SEVERE PHOSPHORUS DEFICIENCY IN SUNFLOWER — THE CAUSE OF FOLIAR SYMPTOMS SIMILAR TO THOSE PRODUCED BY SOME FUNGAL PATHOGENS

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INTRODUCTION

The expression of a characteristic symptom is often used to identify the casual factor of a nutrient disorder (Sprague 1964). Subsequent positive identification of the disorder following the application of, and positive response to, a specific nutrient is desirable but rarely achieved. The provision of an accurate description of symptoms characteristic of a nutrient disorder thus provides an invaluable guide to the likely casual factor and its subsequent amelioration.

Phosphorus (P) deficiency of soils in central Queensland limits the production of sunflowers (*Helianthus annuus* L.) (Hunter and McCosker 1982, D. E. Hibberd, personal communication). Although vegetative and reproductive growth is reduced by P deficiency the expression of foliar symptoms has rarely been reported. Eaton (1949) observed the occurence of dark green leaves of P deficient sunflowers but did not specify any other foliar symptoms of diagnostic use.

In this paper we describe the vegetative growth of sunflowers in soils low in bicarbonate extractable phosphorus (P_B) in pots and in the field. Phosphorus was added to provide a range of P supply conditions. In order to explore the effects of extreme deficiency on growth, the activity of mycorrhiza in promoting plant P uptake was minimised by soil heat treatment (Mosse and Hayman 1980) and by selecting long fallowed (i.e. less biologically active) soil (Black and Tinker 1978). We describe and illustrate P deficiency symptoms in sunflower and emphasize the appearance of concentric band lesions (CBL) which we believe are characteristic of P deficiency in this plant. We suggest that these foliar symptoms may have been incorrectly attributed to infection by pathogenic micro-organisms when P deficiency was the real cause.

MATERIALS AND METHODS

Four experiments were conducted ; three in the glasshouse and one in the field. In all experiments the major objective was to establish the vegetative responses of sunflower following the application of P to soils low in P (< 13 ug g^{-1} P_B). With the exception of the field experiments, single sunflower plants were grown in pots containing approximately 1.2 kg of soil, and watered from a constant water table supplied from an internal reservoir (Hunter 1981). In the pot experiments, soil was treated with basal applications of potassium (K) 30 mg kg^{-1} and zinc (Zn) 5 mg kg^{-1} and continuously supplied with nitrogen (in a ratio of 1 NH_4^+ : 9 NO_3^-) in the irrigating solution.

In experiment I, P was applied to a black cracking clay soil at the rates of 0, 5, 15, 30, 60 mg kg^{-1} as phosphoric acid in the solution initially used to wet up the soil. P was applied in factorial combination with six rates of N, within N always being present in the irrigating solution at concentrations of 0, 10, 30, 60, 100 and 200 mg L^{-1}. Single plants of cv. Hysun 31 were grown in these treatments for 38—39 days and shoots harvested. Three replicates were included.

In experiment II, two black cracking clay soils (Emerald Field Station, P_B 6.5 ug g^{-1} ; B. van Itallie's property in the Callide Valley, P_B 6.5 ug g^{-1}) were used. One half of these soil samples was treated at 80°C for at least 48 hr in order to kill micro-organisms which might affect nutrient uptake by plants. The experiment was designed as a factorial combination of two soils, with and without heat treatment and two levels of P. For the P treatments, P was added in the wetting up solution and on two later occasions (43 and 64 days) at a rate

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of 100 mg kg^{-1} each time. Basal nutrients were added as in experiment I. Nitrogen was added continuously through the constant water supply at a concentration of 100 mg L^{-1}. Hysun 31 was the cultivar grown. One replicate was harvested at flowering, the other at maturity and dry shoot weight determined.

In experiment III, two soils were used — one a black cracking clay from the Emerald Field Station with a P_B of 6 ug g^{-1}, the other the surface fraction of a solodic soil from Brigalow Research Station (location in Z block) with a P_B of 10 ug g^{-1}. Basal nutrients were applied as in experiment I. Ten rates of P (0, 1, 2, 4, 8, 16, 32, 64, 128 and 256 mg kg^{-1} as $Ca(H_2PO_4)_2H_2O$) were placed in single spots 5 cm deep in pots of each of these soils. Seeds of cv. Hysun 31 were germinated and seedlings transplanted four days later and placed in the soil 5 cm to the side of the phosphorus spot. Single plants were harvested 32 days after germination and shoot weight determined. This experiment was not replicated.

Experiment IV was conducted at the Emerald Field Station on a black cracking clay soil (P_B 13 ug g^{-1}) that had been clean fallowed for at least 4 years. Six weeks prior to planting, half the area was treated with a fumigant (Basamid) to reduce microbial activity in the soil. Sunflower cv. Hysun 31) was sown in six metre rows, each row one metre apart. In half the length of these rows a phosphorus band (240 kg ha^{-1}) was present directly below the row of seeds. The area had been pretreated with zinc sulphate. Whole plants were harvested after 69 days and dry weights determined. This experiment was unreplicated.

As the plants in low P treatments exhibited symptoms similar to those caused by some diseases, these were examined, both microscopically and by plant techniques, for pathogens. Some leaves with necrotic lesions were kept in plastic bags in a saturated atmosphere to facilitate the sporulation of any micro-organisms. Other leaves, stems and roots were surface sterilized in calcium hypochlorite for 30 seconds, washed twice in sterile water and dried. Segments were cut aseptically from the margins of C.B.L. and necrotic areas and placed in petri dishes on agar media (water agar, potato dextrose agar), some of which had been amended with streptomycin to retard growth of any bacterial contaminants. Isolated micro-organisms were used to inoculate leaves of sunflower plants growing in pots in the glasshouse. Inoculated plants were placed in a mist chamber overnight and then examined regularly for symptoms for a month.

Table 1

Combined effects of nitrogen and phosphorus on fresh shoot weight of sunflower (38 days) grown under constant water table conditions in a black cracking clay (Experiment I)

Nitrogen concentration (mg L^{-1}) (N)	Shoot fresh weight (g $plant^{-1}$) Phosphorus rate (mg kg^{-1}) (P)				
	0	5	15	30	60
0	5.08[2]	5.98[1]	9.75	11.50	12.43
10	5.57[1]	7.65[1]	11.88	15.90	16.00
30	5.56[2]	10.33	20.16	28.65	30.21
60	6.87	9.45[1]	20.16	28.06	54.02
100	5.34[2]	8.45[2]	18.50[1]	25.55	65.59
200	6.40[2]	9.33	12.22[2]	34.05	73.73

Least sig. diff. (P=0.05) P 3.35, N 3.67 N×P 8.20

Superscripts 1, 2, indicate number of plants out of a possible 3 with necrotic leaf symptoms on first pair of true leaves 20 days after germination.

RESULTS AND DISCUSSION

Dry Matter Production — The dry matter responses to P clearly show that the soils used were very deficient in P (Tables 1—4). It is clear from experiment I (Table 1) that maximum responses to N or P were dependent on adequate levels of the other elements. However, at an intermediate level of P (15 mg kg^{-1}), which was insufficient for maximum growth, the highest rate of N actually depressed growth. The reason for this growth depression is not known. However, N has been shown to reduce mycorrhizal root infection (Chambers et al. 1980) and at a concentration of 114 mg L^{-1} can reduce beneficial microbial development in the root zone Giovannetti et al. 1981), and so reduce the uptake of P. Such a depression in P uptake might explain growth depression in this experiment.

In experiment II (Table 2) the marked depressive effect of soil heat treatment on growth

Table 2

Effect of soil heat treatment and the application of phosphorus on the growth of sunflowers (Hysun 31) in two cracking clay soils (Experiment II)

Soil treatment	Fertilization	Soil	Shoot dry wt. at flowering (g $plant^{-1}$)	Shoot dry wt. at maturity (g $plant^{-1}$)
HT[1]	Nil	E[3]	1.80 (A)	1.00 (C)
		C[4]	0.82 (A)	1.64 (C)
	P added	E	53.97 (B)	92.09 (D)
		C	75.68 (B)	111.73 (D)
NHT[2]	Nil	E	3.45 (A)	5.13 (C)
		C	8.84 (A)	8.61 (C)
	P added	E	61.43 (B)	105.73 (D)
		C	83.33 (B)	107.06 (D)

[1] Heat treated ; [2] Not heat treated ; [3] Emerald Field Station ; [4] Callide Valley. A, B flowering at 100 days and 71 days respectively ; C, D mature at 142 and 114 days respectively.

may be attributed to its depressive effect on soil fungal activity which might have increased plant P uptake. Soil heat treatment has been used often for comparative purposes to kill micro-organisms and thus to prevent any beneficial effects they may have on plant growth (M o s s e and H a y m a n 1980). Where P was applied, soil heat treatment had relatively little effect on subsequent plant growth.

In experiment III (Table 3) dry matter responses to banded P were curvilinear in the two soils, but clearly not asymptotic since the most dry matter occurred at the highest rate of P (256 mg kg^{-1}) in both soils. Furthermore, while the form of the response curves was similar for both soils, plants in the solodic soil were always about twice the size of those grown in the cracking clay soil at similar P rates. Part of this difference may be attributed to the difference in the P status between the two soils.

Table 3

Effect of banded phosphorus on the growth of sunflower in two soils under constant water table conditions in pots (Experiment III)

P rate (mg kg^{-1})	Shoot dry weight (g $plant^{-1}$)	
	Solodic	Black earth
0	0.76^{34}	0.28^{25}
1	0.86^{33}	0.34^{25}
2	1.45	0.22
4	1.55	0.25^{29}
8	2.04	0.89
16	2.82	1.04
32	3.89	2.02
64	3.72	1.98
128	4.23	2.18
256	4.75	3.26

Superscripts indicate days after germination that P deficiency symptoms were first noticed.

In the field (experiment IV, Table 4), the very large dry matter responses to P supported the results obtained in pot culture in similar soil. Where P was not applied, plants grew extremely poorly, being indicative of severe phosphorus deficiency.

Table 4

Effect of P and fumigant treatment (Basamid) on growth of sunflower in the field in a black cracking clay soil after 4 years of clean fallow (mean weights of three plants) (Experiment IV)

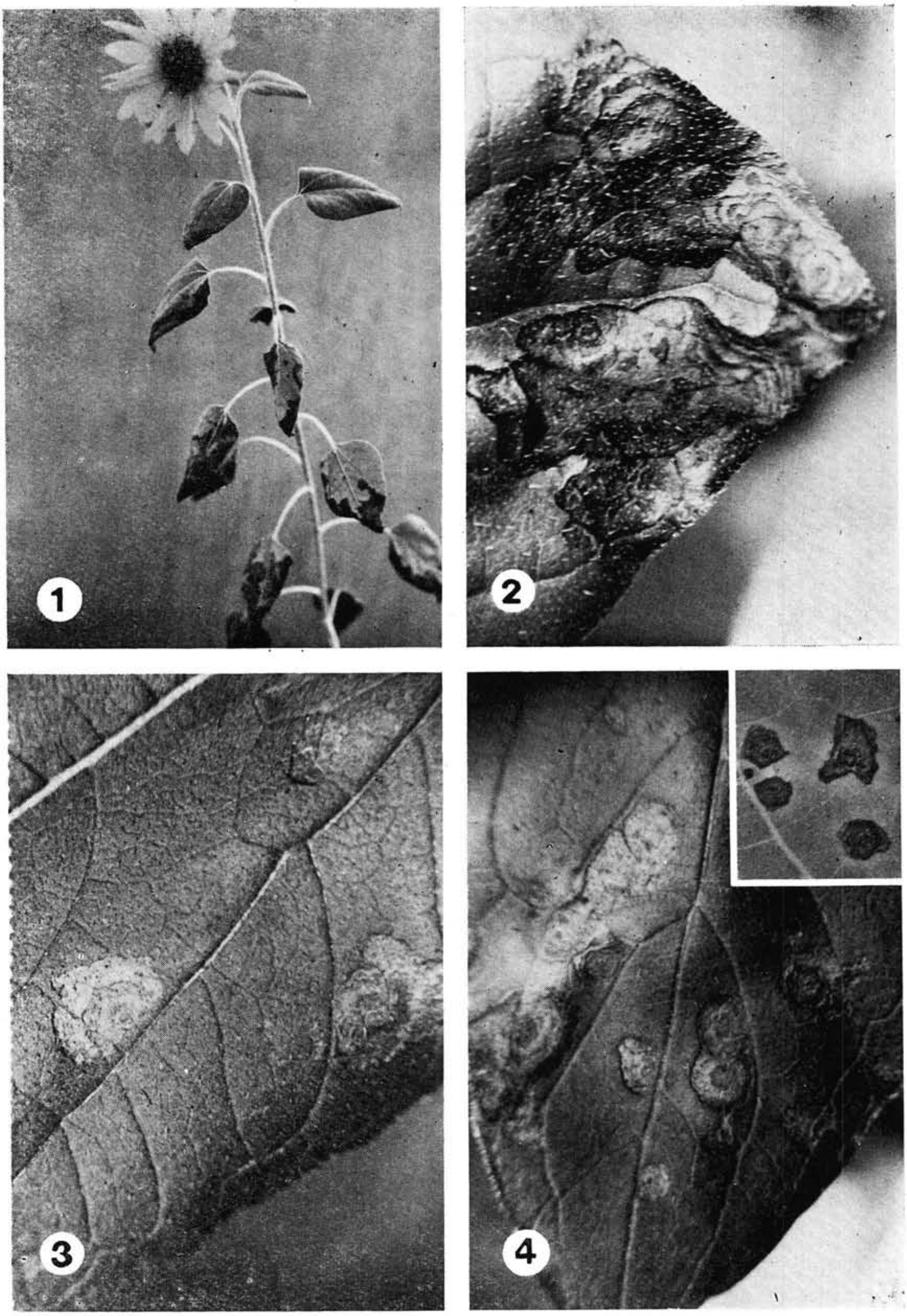
Banded fertilizer treatment	Whole shoot dry wt. (g $plant^{-1}$)		Head dry wt. (g $plant^{-1}$)	
	No fumigant	Plus fumigant	No fumigant	Plus fumigant
Nil P	13	12	8	5
Plus P	122	102	62	64

Symptoms — In both pot and field experiments, foliar symptoms developed in P deficient plants. In many cases discrete areas on the lower leaf surfaces, their margins and tips became water soaked, then chlorotic and necrotic and finally very dark brown and brittle (Fig. 1). These areas spread and in the most severe cases the whole leaf died, but remained attached to the plant. In some cases this necrosis developed in a banded fashion (Fig. 2). Discrete water soaked circular areas also appeared at random over the leaf blade and enlarged rapidly within 24—48 hours to produce concentric bands of lighter and darker tissue (Figs. 3 and 4). Each concerning band appeared to take one day to develop. These concentric bands also develop in large plants supplied with P in experiment II, but further spread was arrested with the third application of P.

The chlorotic and necrotic symptoms on the leaves were not unlike those associated with infection by *Verticillium dahliae* Kleb. This fungus penetrates the roots and eventully becomes systemic when it invades the xylem vessels. It causes prominent chlorotic interveinal patches usually near the margin and centre of the leaf. These chlorotic patches enlarge, coalesce and finally turn brown and necrotic. These symptoms appear first on the lower leaves with progressive development in upper leaves. However, neither *V. dahliae* nor any other soil-borne pathogen was isolated from plants exhibiting similar symptoms in these experiments.

Foliar symptoms may also be produced by a number of air-borne pathogens, with *Alternaria helianthi* (Hansf.) Tubaki and Nishihara being prevalent in Queensland crops (K o c h m l a n, 1977). This fungus produces dark brown to black lesions which can be rounded or angular, on leaves, stems and backs of heads. These lesions may be surrounded by a yellow halo. As the lesions mature, banding may occur within them (Fig. 4 inset) causing a target-like spot. The lesions may coalesce to form large necrotic areas on the leaf which are similar to symptoms shown in Figure 2.

No micro-organisms were isolated from surface sterilized leaves showing early development of C.B.L., but a number of fungi were isolated from older lesions. These were : *Cladosporium* sp. (most consistent), *Aureobasidium* sp. *Drechslera* sp., *Alternaria alternata* (Fr.) Keissler and several yeast fungi. This was the same suite of fungi which were isolated by the second author from some sunflower crops, exhibiting leaf necrosis symptoms, which were growing in the Orion area (Central Highlands) and the southern Darling Downs during 1980. Pathogenicity tests conducted with these organisms, along or in combination, did not produce



Figures 1—4 — 1) Occurrence and distribution of leaf tip and margin necrosis of P deficient plant ; 2) banded development of leaf necrosis ; 3) early stage of development of concentric band lesions on leaf ; 4) random distribution and coalescence of concentric band lesions on a leaf ; insert 4) *Alternaria helianthi* lesions showing concentric banding (target spot) within necrotic areas.

any symptoms on glasshouse grown sunflowers. It is likely that these organisms are saprophytes which have invaded necrotic leaf tissue.

Hence, it would appear that C.B.L. together with chlorosis and necrosis of leaf tissue are the symptoms associated with acute deficiency of P in rapidly growing plants. This pot condition may mimic field conditions where the surface soil contributes most of the plant phosphorus but continues to do so only while the surface is moist. Moist surface conditions for the first few weeks of growth, followed by a rapid drying of the surface soils could give rise to sudden P deficiency and subsequent development of C.B.L. The darker green coloration that has been noted previously in leaves of phosphorus deficient plants grown in sand (Eaton, 1949), was not observed in plants grown in any of these experiments.

CONCLUSIONS

Necrosis of the leaf tips and margins in sunflowers followed by complete leaf death, is probably indicative of initial and continuing poor P supply that is associated with the recycling of P from the lower leaves to the upper meristematic tissue. Saprophytic micro-organisms may increase the rate of necrotic development. Observations suggest that C.B.L. occur most consistently in plants growing rapidly in a medium in which P supply falls very rapidly after a period of adequate supply. This is likely to occur in soil or in solution culture in pots where P is being taken up rapidly without being replaced and also in the field following the rapid decline in the adequate supply of P from the drying surface horizon.

Recent work (Hunter, unpublished) suggests that sunflower, growing in low P soils that are common in Central Queensland, may have an obligate dependence on mycorrhizae. Such a mycorrhizal association could provide enough P in most seasons to allow growth, even in very P deficient soil (5 ug g^{-1} P_B) to proceed free from symptom development. However, such plants may still respond to P particularly in the vegetative phase (Hibberd, personal communication). Assuming the mycorrhizal infection of plants is normal under most field conditions, it is likely that the symptoms described above would be restricted to conditions where mycorrhizal development is likely to be poor (e.g. after long fallows, Black and Tinker 1978) or where P supply declines rapidly (e.g. in pots).

However, it is important to recognize that these symptoms are caused by the lack of P and not by a pathogenic organism. Thus, low yields that have been incorrectly attributed to disease problems caused by micro-organisms, may be increased substantially by appropriate P fertilization.

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DÉFICIENCE SÉVÈRE DU PHOSPHORE CHEZ LE TOURNESOL — LA CAUSE DES SYMPTÔMES FOLIAIRES SIMILAIRES À CEUX PRODUITS PAR CERTAINS CHAMPIGNONS PATHOGÈNES

Résumé

Les plantes de tournesol ont été élevées en pots au sol noir et au champ, en conditions d'approvisionnement différent en phosphore. Ces conditions ont été réalisées en ajoutant des différentes doses de phosphore aux sols à teneur réduite en phosphore (moins de 13 ug g^{-1} phosphore extractible en bicarbonate). Les effets positifs connus de l'association des mycorhizes sur l'absorption du phosphore dans les plantes poussant en sols déficients en phosphore ont été réduits au minimum par le traitement thérmique du sol des pots et par l'utilisation d'un terrain inculte pendant quatre années.

Les symptômes attribués à la déficience en phosphore on été les suivants : vigueur générale faible des plantes, chlorose et nécrose du bout et des bords des feuilles, lésions en bandes concentriques répandues non-uniformément sur la surface de la feuille et la mort complète des feuilles chez les plantes très déficientes.

La chlorose foliaire et les nécroses marginales ont été similaires à celles provoquées par *Verticillium* sp., tandis que les lésions en bandes concentriques étaient apparemment similaires aux lésions causées par certains pathogènes comme *Alternaria* spp. Cependant, tous les microorganismes isolés des plantes élevées en conditions de faible approvisionnement en phosphore présentant tels symptômes n'ont pas été pathogènes au tournesol artificiellement inoculé en serre.

INSUFICIENCIA SEVERA DE FÓSFORO EN GIRASOL-CAUSA DE LAS SÍNTOMAS FALIARES SIMILARES A LOS PRODUCIDOS DE UNOS HONGOS PATÓGENOS

Resúmen

Las plantas de girasol fueron cultivadas en vasos con suelo negro y en campo, en condiciones de abastecimiento diferente de fósforo. Estas condiciones se realizaron añadiendo las diferentes dósis de fósforo a los suelos con contenido reducido de fósforo (menos de 13 ug g^{-1} fósforo extractible en bicarbonato). Los efectos positivos conocidos de la asociación de las micorizas sobre la absorbción del fósforo en las plantas que crecen en suelos deficientes de fósforo fueron minimalizadas por el tratamiento térmico del suelo de los vasos y por el empleo de un terreno nocultivado durante cuatro años.

Las síntomas atribuídos a la deficiencia de fósforo fueron las siguientes: vigor general bajo en las plantas, clorósis y necrósis de la cima y del margen de las hojas, lesiones en forma de cintas concentricas distribuídas con no uniformidad sobre la superficie de la hoja y la muerta completa de las hojas en las plantas muy deficientes.

La clorosis foliaria y las necrósis marginales fueron similares a los causadas por *Verticilium* sp., mientras que las lesiones en forma de cinta concentrica fueron aparentemente similares a las lesiones causadas por unos patógenos como *Alternaria* spp. Sin embargo, todos los microorganismos aislados de las plantas cultivadas en condiciones de bajo abastecimiento con fósforo y que presentaron tales síntomas, no resultaron como patogénicos en el girasol inoculado artificialmente en invernadero.