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Symptomatology, incidence and distribution of *Verticillium* wilt of Olive trees in Andalucía

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Summary - Verticillium wilt (VW) of olive trees, caused by *Verticillium dahliae* kleb., was found in 47 of 122 orchards with about 350.000 trees surveyed in Andalucía, southern Spain, in 1980, 1981 and 1983. The disease occurred in four of seven provinces surveyed, being more prevalent in Córdoba and Jaén than in Sevilla and Granada. Disease incidence and severity was higher in irrigated orchards and also in orchards previously cropped or actually intercropped to cotton or vegetables.

The disease syndromes, namely apoplexy and slow decline, were observed in two experimental plots at the INIA Research Station, Córdoba, where VW-affected trees had been found previously. Apoplexy, wich develops by late winter to early spring, was characterized by a quick dieback of twigs and branches or death of the entire tree. Slow decline was characterized by leaf clorosis and necrosis, necrosis of flower clusters and dieback of twigs, and developed gradually from late spring to early summer. Incidence of apoplexy was higher in trees affected the year before. Overall disease incidence and severity decreased over the years indicating that trees recovered from disease.

Resumen - SINTOMATOLOGIA, INCIDENCIA Y DISTRIBUCION DE LA VERTICILOSIS DE OLIVO EN ANDALUCIA. La Verticilosis del olivo (VO), causada por *Verticillium dahliae* Kleb., fue diagnosticada en 47 de 122 olivares, con alrededor de 350.000 árboles, que fueron inspeccionados en Andalucía en 1981 y 1983. La enfermedad occurrió en cuatro de siete provincias prospectadas, siendo más prevalente en Córdoba y Jaén que en Granada y Sevilla. La incidencia y distribución de la VO fue mayor en olivares de regadío que en los de secano, y en olivares que siguieron a, o estuvieron cultivados intercalarmente de, algodonero o plantas hortícolas.

Dos síndromes, denominados apoplejía y decaimento lento, fueron observados en dos parcelas experimentales del CRIDA-10, INIA, Córdoba, en las que se habian encontrado previamente árboles afectados de VO. La apoplejía se caracterizó per la muerte rápida de brotes y ramas, o del árbol completo, ocurriendo al final del invierno o principio de primavera. El decaimiento lento caracterizado por la clorosis y necrosis de hojas, la necrosis de inflorescencia, y la muerte regresiva de brotes, ocurriò gradualmente desde final de primavera hasta principio del verano. La incidencia de apoplejía fue mayor en los árboles afectados de VO el año anterior, y en general, la incidencia y severidad de la enfermedad disminuyó año tras año, indicando que los árboles se recuperaron de aquélla.

Introduction

Olive (*Olea europaea* L.) is the most important oil crop in Spain with an area around 2,000,000 ha, of which more than 1,000,000, are grown in Andalucía, Southern Spain (Anonymous, 1981). In 1975, symptoms resembling Verticillium wilt (VW) were first observed affecting 4 to 5 year-old olive trees in experimental plots at the INIA Experiment Station at Córdoba. Those plots were established on land which was cropped to cotton previously and irrigated regularly. To our knowledge, VW of olive trees has not been hitherto reported in Spain and information on its relative importance and distribution is lacking. Consequently, we carried out investigations on the prevalence and incidence of the disease in olive orchards in Andalucía. A summary of part of the results reported here has been published elsewhere (Caballero *et al.*, 1980).

VW of olive trees, caused by *Verticillium dahliae* kleb., was first reported in Italy (Ruggieri, 1946) and shortly after it was recorded in California (Snyder *et al.*, 1950). Later it was reported in Greece (Demetriades *et al.*, 1958, Zachos, 1963), Arizona (Boyle, 1963), Turkey (Saydam and Copcu, 1972) and France (Vigoroux, 1975), and now it is considered the most serious disease affecting the crop (Cirulli, 1981; Thanassoulopoulos *et al.*, 1979; Wilhelm *et al.*, 1962; Zachos, 1963).

The symptomatology in VW-affected olive trees has been described in detail by several authors (Cirulli, 1975, 1981; Cirulli and Montemurro, 1976; Ruggieri, 1946; Vigouroux, 1975; Wilhelm and Taylor, 1965; Zachos, 1963). It has been considered not as characteristic as that caused by the same pathogen in vegetables (Thanassoulopoulos and Kistos, 1972), and thus the possibility exists of confusing the disease with

TABLE I. - Disease progress in two plots with Verticillium wilt-affected olive trees at the INIA Experiment Station, Cordoba. (*a*).
TABLA I. - *Desarrollo de la verticilosis del Olivo en dos plantaciones de la Estación Experimental del INIA en Córdoba.*

Plot	Date of observation	Affected trees		Incidence and severity of disease syndromes (*b*)					
				Apoplexy		Slow decline		Apoplexy and slow decline	
		No.	%	Number of trees	Mean severity	Number of trees	Mean severity	Number of trees	Mean severity
1	October 1980	42	7.4	—	—	—	—	—	—
	June 1981	53	9.4	18	2.7	32	1.6	3	2.7
	September 1981	53	9.4	18	2.7	32	2.0	3	3.2
	June 1982	29	5.1	12	1.7	14	2.0	3	2.3
	June 1983	23	4.1	13	1.7	8	1.3	2	2.0
2	December 1980	24	3.6	—	—	—	—	—	—
	June 1981	19	2.8	15	2.2	2	3.0	2	2.8
	August 1981	19	2.8	15	2.2	2	3.0	2	2.8
	June 1982	15	2.2	7	1.6	6	2.1	2	2.8
	June 1983	13	1.9	9	1.1	2	2.8	2	1.8

(*a*) Based on observations on 566 trees in plot 1 and 673 trees in plot 2.
(*b*) Disease severity in affected trees was estimated using a 0-4 index (Thanassoulopoulos *et al.*, 1979).

symptoms of other diseases, mineral disorders or attacks by insects (Thanassoulopoulos and Kistos, 1972; Thanassoulopoulos *et al.*, 1979; Zachos, 1963). Dieback of twigs and branches develops in affected trees and necrosis may extend to the entire crown. Trees as old as 50 to 100 years may be affected, but usually symptoms are much more striking and severe in young trees which die suddenly. Old trees may recover from the disease (Cirulli and Montemurro, 1976; Thanassoulopoulos *et al.*, 1979; Wilhelm and Taylor, 1965) giving rise to fluctuation on disease incidence over the years (Vigoroux, 1975; Thanassoulopoulos *et al.*, 1979). The disease is more prevalent and severe in irrigated orchards (Cirulli, 1981) and especially in those previously planted or intercropped to cotton or vegetables (Cirulli and Montemurro, 1976; Thanassoulopoulos *et al.*, 1979; Wilhelm and Taylor, 1965; Zachos, 1963).

Materials and methods

Disease incidence and distribution. Surveys for VW of olive trees were carried out in the main olive-growing areas of Córdoba, Jaén and Sevilla provinces in May 1980 and June-July 1981, and of Cádiz, Granada, Huelva and Málaga provinces in June 1983 (Fig. 1). Twenty two orchards were surveyed in 1980, including 153 ha and about 25,000 trees with age ranging from less than 10 years in 18 orchards to 25-40 years in four of them. Those orchards had been called to our attention by farmers and Extension Agents as suspected of attacks. In 1981 surveys were systematic including 71 orchards with 1,445 ha and about 300,000 trees. Orchards were choosen at random along itineraries designed according to the extent of olive-growing in the area surveyed. None of them had been examined in 1980. All trees were less than 15-year-old, but in two orchards which had trees up to 30 years. Surveys were also systematic in 1983. They included 29 orchards with 195 ha and 26.000 trees, their ages ranging, from 4 to 15 years.

Orchards were examined by walking through them in an X pattern. Data were recorded on cultural practices, cultivar used and visible symptoms. Disease incidence was estimated in three groups of at least 50 trees each. In most surveys, samples of affected twigs and branches were obtained from several symptomatic trees in every orchard examined. Pieces of sampled tissues were washed in running tap water, peeled off the bark and surface desinfested in 0.5% sodium hypochlorite for 1 to 3 min. Chips cut of wood or entire cross sections were placed onto water agar or potato-dextrose agar (PDA). Plates were incubated at 24°C in the dark for 10 days.

Pathogenicity of an isolate of *V. dahliae* obtained from an VW-affected olive tree was tested on 10, 1-year-old 'Manzanillo' and 'Oblonga' trees propagated as rooted cuttings. The trees were grown in non-sterile soil mixture in 20-cm-diameter plastic pots. The plants were inoculated with a suspension containing 10^7 conidia/ml, by puncturing the main and secondary twigs with a sterile hypodermic syringe. Conidia were obtained from 7-day-old cultures on PDA slants held at 24°C, which were flooded with sterile distilled water. Six trees of each cultivar served as controls. They were treated as inoculated trees except without inoculum. After inoculation plants were kept in a growth chamber adjusted to 18-24°C and with a 14-h photoperiod of fluorescent light of 18,000 lux. One month after

Fig. 1. - Distribution of olive fields surveyed in Andalucia for occurence of Verticillium wilt during the years 1980 (○), 1981 (●), and 1983 (△).
Fig. 1. - Distribución de Olivares inspeccionados en Andalucía durante los años 1980 (○), 1981 (●), y 1983 (△).

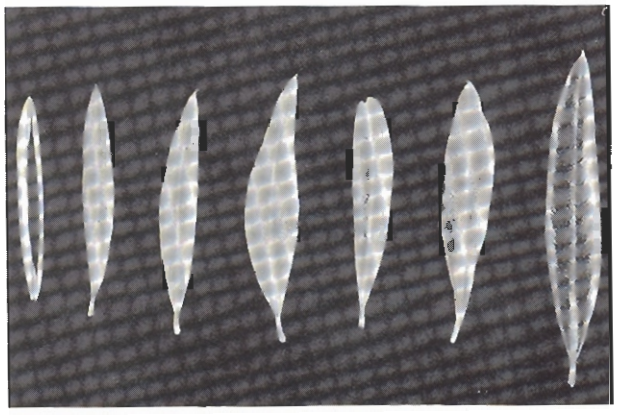

Fig. 2. - Symptomatic leaves from olive tree affected by apoplexy compared with a leaf (right) without symptoms.
Fig. 2. - Evolución sintomatológica en hojas de olivos afectados de apoplejía. A la derecha hoja asintomática.

puncture-inoculation, prior to any visible symptoms, a mixture of mycelia and microsclerotia in PDA of same isolate was added to soil in half of the pots. Control plants were observed daily for the development of symptoms and isolations were made from every plant at the end of the experiment.

Disease symptoms and progress. Detailed observations were made in two experimental plots (plots 1 and 2) at the INIA Experiment Station, Córdoba, with 566 and 673 7-year-old 'Picual' trees, respectively. Trees in plot 2 were drip-irrigated. We had previously isolated *V. dahliae* from some VW-affected trees in those plots. Symptomatic trees were labelled at the initiation of and throughout the study. All trees were examined carefully for symptoms of the disease and isolations were made from affected twigs and branches. Observations were made at variable intervals, 23 times from October 1980 throughout September 1981, and 12 times from January throughout July in 1982 and 1983, in plot 1, and 12 times in each of same periods in plot 2. Disease severity in the affected trees was

TABLE II. - Incidence of apoplexy (A) and slow decline (SD) in Verticillium wilt-affected trees in two plots at the INIA Experiment Station, Córdoba. (*a*)
TABLA II. - *Incidencia de síndromes de apoplejía (A) y decaimiento lento (SD) en dos plantaciones de Olivar de la Estación Experimental del INIA en Córdoba.*

Plot and number of trees affected at given dates (*b*)	Number of trees affected by disease syndromes at given dates															
	June 1981						June 1982					June 1983				
		Affected					Affected					Affected				
		Healthy	Total	A	SD	A+SD	Healthy	Total	A	SD	A+SD	Healthy	Total	A	SD	A+SD
1 October 1980																
Healthy	524	493	31	9	22	—	—	—	—	—	—	—	—	—	—	—
Affected	42	20	22	9	10	3	—	—	—	—	—	—	—	—	—	—
June 1981																
Healthy	513	—	—	—	—	—	498	15	2	10	3	—	—	—	—	—
Affected	53	—	—	—	—	—	39	14	10	4	—	—	—	—	—	—
June 1982																
Healthy	537	—	—	—	—	—	—	—	—	—	—	530	7	3	3	1
Affected	29	—	—	—	—	—	—	—	—	—	—	13	16	10	5	1
2 December 1980																
Healthy	649	642	7	5	1	1	—	—	—	—	—	—	—	—	—	—
Affected	24	12	12	10	1	1	—	—	—	—	—	—	—	—	—	—
June 1981																
Healthy	654	—	—	—	—	—	645	9	2	5	2	—	—	—	—	—
Affected	19	—	—	—	—	—	13	6	5	1	—	—	—	—	—	—
June 1982																
Healthy	658	—	—	—	—	—	—	—	—	—	—	655	3	2	—	1
Affected	15	—	—	—	—	—	—	—	—	—	—	5	10	7	2	1

(*a*) Affected trees were labelled at initiation of the study in October 1980. Afterwards all trees in the two plots were observed for symptoms of the disease at variable intervals.
(*b* Includes trees affected by apoplexy or slow decline.

estimated according to the index used by Thanassoulopoulos *et al.* (1979). Weather during the study was monitored with a hygrothermograph and a pluviometer placed near the experimental plots.

Results

Disease symptoms and progress. Two disease syndromes were observed in VW-affected olive trees, namely apoplexy and slow decline.

The apoplexy syndrome was characterized by a quick dieback of twigs, main and secondary branches, or death of entire tree, the bark of which became purplish during development of necrosis. In same cases, a distinct vascular discoloration occurred in affected branches. Initially, leaves on affected branches lost their deep green color, turning light brown rolling inward to the midrib of lower side (Fig. 2). Necrotic leaves remained firmly attached to affected branches. Apoplexy developed throughout February in 1981, from the end of March to middle of April in 1982, and from the end of January to middle of February in 1983.

Necrosis of flower clusters was the most conspicuous symptom in slow decline-affected trees. Affected flowers momified and persisted or abscised if they were affected early in the flowering period (Fig. 3). Leaves in affected twigs became dull green and usually fell down before dryng up completely, but those at the growing tip remained attached and dry up finally (Fig. 3, 4). Most frequently, symptoms in flower clusters and leaves developed at same time, although in some cases flower necrosis appeared earlier than leaf symptoms. Dieback of twigs followed flower and leaf necrosis, with a brown discoloration usually but not always present in vascular tissues of the non-necrotic part of twigs. In the 3 years of study, symptoms of slow decline were first observed by the end of April, and they continue to develop gradually during the spring and early summer. This contrasts with the development of apoplexy which did not continue after the death of affected branches. Occasionally, some trees were affected sequentially by the two syndromes, which were clearly separated in time (Fig. 5, Table I).

Disease incidence and severity in plots 1 and 2

Fig. 3. - Necrosis of flower clusters in olive tree affected by slow decline.
Fig. 3. - Necrosis de inflorescencias en olivos con decaimiento lento.

decreased steadly throughout the years of study (Table I). Each year, disease incidence was higher in trees already affected the year before than in those that were healthy (Table II). This occurred for both disease syndromes, but in particular for apoplexy. Thus, in 1981 apoplexy developed in 28.6% and 45.8% out of 42 and 24 trees that were affected in plot 1 and 2, respectively, in October 1980. On the other hand, in same plots, only 1.7% and 0.9% out of 524 and 649 trees healthy in October 1980 were affected by apoplexy in June 1981. A similar trend occurred in both plots in 1982 and 1983.

Disease incidence and distribution. Dieback of twigs and branches was the main symptom observed in affected trees. Only in very few cases were dead trees found during the surveys. *V. dahliae* was the only microorganism consistently isolated from affected twigs and branches. The percentage of positive isolations varied widely depending upon cultivar, tissue and date of sampling. In artificial inoculation symptoms developed in 'Manzanillo' but not in 'Oblonga' trees. All inoculated trees showed leaf symptoms by 35 days after puncture-inoculation, and four of them were partially defoliated 15 days later. Inoculated shoots showed severe growth reduction By 70 days after puncture-inoculation. *V. dahliae* was reisolated from all 'Manzanillo' trees and from one 'Oblonga' tree, in 25% and 1.1%, respectively, of sampled tissue. Neither symptoms nor positive isolations, occurred in control trees.

Verticillium wilt of olive trees occurred in 47 of 122 orchards, including about 350.000 trees, which were surveyed in seven olive-growing provinces of Andalucia in 1980, 1981 and 1983. Data on disease occurrence are

Fig. 4. - Flower necrosis and partial defoliation in twigs affected by Verticillium wilt. Leaves near the growing tip remain attached.
Fig. 4. - Necrosis de inflorescencias y defoliación parcial en tallos de olivos afectados con decaimiento lento.

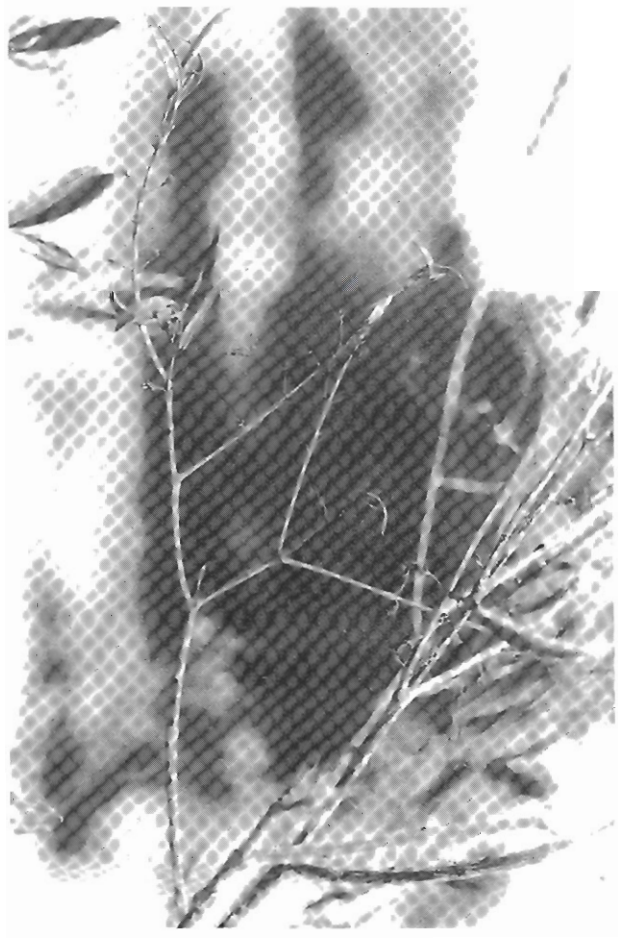

TABLE III. - Distribution and incidence of verticillium wilt (VW) of Olive trees in Andalucia.
TABLA III. - *Incidencia y distribución de la verticilosis del Olivo en Andalucía.*

Characteristics of surveyed orchards	1980 Survey			1981 Survey				1983 Survey		
	Number of orchards		Mean	Number of orchards				Number of orchards		Mean
	Sampled (%)	VW present	incidence	Sampled	VW present	Mean incidence Trace-1 (%)	Mean incidence 1-10 (%)	Sampled	VW present	incidence (%)
Province										
Cadiz	—	—	—	—	—	—	—	1	0	—
Cordoba	15	15	29.7	29	12	7	5	—	—	—
Granada	—	—	—	—	—	—	—	10	3	9.5
Jaen	2	2	70.0	19	7	5	2	—	—	—
Huelva	—	—	—	—	—	—	—	12	0	—
Malaga	—	—	—	—	—	—	—	6	0	—
Sevilla	5	5	9.4	23	3	2	1	—	—	—
Cultivar										
Hojiblanco	—	—	—	16	5	2	3	8	2	12.0
Manzanillo	4	4	5.5	17	4	3	1	2	0	—
Picual	16	16	36.4	35	13	9	4	7	1	1.0
Verdial de Huevar	—	—	—	—	—	—	—	8	0	—
Others	2	2	13.7	3	0	—	—	4	0	—
Irrigation										
Irrigated	11	11	40.2	17	12	9	3	4	3	9.5
Unirrigated	11	11	17.3	54	10	6	4	25	0	—
Previous or interplanted crops										
Cotton, vegetables	13	13	40.3	16	15	9	6	3	3	9.5
Others	9	9	10.6	55	7	5	2	26	0	—

(a) Orchards examined in 1981 and 1983 were randomly choosen along predetermined itineraries. Only orchards suspected of attacks examined in 1980. Disease incidence in each orchard was on counts of affected trees in three growps with at least 50 trees each.

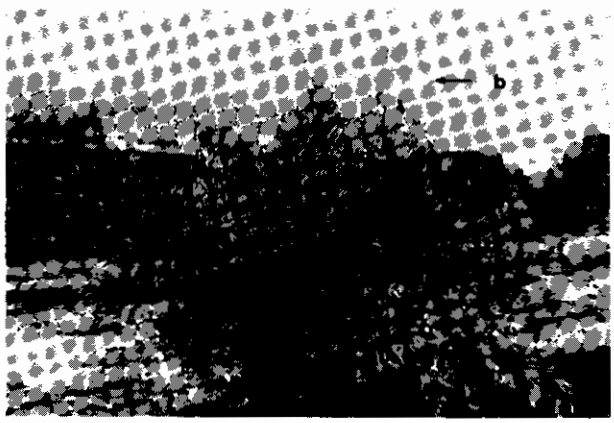

Fig. 5. - Two symptoms in a olive tree: apoplexy (a) and slow decline (b).
Fig. 5. - Arbol enfermo manifestando los dos síndomes: apoplejía (a) y decaimiento lento (b).

summarized in Table III. The disease occurred in four provinces, being more prevalent in Córdoba and Jaén than in Granada and Sevilla. In 1980 VW was found in all orchards called to our attention, with incidence ranging from traces to 100%. In the 1981 survey VW occurred in 31% of 71 orchards surveyed in Córdoba, Jaén and Sevilla, with incidence 10% or less. In some few cases, affected trees were in patches and disease incidence in them ranged to 90%.

Disease prevalence and incidence was higher in irrigated orchards, and also in orchards established on land previously cropped or actually intercropped to cotton or vegetables (Table III). As indicated by disease prevalence and incidence in surveyed orchards, the cultivars 'Hojiblanco' and 'Picual' seem to be more susceptible to VW than 'Manzanillo'.

Discussion

VW of olives, caused by *V. dahliae*, had been observed previously in Andalucia (Blanco-López *et al.*, un-

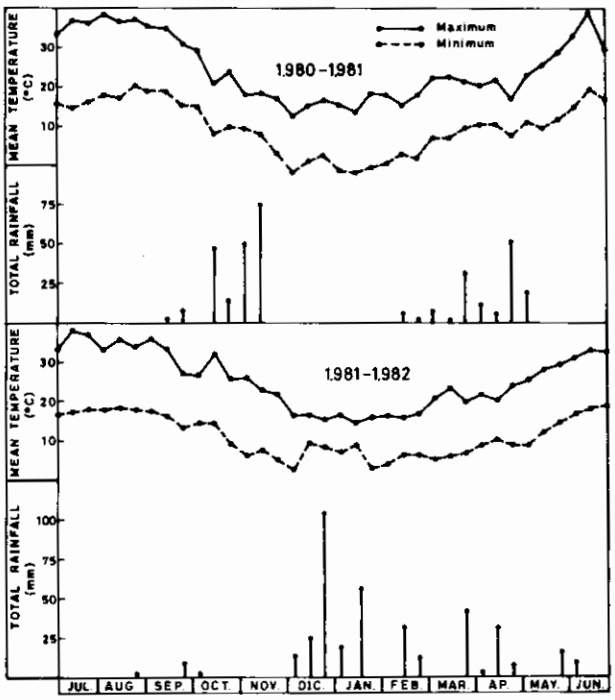

Fig. 6. - Distribution of maximum and minimum temperatures (averages) and rainfall accumulated at INIA Experiment Station, Córdoba, for the yars 1980-1982. Each point represents a ten-day period.
Fig. 6. - Distribución de temperaturas medias de máximas y medias de mínimas y precipitación acumulada durante los años 1980-1982. Cada punto representa la media (temperatura) o suma (precipitación) de un período de dies díaz.

published; Caballero *et al.*, 1980). However, this is the first detailed account of the disease both in Andalucia and Spain. The disease seems to be fairly distributed in the main olive-growing provinces of Andalucía. Data on disease prevalence from systematic surveys in 1981, and on disease incidence from non-systematic surveys in 1980, (Table III), indicate that VW is an important disease of olives in Andalucía, as it is in other olive-growing mediterranean countries (Cirulli, 1981; Thanassoulopoulos *et al.*, 1979).

Results from our disease surveys (Table III) also showed that VW is more severe in irrigated olive orchards, as well as in those planted or intercropped to cotton or vegetables. Those results agree with reports by others (Cirulli, 1981; Cirulli and Montemurro, 1976; Thanassoulopoulos *et al.*, 1979; Wilhelm and Taylor, 1965; Zachos, 1963) and might be of concern in Andalucía, where new plantings are being favoured, since crops susceptible to *V. dahliae* such as cotton, safflower and sunflower are well established and infections by the pathogen occur widespread (Blanco-López, unpublished; Jiménez-Díaz *et al.*, 1980).

Detailed observations on symptom-development in young trees of experimental plots pointed out the occurrence of two disease syndromes differing in severity. The first, a quick decline or apoplexy which develops in late winter or early spring mainly in trees mildly affected the year before, seems to correspond with the apoplexy and the acute decline described by Zachos (1963) in Greece and Cirulli (1981) in Italy, respectively. The second, a slow decline which develops during spring and early summer, resembles the reported gradual desiccation (Zachos, 1963) and chronic decline (Cirulli, 1981). The occurrence of apoplexy in commercial orchards was confirmed in 1982. Eight out of 10 orchards with VW-affected trees in June 1981 were found with apoplexy when examined in April 1982.

Our observations on disease progress in two esperimental plots showed a reduction in disease incidence over the 3 years of study (Table I, II). That reduction resulted from natural recovery of infected trees (Table II), as reported by Thanassoulopoulos *et al.* (1979) and Wilhelm and Taylor (1965), as well as from a decrease of disease incidence in healthy trees (Table II). This seems to be consistent with findings by other authors which pointed out the remarkable influence of weather conditions on progress of VW (Vigouroux, 1975; Thanassoulopoulos *et al.*, 1979). Weather records in our experimental plots (Fig. 6) showed that fluctuations in temperature and precipitation occurred during the years of study.

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