

MANGANESE AND BRITTLE LEAF DISEASE OF DATE PALM TREES

A. Namsi¹, M. Montarone², P. Serra³, O. Ben Mahamoud¹, M.L. Takrouni¹, A. Zouba¹,
O. Khoualdia¹, J.M. Bové⁴ and N. Duran-Vila³

¹Institut National de la Recherche Agronomique de Tunisie (INRAT), rue Hedi Karra, 2049 Ariana, Tunisie and Centre de Recherches Phoenicoles, 2260 Degache, Tunisia

²Unité de Recherches Intégrées (URIH), Institut National de la Recherche Agronomique (INRA), Centre d'Antibes, Route des Colles, 06410 Biot, France

³Departamento de Protección Vegetal y Biotecnología. Instituto Valenciano de Investigaciones Agrarias (IVIA, Apartado Oficial, 46113 Moncada (Valencia), Spain

⁴Laboratoire de Biologie Cellulaire et Moléculaire, IBVM, Institut National de la Recherche Agronomique (INRA) and University of Bordeaux 2, Centre INRA de Bordeaux, BP 81, 33883 Villenave d'Ornon cedex, France

SUMMARY

Five oases affected by “brittle leaf disease”, or “maladie des feuilles cassantes” (MFC) of date palms, and one healthy oasis were chosen in the southern part of Tunisia for mineral analyses of leaflets from adult and juvenile palms, as well as of the soils surrounding the palm trees. In the MFC-affected oases, the following trees were selected: apparently healthy trees (AH), and trees with mild (DS1), moderate (DS2), and severe (DS3) symptoms. Healthy trees (H) were selected in the healthy oasis. Groups of three trees of each category were selected, and the soils from the three trees of each group were mixed. Manganese was the only element for which significant differences were seen between adult leaflets from healthy trees and those from MFC-affected trees. The adult leaflets from the healthy trees (H) had the highest Mn content whereas leaflets from the symptomatic trees DS1, DS2, and DS3 had the lowest Mn contents with values significantly smaller than those for leaflets from H and AH trees. Most trees were growing in soils with a content of exchangeable Mn of around 3 mg/kg or lower, regardless of their disease status. However, the trees of one MFC-affected oasis were growing in soils with a content of exchangeable Mn of around 4.5 mg/kg or higher, regardless of their disease status. From these observations, it is inferred that MFC is apparently not related to the content of exchangeable Mn in the soils of the oases studied, but is inversely correlated with the Mn content in the leaves. The soils of the oases studied were alkaline, with conditions that favour formation of insoluble Mn compounds and therefore render the element unavailable to palm trees.

Leaflets from MFC-affected palm trees have been shown to contain MFC-specific RNAs (MFC-RNAs). Dot-blot hybridization analysis, using a bifunctional

DNA probe that detects the MFC-RNAs, gave positive signals with all preparations from adult symptomatic leaflets collected from DS trees in the affected oases. Some AH trees also gave positive signals, confirming that these symptomless trees are only apparently healthy. No hybridization signals were observed with preparations from symptomless leaflets collected healthy oasis (H) or from the Germplasm Bank of Degache where the disease has never been observed.

Key words: date palm, manganese, dsRNA, brittle leaf.

INTRODUCTION

Brittle leaf disease, or “Maladie des feuilles cassantes” (MFC) in French, has affected or killed up to 40,000 date palm trees in the Djerid region of Southern Tunisia since the 1980s (Takrouni *et al.*, 1988; Triki *et al.*, 2003). Presence of the disease has now also been confirmed in the Biskra region of Algeria (Saadi *et al.*, 2006), and has been reported in the Waddan region of Libya (Ezarug Edongali, unpublished data).

On MFC-affected trees, some fronds have a dull, olive green colour. Leaflets have necrotic streaks, and they become brittle, twisted, frizzled and shrivelled, and they easily break when flexed and squeezed. Affected trees stop growing, have shorter and shorter fronds, and eventually die, so much the quicker when the first symptoms appear in the heart of the crown.

Even though symptoms similar to those of MFC were observed in the 1960s (Mehani, 1988), and possibly earlier, in the 1940s according to farmers, the disease drew renewed attention in the mid 1980s because it seemed to spread epidemically, and its transmission in space was reported as occurring from an affected tree to neighbouring trees in an aggregated manner. These observations suggested the possible involvement of a pathogen, but up to now, and in spite of many efforts (see Triki *et al.*, 2003), no agents such as nematodes, fungi, endoge-

nous and exogenous bacteria, viruses and viroids, could be found to be associated with MFC. Also, no epidemiological studies have been conducted to determine how the disease is transmitted in space and time.

In the late 1980s, brittle leaflets from MFC-affected trees were shown to be deficient in manganese (Mn) (INRAT-Tunis, unpublished data, 1987; McGrath, 1988). Interestingly, Mn deficiency is a common problem in ornamental palms growing in the alkaline soils of south Florida, and is caused by the element's insolubility at high pHs (Broschat and Meerow, 1992).

The soils from the southern Tunisian oases have also high pHs, ranging from 7.4 to 7.8 (Riahi Sassi *et al.*, 1998), and the possibility of MFC being related to unavailability of Mn in the soil gained interest again. Soils from oases free of MFC were found to have ~1 ppm of available Mn and ~69 ppm of total Mn, while oases with MFC-affected trees had less than 0.58 ppm of available Mn and 16 to 28 ppm of total Mn. However, in affected oases, the soils from the vicinity of apparently healthy trees had less available Mn (0.28 to 0.34 ppm) than soils from severely affected trees (0.49 to 0.58 ppm) (Riahi Sassi *et al.*, 1998). Regarding the Mn content of adult leaflets (Deglet Nour variety), those from healthy trees growing at the date palm Research Center at Degache had as much as 23 ppm of Mn (100%); leaflets from apparently healthy trees in MFC-affected oases at Nefta and Tozeur contained 10 ppm of Mn (43.5%), while brittle leaflets from mildly and severely affected trees had approximately the same amounts of Mn, 6 to 8 ppm (26.9 to 34.8%) (Riahi Sassi *et al.*, 1998). Similar values were obtained for leaflets of the Tezer Zit variety.

Even though the above figures indicate that MFC-affected trees grow in soil with low available Mn, and that leaflets from such trees are deficient in Mn, they do not undoubtedly prove that Mn deficiency is the cause of MFC. Indeed, only two MFC-affected oases were studied, Nefta and Tozeur, and the numbers of soil and leaflet samples used were too small to allow statistical analysis of the data. Also, if MFC is caused by Mn deficiency, applications of Mn by trunk injections or foliar sprays, rather than soil applications, must be able to control MFC. Trunk injections of Mn sulphate have been carried out previously (Ben Mahamoud, personal communication), but whether the trees recovered or not has not been clearly documented. For these reasons, large numbers of soil and leaflet samples from one healthy and five MFC-affected oases have now been analysed and submitted to statistical analyses. Leaflets from adult as well as young emerging fronds were used.

Previous studies have indicated that adult leaflets from MFC-affected trees contain two populations of small, host-derived, single-stranded and double-stranded RNAs (MFC-RNAs), the sequences of which are homologous to chloroplast gene sequences. One RNA population carries sequences present in the intergenic

region located between the tRNA-Arg gene and the rRNA 5S gene, while the other has sequences of the *atpE* gene. The MFC-RNAs can be used as molecular markers of the disease (Triki *et al.*, 2003; Namsi *et al.*, 2006). In the present work, all leaflet samples used for chemical analyses were also examined for presence of the MFC-RNAs by molecular hybridization using a bi-functional DNA probe.

MATERIALS AND METHODS

Oases. The healthy date palm oasis was located near Nefta and designated "Nefta –" (Société civile de mise en valeur agricole Essif Lakhdar). The five MFC-affected oases were selected according to different degrees of MFC incidence, and designated "Nefta +" (Remada, moderately affected: 25%), "Tozeur" (Helba, moderately affected: 29%), "Hamma" (Hamman, severely affected: 37%), "Kebili" (Fatnassa, weakly affected: 7%), and "Tamerza" (Brick, very weakly affected: 4%) (Fig. 1).

Selection of palm trees. In each MFC-affected oasis, palm trees with different degrees of symptom severity were identified: apparently healthy, symptomless trees (designated AH), trees with mild symptoms (DS1), moderate symptoms (DS2), and severe symptoms (DS3). In each affected oasis, 18 trees were selected. In the MFC-free Nefta – oasis, 9 healthy trees (H) were chosen. DS1 trees were those in which less than 1/3 of the palms showed symptoms; DS2 trees had 1/3 to 2/3 of the palms showing symptoms, and DS3 trees had more than 2/3 of the palms showing symptoms. Table 1 indicates for each oasis the type, cultivar and number of trees selected. We selected 99 trees in all. Most trees were of the "Deglet Nour" variety, but there were also "Kenta" trees in the Hamma oasis, and "Alig" trees in the Kebili oasis.

Leaflet Samples. On each one of the selected trees, one sample of leaflets was collected from the central part of adult fronds, and one sample from still white, juvenile fronds. It was checked that the adult fronds selected on MFC-affected trees had symptoms, and that their leaflets were brittle.

Soil samples. For each selected tree, one soil sample was collected at 20 cm depth, and a second one at 80 cm depth, where most of the roots occur. The "20 cm" samples from a group of three similar trees were mixed, as were the "80 cm" samples. Each final soil sample was thus combined from a group of three similar trees. For the Nefta – oasis, similar samples were obtained from 3 groups, each of 3 healthy (H) trees. For each one of the following four MFC-affected oases: Nefta +, Tozeur, Hamma, and Kebili, the final soil samples came from 3

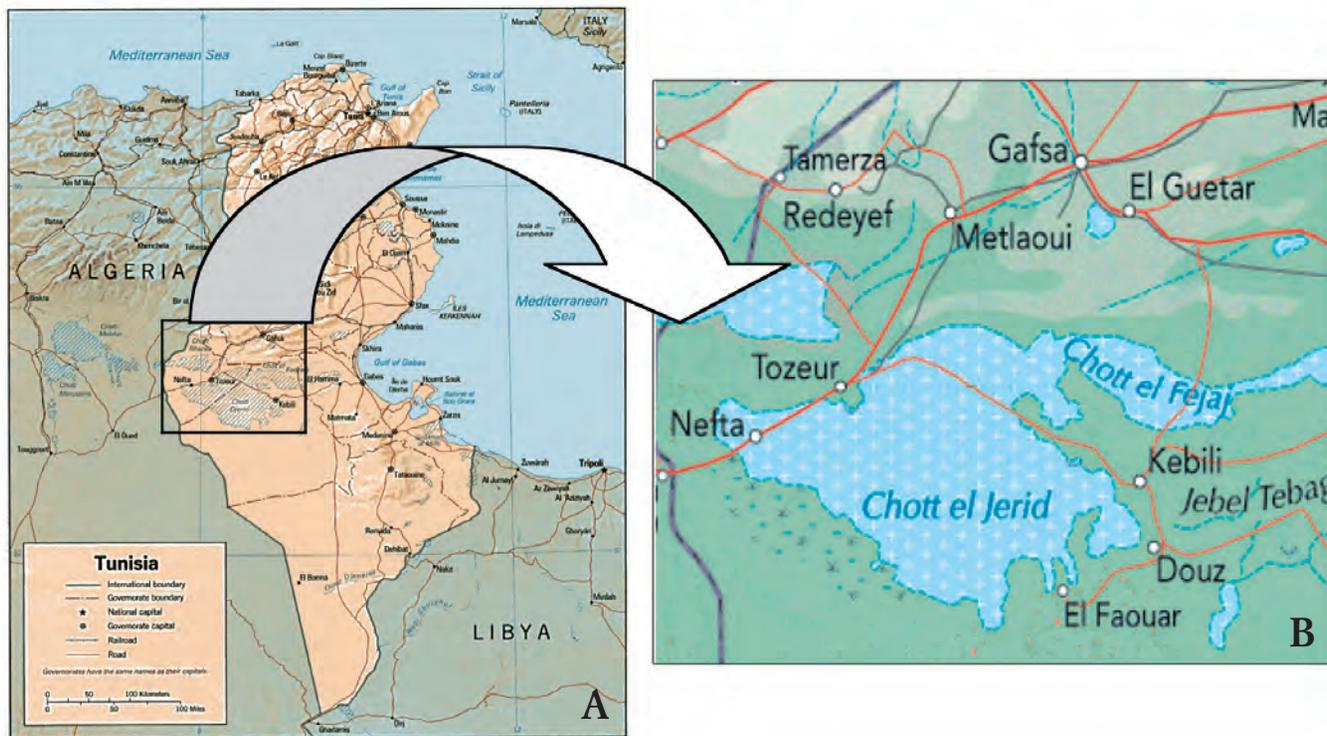


Fig. 1. Map of Tunisia with the Djerid region boxed (A) and enlarged to see the location of the oases studied (B).

groups of apparently healthy (AH) trees and 3 groups of trees with disease symptoms (DS1, DS2, DS3). For the less-affected Tamerza oasis, there were 4 groups of AH trees and only 2 groups of DS trees (DS1, DS2). The total number of final soil samples amounted to 33.

Soil analysis. Soils were analysed by the INRA Soil Analysis Laboratory (273, rue de Cambrai, 62000 Arras, France). The following analyses were done: pH in H₂O and in KCl, total nitrogen, organic carbon, cation exchange capacity, total CaCO₃, extractable P₂O₅, ammonium acetate-exchangeable K₂O, CaO, MgO, Na₂O and exchangeable Mn, EDTA extractable total Mn and boiling water-soluble B.

Mineral analysis of leaflet samples. Leaflet samples were cleaned, dried at the CRPh of Degache and ground at the URIH laboratory, Antibes. After dry mineralisation and solubilization, K, Ca and Mg analyses were done by the URIH laboratory and N, P, Fe, Mn, Cu and Zn by the *Unité de Service et de Recherche en Analyses Végétales et Environnementales* (USRAVE; INRA research centre, 71, ave. E. Bourlaux, BP 81, 33883 Villenave d'Ornon, France). P, K, Ca and Mg, Fe, Mn, Cu, and Zn were determined by ICP-AES, and N, by the Dumas method.

Chemical analysis of irrigation water. Water samples were collected from the wells used for irrigation in the

Table 1. Palm trees from which samples were collected in the six selected oases.

Oases	Type, cultivar and number of palm trees selected						Total
	H	AH	DS1	DS2	DS3		
Nefta -	9 Deglet Nour	-	-	-	-	-	9
Nefta +	-	9 Deglet Nour	3 Deglet Nour	3 Deglet Nour	3 Deglet Nour	3 Deglet Nour	18
Tozeur	-	9 Deglet Nour	3 Deglet Nour	3 Deglet Nour	3 Deglet Nour	3 Deglet Nour	18
Hamma	-	6 Deglet Nour	-	-	-	-	6
	-	3 Kenta	1 Kenta	5 Kenta	3 Kenta	3 Kenta	12
Kebili	-	3 Deglet Nour	-	-	-	-	3
	-	6 Alig	3 Alig	3 Alig	3 Alig	3 Alig	15
Tamerza	-	12 Deglet Nour	3 Deglet Nour	3 Deglet Nour	-	-	18
Total	9	48	13	17	12	12	99

oases studied. The pH, conductivity and mineral analysis of Cl, SO₄, NO₃, H₂PO₄, K, Ca, Na and Mg were determined at the URIH laboratory.

Analysis of RNAs associated with MFC (MFC-RNAs). Total RNAs were extracted from fresh leaflet samples (500 mg), according to the SDS/potassium acetate method (Astruc *et al.*, 1996; Cañizares *et al.*, 1998), and the preparations were subjected to non-ionic cellulose chromatography to obtain dsRNA-enriched preparations (Franklin, 1966; Semancik, 1986). The cellulose was washed twice with STE-17% ethanol, and the dsRNAs bound to the cellulose were eluted with STE. The RNAs were concentrated by ethanol precipitation and resuspended in 50 µl of water. A DIG-labelled bifunctional DNA probe was synthesized by PCR amplification using as template a plasmid containing a 564 bp insert with partial sequences of the intergenic region located between the tRNA-Arg gene and the rRNA 5S gene, and the *atpE* gene (Namsi *et al.*, 2006). Aliquots (4 µl) of dsRNA enriched preparations and two ten-fold dilutions were pre-treated in 33% formamide for 5 min at 100°C, blotted to positively charged Nylon membranes (Roche Diagnostic, Indianapolis, IN, USA), and immobilized by UV crosslinking.

Prehybridization and hybridization were carried out in 50% formamide, and the DIG-labelled hybrids were detected with an anti-DIG-alkaline phosphatase conjugate (Fab fragments) and visualized with the chemiluminescent substrate (CSPD) (Roche Diagnostic, Indianapolis, IN, USA) (Namsi *et al.*, 2006).

Statistical analysis. Data were subjected to ANOVA and the means were compared with Newman and Keuls test. Mn data were also analyzed by ANOVA and scatterplots using the Statgraphics Plus 5.1 package (Stat-Point, Inc., Herndon, VA, USA). Factor analysis was performed with the SPS program (CACI International Inc., Arlington, USA).

RESULTS

Manganese content of leaflets. The manganese contents of the 9 juvenile and the 9 adult leaflet samples from the MFC-free Nefta – oasis, as well as the 18 juvenile and the 18 adult leaflet samples from each of the five MFC-affected oases are summarized in Fig. 2. The Mn content of juvenile leaflets was significantly lower than that of adult leaflets, and ranged from 5.7 to 8.6 mg/kg. No significant differences in Mn content were seen among the juvenile leaflets from the six oases. With adult leaflets, the Mn content was clearly different between the MFC-free Nefta – oasis with 31.4 mg/kg Mn, and the four oases most affected by MFC with only 14.3 to 16.6 mg/kg Mn. The least-affected Tamerza oasis had

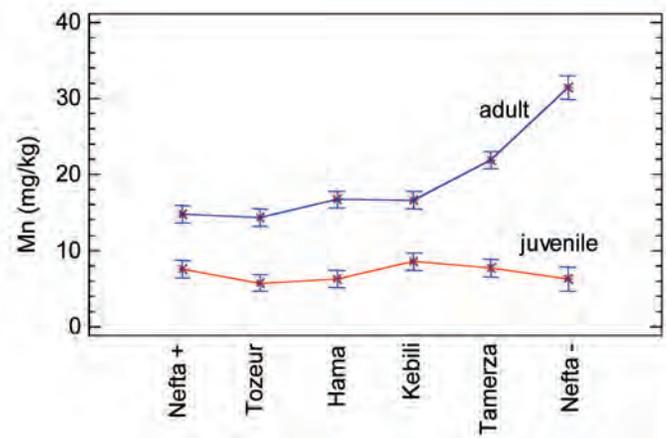


Fig. 2. Manganese content of juvenile and adult date palm leaflets collected in a healthy oasis (Nefta -), and five MFC-affected oases (Nefta +, severely affected; Tozeur, moderately affected; Hamma, moderately affected; Kebili, weakly affected, and Tamerza, very weakly affected). The interaction plot shows the mean values and the 95% confidence LSD intervals.

an intermediate value (21.9 mg/kg).

In Fig. 3A, the Mn data from the 99 adult leaflet samples (see Table 1) have been subjected to ANOVA in order to illustrate the Mn content of leaflets per category of trees.

The leaflets from the healthy trees (H) of the disease-free Nefta – oasis had the highest Mn content (31.4 mg/kg). The leaflets from the apparently healthy trees (AH) in the MFC-affected oases had significantly less Mn (21.7 mg/kg) than the leaflets from the healthy trees (H). The leaflets from the symptomatic trees DS1, DS2, and DS3 had the lowest Mn contents, and these values (average: 11.2 mg/kg) were significantly smaller than those for leaflets from H and AH trees. Similar conclusions could be drawn when the individual MFC-affected oases were considered. As shown on Fig. 3, B to F, in general, the leaflets from the AH trees had significantly more Mn than the DS leaflets, but there was no significant difference among the leaflets from the three categories of DS trees, even though there was a tendency for the DS3 leaflets to have the lowest amount of Mn. In Fig. 3, B to F, the LSD intervals (bar size) are rather large because of the relatively small sample sizes.

Other elements in date palm leaflets. As shown in Table 2, for adult leaflets, there were no significant differences among the six oases regarding N, P, Ca, Mg, and B, but the amount of K in the leaflets was significantly higher in the Tozeur oasis than in the Nefta – and Tamerza oases. Regarding juvenile leaflets, there were differences among oases for N, K, Ca, Mg, and B, but not for P.

Soil texture. As shown in Table 3, the soils of the Nefta -, Nefta +, Tozeur, and Hamma oases were poor

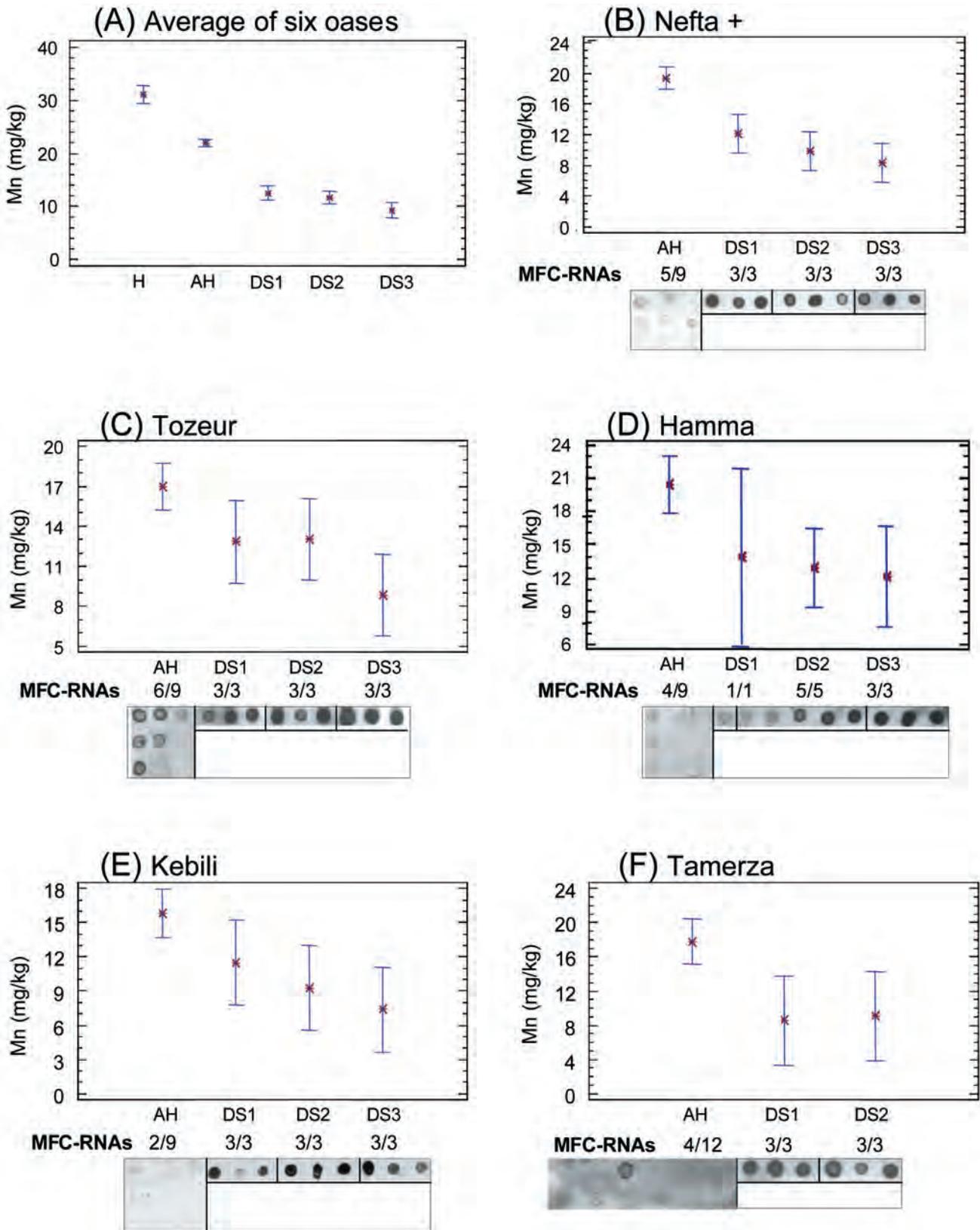


Fig. 3. Manganese content and presence of MFC-RNAs in adult date palm leaflets collected in one healthy and five MFC-affected oases. The interaction plots show the Mn mean values, and 95% confidence LSD intervals, for leaflets collected from: symptomless trees (H) in the healthy Nefta oasis, and from the following trees in the five MFC-affected oases: apparently healthy trees (AH), trees with mild disease symptoms (DS1), moderate disease symptoms (DS2), and severe disease symptoms (DS3). (A) Interaction plot showing the average mean Mn values of the six selected oases. (B-F) Interaction plots showing the mean Mn values of each MFC-affected oasis. The results of MFC-RNAs analyses are summarized below each plot. Figures represent the number of samples with a positive hybridization signal for each disease condition over the total number of samples analyzed in each oasis.

Table 2. Mineral analysis of adult and juvenile leaflets from six oases¹.

Oases	Mineral elements in adult leaflets					
	N (% dw)	P (% dw)	K (% dw)	Ca (% dw)	Mg (% dw)	B (mg/kg)
Nefta -	1.66	0.092	1.22 a	0.48	0.20	33.7
Nefta +	1.55	0.081	1.39 ab	0.47	0.21	25.0
Tozeur	1.43	0.117	1.76 b	0.26	0.18	28.2
Hamma	1.65	0.101	1.46 ab	0.41	0.24	25.3
Kebili	1.74	0.118	1.46 ab	0.44	0.20	22.6
Tamerza	1.43	0.097	1.27 a	0.33	0.22	30.2
F (calculated)	1.14	2.26	3.03	0.89	1.45	2.19
F (P=5%)	2.57	2.57	2.57	2.57	2.57	2.57

Oases	Mineral elements in juvenile leaflets					
	N (% dw)	P (% dw)	K (% dw)	Ca (% dw)	Mg (% dw)	B (mg/kg)
Nefta -	1.02 ab	0.117	1.83 a	0.09 ab	0.087 a	6.38 a
Nefta +	1.00 ab	0.173	1.82 a	0.19 bc	0.133 ab	9.92 b
Tozeur	1.33 b	0.143	2.06 b	0.11 ab	0.117 ab	10.95 b
Hamma	1.15 ab	0.130	1.79 a	0.18 bc	0.148 b	9.02 ab
Kebili	1.13 ab	0.140	1.77 a	0.27 c	0.172 b	10.97 b
Tamerza	0.91 a	0.137	1.98 ab	0.07 a	0.068 a	11.47 b
F (calculated)	3.56	0.430	5.98	7.91	7.91	3.12
F (P=5%)	2.57	2.57	2.57	2.57	2.57	2.57

¹Data were subjected to ANOVA and Newman and Keuls test. Figures followed by the same letter within columns are not significantly different.

in clay and silt, and rich in sand. However, the soil of the Tamerza oasis had more silt than the soils of the other oases. Previous soil analyses (data not shown) have indicated the same to be true also for the Kebili oasis. Thus, these soils range from sandy (Nefta -, Nefta +, Hamma) to sandy silt (Tozeur) and silt (Kebili, Tamerza). With such characteristics and in spite of their differences, the soils of these six oases do not retain much water and are sensitive to drought.

Chemical analysis of soils. The chemical compositions of the soils, subjected to ANOVA and Newman and Keuls test, are given in Table 4. In all cases, the pH was high and most often above 8.0. All soils, but especially those from the 80 cm depth, had a low cation exchange capacity, and were very poor in organic matter and nitrogen. The P₂O₅ concentrations varied from low (0.06 g/kg) to high (0.54 g/kg), and those of K₂O from 0.08 to 0.18, according to the oases. The concentrations of total CaCO₃ were particularly high in the Tozeur and Tamerza oases, and those of CaO, lowest in the Nefta + and Hamma oases. In all oases, the concentrations of MgO were generally high. The smallest concentration of Na₂O occurred in the Tamerza oasis, and the largest in the Tozeur and Kebili oases. The sulphate concentration in the soil of the Tozeur oasis had also been determined in an independent analysis, and was found to be very high, 1.29 mg/kg.

Manganese and boron in the soil. The concentrations of EDTA-extractable total Mn, ammonium acetate-exchangeable Mn, and boiling water-soluble B, are indicated in Table 5.

The soils of the Tozeur oasis, with high MFC incidence, and the Kebili oasis, with low disease incidence, had the highest boron concentrations. Regarding Mn, the "80 cm" soil of the Nefta + oasis, where the incidence of MFC is greatest, had an exchangeable-Mn concentration of 5.56 mg/kg, almost twice as high as that of the Nefta - soil (2.83 mg/kg).

The same trend was shown by the "20cm" soils. Inversely, the Tamerza and Kebili oases, where disease incidence was lowest, had the lowest exchangeable Mn concentration in their soils. The same trends are seen with the EDTA-extractable Mn.

To extend these observations, the exchangeable Mn in the "80 cm" soils from the healthy trees (H trees) of the Nefta - oasis were compared with those from the apparently healthy trees (AH trees) and the symptomatic trees (trees DS1, DS2, and DS3) of the five MFC-affected oases. Fig. 4 A shows that the amounts of exchangeable Mn in the "H" soils and the "AH" soils were, on average, almost identical, and that the values for the "DS" soils were on average, lower than those for the H and AH soils, but not significantly different.

In Fig. 4, B to F, the exchangeable Mn in the "80 cm" soils from the symptomless, apparently healthy

Table 3. Granulometric analysis of soil samples collected at 20 and 80 cm¹.

Oases	Clay (g/kg)		Silt (g/kg)				Sand (g/kg)			
			Fine		Coarse		Fine		Coarse	
	20 cm	80cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm
Nefta -	103.7 b	169.7 c	29.0 b	64.0 b	23.0 b	19.7 b	258.7 c	206.3 b	585.7 d	540.3 c
Nefta +	93.8 b	54.0 b	18.0 ab	5.7 a	37.2 c	9.0 a	518.4 d	396.7 d	332.6 b	535.7 c
Tozeur	152.5 c	162.2 c	50.7 c	60.7 b	29.3 c	26.5 b	335.8 c	287.8 c	431.7 c	462.8 b
Hamma	50.8 a	43.2 a	9.8 a	7.5 a	11.3 a	7.0 a	131.7 a	118.0 a	796.3 e	824.3 d
Kebili	na	na	na	na	na	na	na	na	na	na
Tamerza	235.7 d	194.3 d	217.5 d	232.0 c	108.2 d	108.5 c	198.7 b	194.0 b	240.0 a	271.0 a
F (calculated)	273.1	376.06	309.9	245.51	438.1	223.59	171.2	7.72	242.0	188.39
F (P=5%)	2.43	2.43	2.43	2.43	2.43	2.43	2.43	2.43	2.43	2.43

¹Data were subjected to ANOVA and Newman and Keuls test. Figures followed by the same letter within columns are not significantly different.

Table 4. Chemical analysis of soils sampled at 20 and 80 cm¹.

Oases	pH water		pH KCl		Total CaCO ₃ (g/kg)		Organic matter (g/kg)		Nitrogen (g/kg)		Carbon (g/kg)	
	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm
Nefta -	8.0 a	8.1 a	7.8 a	7.9 a	65.3 b	40.0 b	8.13 b	3.97 b	0.45 b	0.13 a	4.71 b	2.33 b
Nefta +	8.3 c	8.4 c	8.1 b	8.2 c	88.6 c	53.4 b	8.94 b	2.88 a	0.41 b	0.09 a	5.22 b	2.56 b
Tozeur	8.1 b	8.2 b	8.0 b	8.1 bc	119.0 d	129.3 c	12.08 c	4.33 b	0.56 c	0.18 b	7.03 c	2.53 b
Hamma	8.5 d	8.5 d	8.3 c	8.4 d	20.8 a	12.2 a	4.30 a	2.15 a	0.19 a	0.10 a	2.50 a	1.25 a
Kebili	8.1 ab	8.0 a	8.1 b	8.0 b	71.6 b	14.7 a	5.83 a	5.83 c	0.28 a	0.24 c	3.39 a	3.39 c
Tamerza	8.2 b	8.2 b	8.0 b	8.1 bc	348.4 e	356.7 d	22.53 d	9.32 d	1.21 d	0.31 d	13.09 d	5.42 d
F (calculated)	39.06	39.06	34.96	34.96	1578.8	383.2	75.77	81.85	89.6	38.60	75.8	53.02
F (P=5%)	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26

Oases	CEC (cmol/kg)		K ₂ O (g/kg)		CaO (g/kg)		MgO (g/kg)		Na ₂ O (g/kg)		P ₂ O ₅ (g/kg)	
	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm
Nefta -	3.67 c	2.50 ab	0.09 a	0.08 a	31.1 b	67.8 c	0.30 a	0.15 a	0.18 a	0.16 a	0.39 c	0.16 b
Nefta +	4.86 e	2.86 bc	0.15 b	0.08 a	10.8 a	9.2 a	0.48 b	0.25 bc	0.41 b	0.18 a	0.26 b	0.14 b
Tozeur	4.10 d	3.05 c	0.18 b	0.15 b	49.1 c	59.7 b	0.51 b	0.35 d	0.53 c	0.50 c	0.31 b	0.19 c
Hamma	3.22 b	2.53 ab	0.09 a	0.10 a	10.7 a	9.9 a	0.29 a	0.22 b	0.18 a	0.17 a	0.12 a	0.06 a
Kebili	2.42 a	2.38 a	0.09 a	0.10 a	14.7 a	71.4 c	0.32 a	0.29 c	0.41 b	0.40 b	0.13 a	0.06 a
Tamerza	6.20 f	3.65 d	0.17 b	0.16 b	44.1 c	62.1 b	0.71 c	0.44 e	0.12 a	0.09 a	0.54 d	0.30 d
F (calculated)	113.2	104.19	25.24	10.57	156	351.95	156	351.95	32	70.5	43.7	104.19
F (P=5%)	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26	2.26

¹ Data were subjected to ANOVA and Newman and Keuls test. Figures followed by the same letter within columns are not significantly different.

palms (AH trees) within a given MFC-affected oasis was compared with that in the soils from symptomatic trees (trees DS1, DS2, and DS3) of the same oasis. In the Nefta + oasis (Fig. 4, B), the value for exchangeable Mn in the AH soil was significantly lower than that in the "DS" soil, while in the oases of Hamma (Fig. 4, D), Kebili (Fig. 4, E), and Tamerza (Fig. 4, F), it was the opposite, the Mn value for the AH soil being significantly higher than that for the DS soil. A third

situation was observed in the Tozeur oasis (Fig. 4, C), where the values for the AH soil and the DS soil were the same.

Relationship between the manganese content in leaflets and the amount of exchangeable Mn in the soil.

This relationship is shown in the scatterplot of Fig. 5, A in which symptomless (H, AH) trees are labelled in blue and diseased (DS1, DS2, DS3) trees are labelled in red.

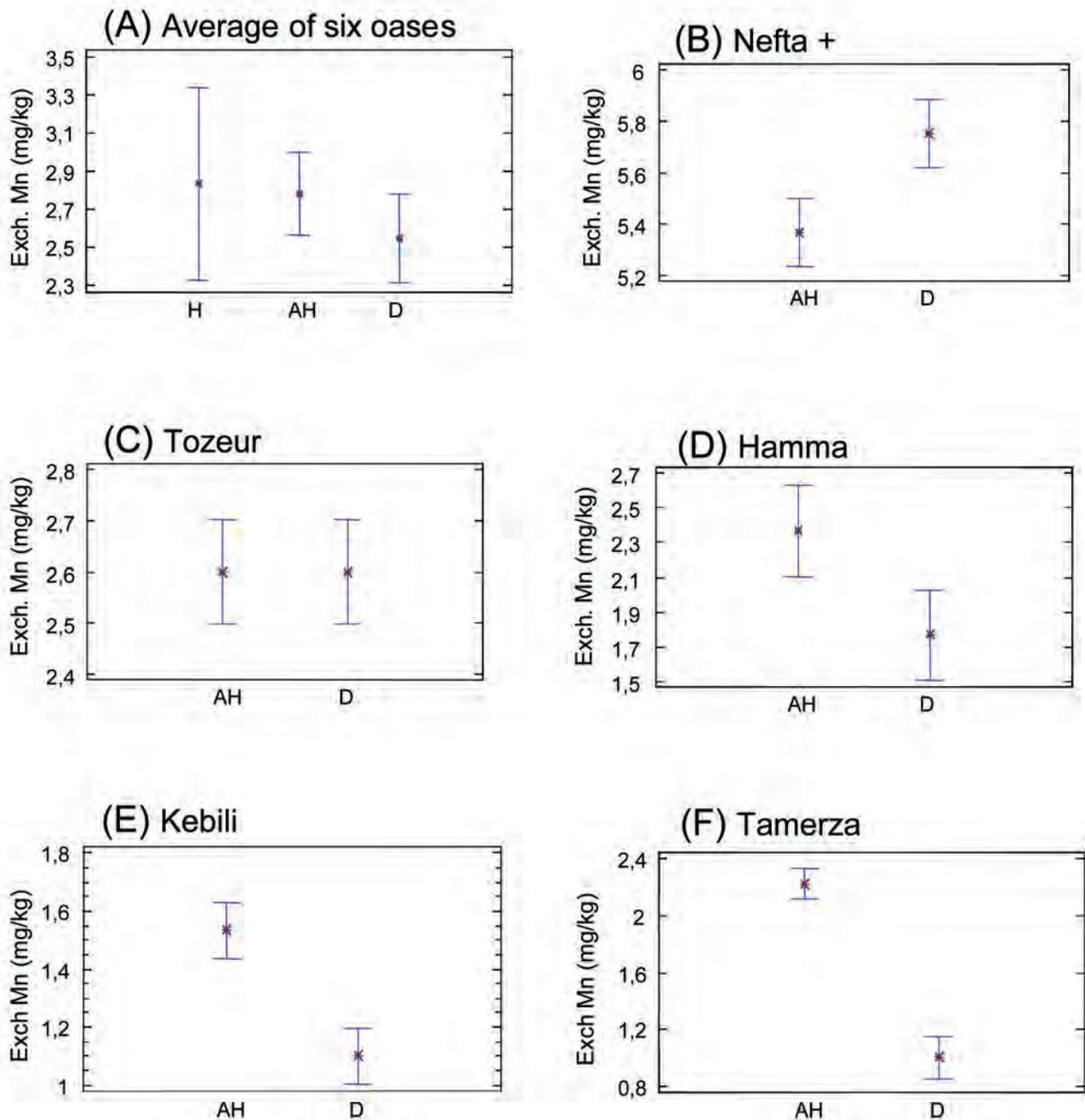


Fig. 4. Amount of exchangeable manganese in soil samples collected at 80 cm depth. The interaction plots show the mean Mn values, and 95% confidence LSD intervals, for soils collected around: symptomless trees (H) in the healthy Nefta oasis, and apparently healthy trees (AH) and MFC-affected trees (D) in the five MFC-affected oases. (A) Interaction plot showing the average mean Mn values of the six selected oases. (B-F) Interaction plots showing the mean Mn values of each MFC-affected oasis.

The scatterplot canonically represents the existence of a relationship between trees with MFC symptoms (in red) and low Mn content in leaflets. Most trees appear to be clustered in the lower half of the scatterplot, indicating that they are growing in soils with a content of exchangeable Mn of around 3 mg/kg or lower, regardless of their disease status. Some trees, however, are clustered in the upper half of the plot, showing that they are growing in soils with a content of exchangeable Mn of around 4.5 mg/kg or higher, regardless of their disease status. The scatterplot of Fig. 5, B, is similar to that of Fig. 5, A, but the trees have been labelled according to the oasis in which they grow: one label for the trees growing in Hamma, a second label for those in Kebili, and so on for the six oases. The scatterplot of Fig. 5, B complements that of Fig. 5, A, and shows that the variability observed in exchangeable Mn in the soil is mostly due to the presence of higher amounts of Mn in the Nefta + soils.

The four major variables studied (MFC status, leaf Mn content, exchangeable Mn and EDTA-extractable total Mn in soil) were subjected to factor analysis with varimax rotation, to obtain a small number of factors which account for most of the variability in the four variables. In our case (Table 6), three factors accounted for 98% of the variability and a fourth factor was considered as residual variability. Factor 1, which accounts for 48.17% of the variability, is marked by high loadings on exchangeable Mn and EDTA extractable total Mn in the soils, and low loadings of the other two variables (Mn content in leaves and MFC status). Factor 2 and factor 3, which account for 25.18% and 24.8% of the variability, respectively, are marked by high loadings of Mn content in the leaves and the MFC status, and low loadings of the other two variables (exchangeable Mn and EDTA extractable total Mn in the soil) (Table 7).

Factor 2 and factor 3 explain the relationship between MFC status and leaf Mn content as shown in Table 7, factor 2 explains this relationship from the Mn

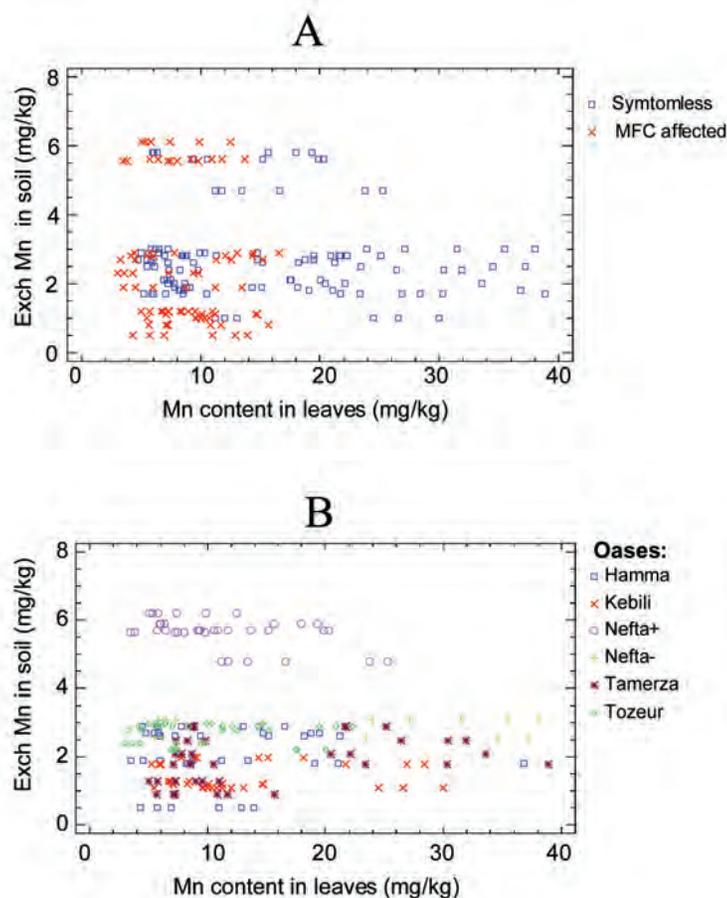


Fig. 5. Scatterplots illustrating the relationship between the manganese content in leaflets and the amount of exchangeable Mn in the soil. **A)** Symptomless trees (H, AH) are labelled in blue, and MFC-affected trees (DS1, DS2, DS3) in red. **B)** The trees of each oasis are labelled differently.

content in the leaves whereas factor 3 does it from the MFC status. In both instances there is an inverse correlation between these two variables, meaning that MFC symptoms are associated with low Mn in leaves. All

Table 5. Manganese and boron analysis of soil samples collected at 20 and 80 cm¹.

Oases	Mn (EDTA) (mg/kg)		Exchangeable Mn (mg/kg)		Boron (mg/kg)	
	20 cm	80 cm	20 cm	80 cm	20 cm	80 cm
Nefta -	6.90 c	3.33 b	3.70 c	2.83 d	0.48 a	0.20 a
Nefta +	7.88 d	7.12 c	5.24 d	5.56 e	0.64 c	0.23 a
Tozeur	5.13 b	3.42 b	3.28 c	2.60 d	1.02 d	0.51 c
Hamma	4.48 b	2.90 ab	2.55 b	2.15 c	0.41 a	0.27 ab
Kebili	3.17 a	2.40 a	1.33 a	1.33 a	0.49 b	0.51 c
Tamerza	7.20 cd	3.02 ab	2.41 b	1.85 b	0.67 c	0.28 b
F (calculated)	47.6	70.50	64.65	343.36	90.49	54.31
F (P=5%)	2.26	2.26	2.26	2.26	2.26	2.26

¹Data were subjected to ANOVA and Newman and Keuls test. Figures followed by the same letter within columns are not significantly different.

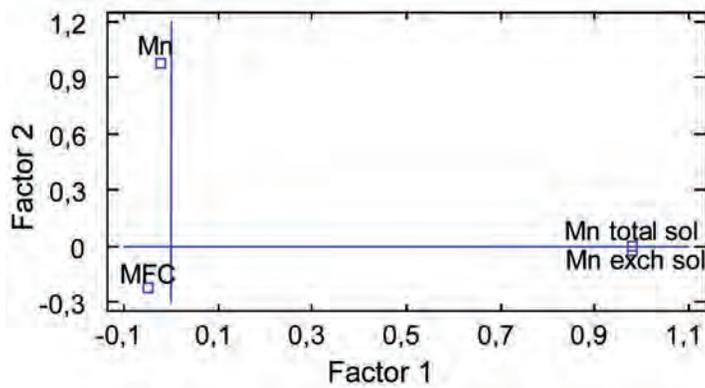


Fig. 6. Plot of factor loadings showing the relative load of factor 1 which accounts for 48.42% of the variability and factor 2 which accounts for 25.18% of the variability.

these considerations are also illustrated in the plot of factor loadings (Figure 6) showing that: i) as expected, a direct relationship exists between exchangeable Mn and EDTA-extractable total Mn in the soil (factor 1); ii) an indirect correlation has been found between the disease status and Mn content in the leaves (factors 2 and 3), and iii) MFC status is apparently not related to the Mn content in the soil.

Chemical analysis of irrigation water. Several sources of irrigation water were analysed. Their pHs range from 7.1 in Kebili to 8.2 in Nefta. Their salinities were high, with excessive amounts of Ca (104 to 426 mg/l), Mg (52 to 149 mg/l) and Na (288 to 393 mg/l), as well as Cl (462 to 710 mg/l) and SO_4 (542 to 1488 mg/l). EC was high, and ranges from 2730 to 4080 $\mu\text{S}/\text{cm}^{-1}$.

Presence of MFC-dsRNAs in palm leaflets. Dot-blot hybridization analysis, using a bifunctional DNA probe that detects the MFC-RNAs, gave positive signals with all preparations from adult symptomatic leaflets collected. In general, strong hybridization signals were obtained, regardless of the symptom intensity (DS1, DS2 or DS3) (Fig. 2 B to F).

Preparations from adult symptomless leaflets collected from apparently healthy trees (AH) in MFC-affected oases also gave positive signals, but in some samples only (Fig. 2, B to F). The hybridization signals were in general weaker than those from symptomatic leaflets. No hybridization signals were observed in preparations from adult leaflets collected from the MFC-free Nefta oasis (H) and from Germplasm Bank of Degache, included as negative controls in the hybridization tests (data not shown).

DISCUSSION

The data on Mn analysis in adult leaflets, as illustrated by the scatterplots of Fig. 5, and the factor load analysis of Fig. 6 clearly show a strong inverse correlation between the concentration of Mn in leaflets and the disease. All symptomatic leaflets are deficient in Mn, and leaflets from the most affected trees (DS3 trees) have in general three to four times less Mn than leaflets from healthy trees (H trees). Even the symptomless trees (AH trees) from the affected oases are only apparently healthy, as their leaflets are already deficient in Mn, and contain only 50 to 65% of the Mn amount present in healthy leaflets. Furthermore, the MFC-dsRNA, which is present in all symptomatic trees, could be detected in several AH trees, and it is to be foreseen that these trees

Table 6. Principal components analysis after varimax rotation.

Statistical factor analysis	Eigenvalues Extraction: Principal components after varimax rotation		
Value	Eigenvalue	% Total Variance	Cumulative %
1	1.927	48.170	48.170
2	1.007	25.178	73.348
3	0.992	24.800	98.148
4	0.074	1.852	100.000

Table 7. Factor loadings (varimax normalized).

Statistical factor analysis	Factor Loadings (rotated) Principal components		
Variable	Factor 1	Factor 2	Factor 3
Exchangeable Mn in soil	0.981	-0.033	-0.017
EDTA-extractable total Mn in soil	0.977	-0.021	-0.079
Mn in leaves	-0.052	0.917	-0.396
MFC status	-0.079	0.079	0.910

will show symptoms soon. On the basis of these observations, one would not expect, within an MFC-affected oasis, the exchangeable Mn (exch-Mn) in the soil from the symptomless AH trees (AH soil) to be very different from that in the soil around the symptomatic DS trees (DS soil). Even though this is true on average (Fig. 4A), it varies among the oases, the exch-Mn in the AH soils being, identical, lower, or higher than that in the different DS soils. It even happens that, unexpectedly, the amount of exch-Mn in the soil of a MFC-affected oasis, the Nefta + oasis, is significantly higher than that in the soil of the healthy Nefta – oasis, and the lowest amount of exch-Mn is found in the soils of the two least affected oases, the Kebili and the Tamerza oases. From these paradoxical observations, as well as the factor load analysis of Fig. 6, it can be inferred that the presence of MFC symptoms is not associated with the amount of potentially exchangeable Mn or the amount of total Mn present in the soil. In fact the availability of Mn does not necessarily correlate with the presence of Mn in the soil but with the ability of the plant to assimilate it.

This situation is to be expected from the soil characteristics of the oases. In all cases, the soils are alkaline, with pHs, usually above 8. They range from sandy soils to sandy silt, and silt. They are very poor in organic matter, and their cation exchange capacity is low. In addition, the irrigation waters have high salinities. These conditions favour formation of insoluble Mn compounds, such as Mn oxides and eventually coprecipitates with CaCO_3 , and render the element unavailable to palm trees. Thus the Mn deficiency which affects the palm trees would probably not be due to lack of Mn in the soil, but to the element's unavailability, as already suggested previously (Riahi Sassi *et al.*, 1998; Worden *et al.*, 2002). Unavailability of Mn is probably the result of purely chemical reactions in the alkaline soils of the oasis, but biotic factors, such as bacterial oxidations of soluble Mn^{2+} to insoluble Mn^{3+} and Mn^{4+} forms (Ben Mahamud and Conforti, 1995), or involvement of mycorrhiza in Mn absorption by the palm trees, cannot be totally excluded at this moment.

In general, the MFC-affected oases are traditional oases, and do not receive as much care as the more recent, "industrial" oases, where nutrition and fertilization are more appropriate. MFC symptoms have been seen only rarely in these more modern oases. Hence, soil management, including use of manure when available, might help overcome MFC. This seems to be the case in Libya, where MFC is apparently not a problem. Also, the oasis of Kebili (Montarone, unpublished results) and Tamerza, contrary to the other oases studied, are relatively rich in clay and silt, which might favor Mn^{2+} even though exchangeable Mn is relatively low, and may explain why these two oases are the least affected. Additionally, recent conditions, such as insufficient irrigation in some traditional oases has probably

resulted in a deterioration of the physiological status of the palms, a situation that could be improved through better water management.

Finally, if MFC does not involve a pathogen, and is nothing else but Mn deficiency due to unavailability of the soil Mn to the palm trees, it should be possible to make affected trees recover solely by providing Mn to the trees by trunk injection or foliar application.

Furthermore, acidification of irrigation water through addition of phosphoric acid, easily available in local mines, would also be beneficial. These experiments are in progress.

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