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# Entomopathogenic fungi from 'El Eden' Ecological Reserve, Quintana Roo, Mexico

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**Abstract**

Entomopathogenic fungi were isolated and identified from insects collected from the tropical forest and an agricultural area at El Eden Ecological Reserve, Quintana Roo, Mexico. These fungi were studied to determine their potential as biological control agents of greenhouse *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae), and to contribute to the knowledge of biodiversity of this area. No pest insects were observed in the tropical forest. In contrast, all insects collected in the agricultural area were considered important pests by the local farmers, with the whitefly, as the most relevant, plentiful in Cucurbitaceae plants. From approximately 3400 collected insects in three different surveys, different anamorphic Ascomycetes were recovered. One isolate of *Aspergillus* sp., two of *Penicillium* sp., three of *Paecilomyces marquandii*, and three of *Verticillium* sp. out of 308 insects (2.9%) from three insect orders, Hymenoptera, Diptera and Isoptera in the tropical forest. In contrast, a higher number of fungal isolates were recovered from the agricultural area: three isolates from *Aspergillus parasiticus*, 100 of *Fusarium moniliforme*, one of *Aschersonia* sp., and 246 of *Fusarium oxysporum* out of 3100 insects (11.3%) from three insect orders, Homoptera, Coleoptera and Lepidoptera. The results of this study show *Fusarium moniliforme* and *F. oxysporum* as highly virulent to infected insects in the agricultural area, with 100 and 246 isolates respectively, out of 350 infected insects of 3100 studied specimens. Laboratory whitefly nymph bioassays with isolates Ed29a of *F. moniliforme*, Ed322 of *F. oxysporum*, and Ed22 of *P. marquandii* showed 96 to 97.5% insect mortality with no significant differences ($P < 0.05$) among them. *F. oxysporum* Ed322 produced no mortality when inoculated on tomato, bean, squash and maize seedlings (with and without injuries) compared to the 100% mortality caused by phytopathogenic strains, *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici*.

**Key words:** ecological reserve, entomopathogenic fungi, pest insects, whitefly bioassays

## Introduction

Tropical biodiversity has figured prominently in the news of the past few years as a potential source of new foods, pesticides, and drugs, which in turn, might contribute to its preservation. Conservation of biodiversity includes the conservation of variety, interactions between species and processes in ecosystems [1]. The discovery of useful products in tropical ecosystems would remind the world of how valuable these resources are [2].

To achieve conservation of biological diversity it is usually necessary to establish natural protected areas of land or water with the intention of permanently preserving certain ecosystems or critical habitats for individual rare, important species, and biotic interactions. In addition, these areas are sources of valuable and rich biological resources, which contain a great diversity of organisms, and shelter many kind of biotic interactions (probably most unknown). In these natural laboratories, the study of organisms, biotic interactions, and ecological processes, can produce useful knowledge that could be applied to solve a sort of ecological and practical problems [3].

Over 70,000 species of fungi and 800,000 species of insects have been described. The abundance of

both fungi and insects, and their contact in a variety of habitats, provide these organisms an opportunity for interaction and coevolution [4]. Relationships between fungi and insects may be mutualistic, through commensal to obligatory pathogenic [5]. Studies on microbiological antagonisms of pest insects in natural protected areas have been conducted in countries like Russia [6] and Ecuador [7], and have identified potential biological control micro-organisms. Detailed studies of mycoparasite population dynamics and their hosts are necessary in order to determine their potential use as biocontrol agents [8].

The use of microbial insecticides in integrated pest management reduces dependence on chemical pesticides [9]. In particular, the use of entomopathogenic fungi as biocontrol agents has several advantages. One such example is their relatively narrow host ranges by targeting specific pest populations while preserving natural predators and other beneficial insects [3].

The aim of the present investigation was to isolate and to identify entomopathogenic fungi in the tropical forest and agricultural areas of El Eden Ecological Reserve, Quintana Roo, Mexico, to determine their potential use against the greenhouse whitefly *Trialeurodes vaporariorum*, and to contribute to the knowledge of biodiversity of this area.

## Materials and methods

### *Collection site*

The study was conducted at El Eden Ecological Reserve in the State of Quintana Roo, Mexico. This reserve is located at the north-eastern portion of the Yucatan Peninsula, at 21°13′ N and 87°11′ W, and an altitude of 5–10 m. The dry season is from December to April and the rainy season from May to November. The major ecosystems present in the reserve include: (1) a semideciduous dry tropical forest dominated by trees (up to 15 m in height), mixed with various species of shrubs, herbs, vines, and epiphytes, (2) secondary plant communities of various higher plants, (3) wetlands that include swamp forest, palm stands, savannahs, cattail, sawgrass swamps, and lagoons, and (4) a poorly developed agricultural area, east of the reserve. The four ecosystems contain 186 species of invertebrates and 136 species of vertebrates [10]. There are 50 rural farms in the agricultural area, each with an additional ∼3 ha home gardens. The area has been clear-cut and cultivated for over 15 years using traditional agricultural practices of slash and burn system. As in much of the Yucatan Peninsula, the production of traditional crops such as maize, bean, squash, and vegetables in El Eden reserve is heavily dictated by the rainy season. Three insect surveys were conducted during the 1996 dry season and the 1997 dry and rainy seasons in the tropical and agricultural areas. The mean rainfall in December 1996 was 48 mm with maximum and minimum temperature of 29 and 8.5 °C, respectively. July and November 1997 had mean rainfall of 122 and 107 mm, respectively, and maximum and minimum temperatures of 36, 33 and 16, 17 °C, respectively. The 1997 rainy season was delayed due to the El Niño phenomenon.

### Insect sampling

In the tropical forest, live insect samples were taken along 500 m transects. Ten random transects per survey were sampled. Samples of foliage, soil (20 cm deep), humus and tree bark were taken every 100 m on both sides of the transect. Foliage samples were taken by shaking the plants or by the help of a net as described by Morris [11]. The ten soil samples taken along each transect were mixed, and 1 kg representative subsample was taken for analysis. The same procedure was applied to bark and humus samples. Soil, humus and crushed tree bark were sieved (5 mm sieve), and insects were collected. A minimum of one hundred live insects was randomly selected from each order/species, in each sampling date in the tropical forest for laboratory studies.

In the agricultural area, 32 interviews were randomly conducted with local farmers to help found the principal pest insects, in December 1996, July and November of 1997. Some farms had no crops at the time of the study and therefore were not surveyed. Local farmers assisted in the surveys and directed us to infestations. To avoid border effect, a 1-ha area in the centre of each farm was sampled. Leaves, soil, and fruit samples were taken every 10 m for a total of 100 m in a zigzag pattern according to Taylor [12]. Samplings of soil and of insects from soil were as described above for the tropical forest. For sampling of insects from plants, these were first shaken and fallen insects collected as described by Morris [11]. Fruits were also collected and searched for insects.

The insects collected were grouped by origin (plant parts or soil). Twenty live individuals per group were selected at random and fixed in 70% ethanol for taxonomic identification. A minimum of one hundred live

insects was randomly selected per species and per sampling date for laboratory studies. The insects were maintained in sterile plastic Petri dishes containing a fragment of host plant material. Dead insects showing mycosis symptoms (i.e., abnormalities in the morphology, colour variation, conspicuous position of appendages) were also collected at random in both areas, and transported to the laboratory in sterile paper bags.

In the laboratory, live insects along with host leaf, bark, or fruit section were placed in sterile plastic vials and incubated at 25–28 °C at a photoperiod of 12:12 (D:L). Plant materials were replenished as needed. Daily observations were made and dead insects were recorded. Insects that died within 4 weeks following collection were considered as initially infected by pathogens. The dead insects were surface-sterilised with 0.5% sodium hypochlorite solution for 60 sec, then rinsed three times in sterile distilled water and dried with sterile absorbent paper. The treated insects were then placed in a moist chamber and periodically examined for fungal growth. Dead insects collected from the field were washed for 60 sec each with 0.5% sodium hypochlorite, 0.02% streptomycin sulphate (Laboratorios Pisa, JC, Mexico) solution, and 0.03% rifampicin (Laboratorios Lepetit, MO, Mexico) solution and then rinsed three times in sterile water, dried and placed in a moist chamber as described above.

**Insect identification**

Insects were identified by species using previously described methods and keys [13–20]. Some insects were identified by Juan Soria from the Departamento de Entomología, Centro Nacional de Referencia Fitosanitaria, Dirección General de Sanidad Vegetal, Ministry of Agriculture, México. Voucher specimens were deposited with the Entomological Collection of this institution.

**Isolation of fungi**

Fragments of mycelia and/or spores from sterilized insects were placed on Sabouraud dextrose agar (DIFCO Laboratories, Detroit, MI) plates supplemented with 0.2% yeast extract (DIFCO) (SDAY medium), with or without antibiotics (0.005% chloramphenicol, La Campana, Cia. Medicinal, DF, Mexico) plus 0.03% rifampicin (Lepetit). Additional SDAY plates were supplemented with 0.02% of rose Bengal (Hycel de Mexico, DF, Mexico). To further assess internal fungal pathogenicity, insects or insect fragments showing fungal growth were retreated with 0.5% sodium hypochlorite and antibiotics (0.02% streptomycin sulphate solution, and 0.03% rifampicin solution), and placed on SDAY plates with and without antibiotics. Plates were incubated at 25 °C in the dark. Fungi were transferred to fresh SDAY plates for colony isolation and identification.

**Identification of fungi**

Fungi were identified according to previously described methods and keys, [21–25]. Isolates were preserved as spores in 10% glycerine (Baker, Mexico) and stored at 4 °C, or in liquid nitrogen at –196 °C. Vouchers of pure cultures established *in vitro* were deposited in the Laboratorio de Alelopatía, Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), and in the Laboratorio de Micología, Instituto de Ecología, A.C., Xalapa, Veracruz. Two atypical *Fusarium* isolates were sent to Dr. W. Gams at the Centraalbureau Voor Schimmelcultures in the Netherlands for identification and were deposited with this institution.

**Entomopathogenic potential of isolated fungi**

Criteria used to select potential microbial control agents among the fungal isolates included mainly, pathogenic interaction with the host by the isolation of the pathogen that caused the death of the insect (considering the pathogen as the fungus inside the insect that surfaces after the insect death according to the mechanism of infection of entomopathogenic fungi) [26]; frequency of isolation; speed of growth evaluated by measurements of the colony diameter in solid media (SDAY) after 15 d at 25 °C; conidial production on SDAY plates after 15 d at 25 °C; and literature review for entomopathogenicity [27]. Since microbial control agents should have an adequate growth in standard media with a high conidial production for future formulations [28], the isolates were evaluated by measurement of the colony diameter on solid media (SDAY) after 15 d at 25 °C; and conidial production on SDAY plates after 15 d at 25 °C. Conidial production was measured by pipetting 10 ml of sterile 0.1% Tween 20 (Sigma Co, St Louis, MO) in each

plate followed by scraping the culture with a sterile blade to harvest conidia. Conidial concentration was determined using a haemocytometer [27]. Determination of speed of growth and conidial production were done in four replicates in preliminary tests for all the isolates obtained (359), but repeated three times in different days for the seven isolates that showed faster growth and higher conidial production: Four isolates of *F. moniliforme*, two of *F. oxysporum* and one of *P. marquandii*. This last isolate was chosen because species of this genus are commonly entomopathogenic [29, 30].

### Whitefly bioassays

Three fungal isolates were finally selected for the whitefly bioassays: *F. moniliforme* Ed29a, *F. oxysporum* Ed322, and *P. marquandii* Ed22. A colony of greenhouse whiteflies (*T. vaporariorum*) was maintained on beans (*Phaseolus vulgaris* L. c.v. Flor de Mayo) at the Instituto de Ecología greenhouse, UNAM. Leaves infested with third-instar nymphs were taken from plants on the day of the experiment, washed in deionized water, and dried with sterile absorbent paper. The sterile leaves infested with whitefly nymphs were examined under the stereomicroscope and third-instar ones were inoculated with 5 $\mu$l of $10^{-8}$ conidia ml$^{-1}$ suspension of each selected fungal isolate. Conidia suspensions were prepared in 0.1% Tween 20 (Sigma). Control nymphs were treated with sterile 5 $\mu$l of 0.1% Tween 20. The experimental unit consisted of 20 whitefly nymphs arranged in a completely randomised design with five replicates and repeated three times. Assays were maintained at room temperature. Insect survival was evaluated 4 d after treatment, because in preliminary observations, one of the tested isolates achieved 100% mortality at day 4. Criteria for nymph mortality were insect mummification (nymph invasion and death by the fungus), and emergence of mycelia from the insect. Nymph's mortality by the fungus was verified by the microscopic observation of fungal specific sporulation from the mummified insects after their incubation in a humid chamber at room temperature. Mortality data were analysed by analysis of variance (ANOVA). When the overall F test was significant, means were separated by the Tukey test ($P < 0.05$).

### Phytopathogenic potential of the most frequently isolated fungus, *Fusarium oxysporum*

Since *F. oxysporum* has been previously reported as a phytopathogenic fungus, we performed an experiment to test the phytopathogenicity of *F. oxysporum* isolate Ed322 against 3 week-old seedlings of tomato (*Lycopersicon esculentum* L. c.v. Rio Grande), squash (*Cucurbita pepo* L.), bean (*Phaseolous vulgaris* L. c.v. Flor de Mayo) and maize (*Zea mays* L. c.v. Chinampero). The experimental unit was a 500-g pot containing four seedlings. The treatments assayed were: (1) *F. oxysporum* Ed322 microconidia, (2) *F. oxysporum* Ed322 macroconidia, (3) positive control, tomato seedlings inoculated with *F. oxysporum* f. sp. *lycopersici* (a tomato phytopathogenic strain), and squash, bean and maize seedlings inoculated with *F. oxysporum* f. sp. *radicis lycopersici* (a phytopathogenic strain for these three crops), and (4) negative control, (seedlings inoculated with deionized sterile water). Within each treatment, two seedlings were injured and the other two were not. A 1-mm wide blade was used to make four injuries to the base of the seedlings. Stem bases or corresponding sites of injuries were inoculated with 1 ml of a $10^6$ conidia ml$^{-1}$ suspension. Care was taken to insure good and sustained contact of conidia suspension with the stem bases and at sites of injuries. Soil in pots was saturated to field capacity with sterilised water before inoculation to prevent absorption of the inoculae by the soil. Pots were maintained in the Instituto de Ecología, UNAM greenhouse, at 25 °C. Experiments were arranged in a completely randomised design with four replicates and repeated three times. Plant survival was evaluated 3 d after treatment.

## Results

*Insects and fungi isolation*

In 1997 the rainy season was delayed due to the El Niño phenomenon and consequently few insects were collected during the July 1997 survey. In contrast, insects collected during the November 1997 survey (after the rainy season) were abundant. A total of approximately 3400 insects comprising 18 species (including two mummies) were collected from the two areas in the three surveys. Four species of insects were from the tropical forest and fourteen from the agricultural area.

From approximately 3400 collected insects in three different surveys, different anamorphic Ascomycetes were recovered. One isolate of *Aspergillus* sp., two of *Penicillium* sp, three of *Paecilomyces marquandii*, and three of *Verticillium* sp. out of 308 insects (2.9%) from three insect orders, Hymenoptera, Diptera and Isoptera in the tropical forest (Table 1). By contrast, numerous insects were collected in the agricultural area during the study period. Fourteen species comprising five orders were identified as pests of crops such as maize, beans and chilli but also of vegetables and fruits (Table 2). As a consequence, a higher number of fungal isolates were recovered from the agricultural area. Four mitosporic Ascomycetes were isolated with high frequency, three isolates from *Aspergillus parasiticus*, 100 of *Fusarium moniliforme*, one of *Aschersonia* sp., and 246 of *Fusarium oxysporum* out of 3100 insects (26.9% of total infected insects (350) or 11.3% of the total sampled insects). All fungi were isolated from 7 insect species in the agricultural area, from which four species belong to families of Homoptera (Membracidae, Cicadellidae, Aphididae and Aleyrodidae), two to Coleoptera (Nitidulidae, Chrysomelidae), and one to Lepidoptera (Noctuidae). The most frequently isolated fungal species was *F. oxysporum*, with 99 isolates out of 200 specimens of the treehopper, *Antianthe viridissima* (Homoptera: Membracidae) collected from chilli crops, 87 isolates out of 400 specimens of the chilli sap beetle, *Carpophilus lugubris* (Coleoptera: Nitidulidae), 30 isolates out of 100 specimens of *Aphis citricola* (Homoptera: Aphididae), and 30 isolates out of 100 specimens of *Dalbulus maydis* (Homoptera: Cicadellidae) (Table 2). *F. oxysporum* represented 70.2% of all fungal isolates from the agricultural area.

*Entomopathogenic potential of fungal isolates*

All the isolated fungi (Tables 1 and 2) had pathogenic relationship with their host insects, produced symptoms such as mummification, and have been previously reported as entomopathogens [23, 24, 28]. However, some species, such as *Aspergillus parasiticus* (anamorphic Trichocomaceae) and *Penicillium* sp. (anamorphic Trichocomaceae) are reported as phyto- and zoopathogenic, as well as human pathogens [31, 32]. For those reasons these species were not included in the present study. Also, *Aschersonia* sp. (anamorphic Clavicipitaceae), although reported as entomopathogenic [24], was not included in the study due to culture difficulties.

Two species of *Fusarium* (anamorphic Hypocreaceae), *F. moniliforme* and *F. oxysporum* were isolated as pathogens from 98.8% of the infected insects in the agricultural area. This high frequency suggested their role as natural enemies of insects. Both species were further tested for their entomopathogenic potential on *T. vaporariorum* whitefly. One species of *Paecilomyces marquandii* (anamorphic Trichocomaceae), isolated from *Solenopsis* sp. in the tropical forest was also selected as mentioned. Following the results of preliminary growth and conidia production tests, we chose four isolates of *F. moniliforme* (Ed134, Ed29a, Ed311a, and Ed313), two of *F. oxysporum* (Ed124 and Ed322), and one of *P. marquandii* (Ed22), to test for growth and conidia production. Table 3 shows the results of this bioassay. *F. moniliforme* Ed29a and *F. oxysporum* Ed322, two isolates with the highest sporulation, were selected for the whitefly pathogenicity assay. *P. marquandii* Ed22 was also selected because of previous reports on entomopathogenic *Paecilomyces* spp., e.g. *P. farinosus* and *P. fumosoroseus* have been reported as biological control agents of pest insects [29, 30].

Results of third-instar nymph *T. vaporariorum* bioassays showed a high insect mortality (96–97.5%) caused by the three isolates tested, with no significant differences ($P < 0.05$) among them (Table 4). Infected nymphs showed different colour changes depending on the fungus, and when the emergent mycelium was again cultured, it showed the specific fungal sporulation of each species.

*Phytopathogenic potential of Fusarium oxysporum isolate (Ed322)*

Micro- and macroconidia of *F. oxysporum* Ed322 produced no mortality on inoculated tomato, bean, squash and maize seedlings (with and without injuries) compared to the 100% mortality caused by phytopathogenic strains, *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *radicis lycopersici* (data not shown).

## Discussion

Complex communities, such as tropical forests, are considered stable. The impact of a sudden population change in one species will be cushioned by the large number of interacting species and will not produce drastic effects in the community as a whole. It has been suggested that such buffering mechanism

*Table 1.* Host insects and entomopathogenic fungi from the tropical forest at El Eden Ecological Reserve, Quintana Roo, Mexico

| Insect species | Habitat sampled | Sample date[a] | Entomopathogenic fungi | % of infection isolates/100[b,c] |
|---|---|---|---|---|
| Musca sp. *Diptera: Muscidae* | Mummy in soil forest | Dec. 1996[d] | *Aspergillus* sp. | 1/3[e] |
| *Solenopsis* sp. [ants] *Hymenoptera:Formicidae* | Soil forest | Dec. 1996 | *Penicillium* sp. | 1 |
| *Solenopsis* sp. *Hymenoptera: Formicidae* | Soil forest | July 1997[f] | *Paecilomyces marquandii* | 3 |
| *Apis* sp. [bees] *Hymenoptera: Apidae* | Mummy in soil forest | Nov. 1997[g] | *Penicillium* sp. | 1/5[e] |
| *Nasutitermes mexicanus* [termites] *Isoptera: Termitidae* | *Sabal* bark | Nov. 1997 | *Verticillium* sp. | 3 |

[a]Dec. = December; Nov. = November.
[b] Number of fungal isolates out of 100 insect individuals analysed, except mummies.
[c]Each fungal isolate corresponds to one individual insect.
[d]December 1996, dry season.
[e]Isolate/total number of mummies.
[f]In July 1997, because of "El Niño" phenomenon the rainy season had just started.
[g]November 1997, rainy season.

operates in tropical forest where insect outbreaks are unknown. This mechanism contrasts with cultivated communities where pest outbreaks are common [33]. In the present study no pest insects were observed in the tropical forest. Coley and Kursar [34] suggest that insect herbivores are rare in tropical forests because are highly regulated by the third trophic level. This fact may explain the lower number of insects found in El Eden tropical forest. In our study, whiteflies and fall armyworm eggs were found along the edge of the tropical forest near to the agricultural area, but none acting as a pest. Whiteflies sheltering in evergreen hosts and migration of adults, when no cultivated plants are present in the agricultural areas have been reported [35]; and may explain the presence of whiteflies and fall armyworm eggs in the tropical forest edge.

In contrast, pest insects were abundant in the agricultural area. The perturbed environmental conditions of this area are ideal for the proliferation of insects. The area has a low biodiversity and consequently pests' natural enemies are scarce. Whiteflies can cause a total loss of tomato and chili production, mainly due to virus infection transmission [36].

Anamorphic ascomycetes were the only entomopathogenic fungi found in insects collected in both study areas. Identified fungi genera were different in each area, except for *Aspergillus*. All of them are first reports of insect pathogens from El Eden Ecological Reserve. *Paecilomyces marquandii* Ed22 isolated from *Solenopsis* sp., grows fast and causes a high nymph whitefly mortality in the bioassays performed. Different species in these genera have already been studied as mycoinsecticides [37, 38]. However, *P. marquandii* has been poorly studied. It has been investigated for its potential as a biological control agent of free living and plant-parasitic nematodes [39]; as a degrader of plastic materials [40]; and for its capability of producing leucinostatin, a peptide antibiotic with cytotoxic activity [41, 42]. Further studies on the isolated Ed22 *Paecilomyces marquandii* will show if this fungus could be a potential biological control agent against whiteflies.

The genus *Fusarium* was the most significant genus of fungi found in insects at El Eden agricultural area. It was isolated from 98.8% of the sampled insects that showed fungal infection (i.e. 350 infected specimens out of 3100 insects [11.3%] studied in this area). *F. oxysporum* was the most frequent species since it represented 70.3% of all fungal isolates (246 isolates out of 350 fungi). This species has received considerable attention from plant pathologists over the past 80 years because of its ability to cause vascular wilt or root rot diseases in a wide range of plants [43]. Despite the broad host range of the species

*Table 2.* Host plants, pest insect species, and entomopathogenic fungi from the agricultural area at El Eden Ecological Reserve, Quintana Roo, Mexico

| Host plants | Pest insect species | Habitat sampled | Sample date[a] | Entomopathogenic fungi | % of infection isolates/100[b,c] |
|---|---|---|---|---|---|
| – | infected insect (non-identified) | Soil | Nov. 1997[d] | *Aspergillus parasiticus* | 3 |
| *Carica papaya* (Papaya)[e] *[Caricaceae]* [f] | *Acanophora femoralis* (Treehoppers) [*Homoptera*: *Membracidae*] | Papaya leaves | Dec. 1996[g] | *Fusarium moniliforme* | 30 |
| *Capsicum annuum* L. (Jalapeño chilli) [*Solanaceae*] | *Antianthe viridissima* (Treehoppers) [*Homoptera*: *Membracidae*] | Chilli leaves | Dec. 1996 | *Fusarium oxysporum* | 52 |
| *Capsicum annuum* | *Carpophilus lugubris* (Chilli sap beetle) [*Coleoptera*: *Nitidulidae*] | Chilli fruits | July 1997[h] | *F. oxysporum* | 18 |
| | | | Nov. 1997 | *F. oxysporum* | 22 |
| *Capsicum frutescens* (Habanero Chilli) [*Solanaceae*] | *A. viridissima* | Chilli leaves | Dec. 1996 | *F. oxysporum* | 47 |
| *Capsicum frutescens* | *C. lugubris* | Chilli fruits | July 1997 | *F. oxysporum* | 23 |
| | | | Nov. 1997 | *F. oxysporum* | 24 |
| *Cucurbita pepo* (Squash) [*Cucurbitaceae*] | *Acalymma trivittata* [*Coleoptera*: *Chrysomelidae*] | Squash leaves | Nov. 1997 | NFI | – |
| *Cucurbita pepo* | *Conotelus stenoides* [*Coleoptera*: *Nitidulidae*] | Squash leaves | Nov. 1997 | NFI | – |
| *Cucurbita pepo* | *Bemisia tabaci* (Whitefly) [*Homoptera*: *Aleyrodidae*] | Squash leaves | Nov. 1997 | NFI | – |
| *Cucumis melo* L. (Melon) [*Cucurbitaceae*] | *B. tabaci* | Melon leaves | Nov. 1997 | NFI | – |
| *Cucumis sativus* L. (Cucumber) [*Cucurbitaceae*] | *B. tabaci* | Cucumber leaves | Nov. 1997 | NFI | – |
| *Lycopersicon esculentum* (Tomato) [*Solanaceae*] | *B. tabaci* | Tomato leaves | July 1997 | NFI | – |
| | | | Nov. 1997 | NFI | – |
| *Sickingia salvadorensis* (Palo de rosa) [*Rubiaceae*] | *Aphis citricola* (aphid) [*Homoptera*: *Aphididae*] | Palo de rosa leaves | Dec. 1996 | *F. oxysporum* | 30 |

*Table 2.* Continued

| Host plants | Pest insect species | Habitat sampled | Sample date[a] | Entomopathogenic fungi | % of infection isolates/100[b,c] |
|---|---|---|---|---|---|
| *Phaseolus vulgaris* (Bean) [*Fabaceae*] | *Zabrotes subfasciatus* (Bean grub) [*Coleoptera*: *Bruchidae*] | Bean fruits | July 1997 | NFI | – |
| *Phaseolus vulgaris* | *B. tabaci* | Bean leaves | July 1997<br>Nov. 1997 | *Aschersonia* sp.<br>NFI | 1<br>– |
| *Zea mays* L. (Maize) [*Poaceae*] | *Spodoptera frugiperda* (Fall armyworm) [*Lepidoptera*: *Noctuidae*] | Maize leaves | July 1997<br>Nov 1997 | *F. moniliforme*<br>*F. moniliforme* | 17<br>13 |
| *Zea mays* | *Dalbulus maydis* (Leafhoppers) [*Homoptera*: *Cicadellidae*] | Maize leaves | July 1997<br>Nov. 1997 | *F. moniliforme*<br>*F. oxysporum* | 40<br>30 |
| *Citrus* spp. (Citrus fruits: Orange, lemon, tangerine) [*Rutaceae*] | *Atta* sp. (Leaf-cutting ant) [*Hymenoptera*: *Formicidae*] | Citrus leaves | Dec. 1996<br>July 1997<br>Nov. 1997 | NFI<br>NFI<br>NFI | –<br>–<br>– |
| *Citrus* spp. | *Anastrepha ludens* (Mexican fruit fly) [*Diptera: Tephritidae*] | Citrus fruits | July 1997<br>Nov. 1997 | NFI<br>NFI | –<br>– |
| *Citrus* spp. | *Carphophilus humeralis* (Sap beetle) [*Coleoptera*: *Nitidulidae* | Citrus fruits | July 1997<br>Nov. 1997 | NFI<br>NFI | –<br>– |
| *Citrus* spp. | *Phyllocnistis citrella* (Citrus leaf miner) [*Lepidoptera:*] *Gracillariidae* | Citrus leaves | Nov 1997 | NFI | – |

[a]Dec. = December; Nov. = November.
[b]Number of fungal isolates out of 100 insect individuals analysed.
[c]Each fungal isolate corresponds to one individual insect.
[d]November 1997, rainy season.
[e]Family/order in brackets.
[f]Common name in parenthesis.
[g]December 1996, dry season.
[h]Because of"El Niñ" phenomenon the rainy season had just started.
NFI = no fungal isolates could be recovered from 100 processed individual insects.

*Table 3.* Growth and conidial production of selected fungal isolates

| Fungal isolate | Insect host | Habitat sampled | Growth[a] (cm)[b] | Sporulationa (conidia $ml^{-1} \times 10^6$)[b] |
|---|---|---|---|---|
| *F. moniliforme* (Ed134)[c] | *Acanophora femoralis* | Papaya leaves | $8.95 \pm 0.3$ | $5.35 \pm 1.2$ |
| *F. moniliforme* (Ed 29a) | *Dalbulus maydis* | Maize leaves | $8.98 \pm 0.26$ | $178 \pm 11.23$ |
| *F. moniliforme* (Ed311a) | *Spodoptera frugiperda* | Maize leaves | $8.92 \pm 0.21$ | $150 \pm 10.59$ |
| *F. moniliforme* (Ed313) | *D. maydis* | Maize leaves | $8.98 \pm 0.22$ | $2.34 \pm 0.56$ |
| *F. oxysporum* (Ed124) | *Antianthe viridissima* | Chilli leaves | $8.89 \pm 0.28$ | $2.77 \pm 1.06$ |
| *F. oxysporum* (Ed322) | *Carpophilus lugubris* | Chilli fruits | $8.96 \pm 0.27$ | $192 \pm 11.47$ |
| *Paecilomyces* marquandii (Ed22) | *Solenopsis* sp. | Soil forest | $8.19 \pm 0.33$ | $31.1 \pm 8.61$ |

[a]Each value represents the mean ± standard deviation of three different experiments. Four replicates were made in each experiment
[b]Growth measurement (colony diameter) and conidia $ml^{-1}$ are reported at 15 d incubation on SDAY medium at 25 °C.
[c](Ed = El Eden; first number = date of collect; subsequent numbers=internal keys).

*Table 4.* Percentage of mortality in third-instar nymphs of Trialeurodes vaporariorum caused by selected fungal isolates

| Fungal isolates | Mortality (%)[a,b] |
|---|---|
| Control[c] | 0 |
| *F. moniliforme* (Ed29a)[d] | $96.6 \pm 3.84$ |
| *F. oxysporum* (Ed322) | $97.5 \pm 3.19$ |
| *Paecilomyces marquandii* (Ed22) | $95.94 \pm 3.12$ |

[a]Each value represents the mean ± standard deviation of three different experiments. Four replicates were made in each experiment.
[b]Evaluated with a suspension of $10^8$ conidia $ml^{-1}$.
[c]Sterile 0.1% Tween 20.
[d](Ed = El Eden; first number = date of collect; subsequent numbers = internal keys).

as a whole, host specialization of individual isolates is more circumscribed. Isolates with the same or similar host ranges are assigned to special forms (f. sp.) and more than 70 f. sp. have been described for *Fusarium* [44]. More often, host range is restricted to a few plant species. For example, although many plants may be symptomless carriers of *F. oxysporum* f. sp. *lycopersici*, it causes disease only in plants of the tomato genus *Lycopersicon* [45]. However, some f. sp. have broader ranges, such as *F. oxysporum* f. sp. *radicis-lycopersici*, which in greenhouses can cause disease in members of several plant families other than tomato [46]. For example, *F. oxysporum* f. sp. *orobanche* was developed in the URSS as a weed killer in the seventies [47]. One isolate of *F. oxysporum* has been evaluated as a *Striga* killer in the dryland zones of Africa where this parasitic plant causes losses of 70% in sorghum and maize. In 1995 the results were dramatic, 85% of the *Striga* were wiped out at the seedling stage by this *Fusarium* isolate with the added advantage that it is not toxic to humans and causes no harm to cereal crops [48].

A large number of *Fusarium* spp. are entomopathogenic; some are weak, facultative pathogens, especially of Lepidoptera and Coleoptera, and they

colonise their dead host as saprophytes. Kuruvilla and Jacob [49] found that in many cases all insect life stages (*Nilaparvata lugens*), including eggs, are susceptible to *Fusarium*. Highly entomopathogenic species are reported primarily from Homoptera and Diptera [50]. *F. moniliforme* is an extremely common species. Feng-Yan and Qing-Tao [51] reported that 21% of 180 *Fusarium* isolates from 150 dead insects were identified as *F. moniliforme*. *Fusarium oxysporum* is also very common. It has been reported to be highly virulent to larvae of the mosquito, *Aedes detritus*, to larvae of the rice green-horned caterpillar, *Melanitis leda*, and to eggs of the European corn borer, *Ostrinia nubilalis*. It has been also isolated from the aphids *Brevicoryne brassicae*, whiteflies on citrus, and scarab larvae [51].

*Fusarium* isolates from El Eden were principally from homopteran hosts. Isolates *F. moniliforme* Ed29a and *F. oxysporum* Ed322 were highly virulent to third-instar nymphs of *T. vaporariorum* in the laboratory. Research on the adverse effects of this fungus on beneficial organisms (including mammals and plants) revealed both harmful as well as safe *Fusarium* isolates in nature [50]. In the present study we observed that Ed322 isolate of *F. oxysporum* was safe for tomato, squash, bean, and maize seedlings. Studies on the potential use of entomopathogenic *Fusarium* species as insect controllers are rare. We concur with previous reports [50] of a need for further studies of *Fusarium* species as potential microbial agents.

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