Microbial and size characterization of airborne particulate matter collected on sticky tapes along US–Mexico border

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ARTICLE INFO

Article history:
Received 23 May 2015
Revised 10 October 2015
Accepted 26 October 2015
Available online 3 June 2016

Keywords:
Fungal spores
Sticky tape
Particle size
Microorganism
Dust

ABSTRACT

Particulate matter (PM) emissions from various sources can affect significantly human health and environmental quality especially in the Chihuahuan Desert region along US–Mexico border. The objective of this study was to use the low-cost sticky tape method to collect airborne PM for size characterization and identification of fungal spores. Sticky tape samplers were placed at 1.0 and 2.0 m above the ground surface at experimental sites in Ciudad Juárez, Mexico and at 0.6, 1.2 and 1.8 m at New Mexico sites, USA. Soil samples were collected in both countries to determine fungal diversity, texture and moisture content. Dust particles collected from all of the experimental sites had a dominant texture of clay (<0.002 mm). The dominant textures identified from soil samples collected from the US and Mexican sites were loam and sandy clay loam, respectively. Alternaria, Penicillium and Fusarium were frequently found funguses in the US sites while Alternaria and Aspergillus were commonly observed in the Mexican sites. The sticky tapes also showed a similar diversity of fungal microorganisms present in the airborne PM at both Mexico and US sites. Alternaria, Penicillium and Aspergillus were the three groups of airborne fungal microorganisms consistently present in the US and Mexican sites. The low-cost sticky tape method has the potential to be used for characterizing different airborne microorganisms and dust particles.

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Introduction

The components of particulate matter (PM) emitted during dust storms and wind erosion events are inorganic particles consisting of the mineral fraction of the soil, chemicals present in the soil, and organic materials. The organic components of the PM may include microorganisms such as bacteria, viruses and fungi that can be transported over long distances (Després et al., 2012; Waisel et al., 2008). Previous studies have reported the transport of bacteria and fungi from the eastern hemisphere to the western hemisphere (Kellogg et al., 2004; Prospero et al., 2005; Smith et al., 2012). The atmospheric transport over long distances of some microorganisms is attributed to their capacity of forming spores and resisting desiccation (Kellogg et al., 2004; Al-Subai, 2002).

Large masses of fungal spores released into the air represent an important component of bio-aerosols and are considered elements of atmospheric contamination around...
the world. They constitute a significant fraction of bioparticles in air and can have implications for human health by causing allergies and a variety of diseases (Li and Kendrick, 1994; Abu-Dieyeh et al., 2010). However, not much is known on the relationship between fungal spores and their effects on health. Also, the knowledge of types of fungal species and their relative frequencies under different scenarios is important for understanding the exposure to the population (Shelton et al., 2002).

Human exposure to fine dust particles and airborne microorganisms is not uncommon in arid regions where fine soil particles are suspended in the air as result of strong winds over soils with low moisture content (Al-Dabbas et al., 2011; Griffin and Kellogg, 2004). Cornelis and Gabriels (2003) and Madden et al. (2010) reported that the emission of PM is negatively correlated with soil moisture content after conducting experiments under laboratory conditions using a closed blowing-type wind tunnel and mechanical laboratory dust generator, respectively. Similar results were reported by Funk et al. (2008) after conducting laboratory and field experiments. Vehicular movement through unpaved roads contributes to PM emissions in arid regions (Flores-Márgez et al., 2011; Gillies et al., 2005; Pinnick et al., 1985; Williams et al., 2008). PM emissions are produced from agricultural fields as a result of particles ≤32 μm in diameter that are transported primarily by suspension (Sharratt, 2011; Shukla and Flores-Márgez, 2014).

Agricultural activities such as tillage and harvesting are sources of fungal spores that are transported from agricultural fields to adjacent rural and urban areas. Lee et al. (2006) evaluated the exposure to dust and bioaerosols collected on farms and reported that farmers were exposed to fungal spores during the harvest. Awad (2005) evaluated the role of vegetation as a source of airborne fungi and identified the common airborne fungal genera in rural and urban areas of Egypt. Alternaria, Aspergillus, Penicillium and Cladosporium were the dominant fungal genera found at all sampling sites, however Alternaria was dominant in cultivated areas while Aspergillus, Penicillium and Cladosporium were frequently detected in urban areas (Awad, 2005). Cladosporium, Penicillium, Aspergillus and Alternaria were the dominant airborne fungal genera in a study conducted by Medrela-Kuder (2003) which evaluated the seasonal variation in the occurrence of airborne fungi in outdoor and indoor environments in Poland. Medrela-Kuder (2003) reported that Cladosporium was the dominant fungus in air samples collected indoor and outdoor during summer, while Penicillium and Aspergillus were the dominant funguses in both test sites during winter. Shelton et al. (2002) reported that Cladosporium, Penicillium and Aspergillus were the most common culturable airborne fungi found both indoors and outdoors during all seasons and regions of the United States.

There is a vital need to quantify the inorganic, organic, chemical, and biological components of airborne PM because of their potential to adversely impact human health. Low-cost methods to measure organic, inorganic and microbial components of airborne PM are needed to monitor particle emissions. The combination of sticky tape samplers and rotorods is a low-cost method previously used to characterize only PM from unpaved roads (Williams et al., 2008). The objective of this study was to identify different types of fungi in the soil and in the PM collected on sticky tapes in the Chihuahuan Desert region along US–Mexico border. The data generated can be used to calibrate air quality models for forecasting the concentration of airborne fungal spores during dust storms.

1. Materials and methods

1.1. Experimental sites

Experimental sites are located in New Mexico, USA and Ciudad Juárez, Mexico along the USA–Mexico border. Experiments were conducted from January to September 2011 at four of the experimental sites, Anthony, Leyendecker Plant Science Research Center south of Las Cruces, Deming and Columbus, USA. Experiments were also conducted at three experimental sites, site 1 (Juárez 1), site 2 (Juárez 2) and site 3 (Juárez 3) in Ciudad Juárez, Mexico from October 2012 to April 2013. Juárez 1 and Juárez 2 are separated by 12 km while there is a distance of 4 km between Juárez 2 and Juárez 3 moving from north to southeast. Experimental sites in New Mexico were located in rural areas with unpaved roads, while experimental sites in Ciudad Juárez were located in urban areas with paved (Juárez 1) and unpaved (Juárez 2 and Juárez 3) roads. All the experimental sites were located in populated areas in the Chihuahuan Desert region along the US–Mexico border. Therefore, the microbial and size characterization of airborne PM from these sites is important for assessing impacts on the health of the people living in these areas in both countries.

1.2. PM and soil sampling

The low-cost sticky tape method (Williams et al., 2008) with rotorods was used to measure PM from unpaved roads at each experimental site. Double-sided sticky tapes (STR tape 0.076 mm thick; Shinto Paint Company Ltd.) and rotorods of a constant speed motor, U-rods (Sampling Technologies Inc., 1989) were combined to develop the sticky tape method. The sticky tape method consists of two rotorods installed on a steel tower of 1-inch in diameter. One of the rotorods was placed at 1.0 m and another at 2.0 m height above the ground surface in the experimental sites of Ciudad Juárez, while rotorods were placed above the ground surface at 0.6, 1.2 and 1.8 m in experimental sites of New Mexico. Each rotorod had two wings, and on each wing a transparent microscope glass slide (Microscopes Plus Ltd., Hertfordshire, UK) and a double-sided sticky tape were attached (Fig. 1).

Before conducting the dust monitoring, each glass slide was weighed using an analytical balance (OHAUS Adventure Pro, Parsippany, NJ, USA) with precision of four decimal points. The glass slides were stored in a box (HS15989RF, Fisher Durable slidebox) for microscope glass to avoid dust contamination before and after their use. A dry pre-labeled glass slide with sticky tape was placed at each wing of the rotorod. The rotorods were attached to a 9 V battery installed on the tower. Before turning on the rotorod, the adhesive tape was carefully peeled off and stored in a clean plastic Ziploc
Fig. 1 – Rotorod with double-sided sticky tape on two transparent glass slides.

Table 1 – Soil particle size distribution and soil moisture content for each experimental site.

<table>
<thead>
<tr>
<th>Country</th>
<th>Experiment site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Clay</th>
<th>Silt</th>
<th>Sand</th>
<th>Soil moisture (%)</th>
<th>Soil texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>Anthony</td>
<td>32°17'00&quot;</td>
<td>106°45'24&quot;</td>
<td>1.39</td>
<td>2.85</td>
<td>95.95</td>
<td>0.22</td>
<td>S</td>
</tr>
<tr>
<td>USA</td>
<td>Columbus</td>
<td>31°50'01&quot;</td>
<td>107°37'11&quot;</td>
<td>17.2</td>
<td>31.95</td>
<td>50.85</td>
<td>1.55</td>
<td>L</td>
</tr>
<tr>
<td>USA</td>
<td>Deming</td>
<td>32°11'59&quot;</td>
<td>107°45'46&quot;</td>
<td>14.02</td>
<td>36.25</td>
<td>49.73</td>
<td>2.07</td>
<td>L</td>
</tr>
<tr>
<td>USA</td>
<td>Leyendecker</td>
<td>32°11'59&quot;</td>
<td>106°44'14&quot;</td>
<td>16.94</td>
<td>21.93</td>
<td>61.12</td>
<td>0.47</td>
<td>SL</td>
</tr>
<tr>
<td>Mexico</td>
<td>Juárez 1</td>
<td>31°39'17&quot;</td>
<td>106°21'33&quot;</td>
<td>8.62</td>
<td>0.76</td>
<td>90.62</td>
<td>1.90</td>
<td>S</td>
</tr>
<tr>
<td>Mexico</td>
<td>Juárez 2</td>
<td>31°37'40&quot;</td>
<td>106°20'33&quot;</td>
<td>26.62</td>
<td>6.76</td>
<td>66.62</td>
<td>7.33</td>
<td>SCL</td>
</tr>
<tr>
<td>Mexico</td>
<td>Juárez 3</td>
<td>31°36'01&quot;</td>
<td>106°24'29&quot;</td>
<td>32.62</td>
<td>8.76</td>
<td>58.62</td>
<td>6.66</td>
<td>SCL</td>
</tr>
</tbody>
</table>

Abbreviation of soil texture: S, Sand; L, Loam; SL, Sandy loam; SCL, Sandy clay loam.
to remove bubbles, scratches and smears from the image. Visible overlaps of particles were manually corrected. Once all particles were within the threshold range, each image was processed into binary image. The particle area, perimeter, and limit to threshold were checked using ImageJ’s particle analyzer tool and the area was calculated as the number of pixels forming the 8-neighbor connected particle (Williams et al., 2008). The pixel range varied from 0 to infinity to account for all particle sizes. The SPSS 19.0 software was used to conduct the analysis of variance of the percentage of particles per mm² with different sizes and textures retained on sticky tapes at each of the seven experimental sites.

1.4. Fungal analysis of soil samples

The standard serial dilution method was used for identifying fungal microorganisms (Fernández-Linares et al., 2006). Ten grams of soil was poured into a 250 mL flask containing 100 mL of distilled water. The flask was placed on a stir plate (Thermo Scientific, USA) and mixed at medium speed for 3 min. Four test tubes were obtained, and 9 mL of distilled water was dispensed in each of them. These tubes were labeled as $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ to indicate dilutions. One milliliter of solution from the flask was taken and added to the tube labeled “$10^{-2}$” and thoroughly mixed. Then, 1 mL of solution from this tube was added to the tube labeled as “$10^{-3}$”, and so on until all four dilutions were created. One milliliter of each dilution was pipetted onto an Acidified Potato Dextrose Agar (APDA) and was spread over the surface using a glass rod. Each plate was labeled with appropriate dilution, treatment, and date. Three plates per dilution level were incubated at room temperature (20–24°C), and on the 10th day the fungal colonies were counted. To compute the number of microorganisms, the number of colonies (NC) was counted on all plates at each dilution level, and an average number of colonies (ANC) were obtained as the ratio of NC and the number of plates used. The ANC was multiplied by the dilution factor to obtain the NC per gram of soil. The average of colonies of different fungal microorganisms observed in the four dilutions was used to calculate the frequency of each of the fungal genera.

1.5. Fungal analysis of sticky tapes

Similar to the fungal analysis for soil, the standard serial dilution method and APDA media were used to grow fungal microorganisms on sticky tapes. To analyze fungal PM on the sticky tapes, the dust-coated sticky tapes were cut into small pieces (1.0 cm²) and, four pieces per plate were placed onto APDA media, and then spread over the surface with a glass rod. Plates were tagged with appropriate dilution, treatment, and date. All plates were incubated at room temperature (20–24°C), and fungal colonies were counted from 10 to 20 days.

Fig. 2 – Average PM concentrations at different heights in (a) Leyendecker, (b) Columbus, (c) Deming and (d) Anthony in New Mexico, USA. Bars are standard errors. PM: particulate matter.
The ANC and frequency of fungal genera from dust particles retained on sticky tapes were calculated using the procedure for the fungal analysis of soil samples.

Portions of 5 mm diameter from the colonies were collected and stained with lactophenol cotton blue to examine the morphology of fungi in soil samples and sticky tapes. A microscope (Microscopes Plus Ltd., Hertfordshire, UK) was used to observe the color, mycelium and presence of septa at 40× magnification. The size, color, grouping, unicellular/multicellular morphology of spores and connection of spores to the hyphae were also considered for the identification of the fungi. Macroscopic and microscopic characteristics of fungal microorganisms and standard taxonomic texts were used to identify each of the fungal microorganisms (Navi et al., 1999; Serrato-Diaz and French, 2010).

2. Results and discussion

2.1. Particle matter concentrations in sticky tapes

Generally, total PM concentrations decreased with increasing elevation at each experimental site in New Mexico (Fig. 2). Flores-Márgez et al. (2014) and Williams et al. (2008) also reported that the height and width of PM plume increased with increasing speed of vehicles but concentrations decreased with elevation on unpaved roads. The decrease in PM concentration with increasing height could be explained by the deposition effect (Li and Bai, 2014). PM concentrations were consistently higher at 0.6 m above the ground surface compared with other elevations. The highest PM concentrations at 0.6 and 1.2 m above the ground surface were obtained for the Columbus site while the lowest PM concentrations were measured in Anthony. High PM concentrations at 0.6 m were in agreement with the percentage of clay content in Columbus (17.20%), Leyendecker (16.94%), Deming (14.02%) and Anthony (1.39%). PM concentrations also decreased with decreasing clay content of soil. Funk et al. (2008) reported that
the PM emission increases with increasing silt and clay content of air-dried soils but decreases with increasing soil moisture contents.

PM concentrations also decreased with elevation above the ground surface in two of the experimental sites of Ciudad Juárez (Juárez 1 and 2), however, PM concentrations were similar at both elevations in Juárez 3 site (Fig. 3). PM concentrations at 2.0 m of elevation were the lowest from Juárez 2 site compared with Juárez 1 and Juárez 3. These observations were in agreement with wind speeds in Juárez 3 (37 km/hr) and Juárez 1 (38 km/hr) which were greater than that measured in Juarez 2 (33 km/hr) during the experiments.

2.2. Particle size analysis using electron microscope

In general, the size of particles retained on the sticky tapes ranged from silt to clay among all sites. Sticky tapes mostly retained clay sized particles and particles retained were always lower than 0.05 mm size. Particle size distributions on sticky tapes were classified as clay (<0.002 mm), very fine silt (0.002 to 0.005 mm) and silt (0.005 to 0.05 mm), and their proportions ranged from 85% ± 7%, 11% ± 6%, and 3.8% ± 2%, respectively (Fig. 4a). The percentage of particles less than 2.5 μm (PM2.5) was greater compared to the percentage of particles greater than PM 2.5 retained on the sticky tapes (Fig. 4b). The lower percentage of particles of size between 200 and 2.5 μm on the sticky tapes was due to the faster deposition of larger sized particles and low suspension height not enough to reach the sticky tapes (Li and Bai, 2014; Williams et al., 2008). The analysis of variance showed that the percentage values for each particle size distribution class was not significantly different among experimental sites, however, the PM2.5 values were significantly different among some sites (Table 2).

Particle size analysis using the electron microscope indicated that the highest count of PM2.5 was obtained in Juárez 2. PM2.5 values greater than 5% particles per mm² were obtained in Juárez 1, Juárez 2, Juárez 3, Deming, Columbus and Leyendecker sites (Fig. 4b). The highest count of PM2.5 particles in Juárez 2 was in agreement with the higher clay content of the soil than the Juárez 1 and all the experimental sites of New Mexico, except Juárez 3. The lowest count of PM2.5 particles was observed in Anthony where the sand content of the soil was greater than 90%.

2.3. Fungal analysis of soil and PM samples on sticky tapes from USA

Several different fungal microorganisms were isolated in soil samples collected from each site. Representative fungal microorganisms are shown in Fig. 5 in pure culture on growth media APDA in Petri plates. Fungal microorganisms identified to genus or species level and isolated at high frequency (>10%) in soils of southern New Mexico included Alternaria alternata, species of Penicillium, Rhodotula minuta, Fusarium oxysporum, Fusarium brachygibbosum, species of Rhizopus, Rhizopus microsporus, and Sordaria fimicola (Fig. 6). Alternaria, Aspergillus, Cladosporium, and Penicillium are common fungal microorganisms in the air around the borderland from El Paso, TX, USA to Ciudad Juárez, Chihuahua, MX (Olivas et al., 1993). Of the

![Image](image_url)

**Fig. 5** – A sample of the fungal population diversity from soil samples (a) top view and (b) bottom view. 1. *Fusarium*, 2. *Penicillium*, and 3. *Alternaria*.

| Table 2 – Analysis of variance of percentage of particles per mm² of different textures retained on sticky tapes at seven experimental sites. |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Source of variation | Degrees of freedom | Clay | Very fine silt | Silt | PM2.5 | PM10 | PM200 |
| Experimental sites | 6 | 0.565 * | 0.556 | 0.591 | 0.000 ** | 0.470 | 0.401 |

Clay: <0.002 mm; very fine silt: 0.002–0.005 mm; silt: 0.005 to 0.05 mm; PM2.5; PM10: particles between the size of 2.5 and 10 μm; PM200: particles between the size of 200 and 10 μm.

* P = P (F > Fo); no statistical significance;

** p-Value indicates a significance at 0.001 level.
fungal microorganisms isolated in this study, many have been reported to be associated with diseases on agricultural crops and human health. For example, *Alternaria alternata* is a known pathogen causing leaf spot disease on more than 100 host plant species, and is also opportunistic on many other host plant species. Additionally, *Alternaria alternata* is associated with allergic diseases including asthma. *Fusarium oxysporum* is a plant pathogen responsible for causing vascular wilts on horticultural crops and it also causes a broad spectrum of infections in human (Nelson et al., 1994). *Penicillus* is a known allergen existing in different regions of the world (Abu-Dieyeh et al., 2010). Species of *Alternaria*, *Fusarium* and *Penicillus* were the most predominant microorganisms since they were isolated from nearly all samples. These three groups of microorganisms were isolated at higher frequency than other microorganisms such as *Rhizopus*, *Rhodotorula*, *Sordaria*, *Paecilomyces* and *Verticillum*.

Mycelial colonies emerging from each piece of sticky tape were identified to genus level based on morphological attributes (Fig. 7). *Alternaria*, *Fusarium* and *Penicillus* had higher frequency of isolation compared to *Rhizopus*, *Aspergillus* and other unidentified fungal microorganisms collected from sticky tapes. *Alternaria*, *Fusarium* and *Penicillus* were consistently the three dominant fungal microorganisms present in the soil and dust samples collected from experimental sites in USA, however, a greater diversity of fungal microorganisms were observed in soil samples than dust samples.

Airborne fungal microorganisms were detected at each of the experimental sites in New Mexico (Fig. 8). *Fusarium* was the dominant fungal microorganism at Leyendecker and Columbus in PM samples collected at 1.2 m above the ground surface. *Alternaria* and *Fusarium* had the higher frequency of isolation compared with *Aspergillus* and *Rhizopus* in PM samples collected at 1.2 m above the ground surface in Deming, while *Alternaria* was consistently dominant at every height in Anthony. There are limited data on airborne PM and fungal microorganisms along the Chihuahuan Desert region (Flores-Márgez et al., 2014). Therefore, it is difficult to identify the most dominant factors responsible for differences in PM emissions, and variations of PM concentrations and airborne fungal microorganisms observed at each site. Variations in PM concentration and distribution of airborne fungal microorganisms are attributed to the combination of atmospheric conditions, vegetation densities, and types of soil (Abu-Dieyeh et al., 2010). Rodríguez-Rajo et al. (2005) reported that fungal spore concentrations increased with elevation after evaluating the distribution of fungal spores in geographically different areas located approximately at 0, 200 and 1000 m
elevation. The mean elevation of 1240 ± 71 m at the experimental sites in New Mexico, USA and Ciudad Juárez, MX suggested that the elevations of the sites did not vary as much as those reported by Rodríguez-Rajo et al. (2005). Therefore, the variations in distribution of fungal microorganisms at the experimental sites could be due to the variability in the local vegetation, soil, wetting and drying patterns of soils, and atmospheric conditions. Some of these are also reported by Sabariego et al. (2012).

2.4. Fungal analysis of soil and PM samples on sticky tape from MX

The morphology of fungal microorganisms consistently present in both US and MX is shown in Fig. 9. Fungal microorganisms in PM were identified to genus. Frequency of fungal microorganisms detected in soil samples is presented in Fig. 10. Species of *Aspergillus* and *Alternaria* were the most predominant microorganisms in all sites, *Bipolaris* and *Rhizopus* were also detected in sites Juarez 2 and Juarez 3.

The dominant fungal microorganism in sticky tapes was *Alternaria* (Fig. 11). *Alternaria* was consistently present in Juárez 1, Juárez 2 and Juárez 3 sites (Fig. 12). Only *Alternaria* was detected in Juárez 1 at both heights and the largest frequency of isolation for *Alternaria* was obtained in Juárez 1. In Juárez 2, *Alternaria* had larger frequency of isolation at both heights whereas *Bipolaris* and *Penicillium* were detected at 1.0 and 2.0 m, respectively. In Juarez 3, *Alternaria* was detected at both heights while *Penicillium* and *Aspergillus* were detected at 1.0 and 2.0 m height, respectively.

In general, *Alternaria*, *Penicillium* and *Aspergillus* were the three groups of airborne fungal microorganisms consistently present in US and MX sites. *Alternaria*, *Penicillium* and *Aspergillus* are known to produce dark-colored spores which are resistant against irradiation and desiccation (Al-Subai,
2002). These characteristics allow the successful dispersal of Alternaria, Penicillium and Aspergillus throughout different regions of the world (Prospero et al., 2005).

The presence of Alternaria in rural areas as Leyendecker, Columbus, Deming and Anthony is in agreement with the vegetation present in those areas that may have served as source of fungal spores. Awad (2005) reported that Alternaria was the common fungal genera in cultivated areas after studying the occurrence of fungal microorganisms in urban and rural areas. Fusarium, a known plant pathogen, was present in PM samples collected from every experimental site in the US. However Fusarium was not present in PM samples, but it was present in soil samples from Ciudad Juárez, MX. The consistent occurrence of Alternaria in urban areas as Juárez 1, Juárez 2 and Juárez 3 could be explained by the dispersion of spores from rural areas with vegetation surrounding the experimental sites.

3. Conclusions

This study was conducted in the Chihuahuan Desert area along the US–Mexico border. PM concentrations decreased with increasing height of the sticky tape above ground level at experimental sites in both countries. The counts of PM$_{2.5}$ were greater than the particles with larger size (>PM$_{2.5}$ and >PM$_{10}$) at every experimental site. The largest count of PM$_{2.5}$ was observed in Juárez 2 while the lowest was observed in Anthony and corresponded well with the clay content of the soil. Among the fungal microorganisms, Alternaria, Penicillium and Fusarium were relatively more frequent than other fungal microorganisms in soil samples collected from the US sites while Alternaria and Aspergillus showed a higher frequency of isolation in soil samples from Mexican sites. Alternaria, Penicillium and Aspergillus were consistently present in US and MX sites. Differences in the distribution of fungal microorganisms in the experimental sites could be attributed to the variability of vegetation density, soil types, variations in soil moisture content patterns, and climate at each of the sites. The sticky tape method has the potential to be used for detecting different microorganisms and future attempts can be made to identify other fungal microorganisms including coccidioides, which are reported to cause Valley Fever in parts of California, Arizona and New Mexico.

Acknowledgments

This study was funded by the Southwest Consortium for Environmental Research and Policy (SCERP) (Project Number: NMSU-02-2012). Authors thank New Mexico State University Agricultural Experiment Station, Nakayama Chair endowment and New Mexico Department of Health. We would like to extend our appreciation to graduate and undergraduate students at NMSU and the Autonomous University of Juarez for their help during field campaigns.

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