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SAPROPHYTIC FUNGI ON TOMATO PHYLLOPLANE: EFFECT OF FUNGICIDES AND LEAF POSITION ON ABUNDANCE, COMPOSITION AND DIVERSITY

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Fungal isolations were made from leaves of tomato plants cultivated in greenhouses in an area close to La Plata, Argentina. Three different schemes of fungicide application were evaluated: high frequency preventive sprayings (Commercial Greenhouse I), low frequency preventive applications (Commercial Greenhouse II) and no fungicide spraying (Control Greenhouse). Leaves were sampled immediately after second fruit formation from three levels of the foliage: low, medium and high. Plating dilution was used to isolate fungal species. Total c.f.u. number and species composition and diversity were assessed by the plating dilution technique. Fungal populations were most abundant on leaves from lower parts of the foliage in the Control Greenhouse. Diversity varied according to fungicide application frequency and leaf position in the canopy. Higher values were recorded for lower leaves in the Control Greenhouse compared with upper leaves from Commercial Greenhouse II. Likewise position in the canopy influenced the frequency of some species. The implications for natural biological control are discussed.

Key words: biodiversity, biological control, phylloplane, tomato

Introduction

Tomato is one of the most important vegetable crops cultivated in the area of La Plata, Argentina (35º W, 58º S). In commercial greenhouses growers usually develop high input cropping systems in which foliar fungal diseases are controlled by high frequency spraying with preventive fungicides. It has been suggested that the excessive use of fungicides may result in a human health risk both for consumers and for farmers living in the area (De Waard et al., 1993). According to Bollen (1971), fungicide application has two main drawbacks: the introduction of large quantities of toxic substances into ecosystems and the generation of fungicide-resistant strains.

Biological control has been suggested as a suitable method for decreasing the incidence and severity of several foliar diseases (Mónaco et al., 1999). The use of biological control in integrated disease management programmes might result in a decrease in fungicide applications, thus leading to a reduction in pollution.

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Regarding biological control in the phylloplane most research has addressed the introduction of foreign antagonistic microorganisms (Blakeman and Fokkema, 1982). Other authors have outlined the importance of indigenous antagonists in suppressing disease and the managing of ecosystem conditions, in order to enhance biological control (Marois and Rouse, 1991). Saprobic fungi inhabiting the phylloplane may exert antagonistic effects on pathogenic fungi, by competition, antibiosis and/or parasitism (Marois and Coleman, 1994). Therefore, microscopic fungi may play an important role not only in pathogenesis but also in the control of foliar diseases (Fokkema, 1993; Andrews and Kenerly, 1978). Restriction or delay in disease onset may be due to the activity of antagonists, which is why the saprophytic fungi of the phylloplane are of interest for natural biological control.

The ability of the indigenous microbial population to prevent pathogenic infections depends mainly on its abundance and diversity. Diversity is important for the effective colonization of different microniches in ecosystems where pathogenesis might take place (Baker, 1991).

Many events that take place on leaf surfaces affect the native saprophytic fungi, quantitatively and qualitatively. A knowledge of the factors that affect the diversity and growth of saprophytic fungi on leaf surfaces would allow us to design a cropping system that would enhance the abundance and diversity of natural antagonists.

The purpose of the work reported here was to evaluate and identify species of fungi inhabiting the phylloplane of tomato from greenhouses with different kinds of management (high input, low input and no pesticide application) and to assess the effect of fungicide application schemes on the abundance and diversity of saprophytic fungi at different canopy levels.

Materials and methods

Sampling of phylloplane fungal flora

Samples were collected from tomato plants cultivated in greenhouses at three different locations in the La Plata green belt. The first sampling was made in a highly technified farm, with five commercial greenhouses devoted to tomato and pepper, managed under a large-scale commercial scheme (Commercial Greenhouse I). The second sampling location was at a smaller farm managed by a family with two greenhouses for tomato cropping (Commercial Greenhouse II). The fungicide application schemes in the two locations are detailed in Figure 1. The third sampling was made in the experimental greenhouse of the Facultad de Ciencias Agrarias y Forestales in La Plata with no pesticide application. Samples were collected in five places equidistantly located along an oblique line with respect to the rows. One plant was selected at each sampling site and three leaves were detached from each: a lower leaf from the third node, a middle leaf from the sixth node and an upper leaf from the ninth node. The combination of fungicide application schemes and leaf canopy positions resulted in nine different treatments.

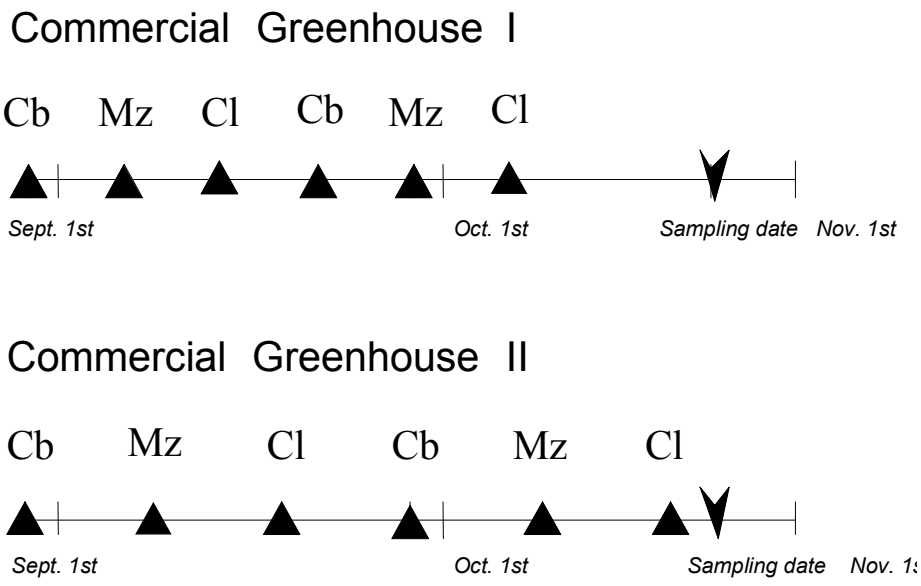

Fig. 1. Fungicide application schemes in the two Commercial Greenhouses. [Cb: Carbendazim (BENCARB®, WP 50%); Mz: Mancozeb (DITHANE® WP 80%); Cl: Chlorotalonil (DACONIL® F 72%)]

Isolation of fungal species

Each leaf sample was dissected into four folioles (subsamples). Then five disks 2 cm^2 in size (16 mm in diameter) were cut from each subsample. These five disks (10 cm^2) were put in 100 ml of sterile distilled water and the mixture was shaken in a magnetic shaker at 120 rev. min^{-1} for 30 min. The suspension was diluted tenfold in sterile distilled water. One ml of this dilution was poured into a Petri dish (9 cm in diameter) to which 10 ml of 2% PDA, oxgall (4 g/l) and 1 ml of streptomycin sulphate solution (250 mg/l) were added. The dishes were incubated at 25±2ºC in the dark for 7 days, after which the number of colony forming units (c.f.u.) was assessed and the species were identified.

The experiments were repeated at least twice with at least four replicates. As the results were the same among experiments, statistical analyses were applied to the last experiments.

The data were statistically analysed by ANOVA and the Tukey multiple range test ($P<0.05$).

Diversity

In order to assess the biodiversity of the fungal populations the Shannon and Weaver diversity index (Odum, 1985) was calculated based on the replicates of each treatment. The results were statistically analysed as described above.

Results

The highest number of fungi was found on the lowest leaves of plants grown in greenhouses that had not been treated (Control Greenhouse) (Figure 2). The other treatments resulted in lower levels of fungal populations, except for leaves located in the upper part of the canopy from Commercial Greenhouse I. Leaves from the medium and lower strata of the canopy from the Control Greenhouse showed the highest diversity indexes, while upper leaves from Commercial Greenhouse II had the least diverse fungal populations (Figure 3).

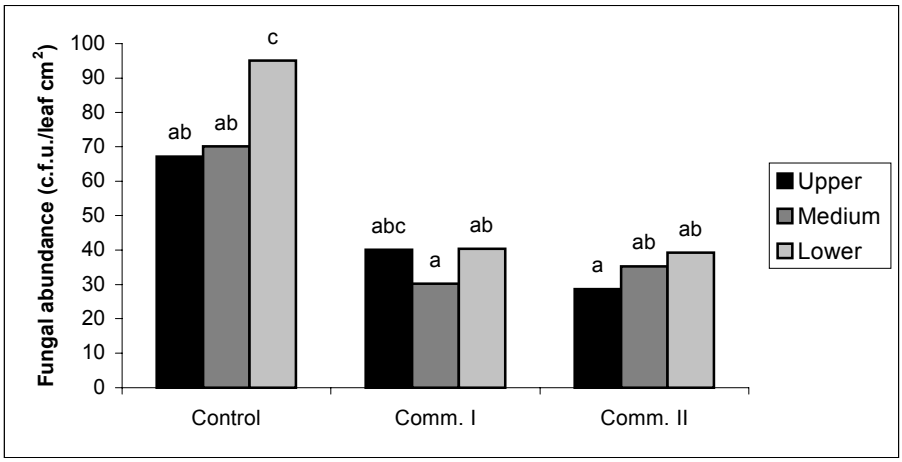

Fig. 2. Abundance of fungal populations on tomato phylloplane. Columns with the same letter do not differ significantly on the basis of Tukey´s multiple range test (P<0.05)

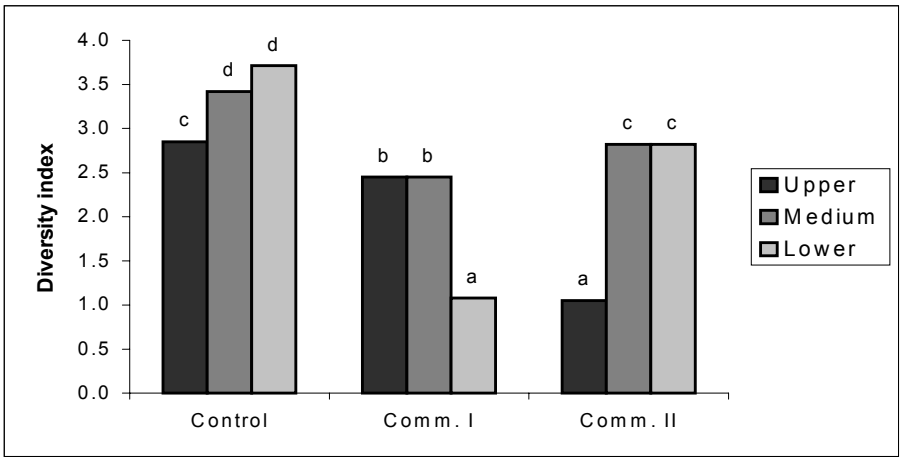

Fig. 3. Diversity of fungal populations (Shannon & Weber index) on tomato phylloplane. Columns with the same letter do not differ significantly on the basis of Tukey´s multiple range test (P<0.05)

Most of the isolated species were more abundant on tomato leaves from the Control Greenhouse and Commercial Greenhouse II (Table 1). *Cryptococcus luteolus, Rhodotorula* sp., *Arthrinium phaeospermum*, *Aspergillus* sp (1), *Trichoderma harzianum* (1)*, T. polysporum, Penicillium* sp. (1), *Alternaria alternata* and *Cladosporium cladosporioides* had a significantly greater c.f.u. number in the Control Greenhouse than in the other locations. However, the c.f.u. number for some *Penicillium* and *Aspergillus* species was greater in Commercial Greenhouse I.

Leaf position in the canopy influenced the fungal population structure. Some species, such as *Epicoccum nigrum, Chaetomium globosum, Aspergillus* sp. (1), *Trichoderma harzianum* (1)*, T. polysporum* and *Penicillium* spp., were more abundant on lower leaves, while *Alternaria alternata, Cryptococcus luteolus, Rhodotorula* sp., *Pleospora herbarum*, *Fusarium semitectum, F. oxysporum* and *Cladosporium cladosporioides* were present in greater numbers on leaves located in the upper and medium levels.

Table 1
Frequency of saprophytic fungus species on the phylloplane

Species	c.f.u./leaf cm^2 (1)								
	Upper leaves			Medium leaves			Lower leaves		
	CG I	CG II	Control	CG I	CG II	Control	CG I	CG II	Control
Alternaria alternata	0	0	10	0	1.1	2.6	2.6	0	3.6 *
Arthrinium phaeospermum	0	0	0	0	1.6	1.8	0	2.4	3.2 *
Aspergillus niger	0	0	2.6	0	2.3	3.0 *	0	3.6 *	2.7
Aspergillus sp. (1)	3.7	1.1	0	1.4	1.5	1.4	1.2	2.8	2.7
Aspergillus sp. (2)	0	0	0	0	0	0	0	0	3.6 *
Cladosporium cladosporioides	1.5	5.04	5.6	0	3.0	4.7	1.3	4.6	6.4
Cryptococcus luteolus	0	0	10.6	0	2.6	9.5 *	0	1.04	5.4 *
Chaetomium globosum	0	0	0	0	0	3.6	0	1.24	5.6 *
Epicoccum nigrum	0	0	1	0	1.2	1.9	2.4	0	3.6 *
Fusarium oxysporum	0	0	3.7	0	0.98	1.9	0	0	1.16
F. semitectum	0	6.2	0.4	1.4	0	1.9	0	0	2.7 *
Nigrospora sp.	1.4	0	0	0	0.8	1.4	0	1.6	2.7 *
Penicillium sp. (1)	28	13.25	32.6	10.2	12.3	10.4	29	13.7	20.4 *
Penicillium sp. (2)	19	12	4.6	19.1 *	9.8	19.1 *	20.4 *	14.8	17.06
Rhodotorula sp.	0	0	3.7	0	0.8	5.04 *	0	0.5	3.0
Pleospora herbarum	0	0	2.9	0	0	3.6 *	0	0	0
Trichoderma harzianum (1)	0	0	0	0	0	2.1	0	0.8	5.6 *
T. harzianum (2)	0	0	0	0	0	1	0	0	3.1 *
T. polysporum	2.6	0	2.8	5	0	3.6	0	5.8	10.2 *

(1) Values expressed in thousands; CG I: Commercial Greenhouse I, CG II: Commercial Greenhouse II.; *Values significantly greater than others in the same row according to the Tukey test ($P<0.05$).

Discussion

Both population diversity and fungal abundance were affected by leaf position in the canopy. Fluctuations in temperature and relative humidity decrease towards lower positions in the canopy (Blakeman, 1985). Andrews (1991) suggested the importance of UV light intensity in the regulation of fungal populations in the canopy. This might explain the lower number of fungi recorded on leaves in the upper part of the canopy in both the Control Greenhouse and Commercial Greenhouse II. According to Odum (1985) biodiversity is low in physically controlled ecosystems, i.e. in those subjected to strongly limited physical factors. Leaves located within the canopy might provide a less restricted environment, thus resulting in more diverse communities. Based on the Control Greenhouse and Commercial Greenhouse II, it was concluded that the fungal population was more abundant on lower leaves, while medium and upper leaves had similar fungal populations. According to Andrews (1991) the presence of abundant nutrient sources in lower leaves due to the proximity of the soil might also explain the presence of higher numbers of fungi. On the other hand, Ruinen (1961) asserted that the successional climax of phylloplane microbial populations could not be reached until the leaves reached maturity, and the lower leaves are the most mature ones on the plant. These findings regarding abundance and diversity and their association with position within the canopy, confirm the results obtained for the Control Greenhouse and Commercial Greenhouse II, but not those found for Commercial Greenhouse I, where the upper leaves had more developed fungal populations than any of the other sprayed leaves. The longer time which elapsed (30 days) from the last application in Commercial Greenhouse I might explain this phenomenon. It is important to consider both abundance and diversity when planning natural biological control (Cook and Baker, 1989). So the abundance and diversity of saprophytic fungi achieved in the Control Greenhouse might be more suitable for appropriate disease suppression.

The qualitative composition of the fungal population was also affected by the fungicide application scheme and leaf position in the canopy. Most of the yeasts and fungi found in the upper leaves belong to the naturally-occurring resident microorganisms on aerial plant surfaces listed by Blakeman and Fokkema (1982). The phyllosphere of the lower leaves is inhabited by a number of soil fungi, mainly *Trichoderma* spp. and *Chaetomium globosum*. The ubiquitous genera *Aspergillus* and *Penicillium* have a distribution profile unrelated with leaf position in the canopy. The effect of the fungicide application scheme on the saprophytic fungus composition allows a distinction to be made between fungicide-tolerant species, i.e. those that prevailed and persisted on the leaves from the commercial greenhouses, and susceptible species, which were present only in the Control Greenhouse. The results demonstrated that fungicide application led to the suppression of many potential antagonists of foliar

pathogens. Resident yeasts, described by many authors as effective biocontrol agents in the phylloplane (Suzzi et al., 1995; Fokkema and Van der Meulen, 1976) were the most affected by fungicide application. The same was valid for *Trichoderma* spp., an acknowledged biocontrol agent whose population was particularly affected by fungicide applications.

The prevalence of *Aspergillus* spp. and *Penicillium* spp. on leaves from Commercial Greenhouses I and II suggests that fungicide resistance may be the result of the selection pressure performed on both genera (Bollen, 1971). The capacity of some indigenous saprobic phylloplane fungi to develop resistance to fungicides is a desirable characteristic which could be utilised in biocontrol methods (Fokkema and Naaj, 1981; Fokkema, 1993).

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