Susceptibility of South African Cactus Pear Varieties to Four Fungi Commonly Associated With Disease Symptoms♦

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ABSTRACT

Ten of the commercially most important cultivars of spineless cactus pear (*Opuntia ficus-indica*) were screened in the glasshouse and field for their susceptibility to four fungal pathogens (*Phialocephala virens*, *Lasiodiplodia theobromae*, *Fusarium* sp1 and *Fusarium* sp2) commonly associated with cladode and fruit diseases in South Africa during surveys conducted over the last three years. The fungi were artificially inoculated by means of toothpicks that had been colonised by the respective fungi prior to being inserted into the cladode or fruit. Control treatments consisted of sterile toothpicks. One trial was conducted with detached cladodes in the glasshouse and a second trial with cladodes of mature plants in a cactus pear orchard. A trial was also conducted with mature fruit of each cultivar in the laboratory. Following inoculation, cladodes and fruit were incubated at room temperature for 14 days to allow for lesion development around the inoculation site, whereafter, the diameters of lesions that resulted from inoculation were measured. In all three trials, considerable variation among cultivars in their susceptibility to each of the four pathogens was evident. Cladode inoculations in the glasshouse and field revealed that Nudosa and Algerian generally were the two most susceptible cultivars while Gymno Carpo, Zastron, and Malta generally were the most resistant. These results were consistent with those of inoculations conducted on fruit. Control treatments in all three trials did not develop lesions around inoculation wounds. Cluster analysis grouped cultivars into two clusters with Nudosa in its own cluster and the remaining cultivars in a second cluster.

Keywords: cactus pear, diseases, fungal pathogens, *Opuntia ficus-indica* (L) Mill.

INTRODUCTION

Spineless cactus pear (*O. ficus-indica*) is an important crop in arid and semiarid regions of the world (Brutsch, 1979; Pimienta-Barrios, 1994; Russel and Felker, 1987, 1985). Despite the fact that it is a native of regions with very hot and dry climates, cactus pear harbours many fungal pathogens, which attack the moisture-rich fruit and cladodes. Apart from a few reports (Varvaro *et al*., 1993; Granata, 1995; Granata and Sidoti, 2000), surprisingly few publications pertaining to diseases of *Opuntia* spp. have appeared worldwide.

The most comprehensive reports of infectious diseases (bacterial and fungal as well as other agents) of spineless cactus pear are by Granata (1995) and Granata and Sidoti (2000). A severe blight on cladodes and fruit caused by *Alternaria alternata* (Fries: Fries) von Keissler was identified by Granata and Sidoti (1997). The natural penetration site for the pathogen seems to be lesions caused by damage, such as from hail, because the first symptoms are usually found in October, especially after heavy rains (Granata and Sidoti, 1997). Infected plants display chlorotic spots around the spines, which developed into yellow scabs characterised by a necrotic centre surrounded by a yellowish halo. A fruit rot caused mainly by

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Alternaria spp. was also reported by Chessa and Schirra (1992). Saad *et al.* (1998) described a bacterium causing wet rot and necrosis in cactus pear as *Erwinia carotovora* subsp. *carotovora* and Varvaro *et al*. (1993) also reported a wet rot caused by *Erwinia* sp. on cactus pear in Italy. Cactus pear is very vulnerable to root rot. One of the earliest reports of root disease is from Mexico where the causal agent was identified as *Fusarium solani* (Pettinari, 1951) but *Phytophtora nicotianae* has also been associated with root rot (Cacciola and Magnano, 1988). Scab caused by *Phyllosticta opuntiae* is, reportedly, one of the most aggressive and noxious diseases of *O. ficus-indica* (Barbera *et al*., 1992).

The commercial cultivation of spineless cactus for its fruit is a relatively recent undertaking in South Africa but has been shown to possess huge export potential. Until recently, only one fungal pathogen, *Didymosphaeria opulenta* (De Not.) Sacc. was reported on the entire genus *Opuntia* in South Africa. However, the report is about *Opuntia stricta* Haw., not *O. ficus-indica* (Crous *et al*., 2000). More recently, three fungal pathogens associated with diseases of cladodes of *O. ficus-indica* were reported (Swart and Kriel, 2002). The need for research on the diseases of *O. ficus-indica* in South Africa recently has become very important because local growers are increasingly reporting disease-related yield losses. The aim of the present study was to systematically evaluate susceptibility of South African cactus-pear varieties to four fungi, *Phialocephala virens* (Siegfried and Siefert, 1992), two unidentified *Fusarium* species, *Fusarium* sp1 and *Fusarium* sp2, and *Lasiodiplodia theobromae* (Pat.) Giff. and Maubl (Latham and Dozier, 1989) that regularly have been isolated from diseased cladodes obtained from various parts of the country over a period of three years.

MATERIALS AND METHODS

Cladode Inoculations

Mature cladodes from 10 commercially important cultivars of *O. ficus-indica* were obtained from Potgietersrus, Limpopo Province, South Africa (23° 50' S) and maintained at room temperature for five days prior to artificial inoculation. Single spore isolates of the four fungi were maintained on potato dextrose agar (PDA) at 5^{o}C. Wooden toothpick sections (20 mm long) were autoclaved for 20 minutes in 250 ml distilled water, removed, blotted, and re-autoclaved in additional water to remove inhibitory substances. Toothpick pieces were then cooled in a sterile petri dish and transferred individually to margins of fast-growing colonies of the four chosen fungi on potato dextrose agar (PDA). After incubation for 72 hours, infested toothpick tips were removed and used as inoculum.

Toothpick pieces were inserted up to 10-mm deep into cladodes in the top older portion of the cladode. Insertion holes were covered with masking tape to prevent desiccation and contamination. Six detached cladodes of each cultivar (replications) were inoculated with each isolate. Control treatments consisted of autoclaved toothpick tips that were inserted into cladodes as described previously. Cladodes were incubated at room temperature for 14 days before the diameter of necrotic lesions that had developed around each inoculation site was measured. In field inoculations, three mature cladodes of each cultivar (replications) were similarly inoculated with toothpicks infested by the four respective fungal isolates. The diameter of the lesion around each inoculation wound was measured and data was subjected to statistical analyses. Koch's postulates were confirmed by re-isolation of the original pathogens from artificially inoculated cladodes.

Fruit Inoculations

In the second trial, three mature fruit of each cultivar (replications) were inoculated with each isolate. To prevent accidental contamination, fruit were wiped with a cloth dipped in ethanol prior to the toothpicks being inserted. Control treatments consisted of autoclaved toothpick tips. Fruit were incubated at room temperature for 14 days before the diameter of the resulting lesion around each insertion wound was

measured and the data was subjected to statistical analyses. Koch's postulates were confirmed by re-isolation of the original pathogens from artificially inoculated fruit.

Statistical Analyses

A two-way analysis of variance (ANOVA) was performed on the data of each inoculation trial and treatment means were separated using the software program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Cluster analysis of data from all three trials, using the UPGMA method, was conducted to generate a dendogram showing relationships between cultivars with regard to their susceptibility to the four pathogens using the NCSS 2000 software (Hintze, 1998). Linear regression analyses between the treatment means of each cultivar for the three trials was performed using Agrobase 2000 (Agronomix Software Inc.).

RESULTS

Tissue decay and mycelium growth became visible at most inoculation sites on cladodes and fruit 60 hours after inoculation (Figures 3 through 8). Control treatments showed very little necrosis on either cladodes or fruit. Virulence of the four fungal pathogens to each of the 10 cultivars differed significantly ($P < 0.05$), and there also were significant interactions ($P > 0.05$) between cultivars and the four isolates (Table 1; Figures 2 through 4). The results of the three respective trials with regard to the relative susceptibility of the 10 cultivars corresponded reasonably to very well. Although correlations between detached cladode and fruit inoculations ($r = 0.692$; $P < 0.05$) and detached cladode and cladode inoculations in the field ($r = 0.727$; $P < 0.05$) were significant, a highly significant correlation ($r = 0.964$; $P < 0.01$) existed between fruit inoculations and cladode inoculations in the field.

In artificial inoculation of detached cladodes in the glasshouse, *L. theobromae* and *Fusarium* sp1 caused the largest lesions in all 10 varieties while *P. virens* generally caused the smallest lesions (Figure 2). Lesions caused by *L. theobromae* on detached cladodes varied between 14.32 mm and 32.95 mm, while those for *P. virens* varied between 3.79 mm and 15.45 mm. Nudosa was generally the most susceptible cultivar to all four pathogens followed by Algerian, Morado, and Meyers (Table 1). In field inoculations, lesions measured after 14 days were considerably smaller than for detached cladodes. *L. theobromae* was also not the most virulent isolate in field inoculations as was the case with inoculation of detached cladodes (Figure 4). *Fusarium* sp1, although causing smaller diameter lesions than in detached cladodes, was equally virulent to *L. theobromae* in field inoculations. Nudosa and Algerian did not differ significantly ($P < 0.05$) in their overall susceptibility to the four pathogens in field inoculations. They were followed by Morado, Meyers, Turpin, and Roedtan whose overall susceptibilities did not differ significantly ($P < 0.5$).

In fruit inoculations, Nudosa was overall the most susceptible cultivar to both *L. theobromae* and *Fusarium* sp1 (Figure 3). The latter isolate displayed a far higher level of virulence to most cultivars, relative to *L. theobromae*, than it did in detached-cladode inoculations. Both *Fusarium* sp1 and *P. virens* were significantly more virulent in fruit inoculations than in detached-cladode inoculations. Algerian was the most susceptible cultivar to *P. virens*. Malta, Zastron, and Gymno Carpo were the three most resistant cultivars to all four pathogens.

Dissimilarity between cultivars in the dendrogram that was generated following cluster analysis ranged from 0.50 to 2.59 (Figure 9). The dendrogram represented two clusters, cluster A and B. Cluster A comprised only one cultivar, Nudosa. Cluster B could be divided onto two groups, I and II. Group I included three cultivars, Zastron, Malta, and Gymno Carpo. Group II included the rest of the cultivars. Malta and Gymno Carpo and Turpin and Skinners Court were clustered closely together. The mean distance between group I and group II in cluster B is 0.73 and 0.91, respectively.

DISCUSSION

Results of the present study represent the first time that South African cultivars of *O. ficus-indica* have been compared with each other with regard to their susceptibility to fungal pathogens. Significant differences were observed between cultivars with regard to their susceptibility to each of the four fungal pathogens for each of the three trials. Significant cultivar-pathogen interactions for both cladode and fruit inoculations were also clearly evident. For example, in field inoculations, *Fusarium* sp1 was more virulent than *L. theobromae* on the cultivars Skinners Court, Nudosa, Morado, Malta, and Turpin, but the opposite was true for Algerian and Meyers. *L. theobromae* was generally the most virulent isolate followed by *Fusarium* sp1. *P. virens* was generally the least virulent isolate in cladode inoculations, but in fruit inoculations it caused lesions on some cultivars, especially Morado and Algerian, that were almost equivalent to *L. theobromae*.

Despite the interactions, there were, nevertheless, definite similarities between the three trials with regard to the overall relative susceptibilities of respective cultivars (Figure 1). The fact that Nudosa and Algerian were consistently the most susceptible cultivars and Malta and Zastron were the most resistant is encouraging as far as the credibility of these results are concerned. The fact that lesions in field inoculations were smaller is probably related to active host resistance of attached cladodes as opposed to detached cladodes. Another factor could be lower mean temperatures during the 14-day period from inoculation than that present in the glasshouse for the detached-cladode inoculations. Varying levels of resistance among cultivars is probably also related to some inherent physiological characteristic of a particular cultivar and the ability of a particular pathogen isolate to overcome that resistance. It is unlikely that morphological characteristics such as thickness of the epidermis could have played a role because wounds were made during artificial inoculations.

Although there are options for chemical control, the search for tolerant cultivars is the safest and most economical alternative for cactus-pear growers (Mondragon-Jacobo and Pérez-Gonzàlez, 2000). Despite the four different pathogens against which the 10 *O. ficus-indica* cultivars were screened in the present study, yielding varying results for each different cultivar, the results are nevertheless useful for the control of cactus-pear diseases in South Africa. For example, Malta and Zastron, the two most consistently resistant cultivars in all three inoculation trials, are probably also relatively resistant to other possible fungal pathogens that may be encountered in South Africa. Fortunately, Morado and Gymno Carpo, two of the most popular cultivars in South Africa, also seem to be relatively disease resistant. By the same token, Nudosa and Algerian are probably the most susceptible of the 10 cultivars and cultivation of these cultivars probably should be avoided when disease pressure is high. Therefore, based on the information provided here, it is hoped that the results of the present study will enable growers to either plant or breed cultivars that are more resistant to disease.

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Table 1. Results of Artificial Inoculation of Detached Cladodes in the Glasshouse, Mature Cladodes in the Field, and Fruit Inoculations in the Laboratory
Values within each column followed by different lowercase letters are significantly different at $P < 0.05$.

DETACHED-CLADODE INOCULATIONS

Cultivar	*P. virens*	*L. theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
Skinners Court	5.06 cb	20.03 def	11.64 b	17.60 bc	2.32	**13.58bcd**
Nudosa	15.45 a	31.04 ab	22.15 a	30.57 a	2.75	**24.80a**
Gymno Carpo	4.86 bc	21.73 de	10.88 b	10.19 ef	1.97	**11.91d**
Morado	5.12 bc	28.69 abc	8.33 b	15.53 bcde	2.51	**14.42bcd**
Zastron	3.79 c	14.32 f	10.88 b	7.57 f	2.77	**9.14d**
Malta	5.93 bc	17.39 ef	9.04 b	16.75 bc	2.28	**14.55d**
Algerian	13.93 a	27.88 abc	10.60 b	13.28 cd	2.73	**16.42bc**
Turpin	6.97 b	25.25 bcd	11.75 b	12.05 cde	2.38	**14.00bcd**
Meyers	7.22 b	24.68 cd	8.94 b	19.17 b	2.14	**14.87bcd**
Roedtan	5.46 bc	32.95 a	9.27 b	12.19 cdef	2.46	**12.46bcd**
Pathogen mean	**7.38**	**24.40**	**11.35**	**15.49**	**2.43**	

FRUIT INOCULATIONS

Cultivar	*P. virens*	*L. theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
Skinners Court	14.33 bc	21.12 b	15.82b	17.21c	2.80	**17.12 c**
Nudosa	13.00 cd	27.11 a	22.00a	28.77a	2.71	**22.72 a**
Gymno Carpo	2.98 f	14.56 cd	8.00c	8.79d	2.61	**8.295 d**
Morado	16.20 b	19.24 bc	19.92a	20.46c	3.00	**18.96 b**
Zastron	4.41 f	13.30 d	8.61c	7.00d	2.36	**8.33 d**
Malta	7.70 e	9.51 d	7.92c	8.91d	3.60	**8.51 d**
Algerian	27.00 a	27.55 a	15.66b	24.78b	4.10	**23.99 a**
Turpin	12.17 cd	18.18 bcd	15.30b	19.23c	2.00	**16.22 bc**
Meyers	11.14 d	29.92 a	15.63b	18.74c	3.20	**19.42 b**
Roedtan	14.00 bc	20.00 b	16.02b	18.82c	3.00	**17.21 bc**
Pathogen mean	**12.29**	**20.05**	**14.49**	**17.27**	**2.92**	

FIELD INOCULATIONS

Cultivar	*P. virens*	*L. theobromae*	*Fusarium* sp2	*Fusarium* sp1	Control	CV mean
Skinners Court	4.36 c	11.33 abc	9.8 c	13.17 b	2.12	**9.66cdefg**
Nudosa	4.4 c	16.8 a	11 b	18.21 a	2.53	**12.6efg**
Gymno Carpo	5.12 ab	9 f	3 e	8.3 c	2.4	**6.36a**
Morado	6.24 a	10.34 bc	8.7 d	11.94 b	2.96	**9.3cdef**
Zastron	3.22 d	8.14 ef	4.3 e	7 c	2.31	**5.67ab**
Malta	4.81 b	7 e	5.51 f	8.48 c	3.06	**6.45b**
Algerian	5.76 a	19.51 cd	8.32 a	18 a	3.3	**12.89cde**
Turpin	4.73 b	9.91 d	8 d	12.37 b	2.12	**8.75cde**
Meyers	4.71 b	18.9 ab	9.9 abc	13.91 b	2.11	**11.88cdefg**
Roedtan	4.51 c	13.96 a	10.81c	13.42 b	2.25	**10.68defg**
Pathogen mean	**4.78**	**12.49**	**7.94**	**12.48**	**25.16**	

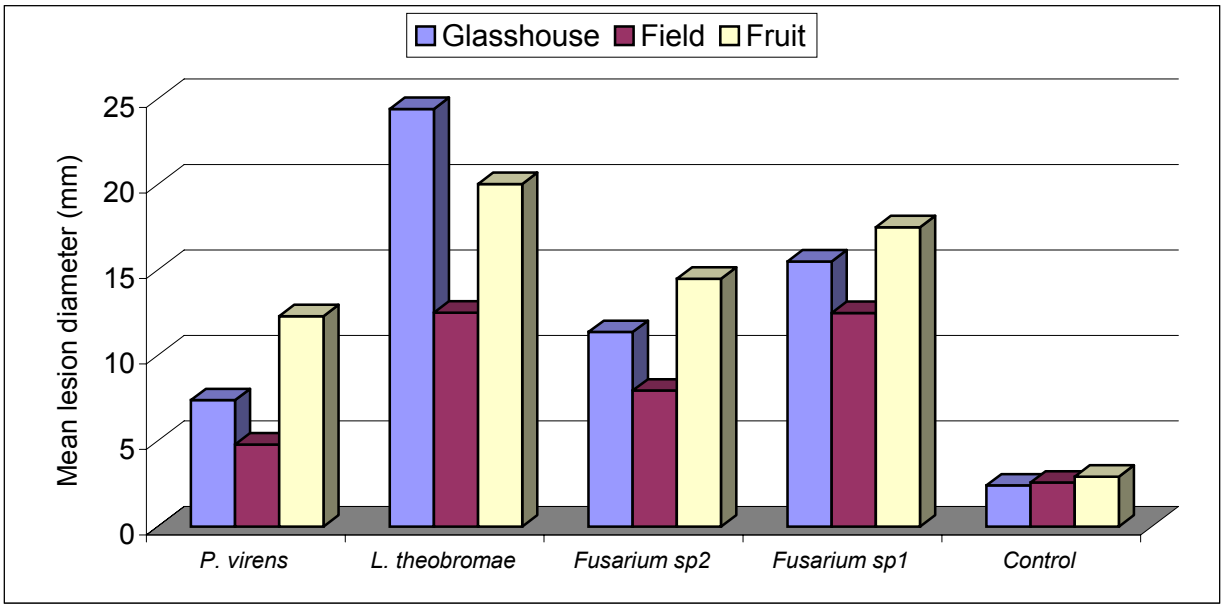

Figure 1 Mean Lesion Diameters on Cladodes Following Artificial Inoculations in the Glasshouse and Field and Fruit Inoculations in the Laboratory

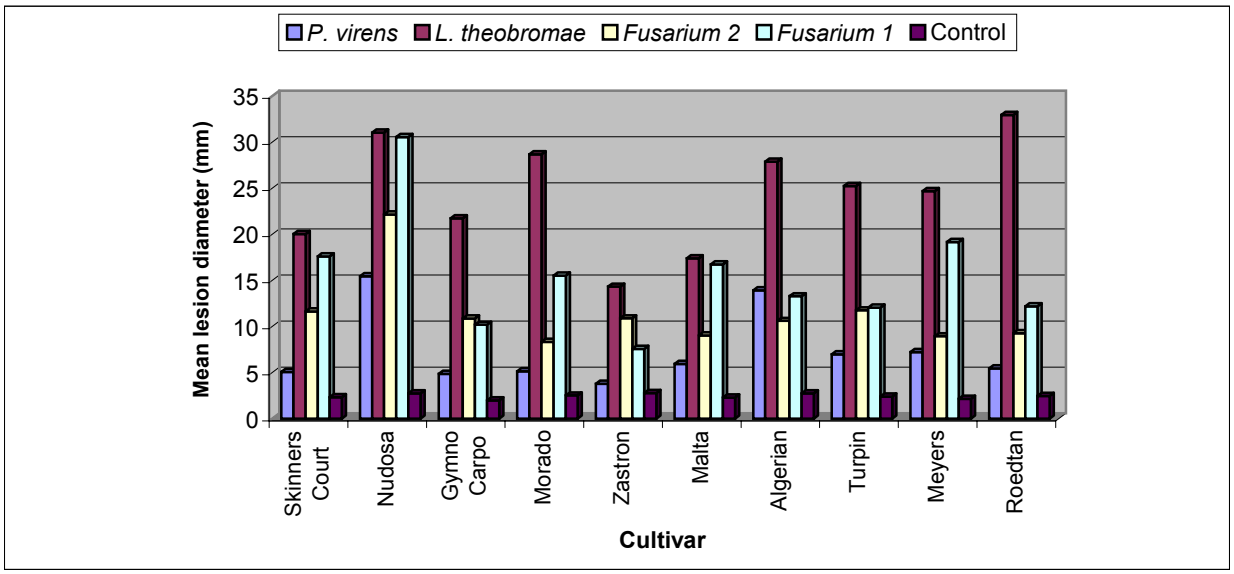

Figure 2 Results of Artificial Inoculations in the Glasshouse on Detached Cladodes of 10 *O. ficus-indica* Cultivars With Four Fungal Pathogens

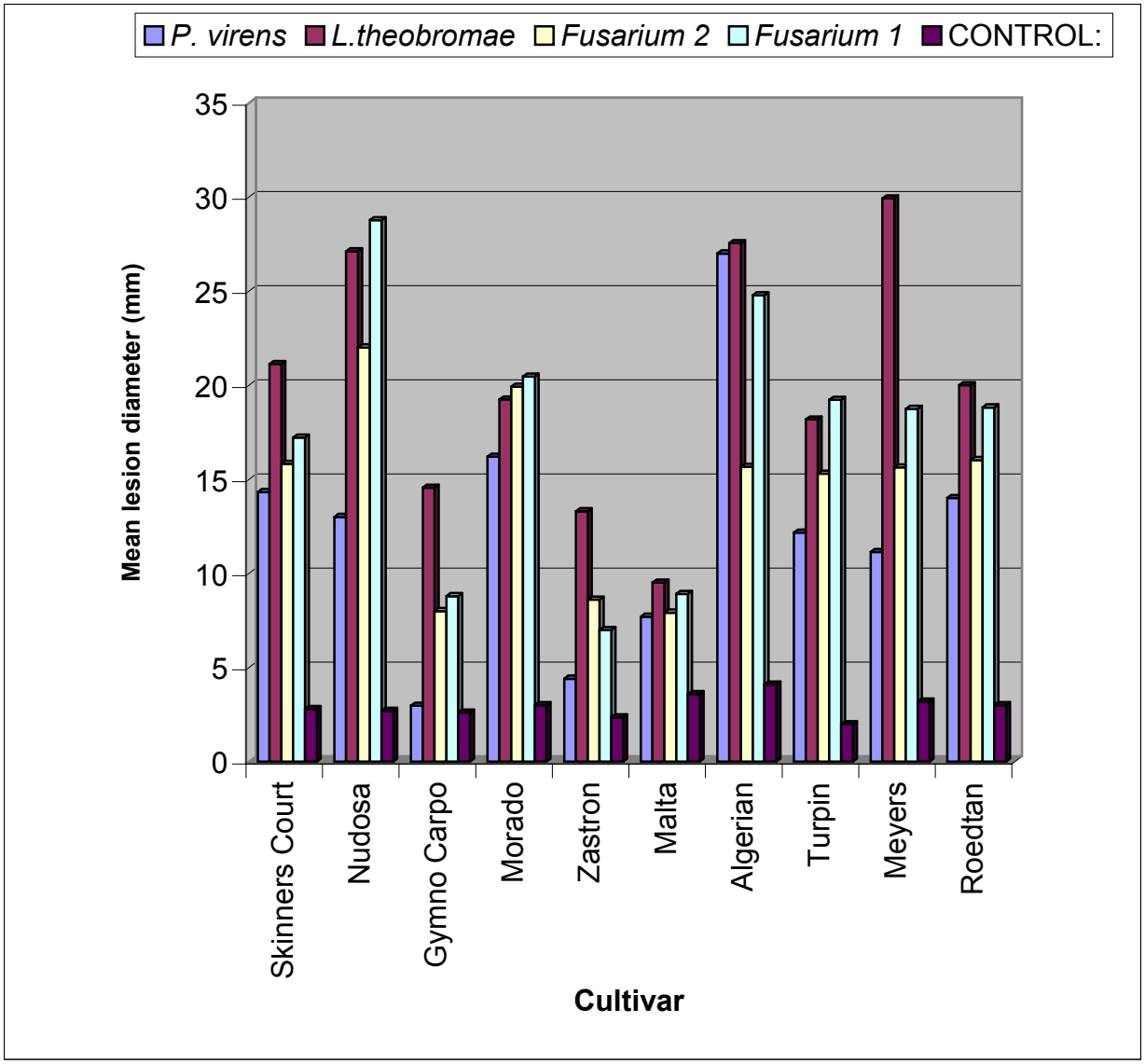

Figure 3 Results of Artificial Inoculations in the Laboratory on Fruit of 10 *O. ficus-indica* Cultivars With Four Fungal Pathogens

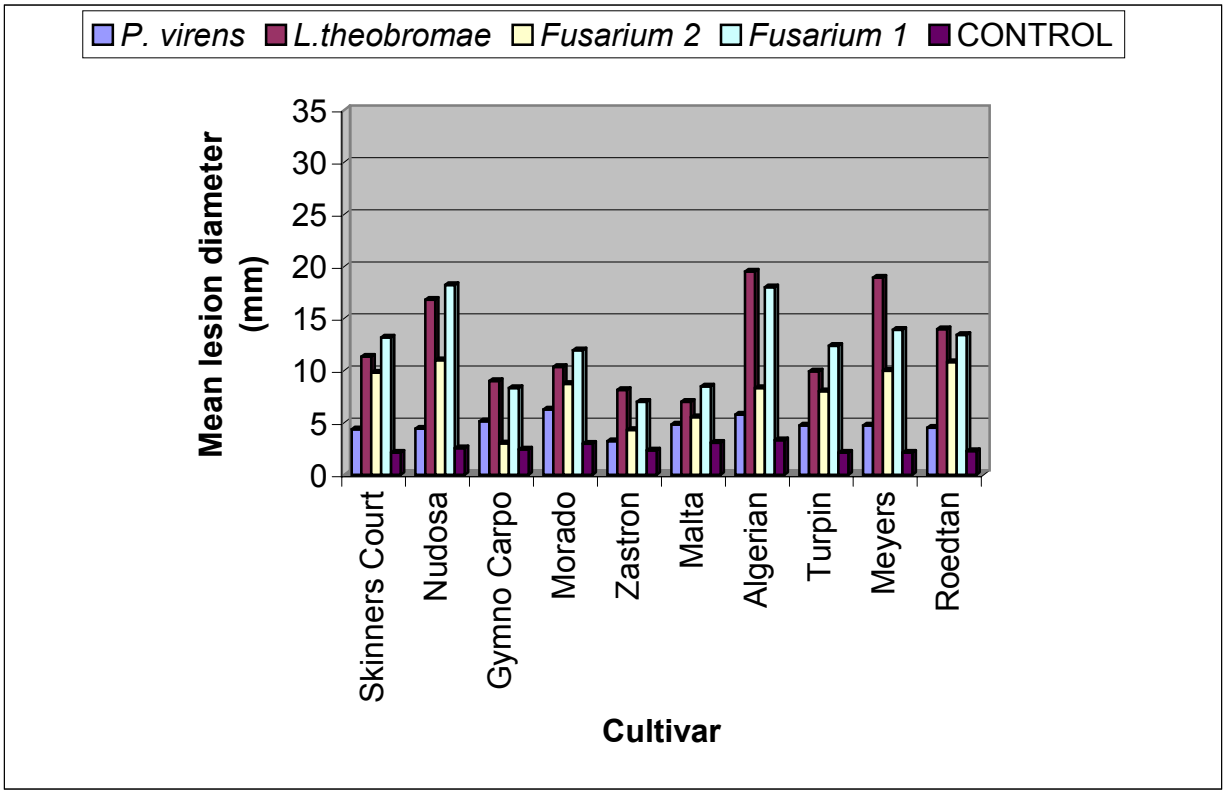

Figure 4 Results of Artificial Inoculations in the Field on Cladodes of 10 *O. ficus-indica* Cultivars With Four Fungal Pathogens

Figure 5 Detached Cladodes of *O. ficus-indica* Inoculated With Toothpick Colonised by Four Fungal Pathogens

Figure 6 Lesion Caused by the Toothpick-Inoculation Technique on a Detached Cladode. Arrow indicates the diameter of the lesion.

Figure 7 Fruit Inoculations Using the Toothpick Technique

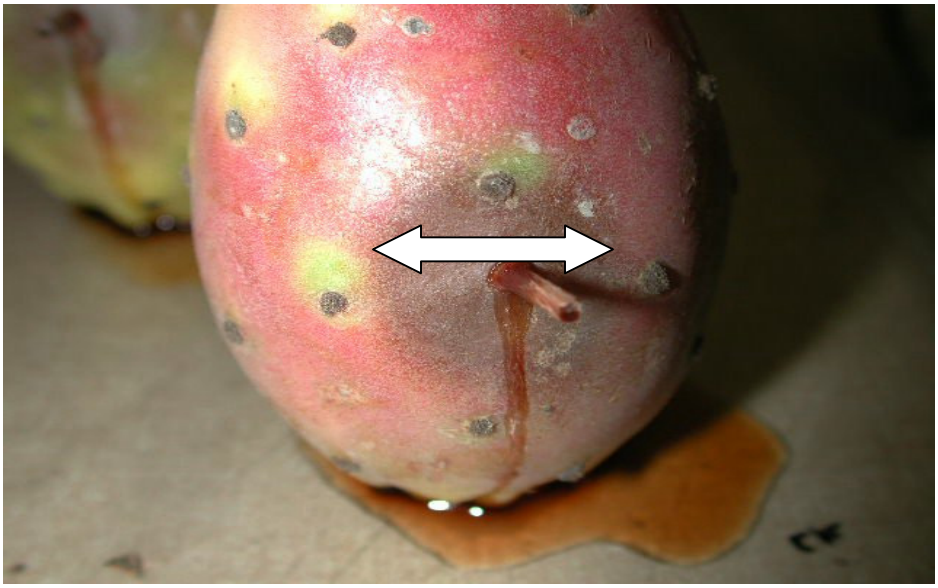

Figure 8 Artificial Inoculation of Cactus-Pear Fruit Using the Toothpick Technique
Arrow indicates diameter of the resulting lesion.

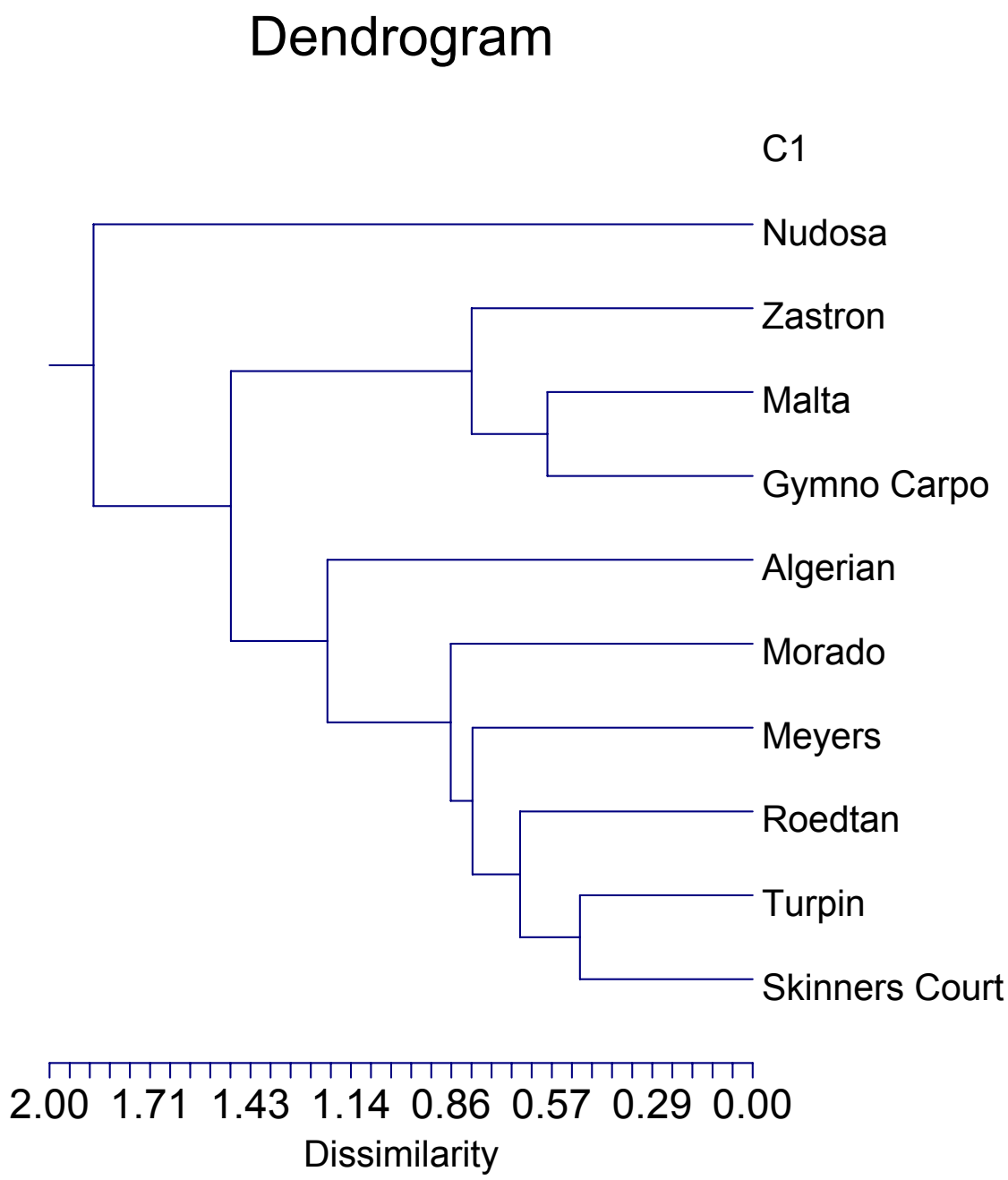

Figure 9 Dendrogram Generated by UPGMA Analysis of the Combined Data Collected From All Three Inoculation Trials