusarium* spp. was detected with a high relative frequency (97.2 %), significant aspect for to suggest management strategies of this phytopathogenic fungus. A recent work reveals some epidemiological aspects of *Fusarium* genus in natural environments and demonstrated the pathogenicity of different *Fusarium* spp. obtained from rainfall water on four plant species (Rodríguez Zafra et al. 2015). On the other hand, *Colletotrichum* spp. showed a 94.4 % relative frequency in this sampling area. In previous studies, spores of *Colletotrichum* spp. have been found

in the atmosphere of a mango orchard affected with anthracnose (Huerta-Palacios et al. 2009). In the case of other phytopathogenic fungi reported in this study (*Verticillium*, *Sphaceloma*, and *Cercospora*), there are reports of their presence in the atmosphere of other agroecosystems (Uddin 2005; Marroquín-Pimentel 1999; Almaguer-Chávez et al. 2012). It is noteworthy that *Alternaria* was found in all sampling (100 %) and showed the highest value of relative density (40.7 %). This finding is in agreement with the results obtained by other authors who reported that *Alternaria* genus is one of the most abundant in the air under different bioclimatic conditions (Rodríguez-Rajo et al. 2005; Aira et al. 2013). Consequently, the widespread presence of *Alternaria* spores has been related to their pigmentation that confers resistance to ultraviolet radiation (El-Morsy 2006).

In this study, the highest concentration of propagules of fungi was found in June (358 CFU m⁻³) for total fungi. Previous investigations, carried out by gravimetric methods in ecosystems of plants with different climatic conditions, reported higher concentrations of spores during warmer months such as May and June (Pepeljnjak and Šegvić 2003). However, few recent studies demonstrated that the number of total viable colonies depends on the culture media and conditions used (Fernández-Rodríguez et al. 2014). Other important aspect, which increased the efficiency

in the aerobiological sampling, was that the culture media in Petri plates at maximum volume collected 56 % more spores than that at half the volume (Keller and Shields 2014). In summary, in this work different meteorological variables have been found to influence the concentrations of the fungal spores collected. Temperature exhibited a statistically significant negative correlation with the total fungal concentrations and that of the genera, *Colletotrichum*, *Fusarium*, and *Sphaceloma*. On the contrary, some studies carried out in other crops such as rice and wheat demonstrated that temperature has a positive influence on the fungal concentrations (Almaguer et al. 2012; Cao et al. 2012). However, our results are in line with those obtained in a tropical rainforest, where cooler temperatures and high relative humidity stimulated the spatial and temporal distribution patterns of fungal spores (Gilbert and Reynolds 2005). We believe that the established microclimate in the forestry systems encourages the dispersion and viability of airborne fungal spores at lower temperatures and higher relative humidity.

Fungal propagules that spread through air cause several diseases that can be identified by the presence of symptoms on the plants. In this study, we observed different avocado diseases, viz. anthracnose, scab, spot, canker trunk, and vascular wilt through visual observations in the study area. During the first sampling (March), only *Cercospora* spot was found, while other diseases appeared subsequently. It is noticeable that June was the only month that showed symptoms of all diseases mentioned above and also presented the highest fungal concentrations. Therefore, there was a positive correlation between fungal concentrations and the symptoms of diseases observed on the avocado orchard. Similar results were reported in the dynamics in concentrations of *Blumeria graminis* f. sp. *tritici* conidia in wheat; the weekly accumulated conidia per cubic meter of air significantly were correlated with disease index (Cao et al. 2012). The pathogenicity of airborne fungal spores has been demonstrated by Schmale III et al. (2012), who showed that isolates of *Fusarium* collected from the air caused *Fusarium* head blight in wheat. These studies are of significance because they could contribute to the better understanding of the airborne fungal community structure and to predict the risk of infection of crops with respect to the weather conditions and plant diseases.

5 Conclusions

This work was focused on aeromycological characterization in an avocado agroecosystem. *Fusarium* spp. and *Colletotrichum* spp. were the most common fungal phytopathogens found in the avocado atmosphere. It was observed the incidence of canker trunk, and anthracnose was present in the same month that these fungal concentrations were highest. This study is significant since it could contribute to the better understanding of the structure of airborne fungal community and to predict the risk of infection of the avocado crop.

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