THE DYNAMICS OF MANGANESE PHYTOTOXICITY: IMPLICATIONS FOR DIAGNOSIS AND MANAGEMENT OF EXCESS MANGANESE IN ACID UPLAND SOILS

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ABSTRACT

Manganese (Mn) in excess of crop requirements is a serious problem when manganiferous soils become acid, waterlogged or amended with organic materials. We investigated the dynamics of manganese phytotoxicity and tested management options for growing crops in acid soils with excess Mn. We hypothesized that Mn phytotoxicity is governed by water use and expressed as continuous negative interaction between current plant/leaf growth rate ($RGR_{PLant/Leaf}$) and future Mn accumulation rate (RAR_{Mn}); and that under growth conditions where $RGR_{PLant/Leaf}$ exceeds RAR_{Mn}, excess Mn can be managed by maximizing $RGR_{PLant/Leaf}$ and minimizing RAR_{Mn}. The parameters $RGR_{Plant/Leaf}$ and RAR_{Mn} were calculated using the conventional growth analysis techniques.

The dynamics of Mn phytotoxicity was investigated by growing Mn-tolerant Lee and Mn-sensitive Forrest soybeans in the greenhouse using *Wahiawa* series, a manganiferous Oxisol in Hawaii. The soybeans were grown at soil pHs 4.78, 5.5, and 6.00 and five growth conditions (control, 80-90% field capacity, 40% shading, green manure and phosphorus at 150 mg kg^{-1}). RAR_{Mn} consistently exceeded RGR_{Leaf} in most treatments. Over the range of soil pH and growth conditions, we found strong positive correlation between RGR_{Leaf} and RAR_{Mn}, this correlation mediated by a more fundamental correlation of both rate processes to plant water use. The dynamics of Mn phytotoxicity, referred to as the 'dual feedback effect' model described a continuous negative interaction between current RGR_{Leaf} and future RAR_{Mn} and between current RAR_{Mn} and future RGR_{Leaf}. Manganese accumulation rate exceeded plant growth rate, leaf

Mn increased with time and growth treatments did not affect growth rate unless soil pH was increased to eliminate excessive Mn in the soil.

Field experiments were conducted in *Rugao* series, an acid Alfisol in Northern Philippines. The soil is acid (pH 4.40) with abundant Fe-Mn concretions within the surface 20-cm. Preliminary field experiment showed Mn phytotoxicity in local soybeans cv. PSB Sy2 and PSB Sy6 as leaf symptoms in addition to low plant growth rates and grain yields associated with leaf Mn exceeding a critical value of 500 mg kg^{-1}. Results of a second field experiment showed that cultivar, liming, and the management of phosphorus (P), manure and mulching modified plant growth rate and enhanced tolerance to excess soil Mn. Lime control (2 t ha^{-1}) neutralized half of the exchangeable Al while keeping saturated paste Mn in excess. Mulching did not affect saturated paste-and increased RAR_{Mn} without affecting RGR_{Leaf}. Increases in RGR_{Leaf} due to P and manure were accompanied by increases in RAR_{Mn}. This increase in RGR_{Leaf} translated to increased yields even when RAR_{Mn} and soil solution Mn were increased as in the case of manure addition. Increases in grain yield due to manure exceeded the increases due to lime or P. Chicken manure was more effective than green manure in increasing grain yield. Plant growth rate exceeded Mn accumulation rate, leaf Mn decreased with time and manure treatments alleviated Mn phytotoxicity despite an increase in soil Mn.

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Chapter 3

DIAGNOSING THE POTENTIAL AND ACTUAL TOXICITY OF MANGANESE IN AN ACID UPLAND SOIL IN BARANGAY SAN ANTONIO, ISABELA, PHILIPPINES

Introduction

The Philippines is an archipelago of more than 7000 islands of varying sizes under a humid tropical climate with annual rainfall of 1200-4600 mm and annual temperature of 25.8 - 27.9 °C. Soils with pH below 5.0 occupy about 56% of the country's 39 M ha land area and includes soils in the uplands as well as the unclassified soils in the hilly and mountainous areas (National land Use Committee, 1985). These soils are mostly Ultisols and Oxisols of the US Soil Taxonomy, as well as other soil orders with *kandic* or oxidic properties (Recel *et al.*, 1990). The Ultisols are estimated to cover 27% of country's land area including the span of the Sierra Madre mountain range bordering the western edge of the Luzon island .

The expanse of acid soils in the Philippines made the country a suitable candidate for testing the Nutrient Management Decision Support systems (NuMaSS) of the Soil Management Collaborative Research Program (SM-CRSP) Decision Aids project. The NuMaSS core experiment has been designed to test predictions of nitrogen, phosphorus and acidity and to develop supporting data to estimate interactions among nitrogen (N), phosphorus (P) and lime rates. Barangay[1] San Antonio in the town of Ilagan, province of Isabela in northern

[1] Barangay is a village of about 600 households; the smallest political unit in the Philippines.

Luzon, Philippines has been selected as the core experiment site for testing the effects of levels of lime, N and P on various crops (rice, corn, peanut, soybean, mungbean) since September 1998. The soil in the testing site is Rugao series, classified according to US Soil Taxonomy as *fine, isohyperthermic Typic Kandiudalf* (Bureau of Soils and Water Management Report, 1999). A description and chemical characteristics of a representative soil profile in the testing site is shown in Table 3.1 & 3.2. Soils collected from four *cabecera*[2] of the *barangay* were characterized by extreme acidity very low P, high exchangeable Al, very low exchangeable bases and considerable amounts of citrate-bicarbonate-dithionite (CBD)-Mn (Table 3.3; adapted from Corton *et al.*, 1998). Black Fe-Mn nodules were likewise visible from the in the soil surface after cultivation.

Manganese toxicity has been suspected in the soil since Mn toxicity symptoms were observed in the 1999 peanut crop (Hue,1999). Extremely low soil pH, combined with the presence of Mn in the form of nodules, concretions and soft masses, and periods of continuous rainfall alternating with dry spells, are important indicators of the potentially toxic amounts of Mn in the soil. Potential toxicity, however, may not be translated to actual phytotoxicity if the plant specie or cultivar is tolerant, or if the growth conditions do not allow excess amounts of available Mn in the soil. The goal of this investigation was to use techniques such as soil and plant analysis, diagnostic symptoms, and plant growth measurements to indicate potential as well as actual Mn phytotoxicity.

[2] The village of San Antonio is further subdivided into smaller units called 'cabecera'.

We hypothesize that the field conditions in San Antonio, characterized by the presence of Fe-Mn nodules (Table 3.1), acid soil pH and episodes of rainfall exceeding plant water use, will favor excess levels of Mn in the soil. This excess of Mn will be indicated by high values of soil tests for "available" Mn and expressed as phytotoxic symptoms and growth as well as grain yield reduction in soybeans. If there is Mn phytotoxicity, then, amelioration with liming will reduce excess Mn in the soil, reduce leaf Mn concentrations and consequently improve growth and grain yield of the crop. Even without reduction of soil Mn, the use of tolerant cv. will be an effective technique to alleviate Mn toxicity. Although green manure addition has been used successfully to ameliorate acidity through neutralization of Al toxicity, it has been shown to increase the level of Mn in the soil solution and, therefore, may prove to aggravate Mn toxicity.

Objectives

1. To assess the effectiveness of soil and plant analysis, phytotoxic symptoms and other soil indicators in diagnosing the potential and actual phytotoxicity of Mn in an acid soil;
2. Measure the effect of cultivar, liming and green manure addition on alleviating phytotoxic symptoms, increasing growth and yield of soybeans grown in an acid soil.

Table 3.1. Soil profile[a] characteristics of a representative soil in *Barangay*[b] San Antonio, Isabela, Philippines.

Taxonomic Classification: *Fine, isohyperthermic Typic Kandiudalf*; 8-18% slopes, slightly eroded

Horizon Symbol	Depth (cm)	Description
Ap	0-18	Yellowish brown (10YR 5/4) moist, sandy clay loam; weak fine breaking to sub-angular blocky structure; slightly sticky, non-plastic, friable; few soft **Mn-Fe concretions**; very few fine tubular pores; few fine roots; clear wavy boundary; soil hardness: 19 kg cm^{-2}; pH 4.6
`B21tc	18-56	Strong brown (7.5YR 4/6) moist, clay loam; no mottles; strong fine breaking to sub-angular blocky structure; sticky plastic slightly firm moist and hard dry; many small and medium soft and hard spherical black **Fe-Mn concretions**; very few fine tubular pores few very fine roots; gradual wavy boundary; soil hardness: 24 kg cm^{-2}; pH 5.2
B22t	56-76	Strong brown (7.5YR 4/8) moist, clay; no mottles; moderate medium and coarse angular to sub-angular blocky structure; sticky plastic firm moist; few small hard spherical black **Fe-Mn concretions**; few medium and coarse partly and highly weathered rock fragments; very few very fine roots; clear smooth boundary; soil hardness; 3 $kgcm^{-2}$; pH 5.0
BCt	76-109	Brown (7.5YR 4/4) mist, clay; no mottles; moderate to strong medium and coarse angular to sub-angular blocky structure; sticky plastic firm; very few very fine tubular pores; common medium and coarse partly and highly weathered rock fragments; gradual wavy boundary; soil hardness: 14 kg cm^{-2} ; pH 4.8
C	109-150	Strong brown (7.5 YR 4/6) moist, clay, no mottles; strong medium and coarse angular to sub-angular blocky structure; sticky plastic slightly firm moist; many medium and coarse partly and highly weathered reddish brown and reddish yellow rock fragments; soil hardness: 10 kg cm^{-2}; pH 4.8

[a]Adapted from Bureau of Soils and Water Management Special Report, (*unpub.*, 1998).
[b]Village of about 300 households, the smallest political unit in the Philippines

Table 3.2. Some chemical characteristics of a representative soil profile (Table 1) in *Barangay* San Antonio, Isabela, Philippines.

Horizon	Depth, cm	Soil pH		OC (%)	Base Saturation,% (Sum of Cations)	Extractable Al, $cmol_+ kg^{-1}$
		1:1 soil:water	1:1 Soil: 0.05 *M* $CaCl_2$			
Ap	0-18	4.6	3.7	0.75	41.8	1.66
B21tc	19 -56	5.2	4.0	0.37	47.4	1.09
B22t	57 -76	5.0	4.0	0.38	66.2	0.90
BCt	77 -109	4.8	4.0	0.35	65.7	1.44
C	110 -150	4.9	4.1	0.29	57.8	2.35

		Exchangeable Bases				Sum of Bases	Exchangeable Acidity	CEC, (sum of cations)
		Ca	Mg	Na	K			
		$cmol_+ kg^{-1}$						
Ap	0-18	0.9	0.1	0.1	T	1.1	1.53	2.63
B21tc	19 -56	1.0	0.2	T	T	1.2	1.11	2.11
B22t	57 -76	1.8	0.2	0.1	T	2.1	1.07	3.17
BCt	77 -109	2.5	0.6	0.1	T	3.2	1.67	4.87
C	110 -150	2.0	0.8	0.1	T	2.9	2.11	5.01

Note: T – trace amounts, <0.1 $cmol_c kg^{-1}$
Adapted from Bureau of Soils and Water Management Special Report, (*unpub.*, 1998).

Materials and Methods

Site

A field experiment was conducted in Barangay, San Antonio in the province of Isabela in Northern Philippines during the cropping season, June-October 2000. San Antonio is a village of about 3000 people residing over 994 ha of land, 350 ha of which is planted to rice and corn (Corton, *et al.*, 1998). The village is within the vicinity of the Sierra Madre Mountain range that runs in the north-south direction and spans the western edge of Luzon island. The Bureau

of Soils and Water Management (BSWM) mapped the soils in this region as Ultisols (US Soil Taxonomy) although soil profiles in the village were later determined as Alfisols (BSWM Report, 1999). The soil is *Rugao* series, characterized by extreme acidity, low P, organic carbon and exchangeable bases and high extractable Al and citrate-bicarbonate-dithionite (CBD) -Mn (Table 3.3). Measured rainfall at the site during the soybean cropping season showed daily rainfall of less than 20 mm in July and August. In the succeeding two months, however, there were at least 5 rainfall events exceeding 20 mm and one rainfall event of about 100 mm (Fig. 3.1).

Table 3.3. Selected properties of surface soil samples from four locations in *Barangay* San Antonio, Isabela, Philippines.

Location	pH (1:1 soil:water)	Bray 2-P, mg kg^{-1}	Exchangeable Cations, $cmol_+$ kg^{-1}				CBD[a]-extractable Mn, g 100 g^{-1}
			K	Ca	Mg	Al	
Centro 1 (lower)	4.45	1.24	0.18	1.21	1.26	0.80	1.2
Centro 1 (higher)	4.32	0.70	0.20	1.07	0.94	0.80	0.1
Centro 2	4.30	1.79	0.22	1.66	1.80	0.70	0.1
Kabisera 18	3.88	9.21	0.34	0.46	0.46	0.40	0.1

[a] Citrate-bicarbonate-dithionite
Adapted from Corton, *et al.* (1999).

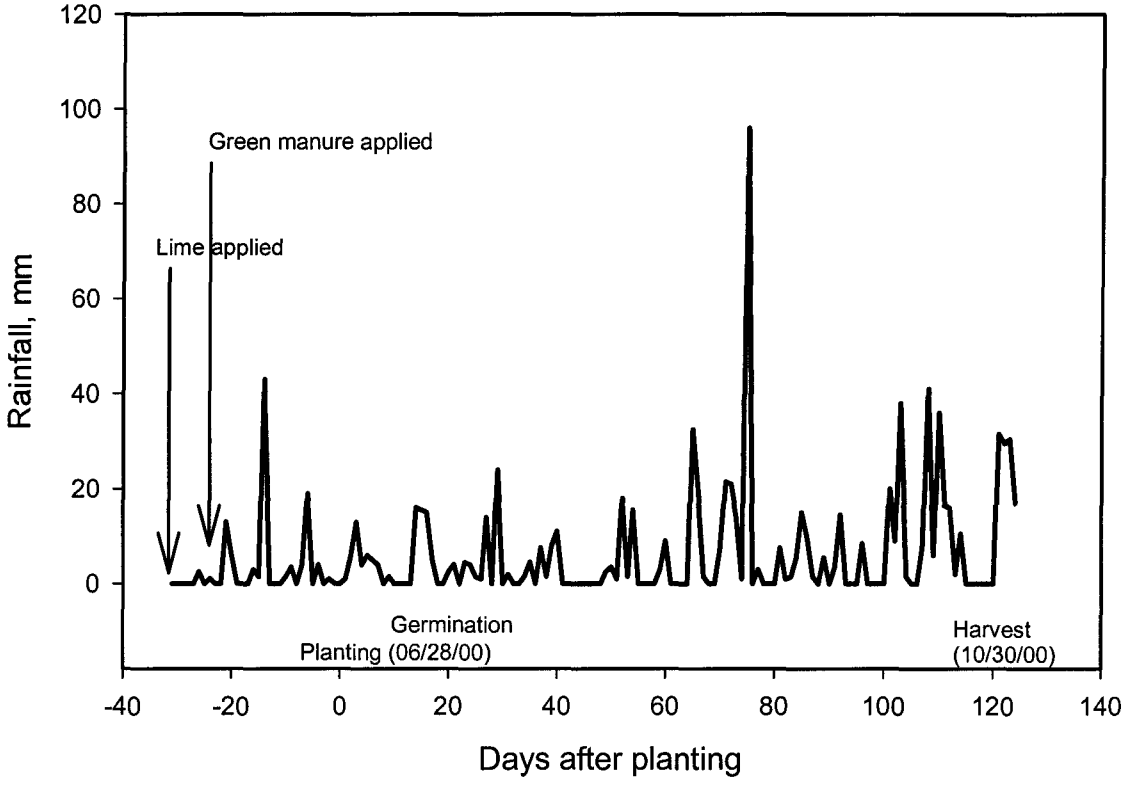


Fig. 3.1. Actual rainfall measured at the experiment site in *Barangay* San Antonio, Isabela, Philippines. Soybean, June-October 2000. Planting date was June 28, 2001.

Treatments and Design of Experiment

Three factors were arranged in split-split plot design in RCB with four replications. The mainplot, subplot and sub-subplot factors were lime, green manure and cultivar, respectively. Two local soybean cultivars PSB Sy2 and PSB Sy6 were the test crop. Two levels of green manure were 0 and 7 t ha^{-1} (air-dry weight basis) while the levels of $CaCO_3$ lime were 0 and 5 t ha^{-1} to raise soil pH from unamended pH of 4.30 to 5.5. Aboveground biomass (leaves and

stem) of 45-day old peanut plants were chopped into small pieces and incorporated as green manure.

Cultural Practices for Growing Soybeans

Soybean seeds were mixed with rhizobium inoculant obtained from the Institute of Plant Breeding, University of the Philippines. The soybeans were then planted at 50 seeds per linear meter along furrows 50 cm apart. The seedlings were thinned at 25 plants per linear meter at 7 days after emergence (DAE). Nitrogen, phosphorus and potassium (K) were applied (in kg ha^{-1}) at 90, 60, 60, respectively. These rates were slightly higher than the recommended N-P-K rates of 80-45-45 for soybeans (Aquino, pers. communication). Both P (as TSP, 40% P_2O_5) and K (as KCl, 60% K_2O) were applied basal; *i. e.*, during the last harrowing 2 days before planting. The fertilizers were mixed within 20 cm of the soil surface by cultivating the soil with a hoe during application. Nitrogen as urea (46% N) was topdressed in two splits, 1/3 of the total amount (30 kg ha^{-1}) at 10 DAE and the remaining 2/3 (60 kg ha^{-1}) at 30 DAE. Pesticides[3] Lannate® (Dupont, Canada, Crop Protection) and Decis® (Bayer CropScience) were sprayed at 35 DAE and 45 DAE to control leaf borers and leafhoppers. Weeds were removed by hand at 25 DAE and 50 DAE. Harvesting was done by hand-picking the soybean pods at maturity, about 120 days after sowing.

[3] The use of these pesticides in our experiment does not constitute any recommendation in our part for their use in treating pests of soybeans.

Soil and Plant Measurements

Soil samples from each plot were collected at 21 DAE and analyzed for pH and "available" Mn using four extractants: water, 1 *M* KCl, DTPA and Mehlich I. Details of the KCl method are described in Bertsch and Bloom (1996) while details of the other three methods can be found in Gambrell (1996).

Eight plants were collected randomly every two weeks from each plot. The leaves were detached for leaf area measurement using a Li-COR leaf area meter (LI-3100a). The leaves and stem samples were then oven-dried at 70°C for about three days (or until a constant moisture was attained) and weighed for the determination of leaf, stem and total plant biomass. Dried leaves were dry-ashed in a muffle furnace at 500 °C for 4 hours. Ashed samples were digested further with 5 ml 2 *M* HNO_3 at 120°C until the acid had evaporated. The residue was then diluted in 0.1 *M* HCl and filtered using Whatman #42 filter paper. The concentrations of Mn, Ca, Mg, K and P in the extracts were analyzed using Inductively Coupled Plasma Emission Spectroscopy (ICP) at the Agricultural Diagnostic Services Center (ADSC) of the University of Hawaii. The severity of Mn phytotoxicity was rated by weekly observations on the general health of the plants, taking note of the appearance of brown spots, necrosis, interveinal chlorosis and crinkling of the leaves (Fig. 3.2). The rating scale is shown in Table 3.4. The symptoms were also documented by taking digital photographs of the plants. Sample rating done at 28 DAE is shown in Fig. 3.3.

Fig. 3.2. Phytotoxic symptoms of excess Mn in field-grown soybeans.

Table 3.4. Rating scale used to score Mn phytotoxicity status of field-grown soybeans.

Rating	Description of symptoms
1	generally healthy, without symptoms of toxicity
2	appears healthy, with few brown spots on leaves, slight interveinal yellowing of leaves
3	stunted, common brown spots on leaves, common interveinal yellowing of leaves, slight crinkling of younger leaves
4	severely stunted, many brown spots on leaves that coalesced to bigger necrotic spots, severe interveinal yellowing of leaves, severe crinkling of younger leaves

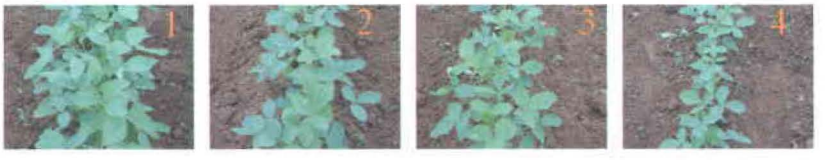


Fig. 3.3. Symptom score of field grown soybeans at 28 DAE.

Growth Analysis and Calculations

Growth analysis techniques were used to calculate rates of plant growth and accumulation of Mn. Relative growth rate of the whole plant (RGR_{Plant}, g g^{-1} d^{-1}) or leaves (RGR_{Leaf}) and the relative accumulation rate of Mn (RAR_{Mn}) were calculated using the following equations from Radford (1967) and Rufty *et al.* (1974), respectively:

$$RGR_{Plant/Leaf} = (lnBm_{T2} - lnBm_{T1}) / (T_1 - T_0) \quad \text{[Eqn. 3.1]}$$

$RAR_{Mn} = \{ln(Bm_{T1} * C_{T1}) - ln(Bm_{T0} * C_{T0})\} / (Bm_{T0} * C_{T0}) / (T_1 - T_0)$ [Eqn. 3.2]

where:

$RGR_{Plant/Leaf}$ is the mean plant/leaf relative growth rate over a given growth interval

RAR_{Mn} is the mean Mn relative accumulation rate over a given growth interval

Bm is the weight of biomass (whole plant, leaves) in g

C is the Leaf Mn concentration in $\mu g\ g^{-1}$

T_0 is a previous sampling date

T_1 is a later sampling date

In Equation 3.1, the $RGR_{Plant/Leaf}$ essentially defines how much additional biomass was gained by the plant per unit of initial biomass per unit of time. This parameter allows the comparison of growth performance of plants subjected to various treatments within an experiment or growth performance of plants between experiments (Hunt, 1990). The conventional growth analysis approach (Radford, 1967) was used to calculate a mean $RGR_{Plant/Leaf}$ over a given growth interval. Incremental $RGR_{Plant/Leaf}$ were calculated for each growth interval represented by sampling dates. Samples were collected every two weeks from 14 DAE until 42 DAE. Two $RGR_{Plant/Leaf}$ were calculated from two growth intervals: 14-28 DAE and 28-42 DAE. The whole growth period $RGR_{Plant/Leaf}$ were calculated as the mean of the $RGR_{Plant/Leaf}$ from the two growth intervals. For example, mean RGR_{Leaf} over the period 14-28 DAE is designated as RGR_{Leaf}, 14-28 DAE while mean RGR_{PLant} over the growth period 14-42 DAE is designated as RGR_{Leaf}, 14-42 DAE.

Only one RAR_{Mn} from growth interval 28-42 DAE was calculated due to the limitation in the number of samples that were analyzed from this experiment. In comparing RGR_{Leaf} with RAR_{Mn}, RGR_{Leaf} at 28-42 days was used.

Statistical analysis

The mainplot, subplot and sub-subplot treatment effects on plant growth and soil chemical properties were analyzed using the Analysis of Variance procedure of the Statistical Analysis Systems (SAS, 1990). Linear relationships (model probabilities and coefficients) between parameters were determined using the Regression procedure of SAS. Probabilities of 5% or less were considered significant. Treatment means were compared by calculating appropriate Least Significant Difference (LSD) for different factors and level of comparisons in a split-split-plot design. Where factor interactions were significant, different LSD values were calculated and used to compare subplot means within a mainplot, subplot means across mainplots, sub-subplot means within a subplot or sub-subplot means across subplots (Gomez and Gomez, 1985). For example, leaf area was significantly influenced by the interaction between green manure and cultivar (Table 3.10). In comparing means of leaf area, LSD^{1}_{05} was used to compare two green manure means at the same or different cultivar, i.e., Gm0 of PSB Sy2 vs. Gm0 of PSB Sy6 or Gm0 of PSB Sy2 vs. Gm7 of PSB Sy2. On the other hand, LSD^{2}_{05} was used compare two cultivar means within a green manure level, i.e., PSB Sy2 vs PSB sy6 at Gm0 or PSB Sy2 vs. PSB Sy6 at Gm7.

Results

Soil pH and extractable Mn

Lime application significantly influenced soil pH and extractable Mn while soybean cultivar and peanut green manure had no significant effect on these soil properties (Table 3.5). The application of 5 t ha^{-1} lime increased soil pH from 4.28 to 5.20. The amount of soil manganese extracted by water, 1 *M* KCl (potassium chloride), DTPA (diethylenetriaminepentaacetic acid) and Mehlich I was significantly reduced by liming (Table 3.6). Water and 1 *M* KCl solubilized similar amounts of Mn. On the other hand, DTPA solution solubilized about twice as much and Mehlich I solubilized 10 times as much Mn as that solubilized by water and KCl. DTPA is a chelator and compared with water and KCL, is expected to solubilize more Mn. Mehlich I contained HCl and H_2SO_4 acids, hence making it the strongest solubilizer of Mn among the four extractants.

Table 3.5. Significance (*Pr*) of the mainplot, subplot and sub-subplot effects and interaction effects of lime, green manure and cultivar on soil pH and soil extractable Mn.

Effects	pH	Extractable Mn			
		Water	KCl	Mehlich 1	DTPA
Lime (L)	*0.0059*	*0.0065*	*0.0092*	*0.0074*	*0.0200*
G. manure (Gm)	*0.6034*	*0.4068*	*0.6594*	*0.3513*	*0.8601*
Cultivar (V)	*0.1477*	*0.4075*	*0.9142*	*0.9266*	*0.4604*
L x Gm	*0.9898*	*0.4324*	*0.5575*	*0.5233*	*0.9815*
L x V	*0.5359*	*0.4693*	*0.2212*	*0.5310*	*0.8225*
G x V	*0.5949*	*0.4144*	*0.7698*	*0.7124*	*0.2729*
L x Gm x V	*0.7221*	*0.3222*	*0.4337*	*0.0889*	*0.0336*

Table 3.6. Soil pH and extractable Mn as influenced by lime and green manure application and soybean cultivar.

Lime t ha^{-1}	Gm t ha^{-1}	Cultivar	Soil pH		Extractable Mn, mg kg^{-1}							
					Water		KCl		Mehlich 1		DTPA	
0	0	PSB Sy2	4.34	*0.16*	4.28	*1.79*	4.44	*1.46*	52.0	*6.2*	11.52	*2.76*
0	0	PSB Sy6	4.28	*0.07*	2.75	*0.52*	3.85	*0.68*	47.9	*7.1*	8.83	*1.32*
0	7	PSB Sy2	4.28	*0.14*	5.07	*1.01*	4.80	*0.47*	52.0	*5.0*	9.58	*0.91*
0	7	PSB Sy6	4.24	*0.09*	5.19	*1.44*	4.73	*0.81*	60.2	*7.7*	11.10	*1.48*
5	0	PSB Sy2	5.33	*0.20*	0.08	*0.04*	0.30	*0.15*	25.1	*4.2*	4.66	*0.80*
5	0	PSB Sy6	5.16	*0.11*	0.11	*0.08*	0.70	*0.17*	27.7	*1.3*	5.05	*0.24*
5	7	PSB Sy2	5.23	*0.11*	0.21	*0.19*	0.33	*0.07*	30.4	*1.6*	5.62	*0.28*
5	7	PSB Sy6	5.16	*0.08*	0.18	*0.04*	0.49	*0.18*	24.8	*2.3*	4.57	*0.42*
MEANS												
Lime 0			4.28	*0.06*	4.32	*0.63*	4.45	*0.43*	53.0	*3.2*	10.26	*0.84*
Lime 5			5.22	*0.06*	0.12	*0.05*	0.45	*0.08*	27.0	*1.3*	4.98	*0.24*
	Gm 0		4.78	*0.14*	1.81	*0.62*	2.32	*0.60*	38.2	*3.8*	7.52	*1.02*
	Gm 7		4.72	*0.13*	2.64	*0.76*	2.59	*0.60*	41.8	*4.3*	7.72	*0.82*
		PSB Sy2	4.79	*0.14*	2.41	*0.75*	2.47	*0.66*	39.9	*3.8*	7.85	*0.99*
		PSB Sy6	4.71	*0.12*	2.03	*0.65*	2.44	*0.54*	40.1	*4.47*	7.39	*0.83*

Numbers in italics are standard error of the mean.

Toxicity Symptoms

The field-grown soybean crop exhibited severe symptoms of Mn toxicity characterized by the following symptoms: appearance of black speckles and lesions in older leaves, irregular yellowing of interveinal tissues of young and old leaves, and crinkling of young leaves. These symptoms started to develop during the unifoliolate leaf stage and were fully expressed at two weeks after emergence when the first trifoliolate has fully expanded. A rating scheme was devised: 1 for plants having no symptoms and 4 for plants showing all the

symptoms described above (Table 3.4). Symptoms were most severe in plots without lime. Soybean cv. PSB Sy2 appeared to be more tolerant of soil acidity compared to PSB Sy6 as shown by less severe symptoms. Plants with severe leaf symptoms were also severely stunted. The plants somewhat recovered, exhibiting fewer symptoms during the next two weeks of growth.

Symptom expression was significantly affected by lime, green manure and cultivar at six weeks after emergence (Table 3.7). Soybean cv. PSB Sy2 generally showed less severe symptoms compared to cv. PSB Sy6. Folding of the leaves of PSB Sy6 was also observed at this stage. Addition of lime markedly alleviated the symptoms. The symptoms were less severe where green manure was applied. The most severe symptoms were expressed in unlimed plots where no green manure was applied, while the least severe symptoms were observed where both lime and green manure were added.

Plant growth

Total aboveground biomass (leaves and stem) and leaf area continuously increased throughout the 42-day sampling period (Fig. 3.4) without indications of leveling off. Leaf area increased from about 50 cm^2 at 14 DAE to a maximum of 660 at 42 DAE. Plant biomass, on the other hand, increased from about 0.20 g at 14 DAE to a maximum of 6.0 g at 42 DAE. The lowest leaf area and biomass were observed in unamended cv. PSB Sy6 while the highest leaf area and biomass were given by cv. PSB Sy2 that received both lime and green manure.

Table 3.7. Lime x green manure and single factor effects on toxicity scores of 6-week old soybean cv..

Lime	Green Manure	PSB Sy2		PSB Sy6	
0	0	3.38	0.239	4.00	0
0	7	2.13	0.515	3.13	0.239
5	0	1.13	0	2.63	0.125
5	7	1.00	0	2.63	0.125

Single factor effects

Effects	Pr	Lime 0	Lime 5	Gm 0	Gm 7	PSB Sy2	PSB Sy6
Lime (L)	*0.0031*	3.16 (*0.22*)	1.84 (*0.21*)				
G. manure (Gm)	*0.0249*			2.78 (*0.28*)	2.22 (*0.24*)		
Cultivar (V)	*<0.0001*					1.91 (*0.28*)	3.09 (*0.16*)
L x Gm	*0.0384*						
L x V	*0.0216*						
G x V	*0.3962*						
L x Gm x V	*0.6678*						

Numbers in italics and parenthesis associated with each mean are standard error of the mean.

Single factor effects of lime, green manure and soybean cultivar as well as the interaction between cultivar and green manure were significant for leaf area, leaf biomass and aboveground biomass at 42 DAE (Table 3.8). The application of lime and green manure significantly increased leaf area, leaf weight and total aboveground biomass. Soybean cv. PSB Sy2 produced more plant biomass and leaves in terms of area and biomass than cv. PSB Sy6. The increase in leaf area, leaf weight and aboveground biomass due to lime exceeded the increases due to cultivar and green manure applications (Table 3.9). The increase in leaf area due to cultivar was greater than the increase due to green manure. In the case

of leaf and total aboveground biomass, the increase due to green manure was greater than the increase due to cultivar (Table 3.9). A significant interaction was found between cultivar and green manure effects on leaf area and biomass. Where green manure was applied, cv. PSB Sy2 produced significantly more biomass and leaf area than cv. PSB Sy6 (Table 3.10).

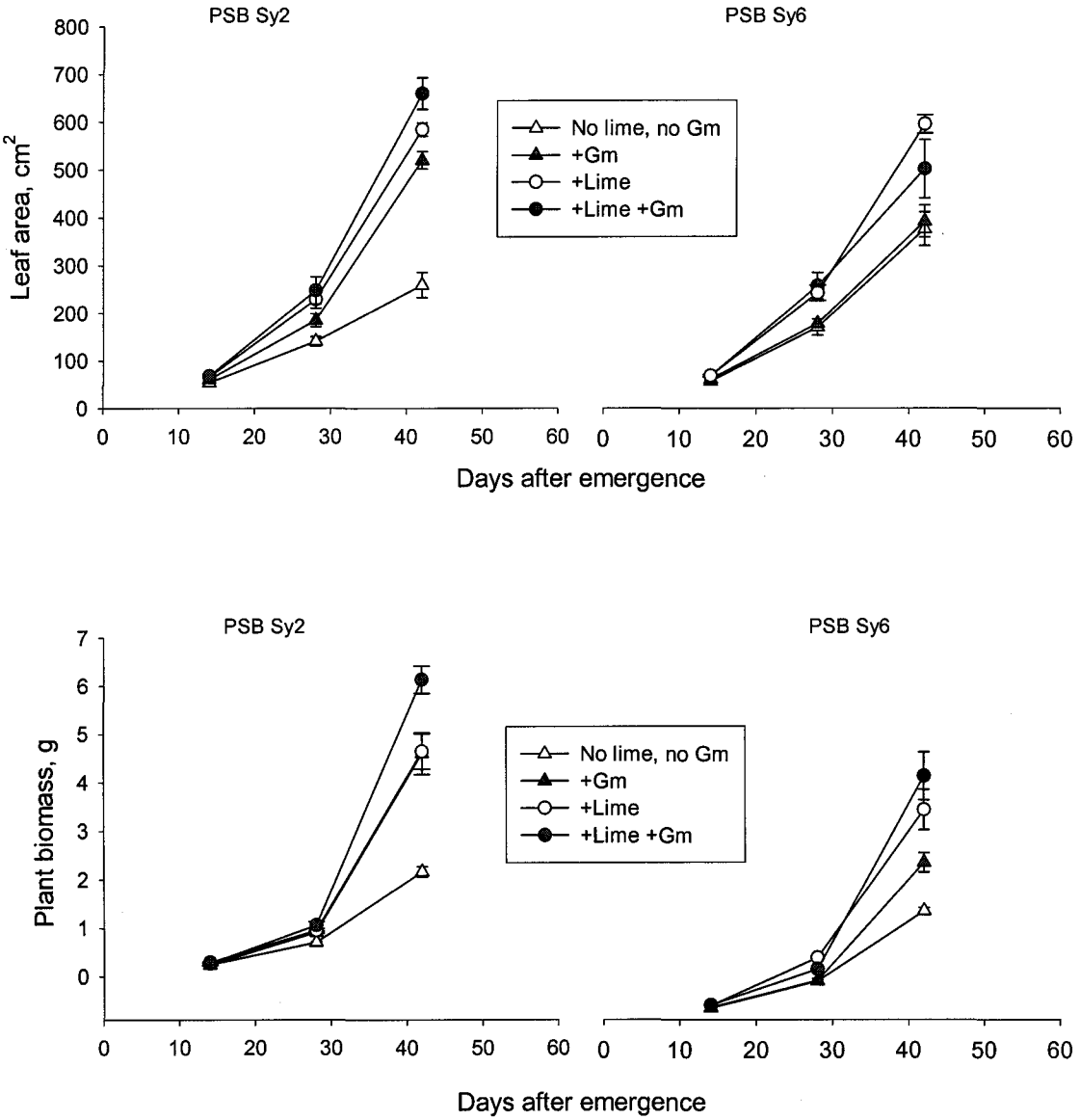


Fig. 3.4. Increase in aboveground biomass and leaf area during the early vegetative stage of two soybean cv. as influenced by lime and green manure addition.

Table 3.8. Significance (*Pr*) of the mainplot, subplot and sub-subplot and interaction effects of lime, green manure and cultivar on leaf area and biomass at 42 DAE, and grain yield of soybeans.

Effects	Leaf area	Biomass per plant		Grain yield
		Leaves	Aboveground	
Lime (L)	*0.0049*	*0.0123*	*0.0114*	*0.0012*
G. manure (Gm)[a]	*0.0468*	*0.0003*	*0.0001*	*0.0072*
Cultivar (V)	*<0.0001*	*0.0011*	*0.0002*	*0.0203*
L x Gm	*0.0394*	*0.3469*	*0.1036*	*0.0131*
L x V	*0.0138*	*0.3317*	*0.4869*	*0.9962*
G x V	*<0.0001*	*0.0217*	*0.0114*	*0.3651*
L x Gm x V	*0.1688*	*0.9394*	*0.4228*	*0.7219*

Table 3.9. Effects of lime, green manure and cultivar on leaf area (42 DAE), biomass (42 DAE) and grain yield of soybeans.

Lime t ha^{-1}	G. manure t ha^{-1}	Cultivar	Leaf area cm^2		Biomass, g plant^{-1} Leaf		Biomass, g plant^{-1} Aboveground		Grain yield kg ha^{-1}	
0	0	PSB Sy2	258	*26*	1.192	*0.033*	2.151	*0.107*	690	*48*
0	0	PSB Sy6	329	*31*	1.102	*0.024*	1.993	*0.057*	569	*65*
0	7	PSB Sy2	519	*18*	2.431	*0.303*	4.586	*0.430*	1499	*128*
0	7	PSB Sy6	343	*29*	1.671	*0.102*	2.879	*0.178*	1148	*164*
5	0	PSB Sy2	583	*13*	2.410	*0.178*	4.631	*0.367*	1848	*64*
5	0	PSB Sy6	521	*17*	2.076	*0.232*	3.844	*0.368*	1663	*76*
5	7	PSB Sy2	658	*33*	3.454	*0.175*	6.122	*0.283*	1945	*227*
5	7	PSB Sy6	439	*54*	2.410	*0.259*	4.461	*0.439*	1660	*109*
MEANS										
Lime 0			362	*27*	1.599	*0.154*	2.902	*0.286*	976	*108*
Lime 7			550	*26*	2.588	*0.164*	4.764	*0.272*	1779	*68*
	Gm 0		423	*36*	1.695	*0.159*	3.155	*0.313*	1192	*150*
	Gm 7		490	*34*	2.492	*0.192*	4.512	*0.335*	1563	*104*
		PSB Sy2	504	*40*	2.372	*0.007*	4.373	*0.395*	1495	*141*
		PSB Sy6	408	*25*	1.815	*0.150*	3.294	*0.277*	1260	*126*

Numbers in italics are standard error of the mean.

Table 3.10. Cultivar x green manure interaction effects on plant growth measurements.

Green manure t ha^{-1}	Cultivar	Leaf area, cm^2		Leaf weight, g $plant^{-1}$		Aboveground biomass, g $plant^{-1}$	
0	PSB Sy2	420	*63*	1.801	*245*	3.391	*0.501*
0	PSB Sy6	425	*40*	1.589	*213*	2.918	*0.390*
7	PSB Sy2	588	*32*	2.942	*252*	5.354	*0.376*
7	PSB Sy6	391	*34*	2.040	*190*	3.670	*0.371*
	LSD^a_{05}	58		0.344		0.528	
	LSD^b_{05}	47		0.403		0.625	
		RGR_{Leaf}		RGR_{Plant}			
0	PSB Sy2	0.0871	*0.0035*	0.0882	*0.0042*		
0	PSB Sy6	0.0862	*0.0031*	0.0873	*0.0034*		
7	PSB Sy2	0.1073	*0.0020*	0.1082	*0.0019*		
7	PSB Sy6	0.0949	*0.0029*	0.0947	*0.0025*		
	LSD^a_{05}	0.0055		0.0047			
	LSD^b_{05}	0.0063		0.0052			

[a]Two green manure means at the same or different cultivar.

[b]Two variety means within a green manure level

Numbers in italics are standard error of the mean.

Concentration of Mn and other nutrients in the leaves

The concentration of Mn in the leaves at 28 DAE and 42 DAE was significantly affected by lime but not green manure or cultivar (Table 3.11). Lime and cultivar interaction influenced leaf Mn at 28 DAE while lime and green manure interaction influenced leaf Mn at the later growth period (Table 3.11). Lime application resulted in a 3-fold decrease in leaf Mn at both sampling dates (Table 3.12). The application of green manure decreased leaf Mn only where no lime was applied.

Lime application significantly influenced leaf Ca and Mg concentrations while green manure influenced leaf Mg, P and K (Table 3.11). The effect of lime was to decrease leaf Mg while increasing leaf Ca (Table 3.14). Green manure, on the other hand, increased leaf Mg, P and K. The soybean cv. differed in their leaf Ca, P and K concentrations. However, cultivar differences were dependent on whether lime was applied or not. Where no lime was applied, cv. PSB Sy2 gave higher leaf Ca and P than cv. PSB Sy6. Where lime was applied, cv. PSB Sy6 gave higher concentration of K compared with cv. PSB Sy2 (Table 3.15).

Table 3.11. Significance (*Pr*) of the mainplot, subplot and sub-subplot and interaction effects of lime, green manure and cultivar on leaf nutrient concentration of soybeans.

Effects	Leaf Mn		Leaf nutrient at 28 DAE			
	28 DAE	42 DAE	Ca	Mg	P	K
Lime (L)	*0.0022*	*0.0012*	*0.0009*	*0.0010*	*0.3378*	*0.5650*
G. manure (Gm)	*0.1752*	*0.0706*	*0.4528*	*0.0144*	*0.0034*	*0.0017*
Cultivar (V)	*0.1297*	*0.6081*	*<0.0001*	*0.5293*	*0.0119*	*0.0001*
L x Gm	*0.1091*	*0.0118*	*0.8515*	*0.7279*	*0.4229*	*0.3924*
L x V	*0.0056*	*0.0781*	*0.0067*	*0.8717*	*0.0023*	*0.0164*
G x V	*0.1182*	*0.9439*	*0.2374*	*0.1102*	*0.1301*	*0.7464*
L x Gm x V	*0.0031*	*0.1703*	*0.9948*	*0.7886*	*0.6878*	*0.2585*

Table 3.12. Single factor and lime x green manure x cultivar interaction effects on leaf Mn concentration of soybeans at two sampling dates.

Lime t ha^{-1}	G. manure t ha^{-1}	Cultivar	Leaf Mn, µg g^{-1}			
			28 DAE		42 DAE	
0	0	PSB Sy2	2175	*220*	1752	*322*
0	0	PSB Sy6	1758	*166*	1601	*212*
0	7	PSB Sy2	1506	*130*	1065	*126*
0	7	PSB Sy6	1521	*190*	1063	*144*
5	0	PSB Sy2	445	*16*	255	*19*
5	0	PSB Sy6	596	*23*	470	*34*
5	7	PSB Sy2	571	*42*	483	*39*
5	7	PSB Sy6	561	*59*	534	*22*
MEANS						
Lime 0			1740	*106*	1370	*125*
Lime 5			543	*23*	435	*31*
	Gm 0		1243	*201*	1019	*192*
	Gm 7		1040	*134*	786	*84*
		PSB Sy2	1174	*192*	888	*169*
		PSB Sy6	1108	*150*	917	*132*

Numbers in italics are standard error of the mean.

Table 3.13. Single factor and lime x green manure x cultivar interaction effects on leaf nutrient concentration of soybeans at two sampling dates.

Lime t ha^{-1}	G. manure t ha^{-1}	Cultivar	Leaf nutrient, g 100 g^{-1}							
			Ca		Mg		P		K	
0	0	PSB Sy2	1.337	*0.069*	0.692	*0.014*	0.256	*0.003*	1.979	*0.050*
0	0	PSB Sy6	1.137	*0.029*	0.663	*0.011*	0.215	*0.011*	2.065	*0.055*
0	7	PSB Sy2	1.319	*0.103*	0.725	*0.047*	0.301	*0.005*	2.146	*0.059*
0	7	PSB Sy6	1.202	*0.079*	0.742	*0.049*	0.241	*0.008*	2.310	*0.108*
5	0	PSB Sy2	2.015	*0.156*	0.559	*0.026*	0.225	*0.013*	1.804	*0.086*
5	0	PSB Sy6	1.594	*0.106*	0.533	*0.052*	0.247	*0.017*	2.249	*0.062*
5	7	PSB Sy2	2.012	*0.040*	0.588	*0.024*	0.264	*0.003*	2.163	*0.065*
5	7	PSB Sy6	1.676	*0.100*	0.595	*0.026*	0.256	*0.005*	2.471	*0.038*
MEANS										
Lime 0			1.20	*0.053*	0.60	*0.021*	0.22	*0.005*	1.80	*0.042*
Lime 5			1.71	*0.072*	0.47	*0.017*	0.21	*0.006*	1.73	*0.062*
	Gm 0		1.39	*0.084*	0.50	*0.021*	0.21	*0.007*	1.65	*0.043*
	Gm 7		1.55	*0.095*	0.66	*0.025*	0.27	*0.004*	2.27	*0.046*
		PSB Sy2	1.60	*0.096*	0.52	*0.025*	0.22	*0.005*	1.68	*0.045*
		PSB Sy6	1.31	*0.069*	0.54	*0.025*	0.21	*0.005*	1.85	*0.054*

Numbers in italics are standard error of the mean.

Table 3.14. Lime and green manure interaction effects on leaf Mn, plant growth rate, Mn accumulation rate and grain yield of soybeans.

Lime, t ha^{-1}	Green manure t ha^{-1}	Leaf Mn, μg g^{-1}				RGR_{Plant}, g g^{-1} d^{-1}		RAR_{Mn}, μg μg^{-1} d^{-1}		Yield, kg ha^{-1}	
		28 DAE		42 DAE							
0	0	1966	*150*	1676	*181*	0.0786	*0.0014*	0.0562	*0.0038*	629	*44*
0	7	1514	*107*	1064	*88*	0.0976	*0.0032*	0.0790	*0.0036*	1323	*117*
5	0	520	*31*	362	*45*	0.0968	*0.0023*	0.0651	*0.0044*	1755	*58*
5	7	566	*34*	508	*23*	0.1053	*0.0029*	0.1095	*0.0051*	1802	*129*
LSD^{a}_{05}		482		387		0.0058		0.0133		338	
LSD^{b}_{05}		438		471		0.0072		0.0161		280	

[a]Two green manure means at the same or different lime level

[b]Two lime means at the same or different green manure level

Numbers in italics are standard error of the mean.

Table 3.15. Cultivar x lime interaction effect on leaf nutrient concentration of soybeans at 42 DAE.

Lime, t ha^{-1}	Cultivar	Leaf nutrient, g 100 g^{-1}					
		Ca		P		K	
0	PSB Sy2	1.328	*0.044*	0.278	*0.010*	2.062	*0.047*
0	PSB Sy6	1.170	*0.038*	0.228	*0.008*	2.187	*0.053*
5	PSB Sy2	2.014	*0.030*	0.245	*0.010*	1.983	*0.078*
5	PSB Sy6	1.635	*0.040*	0.251	*0.005*	2.360	*0.087*
LSD^{a}_{05}		0.014		0.023		0.139	
LSD^{b}_{05}		0.126		0.020		0.197	

[a]Two cultivar means within a lime level

[b]Two lime means at the same or different cultivar level

Numbers in italics are standard error of the mean.

Rates of plant growth and Mn accumulation

The rate by which the soybean plants accumulated biomass was measured as the relative growth rate of the whole plant (RGR_{Plant}) or of the leaves (RGR_{Leaf}). The rate by which the plants accumulated Mn was given by the relative accumulation rate of Mn (RAR_{Mn}). Single factor effects by lime, green manure and cultivar and interaction effects between green manure and cultivar were significant for $RGR_{Plant/Leaf}$. The RAR_{Mn} was influenced singly by lime and green manure but not cultivar (Table 3.16). The interaction effects between lime and green manure was also significant for RAR_{Mn}. The soybean cv. PSB Sy2 and cv. PSB Sy6 gave similar RAR_{Mn}. On the other hand, PSB Sy2 gave a higher RGR_{Plant} and RGR_{Leaf} than PSB Sy6 (Table 3.17). Lime and green manure application significantly increased RGR_{Plant} , RGR_{Leaf} and RAR_{Mn} (Table

3.17). The RAR_{Mn} was increased by green manure only where lime was applied (Table 3.13). On the other hand, $RGR_{Plant/Leaf}$ of cv. PSB Sy2 was significantly higher than that of cv. PSB Sy6 only where green manure was applied (Table 3.10). The increase in RGR_{Plant} due to green manure application was significant regardless of whether lime was applied or not (Table 3.10).

The rate of plant growth far exceeded the rate of Mn accumulation where no amendment was applied or where only lime or only green manure was applied (Fig. 3.5). The addition of both lime and green manure caused an increase in RGR_{Leaf} accompanied by an increase in RAR_{Mn} so that RGR_{Leaf} and RAR_{Mn} were either similar, or RAR_{Mn} exceeded RGR_{Leaf} as in the case of cv. PSB Sy6 (Fig. 3.5). This implied that RGR_{Leaf} and RAR_{Mn} maybe correlated. Fig. 3.6, in fact, shows a significant and considerably strong correlation between RGR_{Leaf} and RAR_{Mn}.

Table 3.16. Significance (*Pr*) of the mainplot, subplot and sub-subplot and interaction effects of lime, green manure and cultivar on plant growth rate and Mn accumulation rate.

Effects	RGR, g g^{-1} d^{-1}		RAR_{Mn}, µg µg d^{-1}
	Leaves	Aboveground	
Lime (L)	*0.0407*	*0.0152*	*0.0405*
G. manure (Gm)	*0.0002*	*0.0001*	*<0.0001*
Cultivar (V)	*0.0066*	*0.0011*	*0.4718*
L x Gm	*0.1199*	*0.0163*	*0.0255*
L x V	*0.8247*	*0.8623*	*0.7377*
G x V	*0.0159*	*0.0030*	*0.2553*
L x Gm x V	*0.6339*	*0.3206*	*0.6393*

Table 3.17. Single factor and lime x green manure x cultivar interaction effects on rates of plant growth and Mn accumulation of soybeans.

Lime t ha^{-1}	G. manure t ha^{-1}	Cultivar	RGR, g g^{-1} d^{-1}				RAR_{Mn}, µg µg d^{-1}	
			Leaves		Aboveground			
0	0	PSB Sy2	0.0793	*0.0015*	0.0780	*0.0020*	0.0541	*0.0056*
0	0	PSB Sy6	0.0798	*0.0028*	0.0792	*0.0021*	0.0583	*0.0059*
0	7	PSB Sy2	0.1038	*0.0025*	0.1051	*0.0017*	0.0824	*0.0022*
0	7	PSB Sy6	0.0908	*0.0036*	0.0902	*0.0028*	0.0755	*0.0069*
5	0	PSB Sy2	0.0949	*0.0038*	0.0983	*0.0032*	0.0657	*0.0054*
5	0	PSB Sy6	0.0925	*0.0034*	0.0954	*0.0028*	0.0645	*0.0077*
5	7	PSB Sy2	0.1109	*0.0022*	0.1113	*0.0028*	0.1125	*0.0088*
5	7	PSB Sy6	0.0990	*0.0040*	0.0993	*0.0028*	0.1065	*0.0062*
Single factor effect					MEANS			
Lime 0			0.0884	*0.0028*	0.0881	*0.0030*	0.0676	*0.0039*
Lime 5			0.0993	*0.0024*	0.1011	*0.0020*	0.0873	*0.0066*
	Gm 0		0.0867	*0.0023*	0.0877	*0.0026*	0.0606	*0.0030*
	Gm 7		0.1011	*0.0024*	0.1015	*0.0023*	0.0942	*0.0050*
		PSB Sy2	0.0972	*0.0033*	0.0982	*0.0034*	0.0787	*0.0063*
		PSB Sy6	0.0905	*0.0024*	0.0910	*0.0023*	0.0762	*0.0056*

Numbers in italics are standard error of the mean.

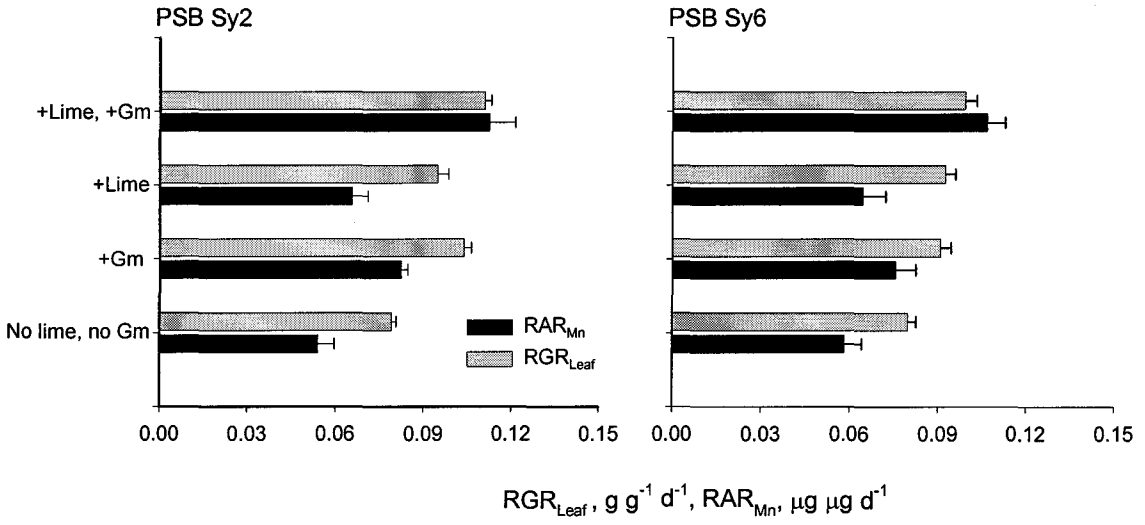


Fig. 3.5. Comparison between RAR_{Mn} and RGR_{Leaf} of two soybean cultivars as influenced by lime and green manure applications.

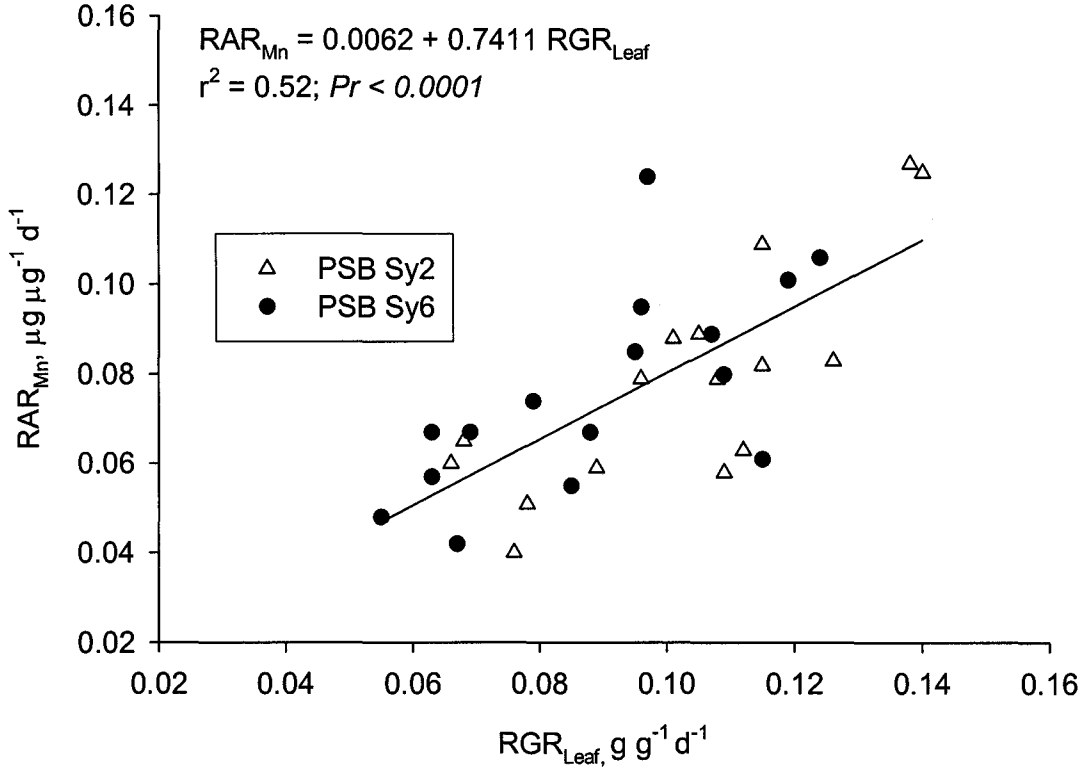


Fig. 3.6. Correlation of leaf growth rate and Mn accumulation rate.

Grain yield and biomass and determinants

Grain yield was influenced singly by lime, green manure and cultivar (Table 3.8). The effect of green manure, however, was dependent on whether lime was applied or not. The soybean cv. PSB Sy2 gave higher yields than PSB Sy6 (Table 3.9). Both lime and green manure application generally increased grain yields. The increase in grain yield due to green manure application was significant only where no lime was applied (Table 3.14).

A significant correlation between leaf Mn and aboveground plant biomass was observed (Fig. 3.7). Likewise, grain yield was significantly and positively correlated with leaf Mn (Fig. 3.8). A similar correlation was observed between symptom score and grain yield (Fig. 3.8). Leaf biomass was expected to be correlated with the final grain yield and by this correlation, one would expect relative growth rate of the leaves to be likewise correlated with grain yield. A significant correlation was found between early leaf growth rate and the final grain yield of both soybean cv. PSB Sy2 and cv. PSB Sy6 (Fig. 3.9). The correlation of grain yield and RAR_{Mn} was statistically significant although somewhat weak.

Soil tests for Mn which include water and Mehlich 1 as extractants did not correlate well with grain yield of both soybean cultivars (Fig. 3.10). Although water-Mn did not seem to be related to grain yield, high grain yields of 1500-2500 kg ha^{-1} were possible only when water-Mn was below 2.0 mg kg^{-1} (Fig. 3.10).

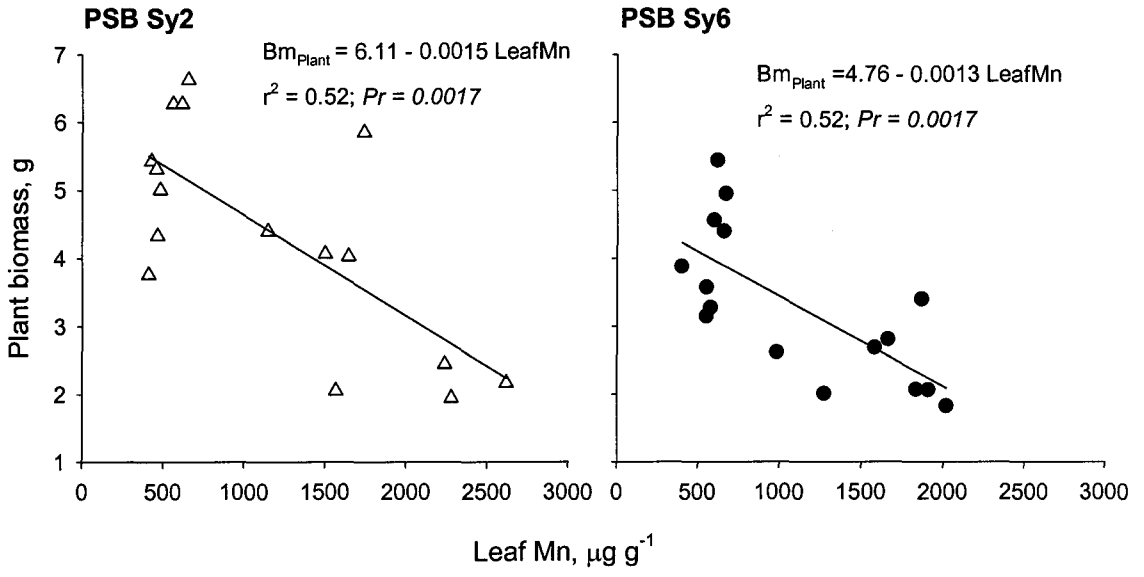


Fig. 3.7. Effect of leaf Mn on aboveground biomass production of two soybean cultivars.

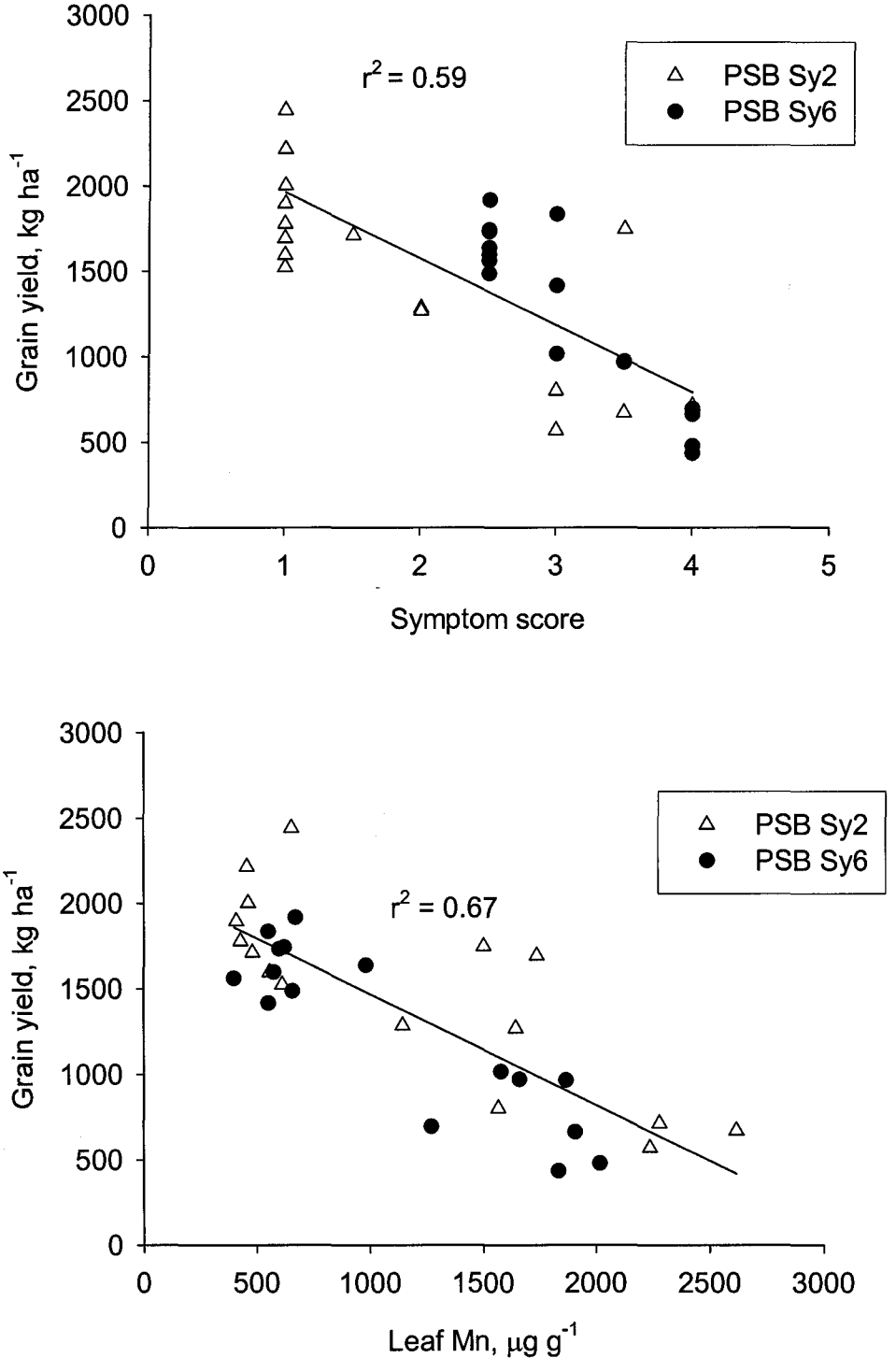


Fig. 3.8. Correlation of Mn phytotoxic symptom score and leaf Mn to grain yield of two soybean cultivars.

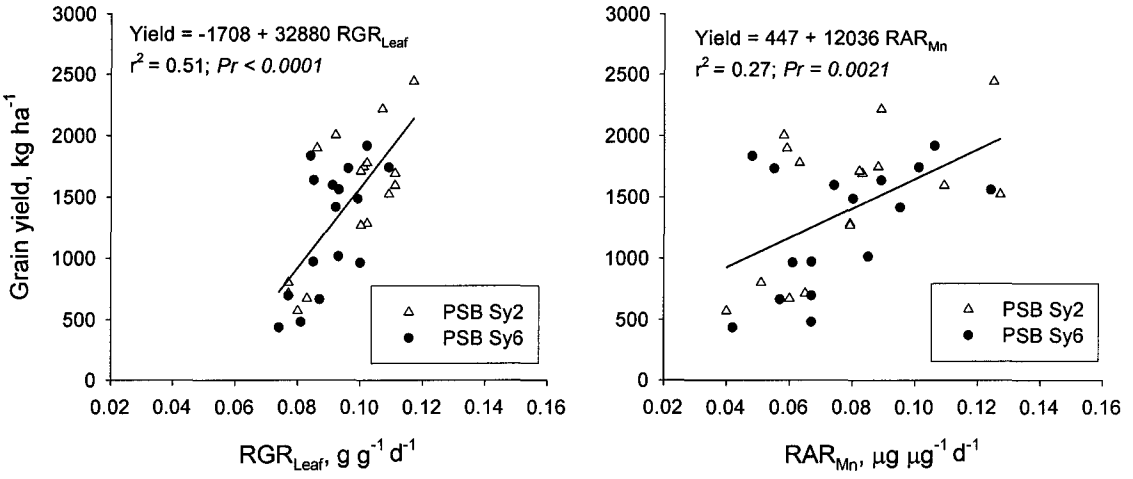


Fig. 3.9. Correlation of plant growth rate and relative accumulation rate of Mn to grain yield of two soybean cultivars.

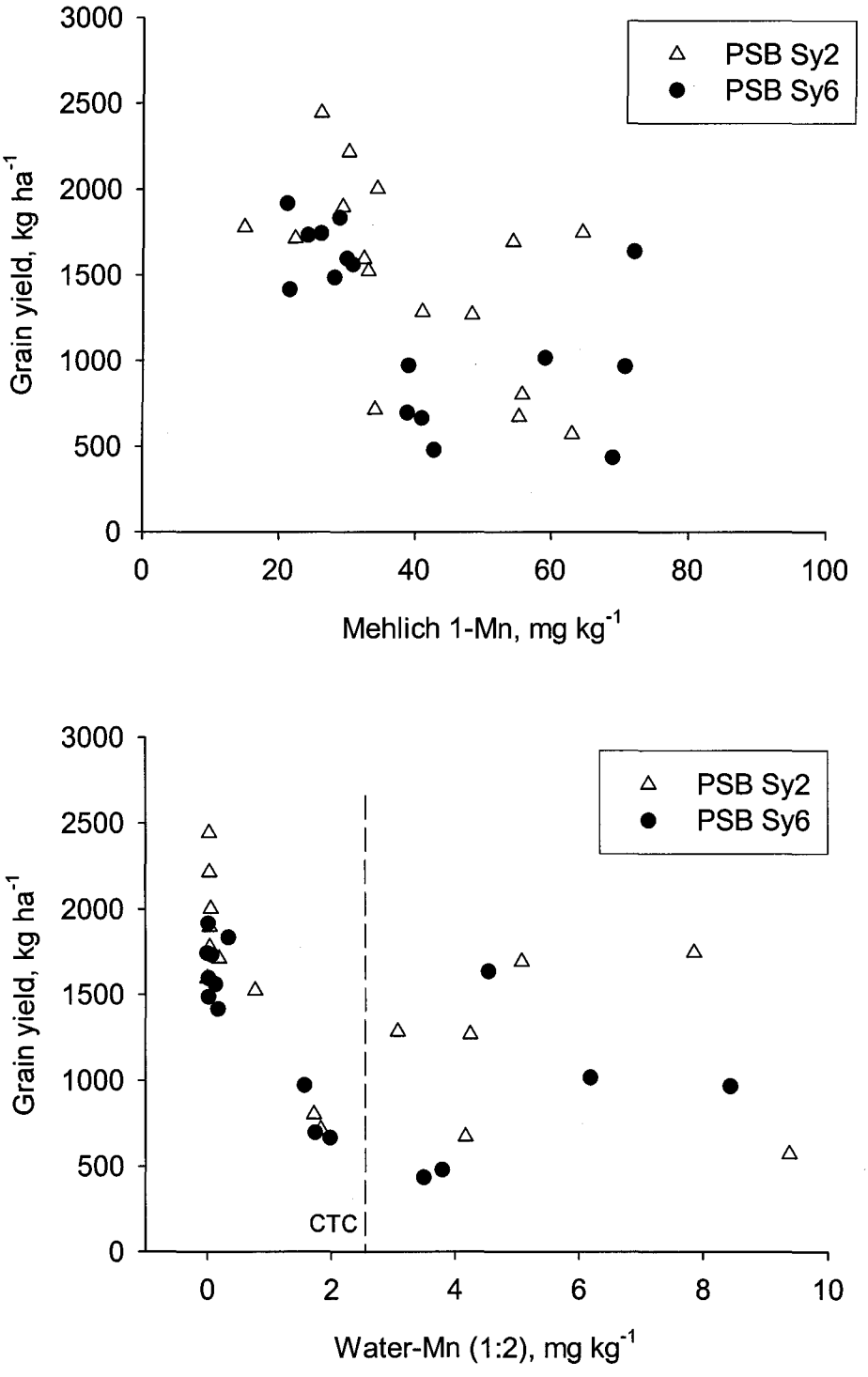


Fig. 3.10. Correlation of Mn soil test values to grain yield of two soybean cultivars.

Discussion

Indicators of potential Mn toxicity

Black, pebble-sized Fe-Mn concretions were visible within the surface 20-cm, which includes the root zone of soybeans. This observation agrees well with the profile description of a representative soil in San Antonio wherein Fe-Mn concretions were observed within 76 cm of the profile (Table 3.1). These concretions strongly effervesce when wetted with 20% hydrogen peroxide. This test has been used in the field to confirm if the observed concretions contain Mn oxide.

Soil pH and redox status are two major soil properties that define solubility of Mn^{2+} in the soil solution (Lindsay, 1991). The soil is extremely acid with pH 4.30, hence favoring high solubility of Mn in the soil. The rainfall pattern in the experiment site is such that there are rainfall events exceeding 300 mm during the typhoon season (Corton *et al.*, 1998) that can potentially saturate the soil. The cropping season, however, occurred during a season of sufficient rainfall to keep the plant from wilting. There were no major episodes of excessive rainfall that led to excess soil moisture except the rainfall event that exceeded 100 mm for one day. In addition the soil was relatively deep and well drained so that the possibility of soil reduction due to water stagnation is very limited.

Very few studies provide critical toxicity concentrations of Mn in the soil and most of the critical concentrations suggested in the literature are very

specific for the plant that has been tested. For example, a critical value for DTPA-Mn was 60 mg kg^{-1} for white clover (Rayment and Verral, 1980) and 306 mg kg^{-1} for cowpea (Vega, *et al.*, 1992). Hue (1998) suggested that saturated paste extract Mn must be kept below 0.5 mg L^{-1} to avoid Mn toxicity. Parker *et al.* (1969) observed definite crinkling of soybean leaves when water-extractable Mn (1:2 soil water) was above 2.5 mg kg^{-1}. The maximum DTPA-Mn measured in the unamended soil was 11.5 mg kg^{-1}, which is way below the critical levels suggested for clover and soybean. Using water or 1 *M* KCl as extractant, soluble Mn was 4.32-4.35 mg kg^{-1}, which is twice as much as the suggested critical value. Among the four extractants used, water indicated a critical value of about 2.0 mg kg^{-1} below which high grain yields were not obtained. The use of this extractant would obviously incur the lowest cost because no expensive chemicals is involved. Also, extraction of Mn can be combined with pH measurement at 1:2 soil to water ratio.

Indicators of phytotoxicity

Manganese phytotoxicity was expressed as symptoms in the leaves and high concentrations of leaf Mn accompanied by reductions in plant growth and grain yield. The early vegetative stage represents the critical period of Mn toxicity effect. Wu (1994) observed the initial symptom of toxicity, which is brown spots on the leaves of soybeans after 7 days of Mn treatment, which indicates that plant response to excess Mn is relatively fast. The symptoms typical of Mn phytotoxicity include brown speckles of older leaves, interveinal chlorosis and

necrosis, and crinkling especially of younger leaves. These symptoms were observed in the emerging unifoliolate leaves in very young soybeans in unamended soil. The succeeding trifoliolate leaves also showed these symptoms. Soybeans grown in limed soil showed brown spots and occasional interveinal chlorosis in the leaves, although much less severe than those in the unlimed soil, and no crinkling of young leaves was observed. Severe symptoms in unlimed soybeans were accompanied by leaf Mn concentrations of 1500-1970 mg kg^{-1} after 28 days growth. These concentrations far exceed the 600 mg kg^{-1} critical toxicity concentration for soybeans reported by Marschner (1990), or the 160 mg kg^{-1} critical value for a sensitive soybean cultivar (Foy *et al.*, 1978). Leaf Mn of limed soybeans ranged from 520-566 mg kg^{-1}, associated with brown spots in the leaves. The local cv. PSB Sy2 and cv. PSB Sy6 used in the study have unknown tolerance to excess Mn in the soil. Moderate symptoms observed at leaf Mn concentrations exceeding 500 mg kg^{-1}, indicates that they are likely to be moderately tolerant. A severely susceptible soybean cultivar that can tolerate only up to 160 mg kg^{-1} Mn in the leaves is expected to show severe symptoms at concentrations of 500 mg kg^{-1}.

The phytotoxic symptoms accompanied by high leaf Mn concentrations were further reflected in the reductions in leaf area, biomass production and grain yield. Leaf Mn concentration was negatively correlated with grain yield and aboveground biomass at 42 days after emergence. This suggests the possibility of using leaf Mn concentration and symptom scores for diagnosing phytotoxicity. These criteria, however, have two important drawbacks. First, the diagnosis

comes after plant damage has occurred. Secondly, plots of relationships between leaf Mn or toxicity scores and grain yield, although indicating a linear relationship, do not suggest a critical value. In addition, these relationships are very likely to be species or cultivar specific because of a wide range of tolerance to Mn between species and cultivar.

Amelioration of the phytotoxicity

The field experiment results indicate that the two soybean cv. differ in their growth rates and inherent capacity to produce biomass (leaf or total aboveground) and grain yield. The cv. PSB Sy2 accumulated biomass at a faster rate than cv. PSB Sy6, leading to more biomass and grain produced across lime or green manure levels. This observation may be interpreted as greater tolerance to excess Mn by PSB Sy2. Greater Mn tolerance in this cultivar was also indicated by less severe leaf symptoms than in cv. PSB Sy6.

Lime was highly effective in increasing biomass accumulation, growth rates and grain yield. This is likely a consequence of increased soil pH, increased availability of Ca, as well as decreased amount of extractable soil Mn and Al due to lime application. Lime application increased leaf Ca with concomitant decrease in leaf Mg. However, leaf Mg concentration was still above the sufficiency level of 0.25%.

The positive effect of green manure application was unexpected. Green manure has been shown in several greenhouse studies to increase the amount

of available Mn in the soil by reduction and complexation processes, hence keeping the Mn^{2+} in solution and available for plant uptake (Hue, 1988; Vega *et al.*, 1992; Porter *et al.*, *in press*). The results in this study do not support this observation, however. Measurements of soil available Mn did not show a significant increase due to green manure application. If extractable Mn increased, which was not captured by soil analysis, it was apparently insufficient to significantly increase Mn phytotoxicity. A decrease in leaf Mn at 42 DAE was even observed in unlimed soybeans where green manure was applied. This reduced concentration coincided with higher biomass accumulation, relative growth rates and grain yield. The organic acid complexes produced during green manure decomposition are very weak complexes and do not serve to immobilize Mn^{2+} but rather keep it soluble in the soil solution (Hue, 1988; Hue, *et al.*, 2001). The Mn toxicity alleviation by the green manure is likely a result of its nutrient content. There were significant increases in leaf P, K and Mg of soybeans in the green manure-treated plots. Lime, on the other hand, increased only leaf Ca, decreased leaf Mg and had no effect on leaf K and P. According to Reuters *et al.* (1997), the deficient to marginal concentrations of P (31 days after sowing), K (26 days after sowing), Ca (21 days after sowing) and Mg (flowering) were 0.29-0.34%, 1.2-1.7%, 0.4-0.9% and 0.19-0.25%, respectively. Green manure addition increased leaf Mg from 0.50% to 0.66%. These concentrations were well above the marginal Mg concentration of 0.25%. Leaf P, on the other hand, increased from 0.21 to 0.27% due to green manure addition. In this case the concentrations were much below the marginal concentration of 0.34%. Leaf K

increased from 1.65% to 2.27%, thus increasing from marginal concentration of 1.7%. Assuming that the suggested marginal concentrations apply to the soybean cultivars planted in acid soil, the results suggest that the green manure was effective in increasing leaf K to sufficiency level and leaf Mg from sufficient to a higher level while failing to increase leaf P above deficiency. Leaf N concentration was not analyzed because we did not expect any N deficiency to occur. Firstly, the rates of blanket fertilizer application of N-P-K (in kg ha^{-1}, 90-60-60) was higher than the local recommendation of 80-50-50 kg ha^{-1} (Aquino, pers. communication). Secondly, the seeds were inoculated with rhizobium before planting. The amount of effective nodules, however, was not measured.

Although liming decreased soil Mn while green manure had no effect, the increased relative growth rate associated with the use of these amendments was accompanied by higher relative accumulation rate of Mn. In fact, RGR_{Leaf} was significantly correlated with RAR_{Mn} . Although there was poor correlation between RAR_{Mn} and grain yield, the correlation between RGR_{Leaf} and grain yield was significant. The increased RGR_{Leaf} and RAR_{Mn} due to the amendments did not increase leaf Mn concentration probably because the difference between the two rates were not sufficient to detect an increase in the concentration. There was an increase in yield and biomass production despite an increase in RAR_{Mn}. This indicates that the growth rate of the plant is a better indicator of tolerance to Mn toxicity than the rate of Mn accumulation given the soil and environmental conditions where the soybeans were grown. In fact RGR_{Leaf} was better correlated than RAR_{Mn} with yield. However, there may be conditions that would

allow RAR_{Mn} to exceed RGR_{Leaf} in which case the RAR_{Mn} might control the RGR_{Leaf} and therefore determine the phytotoxic response. This might be a case where RAR_{Mn} will indirectly predict yield via its impact on RGR_{Leaf}.

Summary and Conclusions

The potential for excess Mn to occur in the acid upland soil in *Barangay* San Antonio, Isabela, Philippines was assessed based on soil indicators and soil tests for extractable Mn. Soil indicators include the presence of Fe-Mn concretions within the root zone of soybeans and the extremely low soil pH. Although the typhoon season brings excessive water, the likelihood of soil saturation for considerable amount of time is very low in the well-drained upland soils of Ilagan. Of the four tests for extractable Mn, only water-soluble Mn and KCl-Mn appeared to diagnose excess Mn in the soil. This is because the relation between soil test values and yield indicate a critical value, which tends to agree with an earlier suggested critical value for this test for soybean crop. This does not mean, however, that Mehlich 1 and DTPA are not useful in diagnosing excess Mn. It is nevertheless, more difficult to interpret a soil test if there is no critical value established for the specific plant in question. Mehlich I and DTPA either had no suggested critical value or that the critical value suggested in the literature was for a different crop. The difficulty of interpreting soil tests results from the fact that there is not one single critical value for a wide range of plant species. Critical values may even differ between cultivars of the same species (Ohki, 1981).

The actual toxicity was indicated by phytotoxic symptoms including brown spots, interveinal chlorosis and crinkling of the leaves, accompanied by high concentrations of leaf Mn and reductions in leaf area, biomass production and grain yield. Leaf Mn and leaf relative growth rate were good predictors of grain yield. Similarly, total aboveground biomass was significantly predicted by leaf Mn. However, the drawback of using symptoms and leaf Mn is that damage has occurred before the diagnosis can be made.

The effect of soil Mn toxicity was alleviated in the cv. PSB Sy2, which showed higher potential for biomass production and grain yield even under excess Mn than the cv. PSB Sy6. Ameliorating the Mn phytotoxicity by liming led to decreased soluble Mn in the soil, decreased leaf Mn and increased relative growth rate, biomass production and grain yield. Although green manure had no effect on soluble Mn in the soil, its effect on biomass, leaf Mn, relative growth rate and grain yield was similar, although of less magnitude than lime. While the main effect of lime is to provide Ca and reduce soil Mn by raising soil pH, the potential for alleviation of Mn toxicity by manures apparently resulted from its nutrient supplying capacity, as shown by increased leaf K, Ca and Mg in the green manure-treated plants. Amelioration of Al toxicity could be an added effect of green manure as commonly observed.

The increase in relative growth rate due to lime and green manure was accompanied by an increase in relative accumulation rate of Mn, which suggests that uptake of Mn is driven by plant growth. On the other hand, plant growth is reduced by leaf Mn concentration. This interaction between growth and Mn

uptake is likely to determine the final biomass or yield under conditions of excess Mn in the soil.

Two lessons have been learned in this experiment. First is that the diagnosis of Mn toxicity is not simply executed by measuring soil Mn and leaf Mn. There has been a difficulty in interpreting soil Mn values because of the scarcity of Mn critical levels for various soil tests that are applicable to a specific crop published in the literature. The ultimate effect of Mn phytotoxicity is reduced biomass production and yield. Although leaf Mn predicted grain yield, the correlation between leaf Mn and grain yield can be improved possibly by using physiologically 'active' leaf Mn instead of total Mn. Secondly, management treatments like green manure addition accelerate Mn accumulation rate but also increases growth rate, with net effects of increasing biomass and yield. It is possible, therefore, to manage excess Mn in San Antonio fields, by increasing the relative growth rate of the plant to increase grain yield. Strategies can be developed so that plant growth rate is maximized even with accompanying increase in accumulation rate of Mn in the plant or increase in Mn level in the soil. This necessitates the conduct of another field experiment focused on management treatments designed to increase plant growth rate. In this first field experiment, we used only one rate of lime application, which perhaps eliminated both excess Al and excess Mn simultaneously and so the treatment effects are reflections of the alleviation of both toxicities. In the future experiment, we can use two rates of lime as controls, a lower lime rate to eliminate excess Al and a higher lime rate, to eliminate excess Mn.

Chapter 4

MANAGING EXCESS MANGANESE IN AN ACID UPLAND SOIL IN ILAGAN, PHILIPPINES: THE 'GROWTH RATE' APPROACH

Introduction

The year-round high temperature and rainfall characteristic of the humid tropics is an asset for continuous crop production if rainfall is uniform. The same asset becomes a liability because it allows faster rates of weathering, leaching of nutrients and decomposition of organic material. Consequently, soils developed under this environment are frequently infertile, acidic and susceptible to erosion, particularly where acid infertility restricts vegetation cover for soils situated in steeply sloping uplands.

Acid soils comprise a large block of arable lands worldwide and even a larger block of agricultural land in the tropics. The acid soils of the tropics of Asia, which belong to the orders Ultisols and Oxisols of the US Soil Taxonomy cover about 1675 M hectares or 38% of the total land area. In the Southeast Asia, 295 M of the total regional land of 446 M hectares are acid uplands (Phosphate and Potash Institute, 1992). An estimated 17M hectares or 58% of total land area of the Philippines are acid soils (National Land Use Committee, 1985), which are predominantly found on hilly lands. If these soils are managed to become productive, it will ease the pressure on food production from the more fertile lowland soils. Much of today's agricultural research is targeted towards production in the fertile lowland environment where maximum return on

investment is highly attainable. It is the pressure to increase yield per unit area that drives agricultural technology to advance and adapt the high-input principle whose aspect of sustainability is still unresolved. Even with the fast-paced advancement of technology, however, we expect yield per unit area to reach a plateau. If and when the fertile soils cannot increase its production anymore, and the land area it covers shrinks due to urbanization, the only alternative left is to expand production to the presently unproductive acid soils.

By definition, acid soils have a pH below 7.0. Serious toxicity of Al and Mn often occurs at pHs 5 and below (Kamprath and Foy, 1985). Such low pHs and excesses of toxic metals are accompanied by deficiencies of Ca, Mg, Mo and P (Marschner, 1991). Both deficiencies and toxicities may occur in combinations, which make the soil acidity problem a complex nutritional disorder. The majority of research on soil acidity has focused on the toxicity of Al in soils and plants. This is perhaps due to the more common occurrence of excess Al than Mn in acid soils. Of the known area of acid soils in the tropics, there is no estimate in the literature of the relative percentage of lands affected by Al or Mn solely or in combination. However, as crop production expands to the less favorable acid uplands, the problem of toxic amounts of Mn in the soil may prove to be more common than is perceived at present. Farmers may start to encounter toxicities of Mn in their soil and their crops grown. When this time comes, we need management strategies that would alleviate or correct this problem. Although liming has been shown to correct soil acidity and the accompanying metal toxicities such as that of Mn and Al, there always remains the question of how

much lime to apply so that the farming enterprise in this limited-resource system remains productive and profitable. In this study, we explored and tested options for managing excess Mn in acid soils. The principle in our approach is to manage plant growth rate to enhance tolerance to the potential toxicity in the soil. Our focus is not so much on reducing soil Mn level but on boosting plant growth to alleviate phytotoxic effects of Mn. The main goal was to evaluate management strategies such as varietal selection, manure application, mulching and P management in terms of effectiveness in improving plant growth and yield of soybeans grown in an acid soil with toxic levels of Mn. We hypothesize that the most successful strategy would be one or combination of treatments that maximizes plant relative growth rate while minimizing relative accumulation rate of Mn. The application P fertilizer is expected to increase early stage growth rate of the plant. The nutrients such as Ca. Mg, K and P contained in manures are expected to enhance plant growth rate and tolerance to excess Mn. However, there is always a potential aggravation of Mn phytotoxicity with manure application especially with excess soil moisture. Green manure and chicken manure are the most locally available organic materials that are potential sources of nutrients to supplement mineral fertilizers. *Leucaena* leaves and chicken manure are considerably different in nutrient content and very likely different in terms of decomposition (or nutrient supply) rates. These differences will have significant impact on how these manures affect plant response to excess Mn.

A high growth rate may not be the cause of tolerance *per se*. It is possible that the management treatments will enhance an existing tolerance mechanism

that will allow increased plant growth even in the presence of excess Mn in the soil or in the plant.

Objectives

1. Evaluate the effectiveness of cultivar, mulching, rates of P application, green manure and chicken manure use in enhancing grain yield, maximizing plant growth rate and minimizing Mn accumulation rate of soybeans grown in an acid upland soil with known toxic levels of Mn as well as Al.
2. Compare the effectiveness of the above management strategies against the effects of liming.
3. Investigate the effects of liming and these management strategies on the level of toxic Mn and Al in the soil.

Materials and Methods

Experiment site

A field experiment was conducted in San Antonio, Isabela, Philippines from June 28 to October 26, 2001. San Antonio is a small village in the province of Isabela in Northern Luzon, Philippines. More than 3000 people representing 610 households reside over 994 ha of land, 350 ha of which is planted to rice and corn (Corton, *et al.*, 1998). The village is within the vicinity of the Sierra Madre Mountain range that runs in the south-north direction and spans the eastern edge of the island of Luzon. The Bureau of Soils and Water Management mapped the

soils in this region as Ultisol (US Soil Taxonomy) although the soil next to the experiment site was classified as an Alfisol (Table 3.1).

Historical records show that rainfall in the area is highly seasonal (Corton *et al.*, 1998). The dry months include February to April, with maximum occasional events of 200 mm rainfall, but commonly no measurable precipitation. Rainfall starts between May and July. October and November are typhoon seasons with median rainfall of about 300 mm. Actual measured rainfall at the site during the soybean cropping season showed that daily rainfall did not exceed 20 mm in July and August. There were at least three rainfall events between 70-100 mm throughout the cropping period. Rainfall events during the harvesting period were less frequent, although each event exceeded 20 mm (Fig. 4.1).

Soil properties

The soil in the experiment site is *Rugao* series, classified by the Bureau of Soil and Water Management as *fine, isohyperthermic Typic Kandiudalf*. A reconnaissance soil survey by the Bureau of Soils and Water Management (BSWM) mapped the soils within the vicinity of the Sierra Madre mountains as Ultisol. However, analysis of a representative soil profile in the village of San Antonio showed a base saturation (based on sum of cations) of >47% (BSWM, 1998) below the upper boundary of the *kandic* horizon, hence, failing the <3the 5% base saturation criteria for an Ultisol (USDA, 1998). Analysis of composite samples from the experiment site showed a base saturation of at least 46%

below the plow layer associated with extremely acid pH, high exchangeable Al, low exchangeable bases and high saturated-paste Mn (Table 4.1).

Treatments and experiment design

Two locally bred soybeans cv. PSB Sy2 and cv. PSB Sy6 provided by the Institute of Plant Breeding (IPB) at the University of the Philippines, Los Banos were used as the test crops. These cultivars were the more recent high-yielding entries in the cultivar trials conducted by researchers at IPB and the Department of Agriculture- Ilagan Experiment Station (Aquino, pers. communication). Three levels of lime applied were included as controls. The management treatments, which also received 2 t ha^{-1} lime, were P levels, mulching and manure (chicken dung and *Leucaena leucocephala*) applications (Table 4.2). Phosphorus was applied as triple superphosphate (TSP, 46% P_2O_5). Manures in the form of partly decomposed chicken dung and sun-dried leaves of *Leucaena leucocephala* were applied at 7 t ha^{-1} (air-dry weight basis). The content of major nutrients in the manure is shown in Table 4.3. Approximately 5-cm thick rice straw mulch was applied to the mulch-treatment plots to cover the soil surface and reduce soil water evaporation. The amounts of nitrogen applied were reduced by 15 kg ha^{-1} with manure application to compensate for the N supplied by the decomposing manures. An estimated 2% content of N in the manures would potentially supply 100 kg N from 7000 kg air-dry weight (5600 dry weight). We assumed that out of this potential supply, 15 kg would be absorbed by the soybean crop.

The two factors, cultivar and management, were arranged in a split plot design with three replications and with cultivar as the mainplot and management treatments arranged randomly among the subplots.

Cultural practices for growing soybeans

The soybean crop was rainfed. Although supplemental irrigation was preferable, there was no access to irrigation water in the area. Soybean was planted after an episode of rain that slightly moistened the soil. Soybean seeds were mixed with *rhizobium* inoculant obtained from the Institute of Plant Breeding (IPB) at the University of the Philippines, Los Banos. Inoculated seeds were then planted in furrows 50 cm apart at the rate of 50 seeds per linear meter of the furrow. The seedlings were thinned to 25 plants per linear meter at 7 days after emergence (DAE). Rates of N and K application were 90 and 60 kg ha^{-1}, respectively across all treatments. These rates were slightly higher than the recommended 75 kg ha^{-1} N and 40 kg ha^{-1} K recommended for soybeans (Aquino, personal communication). Both P (as TSP, 40% P_2O_5) and K (as KCl, 60% K) were applied basal; *i. e.*, during the last harrowing 2 days before planting. The fertilizers were mixed within 20 cm of the soil surface by cultivating the soil with a hoe during application. Nitrogen as urea (46% N) was topdressed in two splits, 1/3 of the total amount (Table 2) at 10 DAE and the remaining 2/3 at 30 DAE. Pesticides[1] Lannate® and Decis® were sprayed at 35 DAE and 45 DAE to control leaf borers and leafhoppers. Weeds were removed by hand at 25 DAE

[1] The use of Lannate and Decis does not constitute a recommendation on our part to use these pesticides to control pests of soybeans.

and 50 DAE. Harvesting was done by hand-picking the soybean pods at maturity, which was about 120 days after sowing.

Soil measurements

Soil samples at 0-20 cm depths from each plot were collected at 14 DAE and analyzed for pH (1:1 soil: water), Mehlich 3-Mn (Mehlich, 1984), saturated paste-Mn (Hue *et al.*, 2001) and exchangeable Al (Bertsch and Bloom, 1996). Each sample represented a composite of the two soybean cultivars. Soil moisture was measured gravimetrically. Exactly 20 g of fresh soil samples were weighed in tin cans, oven-dried at 100°C and the dry weight determined. Soil moisture in percent was calculated by the equation:

$$MC, \% = (FW-ODW)/ODW * 100 \quad \text{[Eqn. 4.1]}$$

where:

MC is moisture content

FW is soil fresh weight

ODW is soil oven-dry weight.

Plant measurements

Six plants were collected randomly and weekly from each plot. The leaves were detached for leaf area measurement using a portable leaf area meter (LICOR 3000a). The samples were then oven-dried at 70°C for three days (or until a constant moisture content has been attained) and weighed for the determination of leaf, stem and total plant biomass. Dried leaves were dry-ashed

in a muffle furnace at 500°C for 4 hours. Ashed samples were digested further with 5 ml of 2 *M* HNO_3 at 120°C until all the acids had evaporated. The residue was then dissolved in 20 ml 0.1 *M* HCl and filtered using Whatman #42 filter paper. The concentrations of Mn, Ca, Mg, K and P in the extract were analyzed using Inductively Coupled Plasma Emission Spectroscopy (ICP) at the Agricultural Diagnostic Services Center of the University of Hawaii.

Toxicity symptoms rating

Weekly observations were recorded on the general health of the plants, taking note of the appearance of brown spots, necrosis, interveinal yellowing and crinkling of the leaves. A rating scale was used to score phytotoxic symptoms as shown in Table 4.4. The symptoms were also documented by taking digital photographs of the plants.

Growth analysis and calculations

Growth analysis techniques (Radford, 1967; Rufty, *et al.*, 1979; Hunt, 1990) were used to test our hypothesis on the effects of management treatments on the rates of plant growth and accumulation of Mn in the leaves of soybeans. The growth of soybean was divided into growth intervals according to sampling dates, which roughly corresponded to several vegetative phases of the plant (Fig. 4.2). Calculations of relative growth rates and relative accumulation rates were done as described in Chapter 3 Growth analysis and calculations.

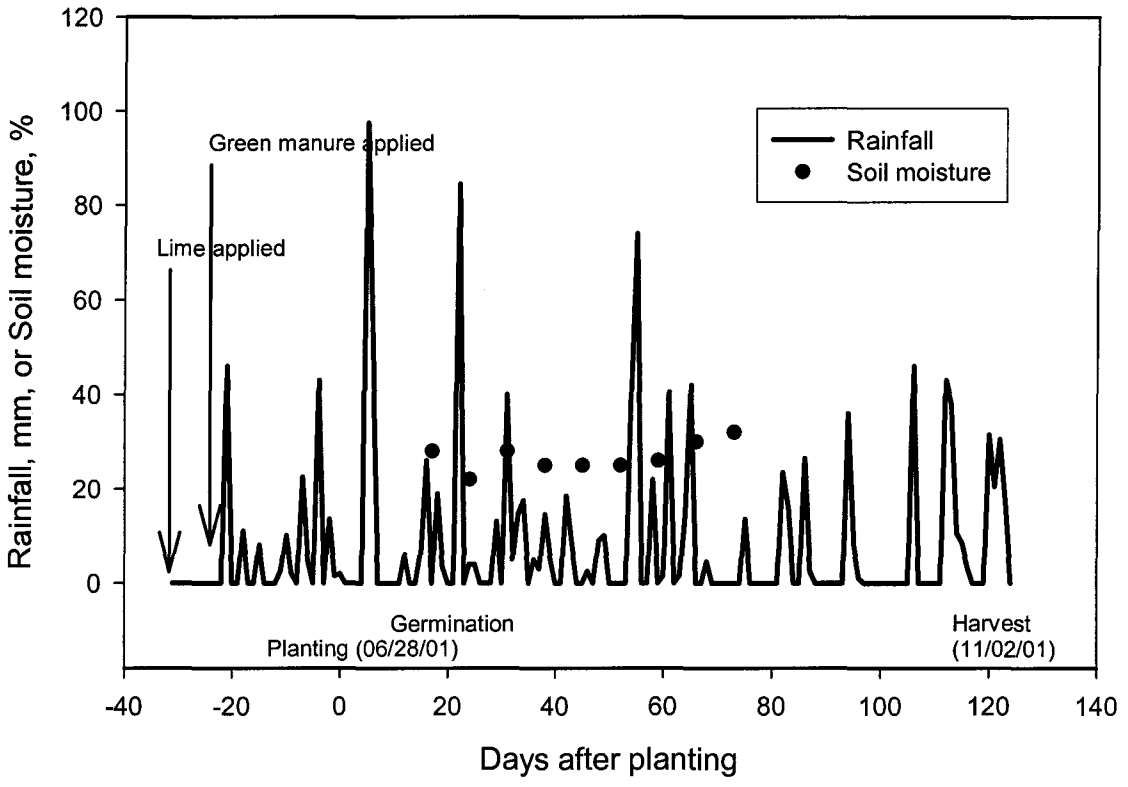


Fig. 4.1. Actual rainfall and soil moisture measurement at the experiment site in San Antonio, Ilagan, Philippines. June-October 2001. Planting date was June 28, 2001.

Table 4.1. Chemical characteristics of the soil at the experiment site in San Antonio, Isabela, Philippines.

Horizon	Soil pH (1:1 soil: water)	Saturated paste-Mn	Exch. Al	Exchangeable Bases			Al Saturation
			$cmol_c\ kg^{-1}$	$cmol_c\ kg^{-1}$			%
				K	Ca	Mg	
A1	4.47	1.34	2.53	0.11	2.00	0.53	47
A2	4.57	1.50	2.58	0.08	2.15	0.41	48
B	4.59		2.78	0.10	1.69	0.58	54

Table 4.2. Soybean cultivars and management factors tested in the field experiment.

Soybean cultivars: cv. PSB Sy2 and cv. PSB Sy6

		Amounts applied					
Treatment	Management	Lime[a]	Manure[a]	Mulch[b]	N[c]	P[c]	K[c]
1	Lime 0	0	0	no	40	30	60
2	Lime 2	2	0	no	40	30	60
3	Lime 5	5	0	no	40	30	60
4	+Mulch	2	0	with	40	30	60
5	P60	2	0	no	40	60	60
6	P100	2	0	no	40	100	60
7	G. manure	2	7 (*Leucaena leucocephala*)	no	25	30	60
8	C. manure	2	7 (Chicken manure)	no	25	30	60
9	P60 +Mulch	2	0	with	40	60	60
10	P100 +Mulch	2	0	with	40	100	60
11	G. manure +Mulch	2	7 (*Leucaena leucocephala*)	with	25	30	60
12	C. manure +Mulch	2	7 (chicken manure)	with	25	30	60

[a, c] Rates in t ha^{-1} and kg ha^{-1}, respectively

[b] Five-cm thick rice straw mulch

Table 4.3. Nutrient content of manures applied to soybeans.

Type of manure	Nutrient, g 100 g^{-1}			
	Ca	Mg	P	K
Chicken manure	3.81	0.98	2.40	1.50
Leucaena leucocephala	2.11	0.37	0.15	0.98

Table 4.4. Rating scale used to score Mn phytotoxicity status of field-grown soybean.

Rating	Description of symptoms
1	generally healthy, without symptoms of toxicity
2	appears healthy, with few brown spots on leaves, slight interveinal yellowing of leaves
3	stunted, common brown spots on leaves, common interveinal yellowing of leaves, slight crinkling of younger leaves
4	severely stunted, many brown spots on leaves that coalesced to bigger necrotic spots, severe interveinal yellowing of leaves, severe crinkling of younger leaves

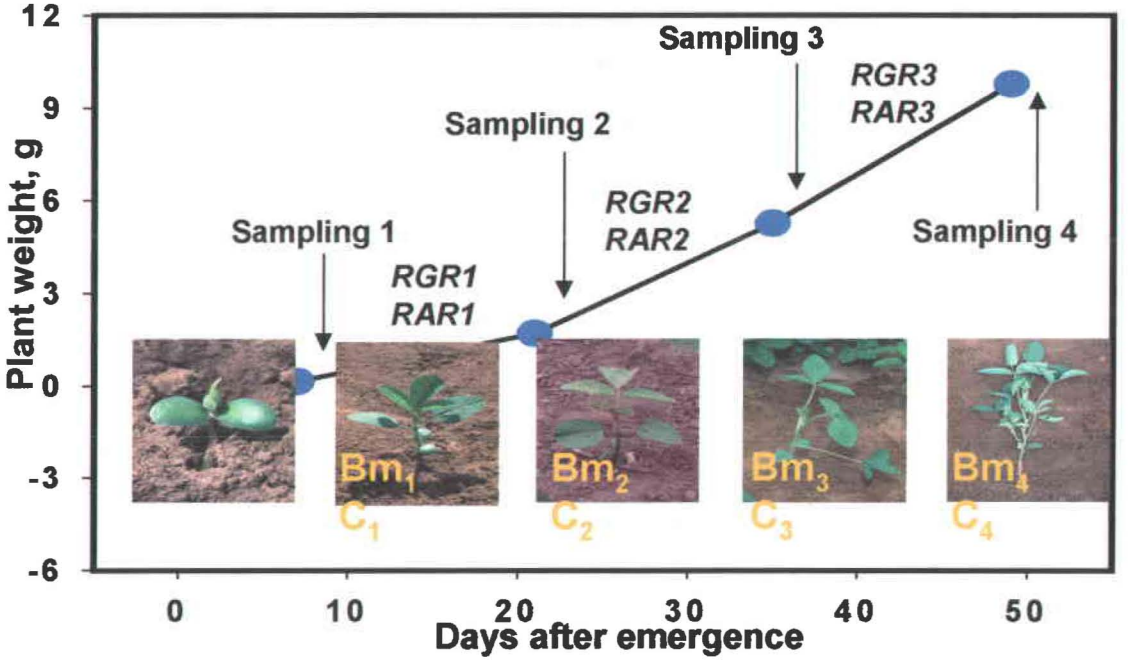


Fig. 4.2. Sampling scheme for the measurement of plant/leaf relative growth rate and relative accumulation rate of manganese.

Relative growth rate for the whole plant (RGR_{Plant}, $g\ g^{-1}\ d^{-1}$) or leaves (RGR_{Leaf}) and relative accumulation rate of Mn (RAR_{Mn}, $\mu g\ \mu g^{-1}\ d^{-1}$) were calculated using the following equations:

$$RGR_{Plant/Leaf} = (lnBm_{T1} - lnBm_{T0}) / (T_1 - T_0) \quad \text{[Eqn. 4.2]}$$

$$RAR_{Mn} = \{ln(Bm_{T1} * C_{T1}) - ln(Bm_{T0} * C_{T0})\} / (T_1 - T_0) \quad \text{[Eqn. 4.3]}$$

where:

$RGR_{Plant/Leaf}$ is the mean plant/leaf relative growth rate over a given interval

RAR_{Mn} is the mean Mn relative accumulation rate over a given growth interval

Bm is the weight of biomass (whole plant, leaves) in g

C is the Leaf Mn concentration in $\mu g\ g^{-1}$

T_0 is a previous sampling date

T_1 is a later sampling date

Incremental $RGR_{Plant/Leaf}$ were calculated at each growth interval represented by sampling dates. Samples were collected weekly from 7 days until 49 days after emergence (DAE). Three $RGR_{Plant/Leaf}$ were calculated from three growth intervals: 7-21 DAE, 21-35 DAE and 35-49 DAE. The whole growth period $RGR_{PLant/Leaf}$ were calculated as the mean of the $RGR_{PLant/Leaf}$ from the three growth intervals.

Only two RAR_{Mn} from growth intervals 14-28 DAE and 28-49 DAE were calculated due to limited number of samples that were analyzed in this experiment. When comparing $RGR_{PLant/Leaf}$ with RAR_{Mn}, $RGR_{PLant/Leaf}$ calculated from 14-28 DAE and 28-49 DAE were used.

The RGR was broken down into 2 components: net assimilation rate (NAR) and leaf area ratio (LAR). These parameters were calculated using the equations from Radford (1967) with an underlying assumption that over the period T_0 to T_1, leaf area and biomass are linearly related.

$$NAR = [(Bm_1-Bm_0)/(LA_1-LA_0)]\ [(ln LA_1-ln LA_0)/T_1-T_0] \quad \text{[Eqn. 4.4]}$$

$$LAR = [(LA_1-LA_0)/(Bm_1-Bm_0)]\ [(ln Bm_1-ln Bm_0)/ln LA_1-ln LA_0)] \quad \text{[Eqn. 4.5]}$$

where LA is leaf area and other terms are as above.

Statistical analysis

The effects of cultivar and management treatments and their interactions on soil and plant measurements were analyzed using the Analysis of Variance

procedure of the Statistical Analysis Systems (SAS, 1990). Probabilities of 5% or less were considered significant. A significant mainplot effect indicated a significant difference between the two soybean cultivars while a significant subplot effect indicated significant differences between management treatments. Single degrees of freedom (*d.f.*) contrast technique was used to compare means or group of means as detailed in Table 4.5. Comparisons between two controls: Unlimed vs. Lime 2 or Lime 2 vs. Lime 5 indicate response to increasing levels of lime while comparison between a management treatment with Lime 2 indicates response to the treatment. The comparison between mulched treatments and their unmulched counterparts indicated the effect of mulching. The comparisons: Manure vs. no manure, Lime vs. P, Lime vs. Manure, manure vs. P, G. manure vs. C. manure and P60 vs. P100 were made across mulch treatments.

Linear relationships (their coefficients and model probabilities) between parameters were determined using the Regression procedure of SAS. To compare slope coefficients describing data relations between cultivars, a separate slope for each replicate within a cultivar was calculated and an analysis of variance was used to test differences in cultivar slopes by treating each replicate slope as an individual observation.

Table 4.5. Single and group mean comparisons made using single *d.f.* contrast technique.

Single *d.f.* contrast	Single means or group of means compared
Un-limed *vs.* Lime 2	Lime 0 *vs.* Lime 2
Lime 2 *vs.* Lime 5	Lime 2 *vs.* Lime 5
Lime 2 *vs.* others	Lime 2 *vs.* all treatments except Lime 0
Mulch *vs.* no mulch	{+Mulch, P60 +Mulch, P100 +Mulch, G. manure +Mulch, C. manure +Mulch} *vs.* {Lime 2, P60, P100, G. manure, C. manure}
Manure *vs.* no manure	{C. manure, C. manure +Mulch, G. manure, G. manure +Mulch} *vs.* {Lime 5, P60, P60+ Mulch, P100, P100 +Mulch}
Lime *vs.* P	{Lime 2, Lime 5, +Mulch} *vs.* {P60, P60 +Mulch, P100, P100 +Mulch}
Lime *vs.* Manure	{Lime 2, Lime 5, +Mulch} *vs.* {G. manure, G. manure +Mulch, C. manure, C. manure +Mulch}
Manure *vs.* P	{G. manure, G. manure +Mulch, C. manure, C. manure +Mulch} *vs.* {P60, P60 +Mulch, P100, P100 +Mulch}
Gm *vs.* Cm	{G. manure, G. manure +Mulch} *vs.* {C. manure, C. manure +Mulch}
P60 *vs.* P100	{P60, P60 +Mulch} *vs.* {P100, P100 +Mulch}

Results

Soil pH, Exchangeable Al and Extractable Mn

Management treatments significantly influenced soil pH, exchangeable Al and saturated paste-Mn (Table 4.6). Lime application of 2 and 5 t ha^{-1} increased unamended soil pH from 4.45 to 4.76 and 5.17, respectively. This was equivalent to a 0.30 unit pH increase at Lime 2 and a 0.72 unit pH increase at Lime 5. The pH increase with 2 t ha^{-1} lime applied was associated with a significant decline in exchangeable Al from 1.61 to 0.9 $cmol_c$ kg^{-1} and a decline in saturated paste-Mn from 1.38 to 1.10 μg ml^{-1}, which was not statistically significant. With 5 t ha^{-1} lime, both exchangeable Al and saturated paste-Mn further declined to 0.42 $cmol_c$ kg^{-1} and 0.10 μg ml^{-1}, respectively (Table 4.7). The application of the two manures and two rates of P as well as mulching did not influence soil pH and exchangeable Al. On the other hand, saturate paste-Mn was increased 2-3 fold by the application of chicken manure and green manure, compared to non-manured treatments. Neither mulching nor P application affected saturated paste-Mn. There was no significant difference in saturated paste-Mn between the two types of manure or between two rates of P.

Soil Mn was also extracted with a Mehlich 3 solution as an alternative to the saturated paste method. Mehlich 3-Mn showed levels ranging from 249-310 μg g^{-1} (Table 4.7) and no significant effects of the management treatments (Table 4.6). Treatment comparison using single *d.f.* contrast, however, showed significant decrease in Mehlich 3-Mn due to 2 t ha^{-1} lime application and increases due to manure and P application.

The amount of soil moisture was also monitored weekly to assess the possible effect of mulching on soil moisture content, which can have considerable impact on plant growth under moisture-limited growth condition. Weekly measurements did not show differences between mulched and unmulched treatments (data not shown) except at 35 DAE where about 3% higher moisture content was detected in the mulched compared with unmulched plots (Table 4.7).

Plant growth and expression of phytotoxicity

The appearance of toxicity symptoms, mainly brown spots started at 10 DAE, when the unifoliolate leaves were completely expanded and the first trifoliolate was halfway expanded. The emerging first trifoliolate showed both brown spots and interveinal yellowing and slight crinkling. These symptoms were fully expressed by 21 days after emergence especially in the unlimed control that had toxicity scores of 3-4. The soybeans in these plots were noticeably stunted compared to the others. While cv. PSB Sy2 showed slight to moderate crinkling of young emerging leaves, cv. PSB Sy6 showed severe crinkling of young leaves and moderate crinkling of old leaves accompanied by drying of leaf tips that extended inward from the edges. Drying of the tips of old leaves were occasionally observed in cv. PSB Sy2. Lime application alleviated the phytotoxic symptoms, decreasing toxicity score to 2-3. There was less severe crinkling of leaves and interveinal chlorosis. The plants, however, remained slightly stunted compared with those that received manures and higher amounts of P. The

application of chicken manure and green manure as well as higher amounts of P reduced the severity of brown spots, crinkling and yellowing. These plants received the lowest score of 1-2. Mulching seemed to have no additional alleviating effect on the expression of phytotoxic symptoms. However, mulching with manure resulted in elongated internodes and, consequently, taller plants.

Biomass accumulation

Plant biomass increased with time (Fig. 4.3, 4.4 4.5) without indication of leveling off except in cv. PSB Sy6 lime controls (Fig 4.3), suggesting continually increasing biomass production beyond 49 days. Differences between the Lime 2 control and management treatments became apparent at 21 DAE and these differences were magnified with time. Biomass accumulation at 49 DAE was significantly influenced by cultivar and management treatment (Table 4.8). Soybean cv. PSB Sy2 produced more leaf and total aboveground biomass than cv. PSB Sy6 across management treatments (Table 4.9.). Both soybean cultivars significantly increased biomass with 2 t ha^{-1} lime applied. Further increases were observed with 5 t ha^{-1} lime applied. The use of mulch as well as the application of P and manure increased biomass production across cultivars. Biomass production was higher in the P treatment compared with lime 2 and lime 5 controls. Manure-treated plots gave higher plant biomass than P- treated plots. Between the types of manure, chicken manure led to higher biomass than green manure application. There was no significant difference in biomass production between P rates (Table 4.8, 4.9).

Increases in accumulated biomass due to the application of P and manure far exceed the increases due to increasing levels of lime. This was observed across soybean cultivars. For example, plant biomass increased from 4.9 g with 2 t ha^{-1} lime to 5.5 g due to lime application up to 5 t ha^{-1} , while P and manure application increased biomass from 4.9 g to 6.4 g (mean across mulch and P rates) and to 8.3 g (mean across type of manure and mulch), respectively (Table 4.9).

Leaf Area

Leaf area of both soybean cultivars increased steadily with time until 49 days after emergence, without indication of a plateau except in the three lime treatments (Fig. 4.6, 4.7, 4.8). Similar to biomass, the differences in leaf area became apparent at 21 DAE and were magnified with time. Leaf area differed with management treatments and cultivars (Table 4.8) at 49 DAE. Leaf area in the lime 2 control averaged at about 779 cm^2 and was increased two-fold in the manure treatments. Leaf area did not increase with lime levels (Table 4.8, 4.9). Increases due to mulching and application of P and manure were significant. Between manure and P, manure application lead to higher leaf area. Between P rates, leaf area did not increase (Table 4.8).

Table 4.6. Significance (*Pr*) of main effect and single *d.f.* contrasts of management treatments on soil pH, exchangeable Al and extractable Mn and soil moisture.

Effects	Soil pH	Exch. Al	Extractable Mn		Soil moisture, %
			Saturated paste	Mehlich-3	
Management (M)	*0.0020*	*0.0465*	*0.0010*	*0.1239*	*0.0464*
Single *d.f.* contrasts					
Un-limed *vs.* Lime 2	*0.0093*	*0.0501*	*0.6621*	*0.0028*	*0.1071*
Lime 2 *vs.* Lime 5	*0.0036*	*0.0086*	*0.0308*	*0.2278*	*0.1119*
Lime 2 *vs.* others	*0.8750*	*0.0563*	*0.6321*	*0.0065*	*0.1128*
Mulch *vs.* no mulch	*0.7319*	*0.4464*	*0.2971*	*0.2464*	*0.0003*
Manure *vs.* no manure	*0.3819*	*0.1559*	*<0.0001*	*0.5627*	*0.7074*
Lime *vs.* P	*0.0104*	*0.8049*	*0.8256*	*0.0074*	*0.8082*
Lime *vs.* Manure	*0.0053*	*0.7290*	*0.0014*	*0.0457*	*0.6063*
Manure *vs.* P	*0.9174*	*0.5622*	*<0.0001*	*0.6417*	*0.9036*
Gm *vs.* Cm	*0.3636*	*0.5917*	*0.1298*	*0.1807*	*0.4769*
P60 *vs.* P100	*0.5836*	*0.7519*	*0.6318*	*0.2744*	*0.9340*

Table 4.7. Effect of lime, P, manure and mulch applications on soil pH, exchangeable Al and extractable Mn.

Management	Soil pH (1:1 soil: water)		Exch. Al $cmol_c\ kg^{-1}$		Extractable Mn			
					Saturated paste, $\mu g\ ml^{-1}$		Mehlich-3 $mg\ kg^{-1}$	
Un-limed	4.45	*0.032*	1.61	*0.133*	1.38	*0.280*	310	*5*
Limed, 2 t ha^{-1}	4.76	*0.018*	0.90	*0.163*	1.10	*0.328*	249	*2*
Limed, 5 t ha^{-1}	5.17	*0.092*	0.42	*0.146*	0.10	*0.050*	272	*13*
Mulch	4.62	*0.064*	0.99	*0.206*	0.86	*0.132*	296	*15*
P60	4.71	*0.088*	0.76	*0.114*	0.84	*0.269*	288	*11*
P60 +Mulch	4.74	*0.057*	0.76	*0.058*	0.75	*0.126*	284	*11*
P100	4.69	*0.078*	0.78	*0.056*	0.58	*0.098*	310	*3*
P100 +Mulch	4.86	*0.088*	0.64	*0.056*	0.57	*0.052*	291	*9*
G. manure (Gm)	4.77	*0.036*	0.69	*0.126*	3.19	*0.709*	292	*5*
Gm +Mulch	4.80	*0.078*	0.64	*0.213*	2.28	*0.281*	304	*7*
C. manure (Cm)	4.60	*0.033*	0.60	*0.161*	2.81	*0.559*	279	*9*
Cm +Mulch	4.80	*0.037*	0.82	*0.066*	2.06	*0.102*	281	*7*

Numbers in italics are standard error of the mean.

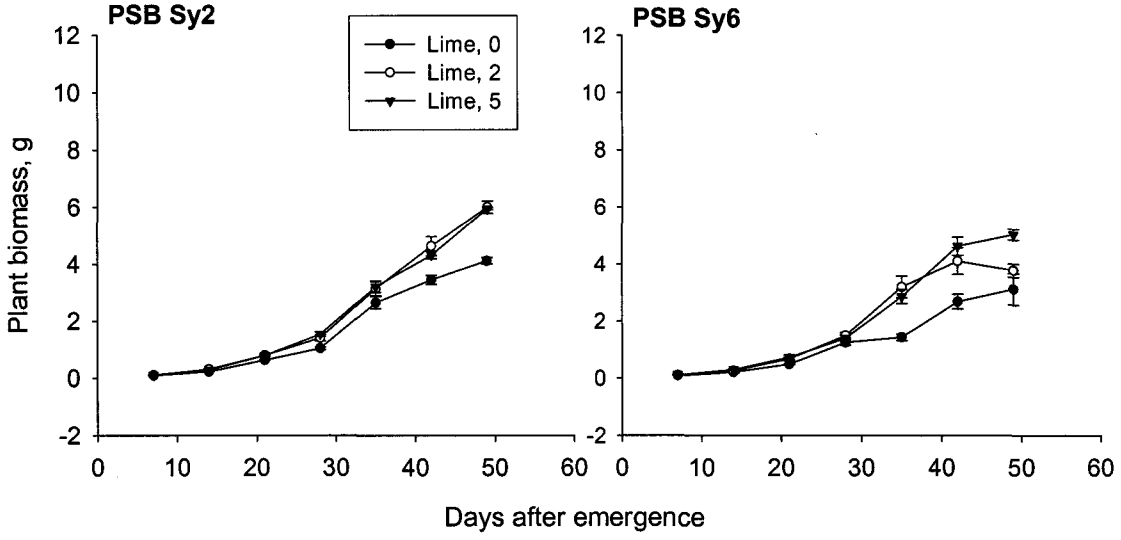


Fig. 4.3. Biomass accumulation of two soybean cultivars as influenced by the amounts of lime added.

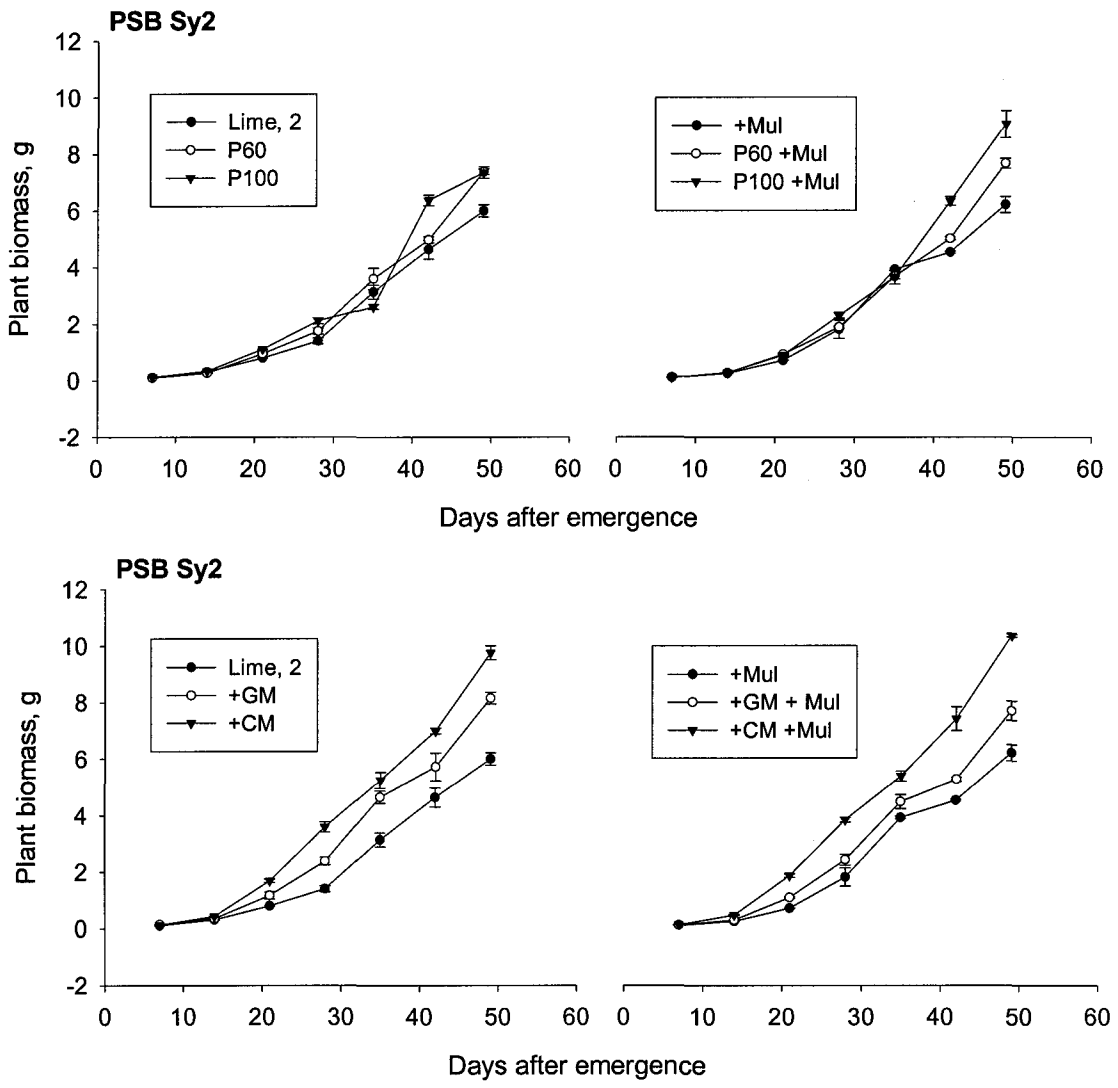


Fig. 4.4. Biomass accumulation of soybean cv. PSB Sy2 as influenced P and manure applications with or without mulch.

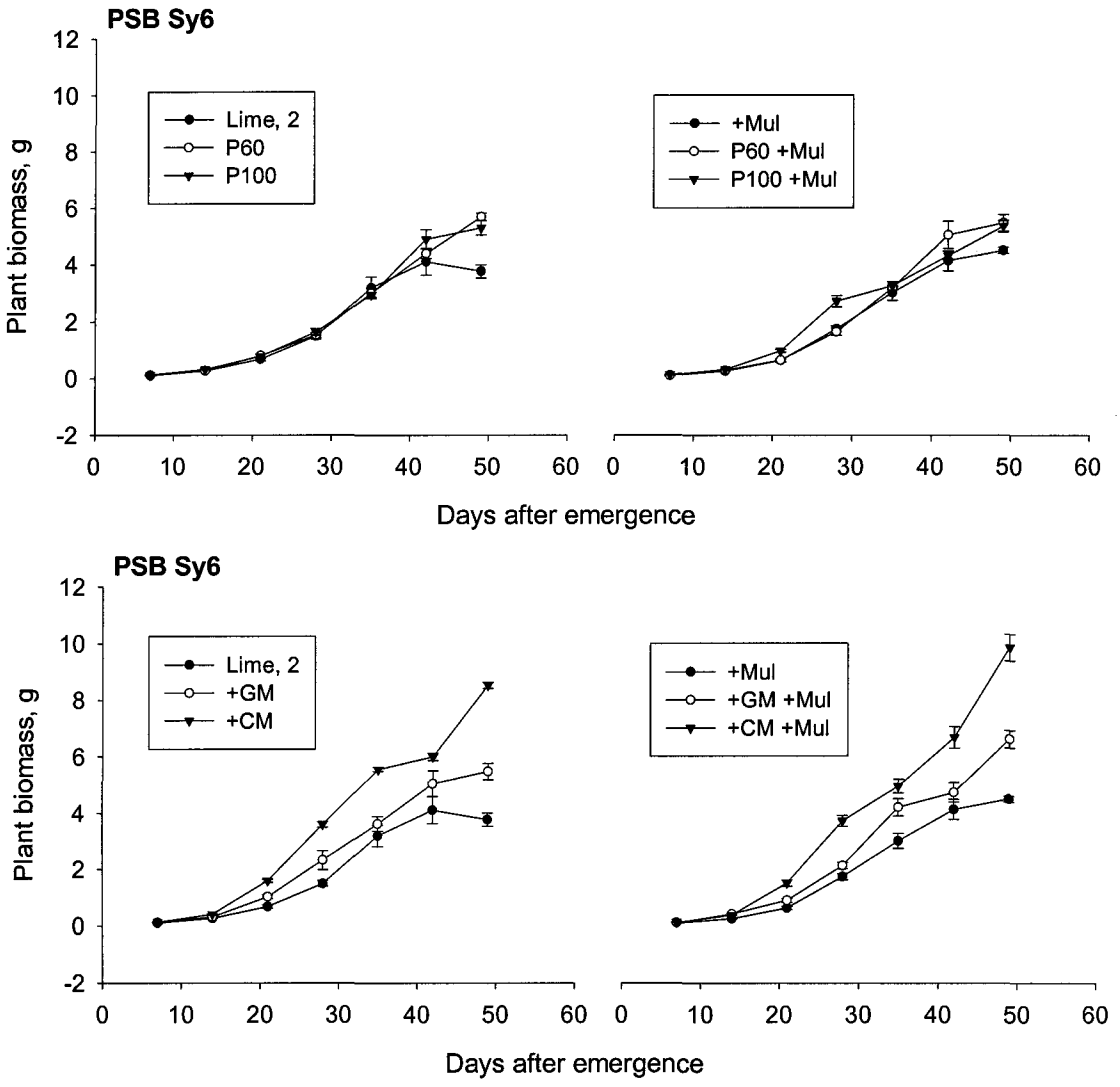


Fig. 4.5. Biomass accumulation of soybean cv. PSB Sy6 as influenced by P and manure applications with or without mulch.

Table 4.8. Significance (*Pr*) of main effect, interaction effect and single *d.f.* contrasts of cultivar and management effects on plant biomass leaf area and grain yield.

Effects	Biomass (49 DAE) g plant^{-1}		Leaf area (49 DAE) cm^2	Grain yield kg ha^{-1}
	Leaf	Aboveground		
Cultivar (Cv)	*0.0303*	*0.0586*	*0.0091*	*0.0310*
Management (M)	*0.0002*	*<0.0001*	*0.0010*	*0.0081*
Cv x M	*0.1371*	*0.0888*	*0.9574*	*0.3824*
Single *d.f.* contrasts		Across Cultivars		
Un-limed *vs.* Lime 2	*0.0159*	*<0.0001*	*0.4075*	*0.0015*
Lime 2 *vs.* Lime 5	*0.0038*	*0.0286*	*0.7088*	*0.0467*
Lime 2 *vs.* others	*<0.0001*	*<0.0001*	*0.0248*	*<0.0001*
Mulch *vs.* no mulch	*<0.0001*	*<0.0001*	*0.0089*	*0.0782*
Manure *vs.* no manure	*<0.0001*	*<0.0001*	*0.0007*	*<0.0001*
Lime *vs.* P	*<0.0001*	*<0.0001*	*0.0491*	*0.0004*
Lime *vs.* Manure	*<0.0001*	*<0.0001*	*0.0010*	*<0.0001*
Manure *vs.* P	*<0.0001*	*<0.0001*	*0.0261*	*<0.0001*
Gm *vs.* Cm	*<0.0001*	*<0.0001*	*0.0594*	*0.0086*
P60 *vs.* P100	*0.2113*	*0.2715*	*0.6978*	*0.0435*

Table 4.9. Effect of management and cultivar on biomass and leaf area of soybeans.

Management	Biomass (49 DAE), g plant^{-1}				Leaf area (49 DAE) cm^2		Grain yield kg ha^{-1}	
	Leaf		Aboveground					
	Mean across cultivar							
Un-limed	1.786	*0.204*	3.621	*0.337*	610	*96*	389	*41*
Limed, 2 t ha^{-1}	2.157	*0.219*	4.887	*0.518*	779	*93*	1026	*97*
Limed, 5 t ha^{-1}	2.608	*0.103*	5.494	*0.219*	855	*86*	1412	*162*
Mulch	2.737	*0.307*	5.366	*0.405*	941	*107*	1285	*116*
P60	2.976	*0.176*	6.539	*0.383*	1037	*188*	1451	*109*
P60 +Mulch	3.112	*0.264*	6.577	*0.513*	1041	*126*	1584	*181*
P100	3.027	*0.284*	6.329	*0.478*	954	*122*	1688	*159*
P100 +Mulch	3.322	*0.411*	7.211	*0.856*	1235	*164*	1901	*97*
G. manure (Gm)	3.196	*0.319*	6.820	*0.612*	1077	*150*	1888	*231*
Gm +Mulch	3.381	*0.209*	7.169	*0.318*	1243	*94*	1991	*141*
C. manure (Cm)	3.697	*0.147*	9.147	*0.319*	1221	*118*	2414	*80*
Cm +Mulch	4.304	*0.299*	10.116	*0.237*	1651	*254*	2199	*128*
Cultivar mean								
PSB Sy2	3.529	*0.128*	7.479	*0.290*	1165	*76*	1694	*106*
PSB Sy6	2.521	*107*	5.734	*0.312*	942	*55*	1511	*97*

Numbers in italics are standard error of the mean.

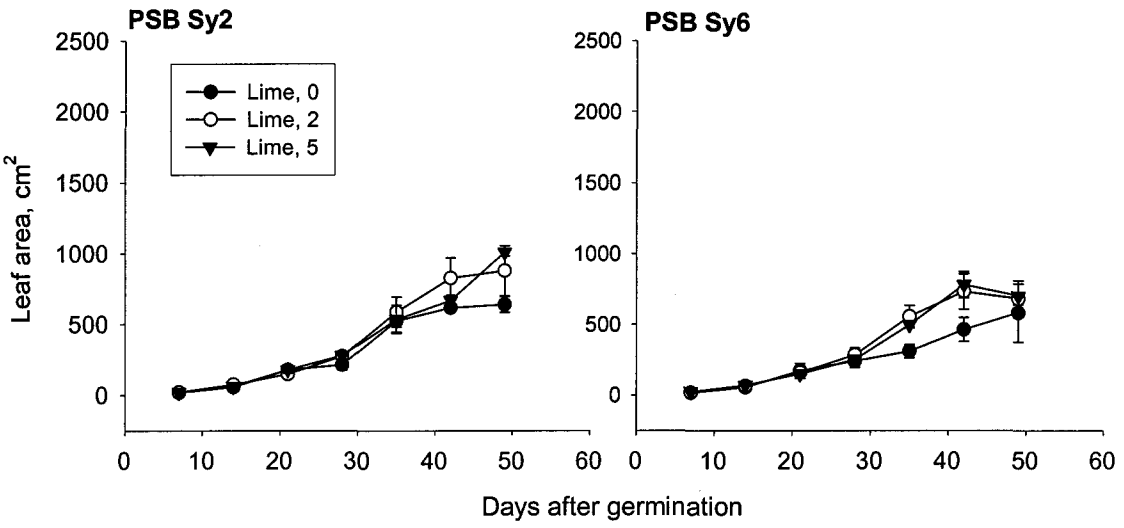


Fig. 4.6. Increase in leaf area of two soybean cultivars as influenced by the amount of lime applied.

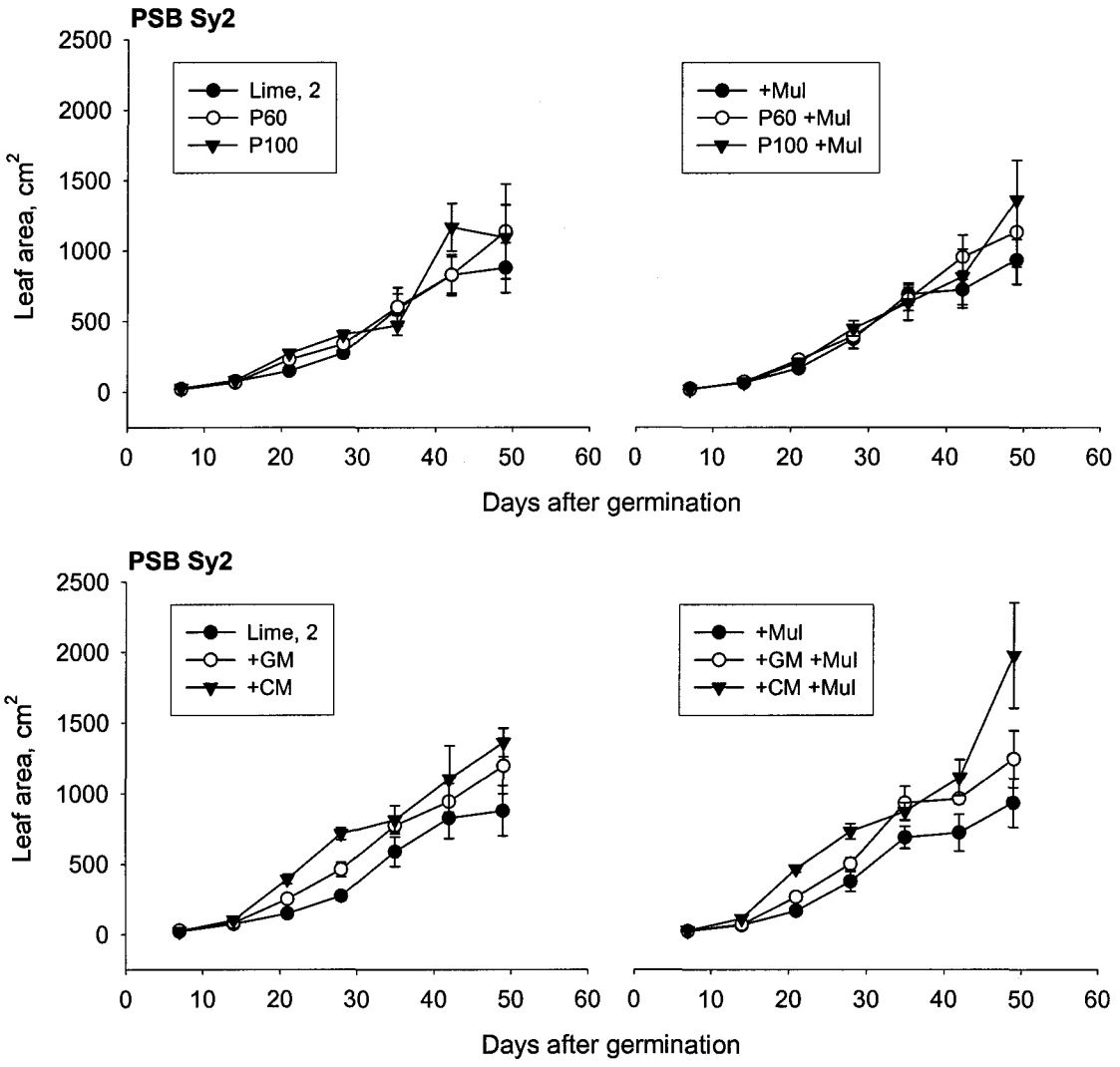


Fig. 4.7. Increase in leaf area of soybean cv. PSB Sy2 as influenced by P and manure application with or without mulch.

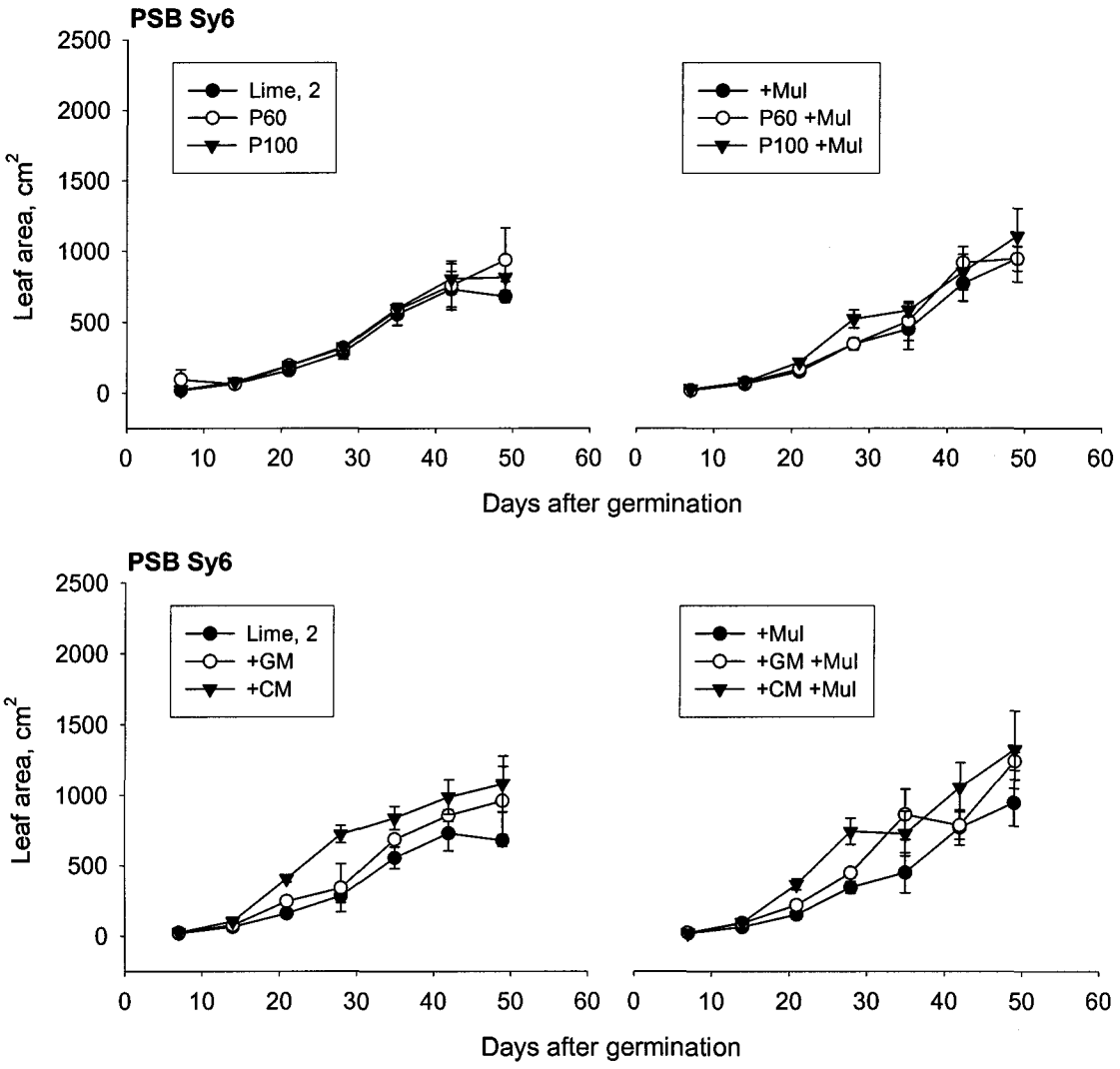


Fig. 4.8. Increase in leaf area of soybean cv. PSB Sy6 as influenced by P and manure applications with or without mulch.

Leaf Mn and other nutrients

Leaf Mn tended to decline with time, with greater decline between 14 and 28 DAE (Fig. 4.9, 4.10, 4.11). Leaf Mn concentration in the unlimed control declined from 1000 to 800 mg kg^{-1} in cv. PSB Sy2 and from 900 to 650 mg kg^{-1} in

cv. PSB Sy6. Leaf Mn concentrations were significantly higher in all management treatments compared with the limed controls at all sampling dates. Leaf Mn at three sampling dates differed with management treatments but not cultivar (Table 4.10). Lime at 2t ha^{-1} consistently decreased leaf Mn across varieties at all sampling dates (Table 4.11). Higher lime rate had no further effect on leaf Mn. Mulching likewise did not influence leaf Mn concentrations in both soybean cultivars. Manure applications significantly increased leaf Mn at all sampling dates while P decreased leaf Mn only at the early growth stage. Between P and manure treatments, manure application gave higher leaf Mn concentrations and between green manure and chicken manure, the latter gave higher leaf Mn concentrations (Table 4.11).

Among leaf nutrients only leaf Ca and P were significantly influenced by management treatments (Table 4.12). Mean comparisons, however, did not show significant effects of lime, P, manure or mulch application. Leaf P concentration was not influenced by lime rates. Mulching and the application of manure and P significantly increased leaf P compared to the limed controls (Table 4.13). Between lime and P treatment, P resulted in higher leaf P concentration. Between manure and P treatments, manure gave higher leaf P concentration. Comparing the two manures, chicken manure resulted in higher leaf P concentration than green manure. A higher rate of P did not further increase leaf P (Table 4.13).

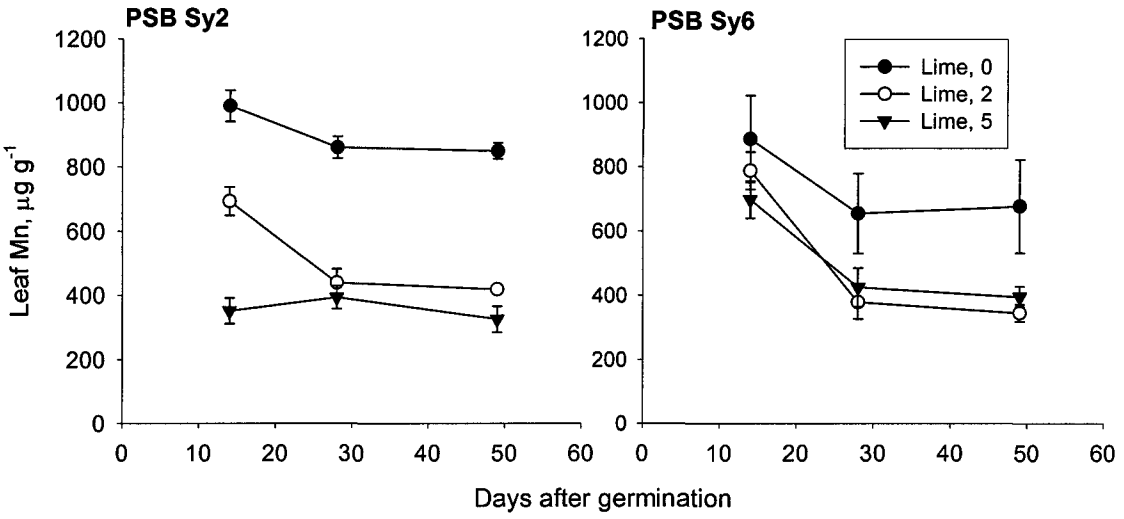


Fig. 4.9. Changes in leaf Mn concentration of two soybean cultivars as influenced by amounts of lime added.

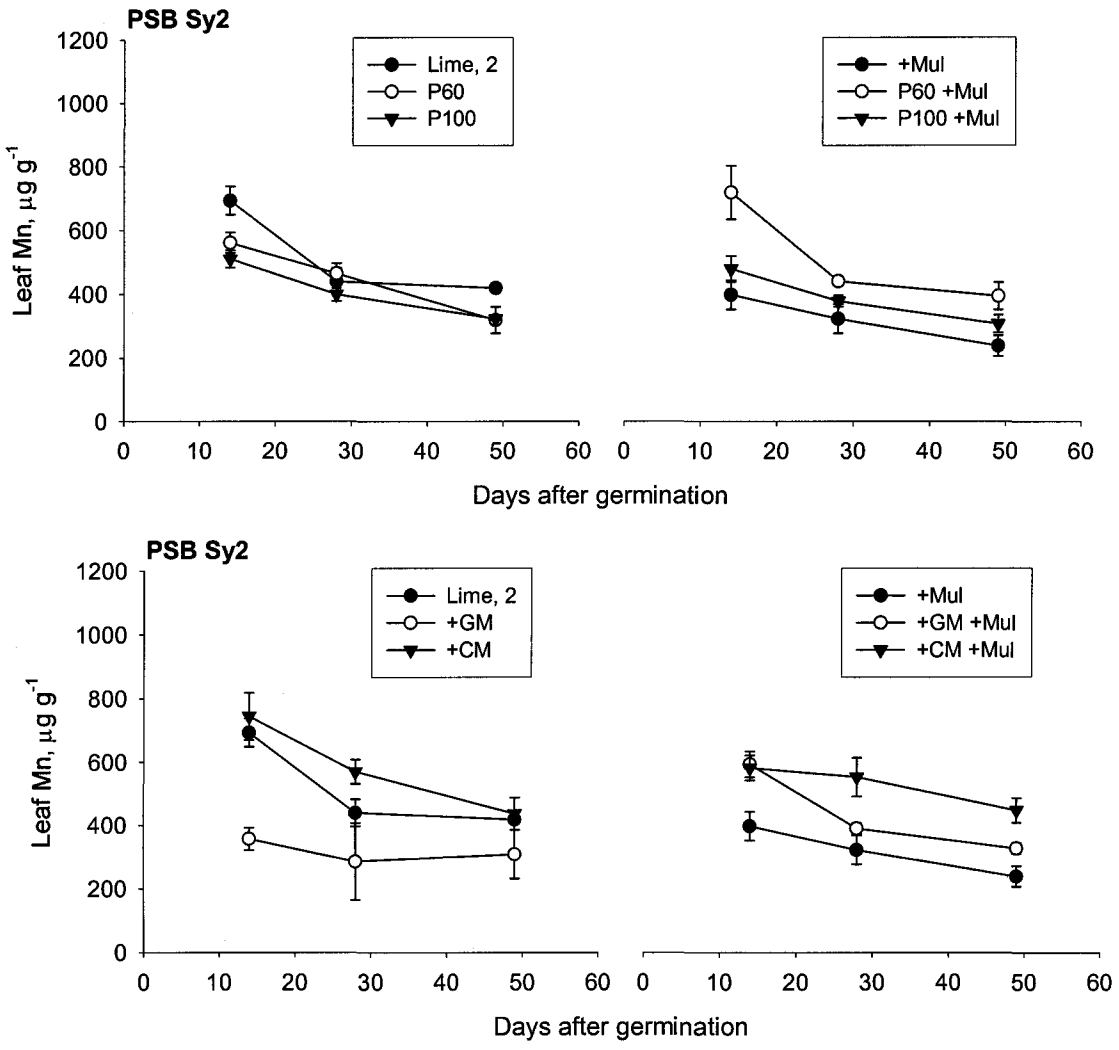


Fig. 4.10. Changes in leaf Mn concentration of soybean cv. PSB Sy2 as influenced by applications of P and manures with or without mulch.

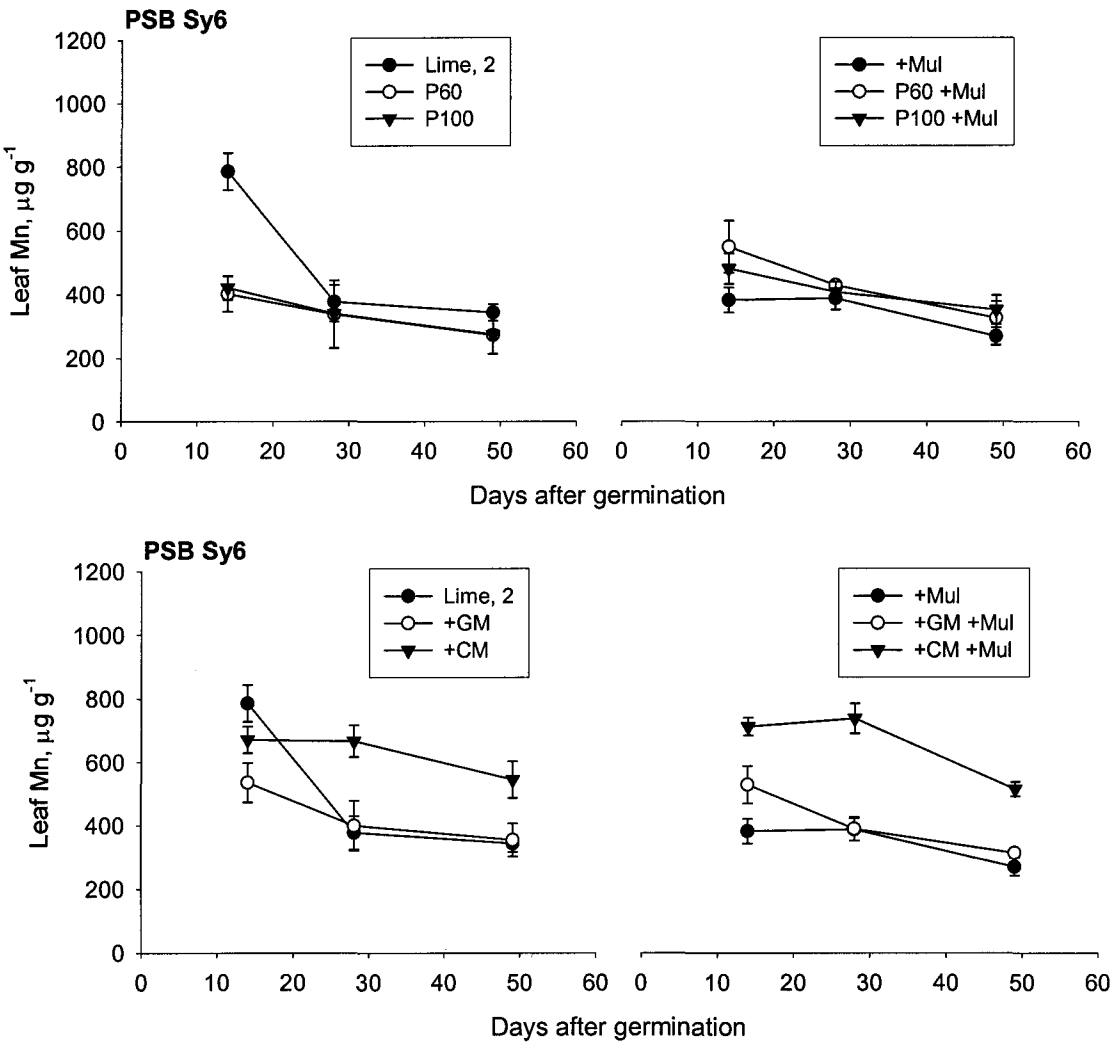


Fig. 4.11. Changes in leaf Mn concentration of soybean cv. PSB Sy6 as influenced by applications of P and manures with or without mulch.

Table 4.10. Significance (*Pr*) of main effect, interaction effect and single *d.f.* contrasts of cultivar and management effects on leaf Mn at 3 sampling dates.

Effects	Leaf Mn		
	14 DAE	28 DAE	42 DAE
Cultivar (Cv)	*0.5063*	*0.5136*	*0.5440*
Management (M)	*0.0040*	*0.0497*	*0.0014*
Cv x M	*0.1858*	*0.1477*	*0.5158*
Single *d.f.* contrasts		***Across varieties***	
Un-limed *vs.* Lime 2	*0.0011*	*<0.0001*	*<0.0001*
Lime 2 *vs.* Lime 5	*0.0004*	*0.9923*	*0.6521*
Lime 2 *vs.* others	*<0.0001*	*0.5340*	*0.4065*
Mulch *vs.* no mulch	*0.4063*	*0.4827*	*0.5811*
Manure *vs.* no manure	*0.0217*	*0.0002*	*0.0004*
Lime *vs.* P	*0.0015*	*0.7799*	*0.0938*
Lime *vs.* Manure	*0.4993*	*0.0064*	*0.1273*
Manure *vs.* P	*0.0109*	*0.0012*	*0.0008*
Gm *vs.* Cm	*<0.0001*	*<0.0001*	*<0.0001*
P60 *vs.* P100	*0.0391*	*0.3730*	*0.6775*

Table 4.11. Effect of management on leaf Mn concentration in soybean leaves at three sampling dates.

Management	Leaf Mn, μg g^{-1}					
	14 DAE		28 DAE		49 DAE	
	Mean across cultivar					
Un-limed	940	*69*	756	*74*	762	*76*
Limed, 2 t ha^{-1}	741	*39*	410	*33*	381	*21*
Limed, 5 t ha^{-1}	526	*83*	411	*32*	360	*27*
Mulch	390	*27*	355	*30*	254	*20*
P60	484	*46*	403	*57*	296	*34*
P60 +Mulch	635	*65*	424	*9*	360	*34*
P100	468	*29*	371	*20*	299	*13*
P100 +Mulch	480	*28*	393	*18*	329	*26*
G. manure (Gm)	449	*55*	344	*38*	332	*36*
Gm +Mulch	561	*35*	390	*18*	320	*10*
C. manure (Cm)	710	*36*	619	*68*	491	*48*
Cm +Mulch	647	*36*	645	*54*	480	*25*

Numbers in italics are standard error of the mean.

Table 4.12. Significance (*Pr*) of main effect, interaction effect and single *d.f.* contrasts of cultivar and management effects on leaf nutrient concentration at 28 DAE.

Effects	Leaf Nutrient g 100 g^{-1}			
	Ca	Mg	P	K
Cultivar (Cv)	*0.2180*	*0.4365*	*0.3872*	*0.3295*
Management (M)	*0.0078*	*0.0724*	*0.0141*	*0.3321*
Cv x M	*0.2852*	*0.2782*	*0.1388*	*0.2434*
Single *d.f.* contrasts	Across Cultivars			
Un-limed *vs.* Lime 2	*0.0636*	*0.0002*	*0.7420*	*0.6491*
Lime 2 *vs.* Lime 5	*0.0523*	*0.0244*	*0.3461*	*0.8314*
Lime 2 *vs.* others	*0.0906*	*0.0002*	*<0.001*	*0.0771*
Mulch *vs.* no mulch	*0.4339*	*0.5329*	*<0.001*	*0.0042*
Manure *vs.* no manure	*0.1312*	*0.8487*	*<0.001*	*0.0005*
Lime *vs.* P	*0.4241*	*0.0012*	*<0.001*	*0.1538*
Lime *vs.* Manure	*0.9747*	*0.0037*	*<0.001*	*0.0008*
Manure *vs.* P	*0.1364*	*0.3250*	*<0.001*	*0.0043*
Gm *vs.* Cm	*0.3730*	*0.0114*	*<0.001*	*0.4035*
P60 *vs.* P100	*0.8390*	*0.4478*	*0.8696*	*0.5126*

Table 4.13. Effect of management on leaf nutrient concentration in soybean leaves at 28 DAE.

Management	Leaf nutrient, g 100 g^{-1}							
	Ca		Mg		P		K	
	Across cultivars							
Un-limed	0.93	*0.056*	0.49	*0.011*	0.21	*0.008*	1.76	*0.133*
Limed, 2 t ha^{-1}	1.12	*0.065*	0.38	*0.023*	0.21	*0.009*	1.84	*0.142*
Limed, 5 t ha^{-1}	1.33	*0.079*	0.44	*0.021*	0.22	*0.006*	1.80	*0.090*
Mulch	1.32	*0.114*	0.47	*0.032*	0.26	*0.011*	2.06	*0.052*
P60	1.28	*0.089*	0.47	*0.029*	0.27	*0.014*	1.86	*0.094*
P60 +Mulch	1.29	*0.046*	0.44	*0.004*	0.30	*0.008*	2.14	*0.114*
P100	1.28	*0.057*	0.48	*0.018*	0.26	*0.007*	1.75	*0.107*
P100 +Mulch	1.26	*0.096*	0.47	*0.018*	0.30	*0.016*	2.09	*0.107*
G. manure (Gm)	1.16	*0.082*	0.44	*0.018*	0.27	*0.009*	2.24	*0.116*
Gm +Mulch	1.18	*0.077*	0.41	*0.012*	0.28	*0.006*	2.26	*0.096*
C. manure (Cm)	1.20	*0.071*	0.47	*0.011*	0.34	*0.010*	2.13	*0.084*
Cm +Mulch	1.26	*0.089*	0.48	*0.021*	0.38	*0.011*	2.18	*0.113*

Numbers in italics are standard error of the mean.

Grain yield

Grain yield of soybean was significantly affected by cultivar and management treatment (Table 4.8). Grain yield of cv. PSB Sy2 averaged across treatments was 1693 kg ha^{-1}, which was significantly higher than 1511 kg ha^{-1} from cv. PSB Sy6. The application of lime and P significantly increased grain yield. Mulching, on the other hand, did not increase grain yield (Table 4.8, 4.9). Grain yields with the application of P or manure were higher than yield with the application of lime. Between P rates, the higher rate of P resulted in higher grain yields. Chicken manure likewise gave higher yields than green manure

treatment. Yield without lime was very low at 389 kg ha^{-1}. Application of 2 t ha^{-1} lime increased yield to 1026 kg ha^{-1}. There was an additional 400 kg ha^{-1} with additional 3t ha^{-1} lime. Yield advantage due to application of P, green manure and chicken manure far exceeded the increase in yield due to additional lime applied (Fig. 4.12). Soybeans which received chicken manure gave the highest grain yield, followed by those that received green manure, 100 kg ha^{-1} P and 60 kg ha^{-1} P in descending order.

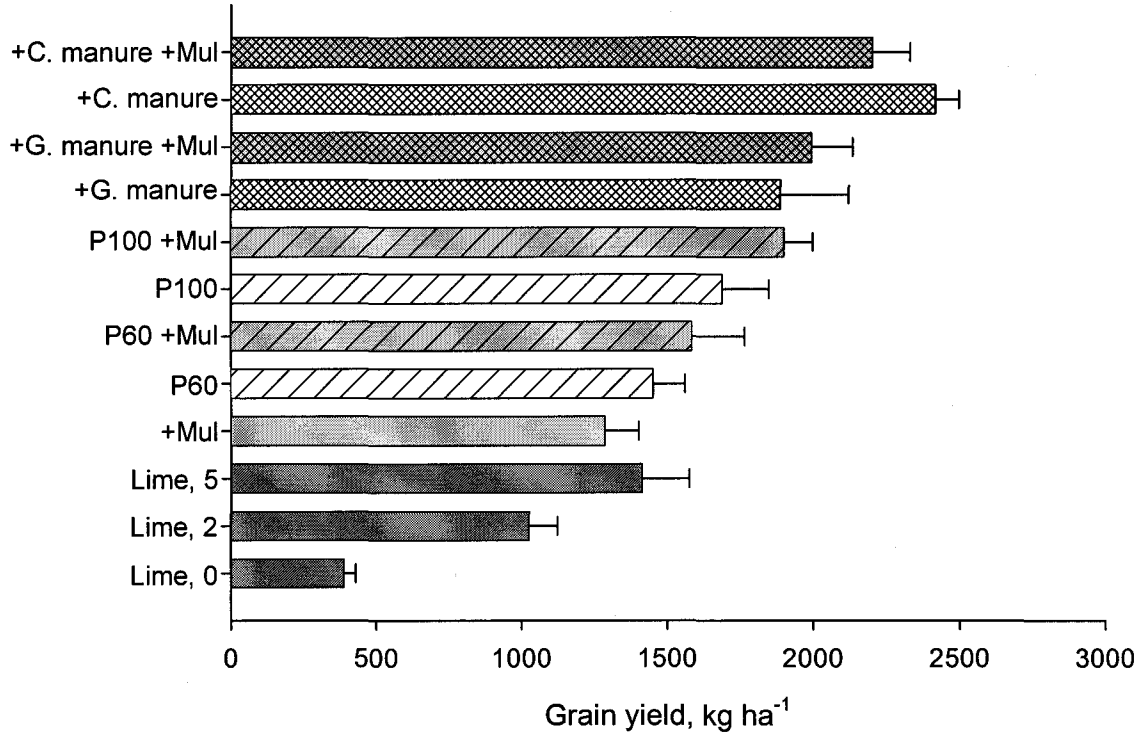


Fig. 4.12. Comparison of grain yield increases due to the applications of lime, P and manure, with or without mulch.

Plant relative growth rates and relative accumulation rate of manganese

At the early growth stage (14-28 DAE), total aboveground plant growth rate (RGR_{Plant}) was influenced only by management treatment. Management treatment and the interaction between management treatment and cultivar significantly influenced relative rates of leaf growth (RGR_{Leaf}), accumulation of Mn (RAR_{Mn}) in the leaves and the difference between RGR_{Leaf} and RAR_{Mn} ($RGRRAR_{Diff}$). (Table 4.14).

Mean plant growth rate of cv. PSB SY2 was 0.133 g $g^{-1}d^{-1}$, which was similar to the plant growth rate of cv. PSB Sy6 amounting to 0.131 g $g^{-1}d^{-1}$. The corresponding leaf growth rates were 0.128 g g^{-1} d^{-1} for cv. PSB Sy2 and 0.128 g g^{-1} d^{-1} for cv. PSB Sy6. Soybean cv. PSB Sy2 accumulated Mn at the rate of 0.112 μg μg^{-1} d^{-1}, which was similar to 0.110 μg μg^{-1} d^{-1} rate of cv. PSB Sy6.

The response of RGR_{Plant} was similar across cultivars. Increasing lime rates did not increase RGR_{Plant} while mulching and manure application significantly increased RGR_{Plant} (Table 4.14, 4.15). Compared with lime, P and manure gave higher RGR_{Plant}. Between manure and P, manure led to higher RGR_{Plant}. Compared with chicken manure, green manure gave higher RGR_{Plant} . The response of RGR_{Leaf} to management treatments differed with soybean cultivar although there were similar responses observed. In both cultivars, mulching did not influence RGR_{Leaf} while green manure application increased RGR_{Leaf}. Comparing lime and P, the latter gave higher RGR_{Leaf} in cv. PSB Sy2

but not cv. PSB Sy6. Between the two types of manure, chicken manure gave higher RGR_{Leaf} than green manure in cv. PSB Sy6 but not cv. PSB Sy2.

The response of RAR_{Mn} to management treatments likewise differed with cultivar (Table 4.14). In cv. PSB Sy2, RAR_{Mn} decreased with 2 t ha^{-1} lime applied, followed by an increase with 5 t ha^{-1} lime applied (Table 4.15). In the case of cv. PSB Sy6, RAR_{Mn} decreased with 2 t ha^{-1} lime with no further change when 5 t ha^{-1} lime was applied. Mulching significantly increased RAR_{Mn} of cv. PSB Sy6 but not cv. PSB Sy2. Manure application increased RAR_{Mn} of both cultivars. Between manure and P, manure led to higher RAR_{Mn} and between the two types of manure, chicken manure gave a higher RAR_{Mn}. This was observed in cv. PSB Sy6 but not cv. PSB Sy2. The absolute difference between RGR_{Leaf} and RAR_{Mn} , called the $RGRRAR_{Diff}$, differed with management treatment, the response to management treatment different for each cultivar (Table 4.14). The $RGRRAR_{Diff}$ was increased by the application of 2 t ha^{-1} lime in both cultivars (Table 4.15). This parameter was decreased by P and manure addition only in cv. PSB Sy6. In cv. PSB Sy6, the $RGRRAR_{Diff}$ was significantly lower with chicken manure than with green manure application.

In both cultivar and most management treatments, RGR_{Leaf} were consistently higher than RAR_{Mn} (Fig. 4.13, 4.14, 4.15). This is reflected in a decrease in leaf Mn concentration with time (Fig. 4.16). In general, an increase in RGR_{Leaf} due to a management treatment was accompanied by an increase in RAR_{Mn}. Also, RGR_{Plant} was highly correlated with RGR_{Leaf} (Fig. 4.17), during two growth intervals of the both soybean cultivars. The relationship between these

RGRs is described by a linear equation with significant and strong correlation coefficients. This observation indicates that the relative growth rate of the leaves alone can be used as a proxy for relative growth rate of the whole plant, thus making whole plant sampling unnecessary for growth analysis. A negative impact of current RGR_{Leaf} on future RAR_{Mn} in both soybean cultivars were observed (Fig. 4.18). The correlation coefficient was somewhat weaker for cv. PSB Sy2 than cv. PSB Sy6. A similar effect between current RAR_{Mn} and future RGR_{Leaf} was observed in both cultivar, although the correlation coefficient was somewhat low.

Net assimilation rate and Leaf area ratio

Net assimilation rate (NAR) and leaf area ratio (LAR) are the two components of relative growth rate (RGR) (Radford, 1967). At any instant in time, RGR = NAR x LAR. However, the equation is not always true for mean values of RGR, NAR and LAR. An exception is where leaf area and biomass weight both increases exponentially with the same exponent (Radford, 1967). When a management treatment has a significant impact on RGR, it is important to identify which component of RGR was specifically affected. Statistical analysis of NAR and LAR values showed that management treatment and the interaction between cultivar and management treatment significantly influenced NAR. Leaf area ratio, on the other hand was similar between cultivars and among management treatments. Soybean cv. PSB Sy2 showed significant difference in NAR between lime levels, with soybeans receiving 5 t ha^{-1} lime giving a higher

NAR than those receiving 2 t ha^{-1} lime. On the other hand, cv. PSB Sy6 showed significant difference in NAR between green manure and chicken manure treatments, with chicken manure application resulting in higher NAR in soybeans compared with those that received green manure.

Yield determinants

In any stress environment, grain yield (or economic yield in general) is the ultimate measure of the success of any intervention to modify the stress environment and alleviate stress effect on plant performance. In the case of Mn phytotoxicity in soybeans, grain yield is an important measure of how much phytotoxicity has occurred. The correlation of several plant parameters with grain yield was determined to identify indicators or determinants of grain yield under conditions of excess Mn over the range of management treatment imposed. Leaf area, leaf biomass and leaf growth rate at early vegetative stage was correlated with grain yield (Fig. 4.19, 4.20, 4.21). Because leaf biomass is correlated with leaf growth rate, we observed significant positive correlation between leaf growth rate and grain yield (Fig. 4.20). However, this observation was true only for cv. PSB Sy2 but not cv. PSB Sy6. The correlation between leaf area, leaf biomass and leaf growth rate and grain yield is expected and likewise suggests that management of these plant parameters at the early growth period translates to managing the final grain yield. Leaf Mn, however, did not show good relation with grain yield and seemed unrelated to leaf area in both cultivars (Fig. 4.20, 4.19). The relative accumulation rate of Mn was likewise positively correlated with grain yield (Fig. 4.21). These relationships were significant for cv.

PSB Sy2 but not cv. PSB Sy6, and possible a consequence of the correlation between plant growth rate and Mn accumulation rate. The absolute difference between plant growth rate and Mn accumulation rate was not correlated with grain yield (Fig. 4.22). The management treatments somewhat provided different amounts of P to the soybean crop. There were two rates of P application (60 and 100 kg ha^{-1}) and two types of manure (*Leucaena* leaves and chicken manure), which provided different amounts of P to the growing crop. Consequently, leaf P was positively correlated with grain yield of both cultivars (Fig. 4.23).

Soil test values for available Mn by saturated paste extract did not show significant correlation with grain yield (Fig. 4.24). A similar observation was made with the use of phytotoxic symptom scores to predict grain yield (Fig. 4.25).

Table 4.14. Significance (*Pr*) of main effect, interaction effect and single *d.f.* contrasts of cultivar and management on plant growth rates, Mn accumulation rate and the difference between leaf growth rate and Mn accumulation rate at early growth stage of soybeans.

Effects	RGR, g g^{-1} d^{-1}		RAR_{Mn}	RGR_{Leaf}-RAR_{Mn}
	Plant	Leaf	μg $μg^{-1}$ d^{-1}	
Cultivar (Cv)	*0.4559*	*0.7884*	*0.6253*	*0.7330*
Management (M)	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Cv x M	*0.3258*	*0.0052*	*0.0003*	*0.0005*
Single *d.f.* contrasts	**Across cultivar**		**PSB Sy2**	
Un-limed *vs.* Lime 2	*0.5743*	*0.9646*	*0.0378*	*0.0102*
Lime 2 *vs.* Lime 5	*0.9415*	*0.1137*	*<0.0001*	*<0.0001*
Lime 2 *vs.* others	*0.0003*	*0.0014*	*<0.0001*	*0.0102*
Mulch *vs.* no mulch	*0.0006*	*0.1000*	*0.4784*	*0.1986*
Manure *vs.* no manure	*<0.0001*	*0.0119*	*0.0097*	*0.9762*
Lime *vs.* P	*0.0003*	*0.0037*	*0.0518*	*0.1157*
Lime *vs.* Manure	*<0.0001*	*0.0015*	*0.0120*	*0.2159*
Manure *vs.* P	*0.0032*	*0.3929*	*0.1499*	*0.4747*
Gm *vs.* Cm	*0.0080*	*0.5879*	*0.0838*	*0.1377*
P60 *vs.* P100	*0.4198*	*0.4549*	*0.0938*	*0.2454*
			PSB Sy6	
Un-limed *vs.* Lime 2		*0.5499*	*0.0173*	*0.0086*
Lime 2 *vs.* Lime 5		*0.4325*	*0.4848*	*0.1251*
Lime 2 *vs.* others		*0.1085*	*<0.0001*	*<0.0001*
Mulch *vs.* no mulch		*0.1013*	*0.0018*	*0.0035*
Manure *vs.* no manure		*0.0034*	*0.0010*	*0.0326*
Lime *vs.* P		*0.0638*	*0.0001*	*0.0002*
Lime *vs.* Manure		*0.0001*	*<0.0001*	*<0.0001*
Manure *vs.* P		*0.0198*	*0.0358*	*0.3946*
Gm *vs.* Cm		*0.0001*	*<0.0001*	*0.0072*
P60 *vs.* P100		*0.1290*	*0.1915*	*0.6422*

Table 4.15. Effect of management and cultivar on plant growth rates, Mn accumulation rate and the difference between leaf growth rate and Mn accumulation rate at early growth stage of soybeans.

Management	RGR $g\ g^{-1}\ d^{-1}$				RAR_{Mn} $\mu g\ \mu g^{-1}\ d^{-1}$		RGR_{Leaf}-RAR_{Mn} ($RGRRAR_{Diff}$)	
	Plant		Leaf					
	Across cultivar				PSB Sy2			
Un-limed	0.1154	*0.0059*	0.1041	*0.0046*	0.0941	*0.0042*	0.0100	*0.0016*
Limed, 2 t ha^{-1}	0.1105	*0.0101*	0.1046	*0.0073*	0.0717	*0.0036*	0.0328	*0.0071*
Limed, 5 t ha^{-1}	0.1111	*0.0039*	0.1219	*0.0007*	0.1301	*0.0034*	0.0082	*0.0033*
Mulch	0.1383	*0.0068*	0.1185	*0.0189*	0.1032	*0.0155*	0.0153	*0.0042*
P60	0.1511	*0.0071*	0.1306	*0.0022*	0.1169	*0.0013*	0.0137	*0.0010*
P60 +Mulch	0.1370	*0.0075*	0.1320	*0.0050*	0.0979	*0.0051*	0.0341	*0.0073*
P100	0.1275	*0.0043*	0.1273	*0.0020*	0.1098	*0.0052*	0.0175	*0.0072*
P100 +Mulch	0.1247	*0.0050*	0.1466	*0.0017*	0.1300	*0.0038*	0.0166	*0.0048*
G. manure (Gm)	0.1354	*0.0077*	0.1327	*0.0089*	0.1183	*0.0123*	0.0144	*0.0056*
Gm +Mulch	0.1569	*0.0062*	0.1407	*0.0080*	0.1114	*0.0063*	0.0295	*0.0070*
C. manure (Cm)	0.1312	*0.0055*	0.1454	*0.0078*	0.1234	*0.0033*	0.0220	*0.0109*
Cm +Mulch	0.1441	*0.0055*	0.1362	*0.0024*	0.1320	*0.0035*	0.0042	*0.0028*
					PSB Sy6			
Un-limed			0.1234	*0.0110*	0.1010	*0.0132*	0.0224	*0.0023*
Limed, 2 t ha^{-1}			0.1181	*0.0015*	0.0649	*0.0092*	0.0532	*0.0095*
Limed, 5 t ha^{-1}			0.1110	*0.0010*	0.0748	*0.0055*	0.0362	*0.0047*
Mulch			0.1321	*0.0032*	0.1351	*0.0119*	-0.0010	*0.0097*
P60			0.1224	*0.0061*	0.1050	*0.0161*	0.0174	*0.0122*
P60 +Mulch			0.1179	*0.0044*	0.1016	*0.0131*	0.0164	*0.0088*
P100			0.1125	*0.0008*	0.0973	*0.0017*	0.0151	*0.0012*
P100 +Mulch			0.1475	*0.0045*	0.1360	*0.0074*	0.0115	*0.0048*
G. manure (Gm)			0.1378	*0.0051*	0.1162	*0.0064*	0.0217	*0.0038*
Gm +Mulch			0.1054	*0.0076*	0.0838	*0.0047*	0.0216	*0.0114*
C. manure (Cm)			0.1486	*0.0113*	0.1476	*0.0110*	0.0011	*0.0050*
Cm +Mulch			0.1527	*0.0031*	0.1551	*0.0034*	-0.0024	*0.0029*

[a]*d.f.* Contrast Across cultivar
Numbers in italics are standard error of the mean.

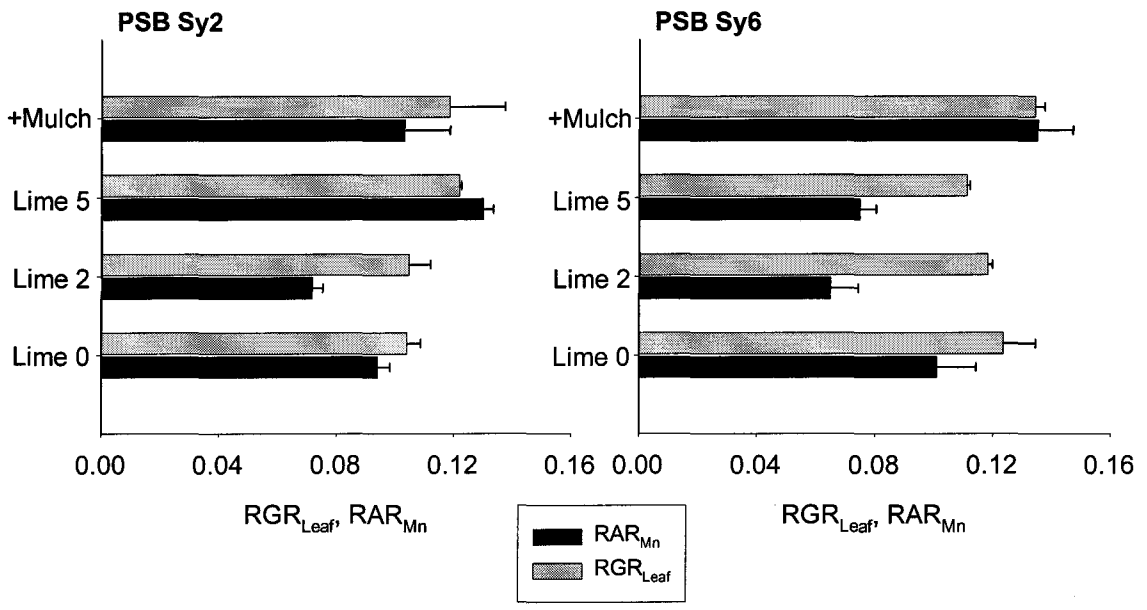


Fig. 4.13. Effect of lime application on rates of leaf growth and Mn accumulation of two soybean cultivars.

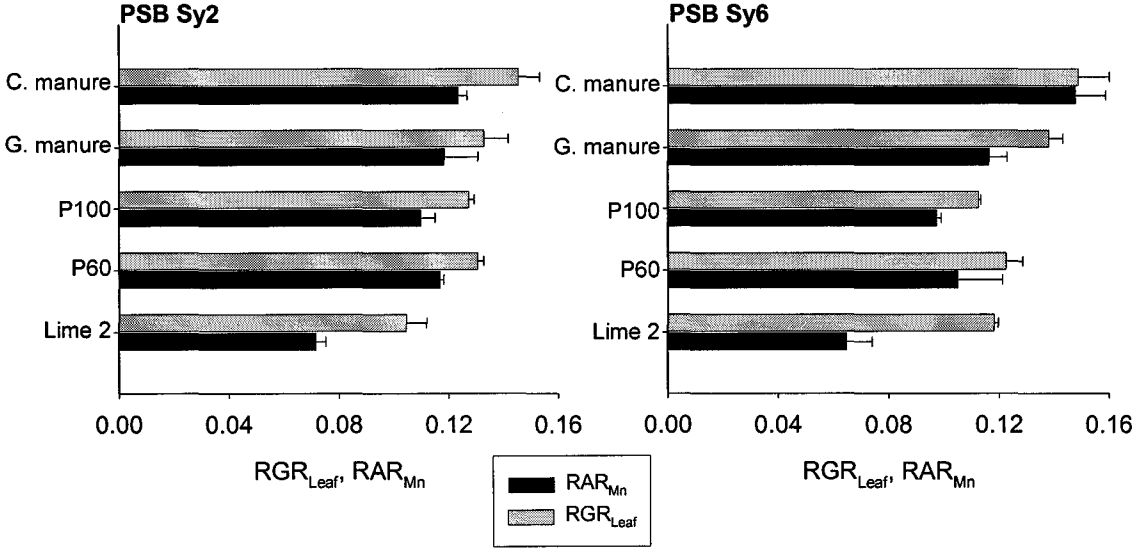


Fig. 4.14. Effect of P and manure applications without mulch on rates of leaf growth and Mn accumulation of two soybean cultivars.

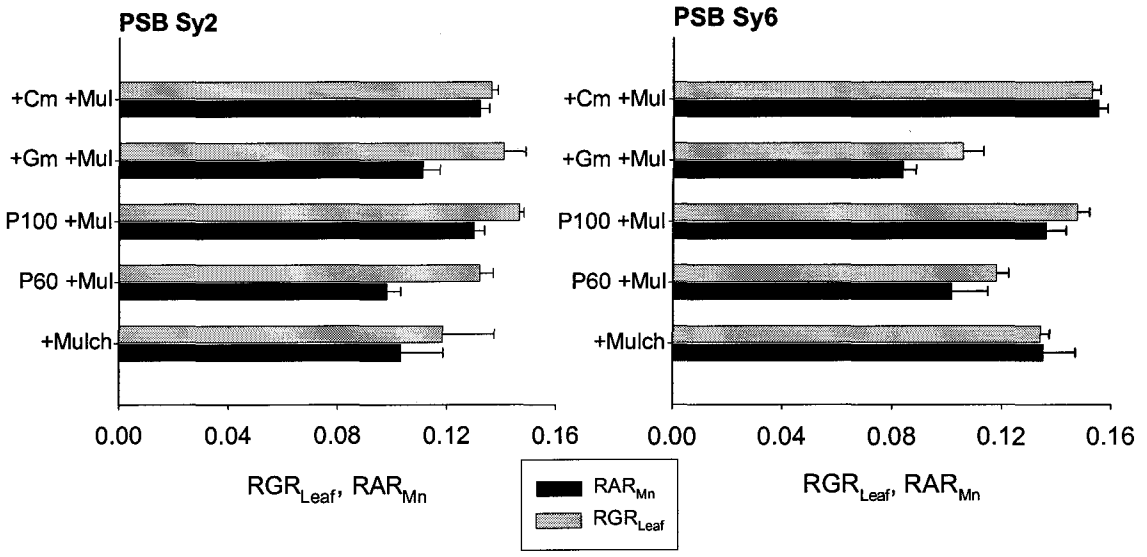


Fig. 4.15. Effect of P and manure applications with mulch on the rates of leaf growth and Mn accumulation of two soybean cultivars.

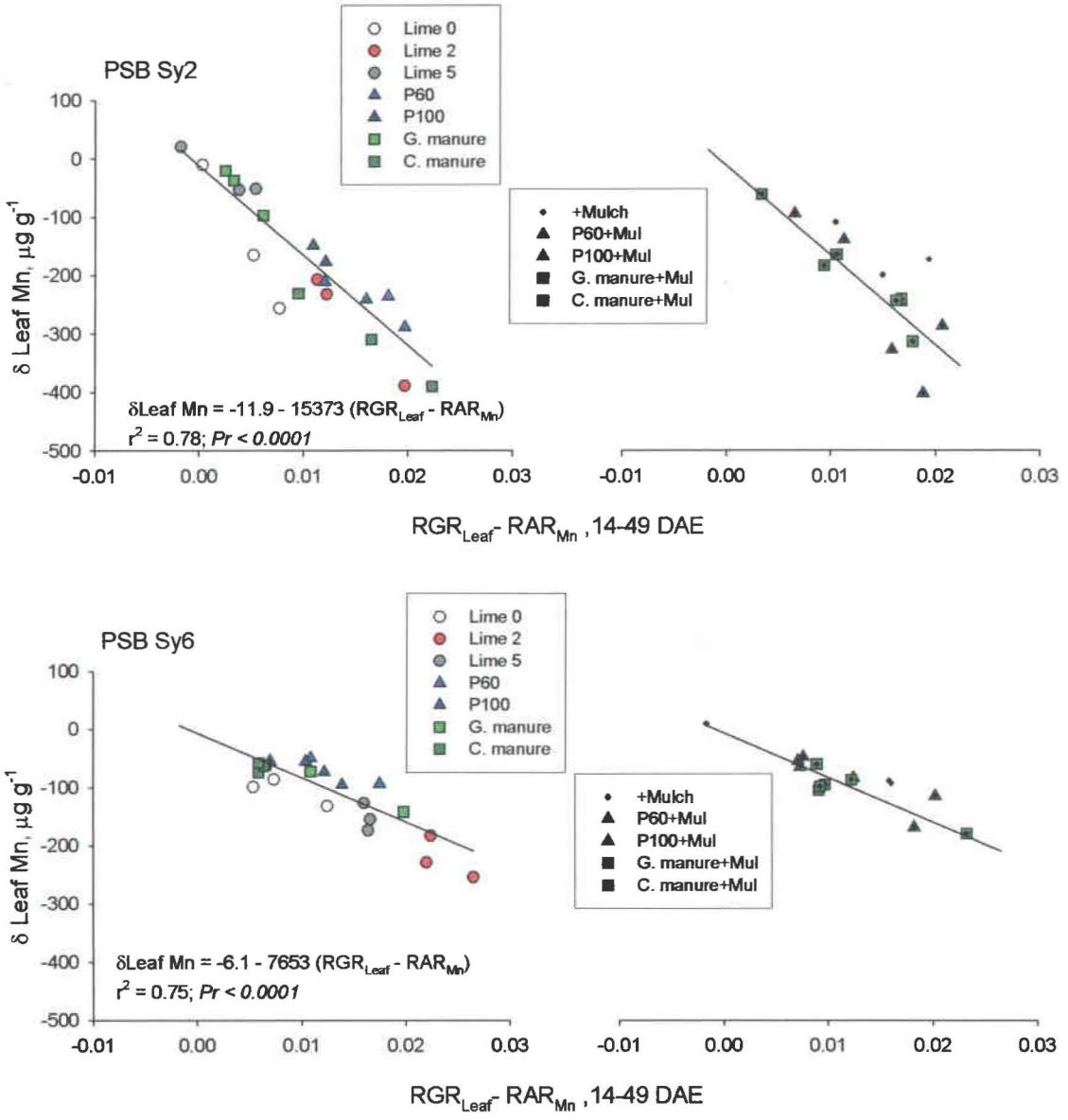


Fig. 4.16. The relationship of the difference between leaf growth rate and Mn accumulation rate to changes in leaf Mn with time in two soybean cultivars. The equation was fitted using all the data points within each cultivar.

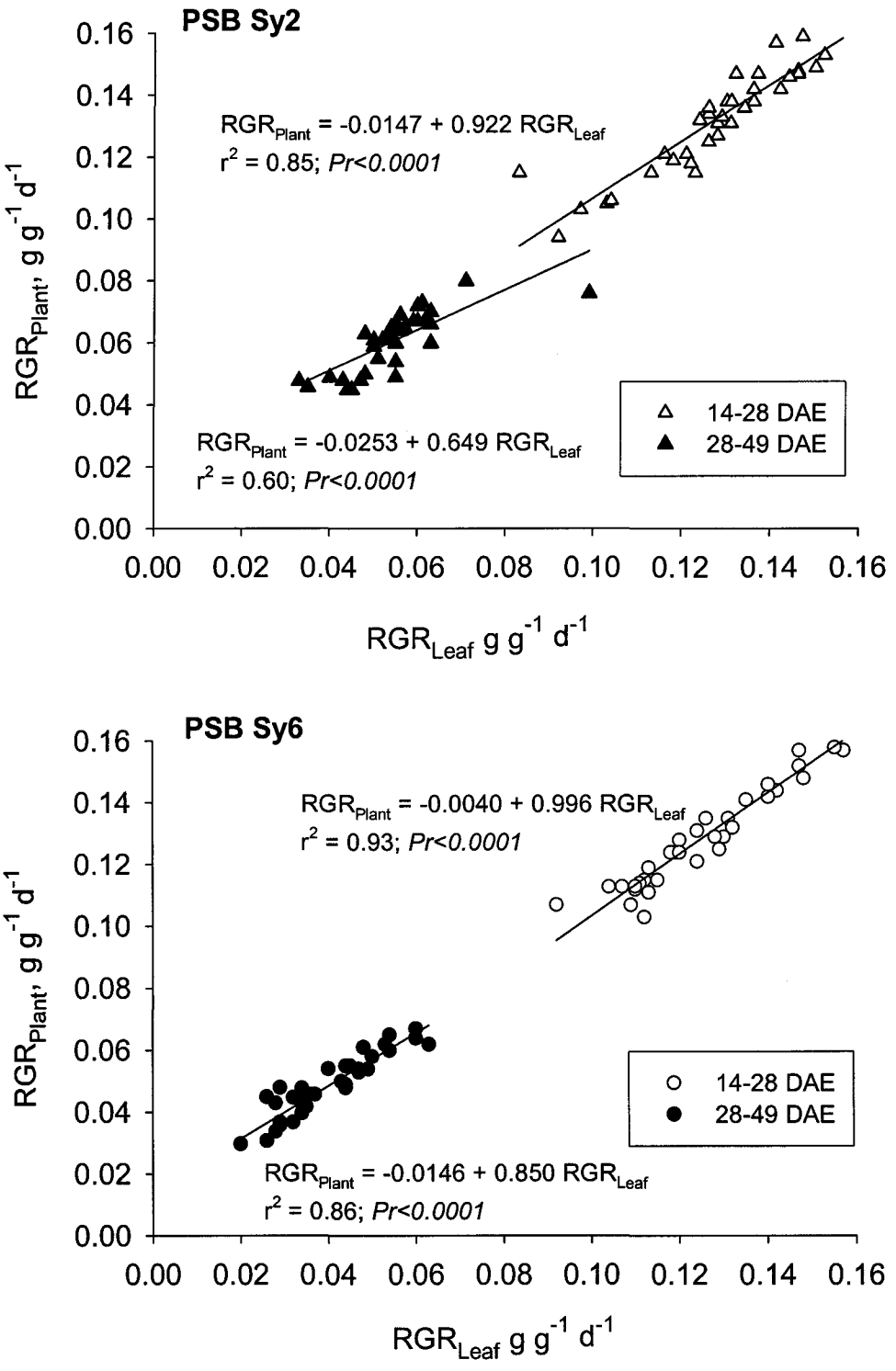


Fig. 4.17. Correlation between plant growth rate and leaf growth rate at two growth intervals in two soybean cultivars.

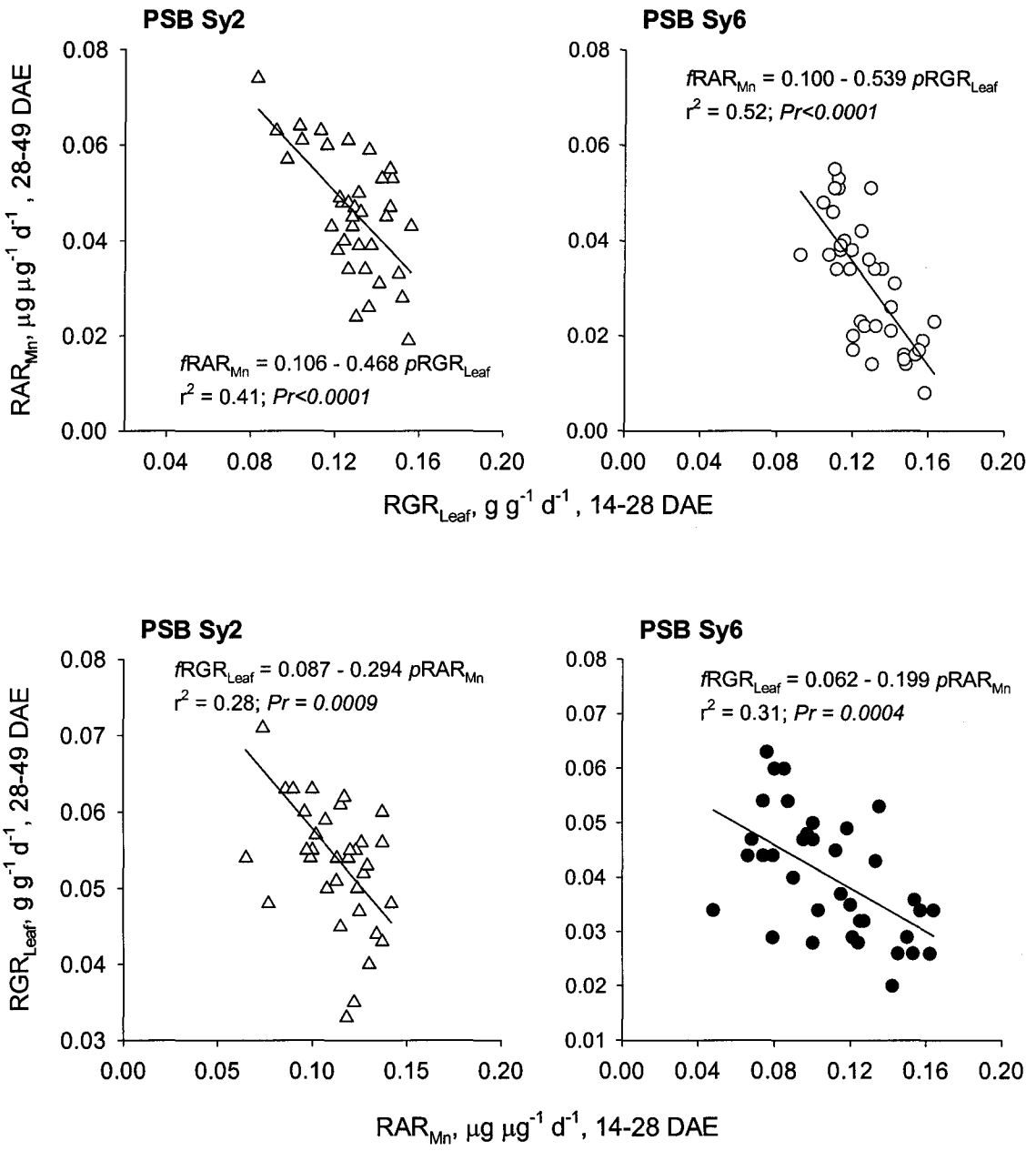


Fig. 4.18. Effect of current leaf growth rate (cRGR$_{Leaf}$) on future Mn accumulation rate (fRAR$_{Mn}$) and the effect of current Mn accumulation rate (cRAR$_{Mn}$) on future leaf growth rate (fRGR$_{Leaf}$) in two soybean cultivars.

Table 4.16. Significance (*Pr*) of main effect, interaction effect and single *d.f.* contrasts of cultivar and management on net assimilation rate, leaf area ratio and leaf area growth rate of soybeans.

Effects	Net Assimilation rate		Leaf Area Ratio
Cultivar (Cv)	*0.3268*		0.4791
Management (M)	*0.0024*		0.1762
Cv x M	*0.0403*		0.5531
Single *d.f.* contrast	PSB Sy2	*PSB Sy6*	
Un-limed vs. Lime 2	*0.9046*	*0.5319*	
Lime 2 vs. Lime 5	*0.0491*	*0.9590*	
Lime 2 vs. others	*0.0175*	*0.5236*	
Mulch vs. no mulch	*0.6977*	*0.2916*	
Manure vs. no manure	*0.1201*	*0.2672*	
Lime vs. P	*0.0942*	*0.8402*	
Lime vs. Manure	*0.1393*	*0.2006*	
Manure vs. P	*0.8802*	*0.2376*	
Gm vs. Cm	*0.9072*	*0.0104*	
P60 vs. P100	*0.1679*	*0.0799*	

Table 4.17. Effect of management and cultivar on the net assimilation rate and leaf area ratio at early vegetative stage of soybeans.

Management	Net assimilation rate[a]				Leaf area ratio			
	PSB Sy2		PSB Sy6		PSB Sy2		PSB Sy6	
Un-limed	27.45	*2.189*	33.64	*5.303*	387	*41*	376	*28*
Limed, 2 t ha^{-1}	27.92	*1.913*	31.50	*1.918*	375	*13*	378	*27*
Limed, 5 t ha^{-1}	36.01	*1.303*	31.68	*1.365*	339	*11*	352	*15*
Mulch	35.79	*2.044*	33.87	*1.356*	370	*6*	407	*12*
P60	37.28	*3.616*	35.96	*2.225*	394	*20*	413	*20*
P60 +Mulch	27.03	*5.904*	35.26	*0.926*	457	*45*	380	*9*
P100	34.83	*0.385*	31.06	*2.552*	375	*4*	396	*16*
P100 +Mulch	34.28	*2.085*	29.52	*1.753*	373	*17*	383	*20*
G. manure (Gm)	36.73	*1.416*	27.58	*0.589*	483	*18*	381	*20*
Gm +Mulch	35.89	*0.120*	38.80	*1.663*	379	*8*	396	*24*
C. manure (Cm)	33.50	*1.948*	28.60	*1.149*	395	*9*	413	*8*
Cm +Mulch	41.89	*4.168*	38.86	*2.870*	356	*29*	383	*23*

[a]Mean and standard error x 10^{-5}; Numbers in italics are standard error of the mean.

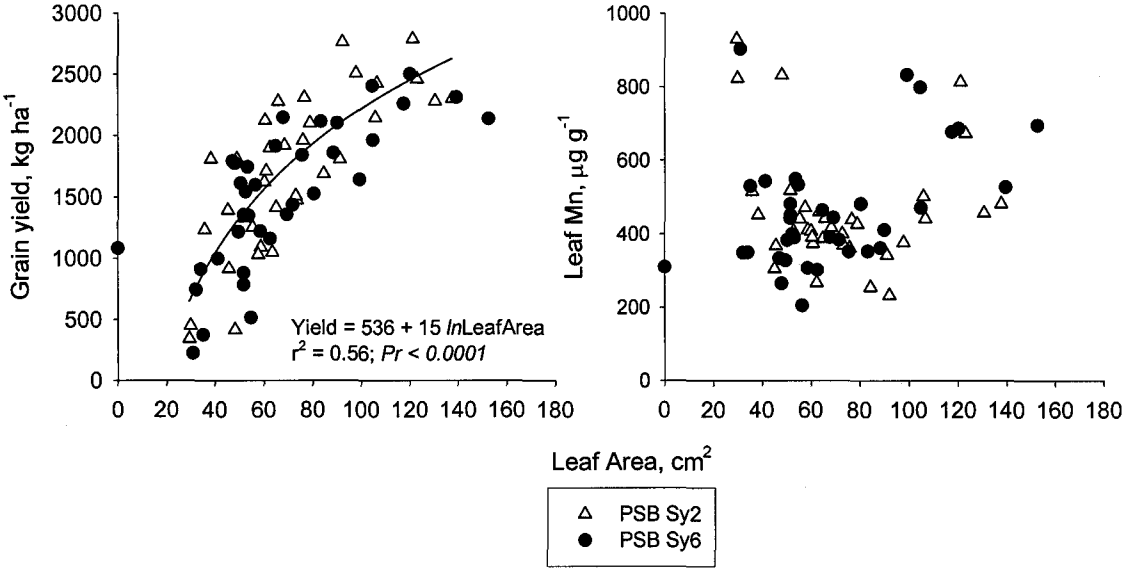


Fig. 4.19. Leaf area as a predictor of grain yield and leaf Mn at early vegetative of two soybean cultivars.

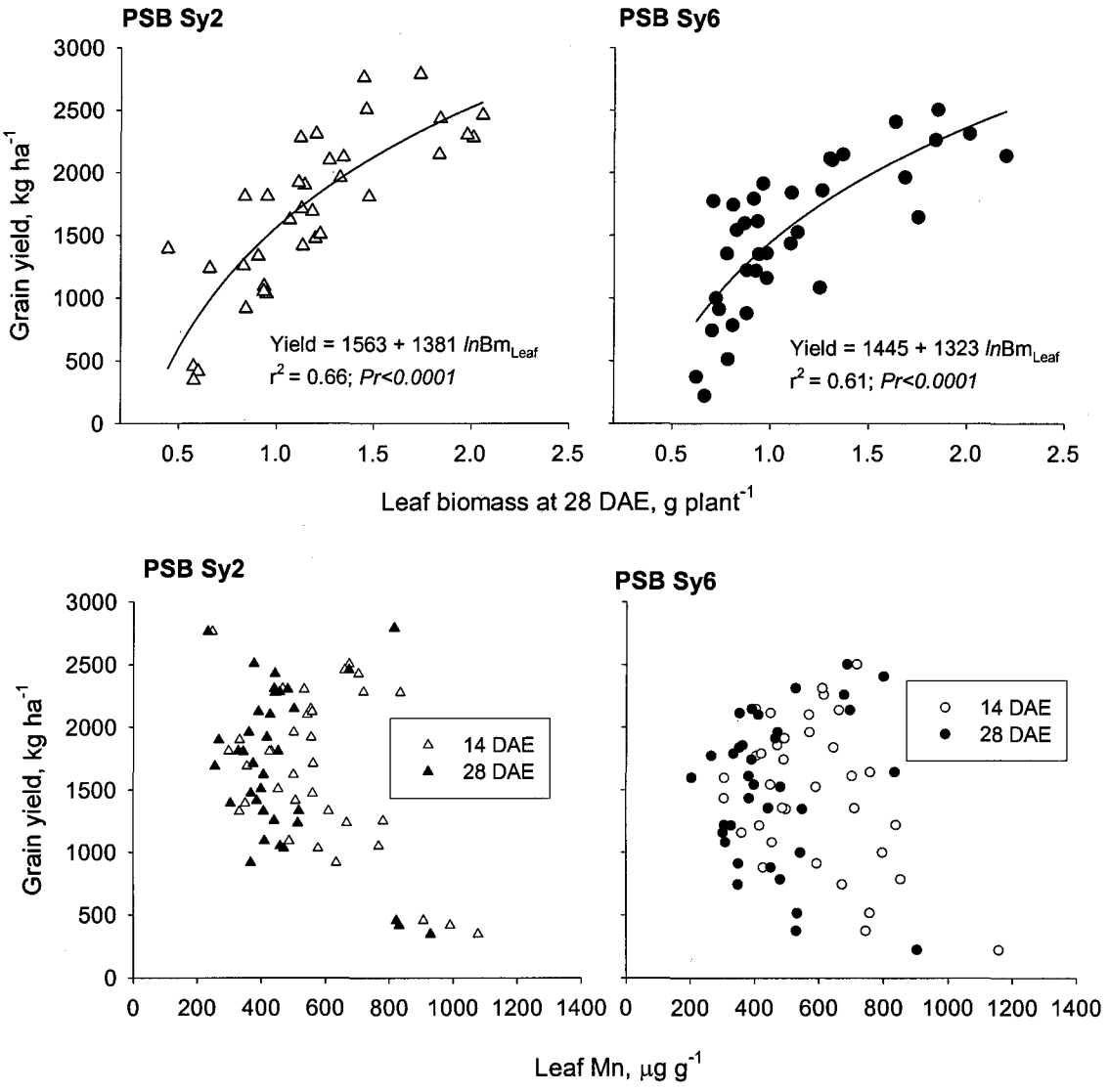


Fig. 4.20. Correlation of leaf Mn and leaf biomass at early vegetative stage as to grain yield of two soybean cultivars.

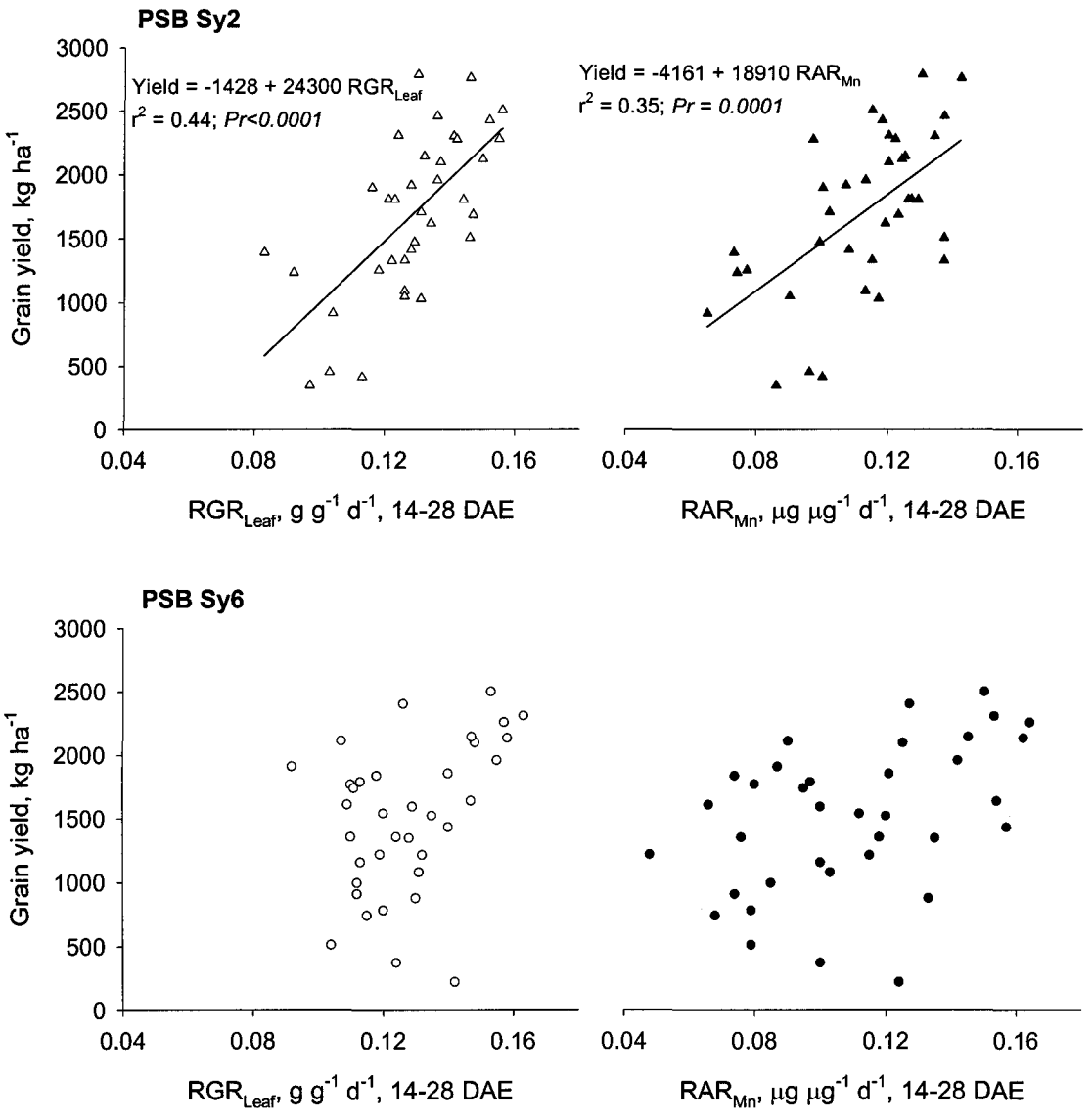


Fig. 4.21. Leaf growth rate and Mn accumulation rate at early vegetative stage as predictors of grain yield of two soybean cultivars.

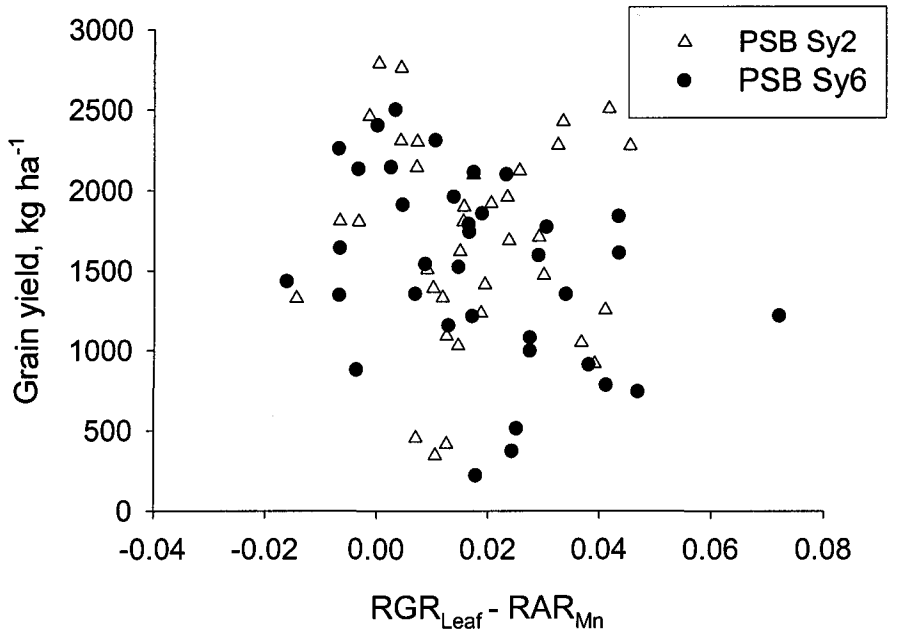


Fig. 4.22. Correlation of the absolute difference between leaf growth rate and Mn accumulation rate to grain yield of two soybean cultivars.

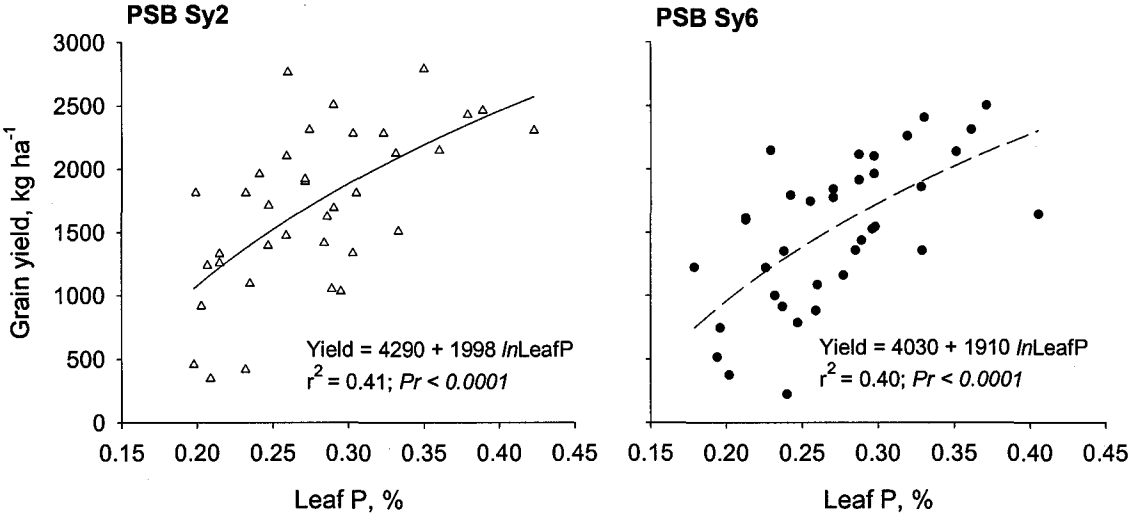


Fig. 4.23. Relation of leaf P at early vegetative stage to grain yield of two soybean cultivars.

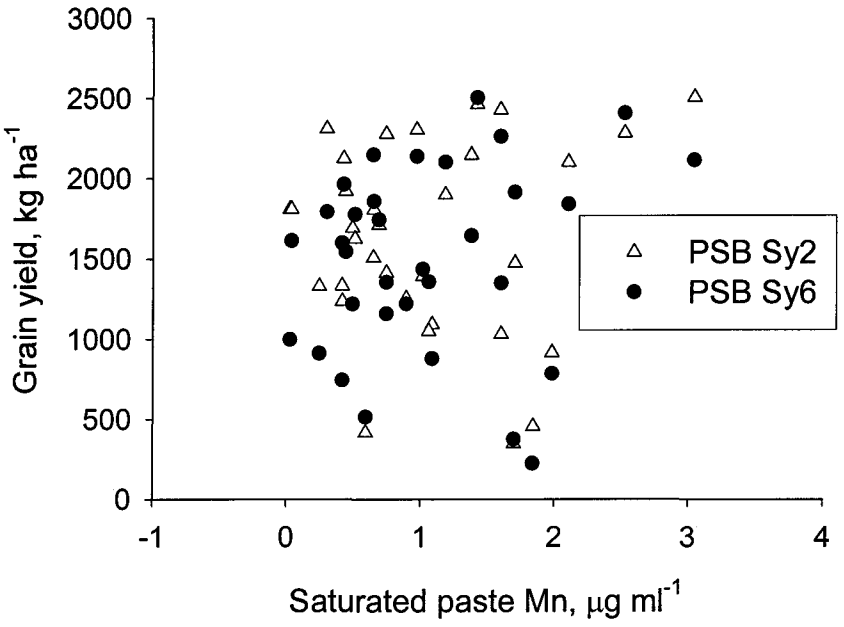


Fig. 4.24. Correlation of saturated paste-Mn to grain yield of two soybean cultivars.

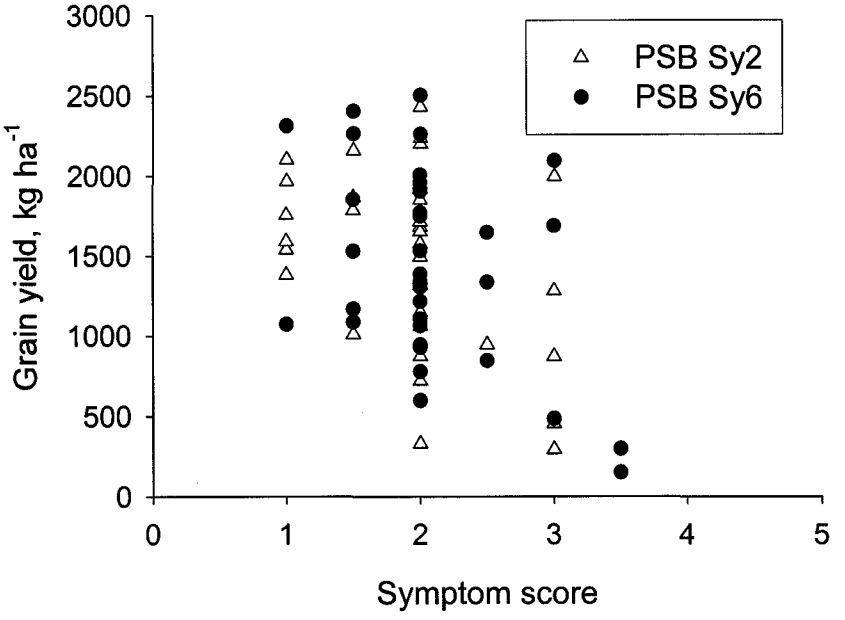


Fig. 4.25. Correlation of symptom score to grain yield of two soybean cultivars.

Discussion

The Approach

The ultimate measure of success of any soil fertility management strategy is crop yield, that portion of harvested crop that has economic value to the farmer. A powerful evidence of alleviating an existing toxicity in an acid soil is to be able to grow a crop on the soil, preferably symptom-free but most importantly able to produce economic yield. The advantages of any field experiment are that it facilitates the testing of management strategies under the condition that is more similar than the glasshouse to the conditions where crops are grown by farmers, and that crops can be grown to maturity. Yield, therefore, can be measured.

The treatments that were imposed in this experiment were conceived based on a principle of integrated management, and taking into account previous results of experiments that show aggravation as well as alleviation of Mn toxicity. In the context of this study, integrated nutrient management implies that we recognized the complex nature of soil acidity, having in mind the nutrient deficiencies and metal toxicities associated with low pH. Manganese phytotoxicity in itself is difficult to predict with any single plant or soil property because in many instances, the interactions between environment, plant genotype and soil characteristics dominates the single effects of each of these factors. An outstanding and interesting theme in the study of Mn phytotoxicity is the enhancement of tolerance due to nutrient and environment manipulation. More interesting is the observation that there seems to be inconsistent effects of these manipulations. For example, high temperature or intense light has been

shown to increase (Rufty, *et al.*, 1979) or decrease (Marsh *et al.*, 1989) tolerance to Mn toxicity. Similar observations have been reported for the application of phosphorus (Morris and Pierre, 1947; Bortner, 1935; Kang and Fox, 1980) or various organic material applications (Nkana *et al.*, 1998; Vega, *et al.*, 1992). Various authors (Marsh, *et al.*, 1989; Rufty *et al.*, 1979) suggested that such inconsistent effects are possible because tolerance is conferred *via* an increase in growth rate, so that environmental and nutrient manipulation would confer enhanced tolerance only when they can enhance plant growth rate. In our exploration of possible options for managing excess Mn, we focused on managing growth rate as opposed to managing the excess Mn in the soil.

Effects of management on soil toxicities

The *Rugao* soil series used in this field experiment is very acid, with evidence of both toxicities of Al and Mn. The amount of exchangeable Al in unlimed soil (1.6 $cmol_c\ kg^{-1}$) is within the typical range of exchangeable Al (1.3-6.6 $cmol_c\ kg^{-1}$) in many acid soils of the tropics according to reports of many investigators (Kamprath, 1984). Likewise the amount of soil solution Mn (1.38 $\mu g\ ml^{-1}$) is about three times as much as the critical toxicity level in soil (0.5 $mg\ L^{-1}$) as suggested by Hue (1998). Short, stubby and brownish roots of soybean in the unlimed soil also indicate the presence of Al toxicity. On the other hand, the presence of brown spots, interveinal yellowing and crinkling of soybean leaves confirm the presence of Mn toxicity. Lime at 2 tons ha^{-1} significantly increased soil pH while decreasing exchangeable Al but not saturated paste-Mn. The

reason for selecting this lower rate of lime application was to minimize exchangeable Al without affecting soil solution Mn and, therefore, to maintain potentially excessive levels in solution. The higher rate of lime further lowered both exchangeable Al and saturated paste-Mn to non-toxic levels. All of the P, mulching and manure treatments received 2 tons ha^{-1} lime so that comparison with the lime 2 t ha^{-1} control indicates the effect of each particular treatment. None of the P, mulching and green manure treatments affected the level of soil pH and exchangeable Al when compared with Lime 2. On the other hand, green manure and chicken manure application caused about 3-4 fold increase in saturated paste-Mn. This effect is consistent with results of various laboratory and greenhouse experiments where the application of organic matter increased the concentration of Mn in the soil solution (Vega, *et al.*, 1992; Hue *et al.*, 2001). This can be explained by the reduction reaction of Mn as shown below (Adams, 1981):

$$MnO_2 + 4H^+ + 2\,e^- \Leftrightarrow Mn^{2+} + 2\,H_2O \qquad \text{[Reaction 4.1]}$$

This equation suggests the availability of electrons from decomposing manure would enhance the reduction of Mn^{4+} to Mn^{2+}. In the previous field experiment, where peanut tops were applied, an increase in extractable Mn due to green manure application was not observed. This observation can be attributed to one or combinations of the quality of the green manure, the time of sampling or the strength of the extractant. The peanut green manure was harvested at about flowering so that the plant tissue possibly contained less easily decomposable compounds. The soil was sampled at 21 days after

emergence, which is about 42 days after green manure incorporation. The period of active decomposition when the maximum amount of Mn oxide were solubilized and reduced could have occurred before the sampling date. It was also possible that compared to saturated paste extract, water and KCl were too weak to extract Mn solubilized by green manure decomposition while DTPA and Mehlich 1 were too strong to accurately represent the Mn pool that is released by green manure decomposition.

Effectiveness of liming

Liming is the conventional and often necessary solution to neutralizing Al and Mn toxicities in acid soils. In the form of $CaCO_3$, it also provides Ca, which was deficient in our test soil. Lime applied at 2 kg ha^{-1} neutralized about half of the soil exchangeable Al and a small amount of Mn in the soil solution. Short, stubby and brownish roots were not observed in the limed soybean as a result. Phytotoxic symptoms of excess Mn such as brown spots, yellowing and crinkling were less severe and leaf Mn concentration in the leaves was significantly decreased. This alleviation of phytotoxic symptoms was accompanied by significant increases in biomass production and yield. The rate of plant growth was unaffected while that of the accumulation of Mn decreased. Application of lime to 5 t ha^{-1} further decreased half of the exchangeable Al and reduced soil solution Mn below the critical level of 0.5 mg L^{-1} suggested by Hue (1998). These changes in soil Mn and Al were accompanied by further significant increases in biomass production and yield. Plant growth rate was likewise

unaffected while the rate of Mn accumulation was increased to the level similar to that of the unlimed soybean.

Effectiveness of phosphorus and manure application

The application of phosphorus did not contribute to the neutralization of saturated paste-Mn and exchangeable Al. We used triple superphosphate (TSP; 40% P_2O_5) fertilizer in this field experiment. It is a form of Ca-phosphate although the Ca content is very small compared with other Ca-phosphates such as mono-, di- or tri Ca-phosphate. This fertilizer was selected to avoid Ca supply that will sufficiently influence plant response to the fertilizer. A significant impact of P application was noted on the amount of plant biomass and grain yield production. The additional biomass and grain yield produced due to P application was significantly higher than that produced due to additional lime. These results are reflected in the increased P concentration in the plant tissue at 28 days after emergence. Consistent with increased biomass production is the increased rate of plant growth, although further increases due to a higher rate of P tended to be insignificant for both cultivars. Increased rate of plant growth was accompanied by significant increases in the rate of Mn accumulation in the plant tissue of PSB Sy6. The increase in leaf Mn due to P was significant only at 14 days after emergence. During the later vegetative stages, leaf Mn with P application was similar with the limed control. The effect of P on Mn phytotoxicity may be attributed to a possible formation of slowly soluble Mn-phosphate minerals identified by Boyle and Lindsay (1986). The authors suggested that

Mn-phosphate may control Mn solubility when strengite-soil Fe or variscite-gibbsite equilibria controls the solubility of P as may be possible in Wahiawa soil. On the other hand, Jones and Fox (1978) found no effect of P on soil Mn availability and concluded that the decreased accumulation of Mn in tomato due to P fertilization is a result of reactions at root surfaces or inside the plant rather than precipitation in the bulk solution. The increased availability of P may have reinforced the production of organic acids inside plant cells that has been reported to neutralize excess Mn.

The use of chicken manure and green manure caused a three- to four-fold increase in soil solution Mn. This is consistent with the reduction of Mn^{4+} to Mn^{2+} aided by the availability of electrons from the decomposing manure. The organic acid by- products also act to solubilize Mn and keep it in solution (Hue *et al.*, 2001). Inconsistent with the increased soil Mn are marked increases in biomass and grain yield. Such increases far exceeded the increases due to the application of lime up to 5 t ha^{-1}. Both rates of plant growth and accumulation of Mn in the plant tissue were increased by green manure and chicken manure application. Chicken manure application, however, was more effective than green manure in increasing biomass, grain yield, and rates of plant growth and manganese accumulation. This observation can be attributed to the greater amount of nutrients contained in the chicken manure (Table 4.3). The concentration of P, Ca, Mg and K is greater in chicken manure than green manure. However, only P and Mg and not K and Ca concentrations were higher in soybeans that received chicken manure (Table 4.13). The proportion of total P

in chicken manure which is soluble can be as high as 29% (Leinweber, *et al.*, 1997). We did not analyze for N content in the manures or in soybean leaves because we did not expect nitrogen to affect growth. The rate of N fertilizer application was 90 kg N ha^{-1}, which is slightly higher that the recommended rate of 75 kg ha^{-1} (Aquino, pers. comm.) and that soybean seeds were inoculated with *rhizobium* before planting.

Effectiveness of mulching

Mulching did not affect either soil exchangeable Al or saturated paste-Mn. Its effect on plant biomass, yield and rates of growth and Mn accumulation differed with the combined management. Mulching significantly increased biomass production, leaf area and plant growth rate of both cultivars. An increase in accumulation rate of Mn was observed only in PSB Sy6. Leaf Mn at all sampling dates was not influenced by mulch treatment. Of all plant and soil measurements, RAR_{Mn} and plant biomass and growth rates were the parameters consistently increased by mulching. This practice is expected to reduce the evaporation of water from the soil and therefore improve availability of water for plant use. Although water conservation was not reflected in higher soil moisture in mulched plots except at 35 days after emergence, we can infer that the increased biomass could have resulted partly from the additional water conserved by mulching. The greater biomass in the mulched pots would have absorbed more water. The soil moisture being similar between mulched and unmulched plots despite higher biomass and more water demand in the mulched

plots could mean that in fact there was more water kept in the soil. The plant used the water so that it was not reflected in the soil moisture measurement.

The cultivar factor

The two soybean cultivars showed similar responses to management treatments. Soybean cv. PSB Sy2, however, was more responsive to liming, P and manure applications than cv. PSB Sy6. In general, cv. PSB Sy2 produced more leaf biomass, leaf area and grain yield than cv. PSB Sy6. Total plant biomass production and leaf Mn concentration were similar for both cultivars. Likewise, the rates by which the plant grew and accumulated Mn at early growth stage were similar in both cultivars. At the later growth stage, PSB Sy2 showed higher growth rate. This indicates a higher growth and yield potential for cv. PSB Sy2 than Sy6 although the measured growth rates were not statistically different at the early growth stage. Being a more productive and more responsive cultivar, cv. PSB Sy2 has obvious advantages if selected and grown in an acid soil and a similar environment like Rugao series in San Antonio, Philippines.

Management costs

Liming is desirable because of its capacity to neutralize both excesses of Al and Mn and for a long period of time. This is in contrast with using green manure whose effects may be limited to the cropping season when it was applied or whose residual effect may extend to the next two cropping seasons at the most. However, in the town of Isabela, lime was unavailable when the

experiments were conducted. The lime used in the experiment was shipped from Leyte, another island in western Philippines. If lime would be available, an interview with the trader indicates that a bag of lime would probably cost half to as much as a bag of urea (46-0-0). However, the actual cost of would depend on shipping from the source and the demand for it. A bag of urea costs P 350 or about $7 in June 2000. Assuming that lime would cost $3 per 50 kg, an application of 5000 kg ha^{-1} (5 t ha^{-1}) would cost $300. In contrast, green manure may be collected from a nearby forest where *Leucaena* grows abundantly. To collect 7000 kg leaves of this tree would entail a labor cost of about $30 and an additional application cost of about $50. Chicken manure, on the other hand, cost 50c per 25 kg and an application of 5000 kg would cost about $100. Considering the residual effect of lime, liming may prove to be cheap in the long run, if it is at all available. The use of a tolerant cultivar would not entail additional cost if readily available. Despite a lowest yield advantage due to cultivar, this practice proves to be a no additional-cost, sure-profit practice. Mulching materials such as rice straw is abundant in the area especially after the harvest season of rice. A labor cost of mulch application over a hectare field is estimated at $30.

The best yield determinant?

The answer to the question of what factor best determined or indicated grain yield of the soybean crop is neither direct nor specific. Except for the three controls, the treatments that were imposed did not correspond to increasing rates

of a factor or set of factors but rather to 'management' treatments, each one a unique "package". The three controls are rates of lime but they are not comparable to all the other treatments in terms of amount of lime. The P60 and P100 are rates of P but they may not be directly comparable to green manure and chicken manure treatments in terms of amount of P applied. Although we know the concentration of P in the organic materials, we had no measurement of the amount of P that was actually released and taken up by the plant. Mulching is another type of management that was combined with P and manure applications and expected to enhance soil moisture conservation and not contribute to the supply of any nutrient although part of the mulch in direct contact with the soil partially decomposed over the crop growing season.

Although leaf Mn was significantly affected by management treatments, it was poorly correlated with the final grain yield. The plot for cv. PSB Sy2 seemed to suggest a decreasing yield with increasing Mn except for some points that lie at the upper right side of the supposed regression line. These points represent manure treatments, which showed high leaf Mn and high yield. This observation is also true for cv. PSB Sy6 leaf Mn at 28 DAE where some manure treatments showed high Mn and high yield.

Among the plant growth measurements, leaf biomass, leaf area and RGR_{Leaf} were correlated with grain yield. Leaf biomass at 28 DAE was positively correlated with grain yield of both cultivars. Leaf growth rate being closely related to total leaf biomass was also correlated with grain yield although the correlation coefficients were lower. The leaf is the main plant organ for

assimilate production and storage. This suggests that for soybean, the production of leaves at the early vegetative stage, or the maintenance of a fast growth rate is critical for future grain yield.

The rate by which Mn accumulated in the leaf tissue was significantly and positively correlated with grain yield of cv. PSB Sy2 but not cv. PSB Sy6. The correlation coefficient, however, was lower than those with RGR_{Leaf}. The positive correlation of Mn accumulation rate to grain yield can be interpreted not as true correlation but as a consequence of the correlation between relative growth rate and Mn accumulation rate. Due to the positive correlation of leaf growth rate to Mn accumulation rate, and the correlation of leaf growth rate to grain yield, it followed that Mn accumulation rate was also correlated with grain yield. The absence of this kind of correlation in PSB Sy6 may suggest non-sensitivity of final grain yield to early growth plant performance.

Leaf P and K were the next best candidates as yield determinants. There were different amounts of P applied in each management treatment and leaf P at 28 DAE differed with treatments. Leaf P was highly correlated with grain yield. Although the amount of K fertilizer was constant in all treatments, there was additional K contained in the manures and leaf K also differed with treatment. Regression analysis showed however, that leaf P but not K was correlated with grain yield. Highest yield from the manures, however, is not fully attributable to P supply because the P contained in the green manure and chicken manure is markedly less than 100 kg ha^{-1}. Assuming a 40% release throughout the growing season, green manure could have supplied about 5 kg ha^{-1} while

chicken manure P could have amounted to 67 kg ha^{-1}. The amount of K supplied assuming the same release rate could have been about 27 kg ha^{-1} for green manure and 42 kg ha^{-1} for chicken manure. In addition to the higher amount, the K in the chicken manure is inorganic, and is therefore immediately available.

The use of growth analysis

The growth analysis technique is simple yet cumbersome. At the minimum, plant growth analysis requires biomass and nutrient concentration data and simple calculations. Compartmentalization of growth rate into net assimilation rate and leaf area ratio, however, requires the user to satisfy certain assumptions. There is obviously more work involved in sampling and analyzing tissue samples. Instead of growing the plant continuously and harvesting at one sampling date to get a correlation between biomass produced and nutrient concentration at that sampling date, we need successive samplings so we can calculate how fast the plant is growing, and how fast the nutrient is being accumulated in the plant tissue over a designated growth interval. This entails growing several plants to accommodate all sampling dates. Destructive sampling in field experiments is not a problem where hundreds or even thousand of plants are grown in each plot. It becomes a big job only in pot experiments where usually only one or two plants can be grown in a pot.

Besides the work involved, growth rates and relative nutrient accumulation rates can be obtained only after a crop is grown and had spent its

vegetative life span. This makes RAR_{Mn} and $RGR_{PLant/Leaf}$ unlikely candidates for diagnosis of nutrient toxicities like that of Mn. Its usefulness lies in the study of the dynamics of the phytoxicity. In our result for example, leaf Mn was the least accurate predictor of yield. If our soil was Mn toxic, which is very likely, based on the appearance of phytotoxic symptoms, the presence of Mn-Fe concretions, and high soil solution Mn, then we expect leaf Mn to predict yield. If we did a simple "grow to harvest" and analyze leaf Mn technique and we get this no-correlation effect, we are left with only the suspicion that perhaps there was really no toxicity. But because we did a growth analysis, we know that in all our treatments, relative growth rate exceeded relative Mn accumulation rate. This indicates and agreed with another observation that leaf Mn, although it started at 'toxic' levels, decreased over time. This means that at one point there was a toxicity and the soybean crop was able to recover from it, most likely due to the management treatment imposed or the growth conditions surrounding the crop. The potential toxicity was present but the full potential was not realized. Other growth conditions may permit higher RAR_{Mn} than $RGR_{PLant/Leaf}$. In such a case, potential toxicity is more equivalent to actual toxicity, and the toxicity situation is chronic, the plant will have difficulty growing out of the phytotoxicity.

Summary and Conclusions

A field experiment was conducted in an acid upland in San Antonio, Isabela, Philippines. The soil is classified as *fine, isohyperthermic Typic Kandiudalf* (US Soil Taxonomy), very acid (pH 4.45), low in exchangeable bases

and high in exchangeable Al and soil solution Mn. Symptoms of Al toxicity were expressed as short, stubby brownish roots while that of Mn toxicity was exhibited as brown spots, interveinal yellowing and crinkling of the leaves of soybean.

Two soybean cultivars cv. PSB Sy2 and cv. PSB Sy6 were used to test management strategies including liming, rate of P application, manure application (chicken dung and leaves of *Leucaena leucocephala*) and mulching. These practices were expected to boost plant growth rate and yield of the soybean crop grown in an acid soil with excess Mn. Results show that cv. PSB Sy2 had higher biomass growth and yield potential, producing more biomass and grain than cv. PSB Sy6 even in the control plots that received no lime. Liming at 2 t ha^{-1} significantly neutralized exchangeable Al but not Mn, thus increasing biomass and yield but not rates of plant growth and Mn accumulation. Mulching had no effect on soil exchangeable Al and Mn or soil moisture level except at 35 days after seedling emergence. However, mulching alone or combined with P and manure applications increased the rate of plant growth. The application of up to 100 kg P ha^{-1} increased biomass and yield production as well as the rates of plant growth and Mn accumulation. Application of manures had similar effects on rates of plant growth and Mn accumulation. Increases in yield and biomass production due to the use of green manure far exceeded the increases due to liming or P application. This is in spite of the three- to four-fold increase in soil solution Mn caused by the application of manures. Chicken manure was more effective than green manure in increasing biomass and yield of both soybean cultivars. Our results indicate that if growth rate is increased, even when

accompanied by increases in accumulation rate of Mn, the crop tends to perform well in terms of yield. In our experiment, relative growth rate of the plant far exceeded the relative accumulation rate of Mn, indicating decreasing concentrations of Mn in the leaf tissue over time. The absolute difference between leaf growth rate and Mn accumulation rate, however, did not appear to be related to grain yield or biomass production. When plant growth rate exceed Mn accumulation rate, plants may have started with toxic levels of Mn, but under the growth conditions that can increase plant growth rate, plants may be able to recover and grow out of the toxicity. In other soil and growth conditions where Mn accumulation rate exceeds plant growth rate, there will be chronic toxicity. In this situation it may be more difficult to manage plant growth rate so that the more feasible option is to manage Mn accumulation rate by decreasing Mn concentration in the soil.

Our results have implications on how Mn toxicity can be managed in addition to decreasing soil Mn level. Our approach was to assemble strategies to increase growth rate under the limit of the environmental condition, so that the crop can tolerate excess Mn in an acid soil. This approach, however, may be successful only where growth conditions permit plant growth rate to exceed Mn accumulation rate, in which case, the focus can be shifted from elimination excess soil Mn to managing plant growth rate that will enhance crop tolerance to an existing level of available Mn in the soil.

Chapter 5

THE DYNAMICS OF PLANT GROWTH, MANGANESE UPTAKE AND PHYTOTOXICITY IN AN ACID MANGANIFEROUS OXISOL

Introduction

Manganese (Mn) phytoxicity is a serious problem in acid soils developed from high-Mn (manganiferous) rocks and minerals. This metal element tends to be retained in soils throughout the weathering process (Laughnan, 1969 as cited by Gilkes and McKenzie, 1988). Weathering of parent rocks is particularly fast under tropical climate and facilitates leaching of bases that eventually leads to soil acidification. Manganese-rich secondary minerals under low pH are highly soluble, thus, Mn availability exceeds plant requirements eliciting phytotoxic response. The occurrence of Mn phytotoxicity, however, is not limited to acid soils. It has been reported even in calcareous soils under conditions of high organic matter and poor drainage in the tropical, sub-tropical and temperate zones (Kamprath and Foy 1971; Moraghan and Freeman, 1978; Giller, *et al.*, 1992). Although reports of occurrences are often isolated and incidental, Mn phytoxicity may prove to be more widespread with the expansion of agriculture onto the acid upland soils, which cover large amounts of land in Asia. Since excess Mn is observed at a soil pH value where Al toxicity is absent, one can expect excess Mn to be encountered before Al toxicity in the process of soil acidification.

The phytotoxicity of Mn is difficult to predict because of interactions among plant, soil and environmental factors influencing plant growth, Mn uptake and phytotoxicity responses under conditions of excess Mn in soils (El-Joual and Cox, 1999). An important consequence of these interactions is that to date, there is no single soil property or measure of soil or plant Mn that can predict Mn phytotoxicity with high probability. The limited success in the use of critical Mn toxicity concentrations in soils and plants has been attributed to the wide variation in genetic tolerance between plant species and between cultivars within species (Foy *et al.*, 1981). Interestingly, environmental factors (sunlight, temperature) and nutrient interactions (Si, Ca, Mg, K, P, Fe) have been reported to confer (intra-specific) tolerance as demonstrated by several studies (Heenan and Carter, 1977; Rufty, *et al.*, 1979; Horiguchi; 1988; Galvez, *et al.*, 1989; Heenan and Carter, 1975; Elamin and Wilcox, 1986; Bortner, 1935; Alam *et al.*, 2002).

Enhanced tolerance attained by manipulating environmental factors and nutrient levels can be explained by an increase in plant growth rate set by the given growth condition. However, high growth rate under excess soil Mn is likely to cause high uptake rate of Mn given the poorly regulated nature of Mn^{2+} absorption (Clarkson, 1988). The uptake of Mn and the resulting phytotoxic response of plants seems to be a very dynamic process likely to be governed by water use and exhibited as a continuous negative feedback between plant growth rate and Mn accumulation rate. A consequence of this feedback interaction between plant growth rate and Mn accumulation rate would be the

possibility of producing similar biomass with different Mn concentrations, or similar Mn concentrations with different biomass, depending on whether the growth condition affect mainly plant growth rate or Mn accumulation or both. This could explain a lack of correlation between plant biomass production and tissue Mn concentrations over a range of growth conditions for the same cultivar.

Objectives

1. Measure the influence of soil pH, reduced water and sunlight, and added P and green manure on soil available Mn; and on the dynamics of soybean growth, Mn uptake and phytotoxicity.
2. Determine the relation between **plant growth rate** and **Mn accumulation rate** over a range of growth conditions and soil Mn levels.
3. Examine the negative feedback mechanism between **plant growth rate** and **Mn accumulation rate** over a range of growth conditions and soil Mn levels.
4. Compare plant growth, Mn uptake and phytotoxicity responses, **plant growth rate – Mn accumulation rate relation**, and **plant growth rate – Mn accumulation rate feedback effects** between Mn-tolerant and Mn-susceptible soybean cultivars.
5. Develop a dynamic model of phytotoxic response to excess Mn.
6. Assess the implications of the feedback effects between **plant growth rate** and **Mn accumulation rate** on developing management strategies for growing healthy plants in acid soils with excess Mn.

Materials and Methods

Treatments and design of greenhouse experiment

To provide answers to our research questions, we conducted a pot experiment at the greenhouse facility of the Tropical Plant and Soil Sciences, University of Hawaii. Wahiawa series [*clayey, kaolinitic Isohyperthermic, Rhodic Eutrustox*] was used to grow soybeans in pots for 56 days. The soil, Wahiawa series from Central Oahu, Hawaii, is acid (pH 4.78) and known to contain such high amounts of total Mn (17 g kg^{-1}) that watermelon failed to grow in this soil (Hue and Mai, 2002). We used soybean as the test plant [*Glycine max*. (L.)] because of the wide range in tolerance for Mn observed in this species. Manganese-tolerant cv. Lee and Mn-susceptible cv. Forrest were selected to represent differing tolerance to Mn. These cultivars were chosen because early studies have established their tolerance/susceptibility to Mn as opposed to the soybean cultivars from the Philippines whose tolerance to Mn has not been evaluated. The Philippine soybean cultivars were not included because it would have increased the experiment beyond manageable size. Three soil pH levels, representing three levels of excess soil Mn, were established by using unamended soil (pH 4.78), and adding 1.2 or 2.0 g kg^{-1} $CaC0_3$ to adjust soil pH to 5.50 and 6.00, respectively. Five 'growth conditions', representing five growth rate potentials were imposed (Table 5.1). These growth conditions involved manipulation in water, sunlight and nutrient availability, which were expected to

reduce plant growth rate as in the case of reduced water availability or increase growth rate as in the case of increased amount of added P.

The treatments were arranged in a 2 (cultivar) x 3 (soil pH) x 5 (growth condition) factorial in a Randomized Complete Block design with 3 replications. Four additional replications were added to accommodate destructive sampling at four dates. The plants to be harvested at each sampling date were pre-determined at the start of the experiment.

Table 5.1. List of soybean cultivars, soil pH levels and growth condition.

Factor[a]	Factor Levels	Description
Cultivar	Forrest	Mn-susceptible
	Lee	Mn-tolerant
Lime[b] addition to soil pH	4.78	No lime added
	5.50	Rate of lime was 1.2 g kg^{-1}
	6.00	Rate of lime was 2 g kg^{-1}
Growth condition	Control	Field capacity 300 ml kg^{-1} No green manure 75 mg kg^{-1} P Full sunlight
	80-90% FC	80-90% Field capacity
	G. manure	10 g kg^{-1} green manure (*Leucaena leucocephala*)
	P150	150 mg kg^{-1} P
	40% Shade	Blocked 40% of sunlight

[a]Factors were combined in a 2 x 3 x 5 factorial in Randomized complete block

[b]Lime material was $CaCO_3$

Soil preparation

Bulk Wahiawa soil was collected from the experimental plots of the Poamoho Experiment Station of the University of Hawaii. The soil was air-dried crushed, and sieved to a 2 mm-size fraction. Two and a half kilograms soil was weighed into each pot. Lime ($CaCO_3$) and triple superphosphate (TSP; 40% P_2O_5) fertilizer were added correspondingly to each lime- and P-treated pot. Lime and P fertilizer were mixed thoroughly with the dry soil. The moisture of the treated and untreated soils was then adjusted to field capacity by adding 300 ml distilled water per kilogram of soil and incubated for two weeks. By the end of the 2^{nd} week, green manure treatments were applied to green manure--treated pots by mixing into the soil 10 g kg^{-1} of dried and ground leaves of *Leucaena leucocephala*. The pots were then incubated at field capacity for another three weeks to allow decomposition of the green manure. Incubation was done under shade to avoid fast evaporation of soil moisture and increasing soil temperature that can drastically affect the availability of manganese. Soil moisture was adjusted to field capacity every three days during the incubation period.

Growing soybeans

Seeds of the two soybean cultivars were soaked overnight in distilled water and then allowed to germinate for 3 days. Five of the pre-germinated seeds were planted in each pot, at about 2 cm below the soil surface. Seven days after emergence (DAE), the seedlings were thinned to two plants per pot and dissolved nutrients were applied. The amounts of each nutrient added in mg

kg^{-1} were 150 N as urea, 150 K as KCl, 50 Ca as $CaSO_4$, 50 Mg as $MgSO_4$, 5 Cu as $CuSO_4$, 5 Zn as $ZnSO_4$, 2 B as Na_3BO_3 and 0.5 Mo as NH_4MoO_4.

Light and water treatments

Both light and water treatments were imposed after seedling thinning, that is at 7 DAE. The shade treatment was established by using shade cloth that blocked 40% of the sunlight. Soil moisture in the 'dry' treatment was kept within 80-90% of the field capacity while the rest of the treatments were maintained at field capacity. Moisture level was maintained by adjusting pot weights daily during the first three weeks and twice daily thereafter. The amount of water added included an excess amount to account for evaporation loss and increase in plant fresh weight. Plant fresh weight was estimated by taking the dry weight and fresh weight of the seedlings after thinning, calculating the moisture content, and assuming a 0.04 g g^{-1} d^{-1} growth rate. From the initial dry weight and an assumed growth rate, a daily dry weight was estimated and converted to fresh weight based on the calculated moisture content.

Soil measurements

The soils in each pot were sampled before planting, and at 28 and 56 DAE for the determination of pH and soil available Mn from Mehlich-3 and saturated paste extracts. Soil sample for each treatment represents the composite sample from two cultivars subjected to that treatment. Soil analysis was not replicated within a pot. Since each treatment was replicated three times, it follows that the

soil measurements were also replicated three times for each treatment. Soil solution pH was measured from a 1:1 soil/water ratio by weight. Mehlich-3 extracts were prepared by weighing 2 g soil, adding 20 ml Mehlich-3 solution (Reed and Martens, 1996), shaking for 5 min and filtering the suspension. Saturated paste was prepared by adding distilled water (in small lots) to 100 g soil, while stirring until the soil is saturated and a paste consistency is attained. The saturated paste was incubated for 30 minutes after which the soil solution was extracted with suction. Manganese in the extract was analyzed by Inductively Coupled Plasma Spectrometry at the Agricultural Diagnostic Services Center (ADSC) of the University of Hawaii.

Plant measurements

Plant measurements were carried out every 14 days. Rates of transpiration and CO_2 assimilation were measured using a portable infrared gas analyzer (CIRAS-1, PP Systems, 1993). The middle leaflet of the youngest fully mature trifoliolate (about the third trifoliolate from the top) was selected for the transpiration and assimilation rate measurement. The measurements were carried out from 12:00 NN to 2:00 PM, the time of day when temperature was maximum and sunlight was most intense. Plant water use was estimated by multiplying transpiration rate with the total leaf area of the plant.

The greenness of the leaf was determined using a portable chlorophyll meter (Minolta SPAD 502). For each pot, measurements were done on all leaflets of the youngest and oldest fully mature trifoliolate, giving six

measurements from each plant, which were averaged to represent the SPAD reading for that pot.

Two soybean plants per pot were sampled every 14 days for the measurement of leaf area, leaf dry matter, total aboveground dry matter and leaf Mn. Sample pots for each sampling date were pre-determined at the start of the experiment. Leaf area was measured using a portable LI-COR leaf area meter (LI 3000a). Leaf samples were separated into young and old leaves. Young leaves refer to the first three trifoliolate from the top, and includes the youngest fully mature trifoliolate. Old leaves refer to the rest of the trifoliolates below the young leaves and includes, if present, the unifoliolate. The stem and leaf samples were oven-dried at 70°C for three days (or until a constant moisture content has been attained) and weighed for the determination of leaf, stem and total plant biomass. Dried leaves were dry-ashed in a muffle furnace at 500°C for 4 hours. Ashed samples were digested further with 5 ml of 2 *M* HNO_3 at 120°C until all the acids have evaporated. The residue was then dissolved in 20 ml of 0.1 *M* HCl and filtered using Whatman #42 filter paper. The concentrations of Mn, Ca, Mg, K and P in the solution were analyzed using Inductively Coupled Plasma Emission Spectrometry (ICP) at the Agricultural Diagnostic Services Center (ADSC) of the University of Hawaii.

Toxicity symptoms rating

The severity of Mn phytotoxicity was scored based on the percent of the leaves damaged due to observed symptoms such as brown spots, crinkling,

necrosis, interveinal chlorosis and leaf vein lesions (Fig. 5.1). A symptom-scoring scheme (Table 5.2) was developed for each of these five symptoms. A maximum possible score for each symptom was 3. Thus, for five symptoms, the maximum total possible score was 15. In addition, the phytotoxicity symptoms were documented by taking digital photographs of the plants at each sampling period.

Table 5.2. Symptom-scoring scheme.

Score	Percent of leaves affected
0	none
1	<20
2	21-50
3	>50

The scoring system used in the greenhouse experiment was slightly different from the field experiment. The objective of the rating system in this experiment was to make a more detailed scoring of the symptoms that will better differentiate phytotoxicity status based on what specific symptoms are present and how much of the plant part was damaged by the symptoms.

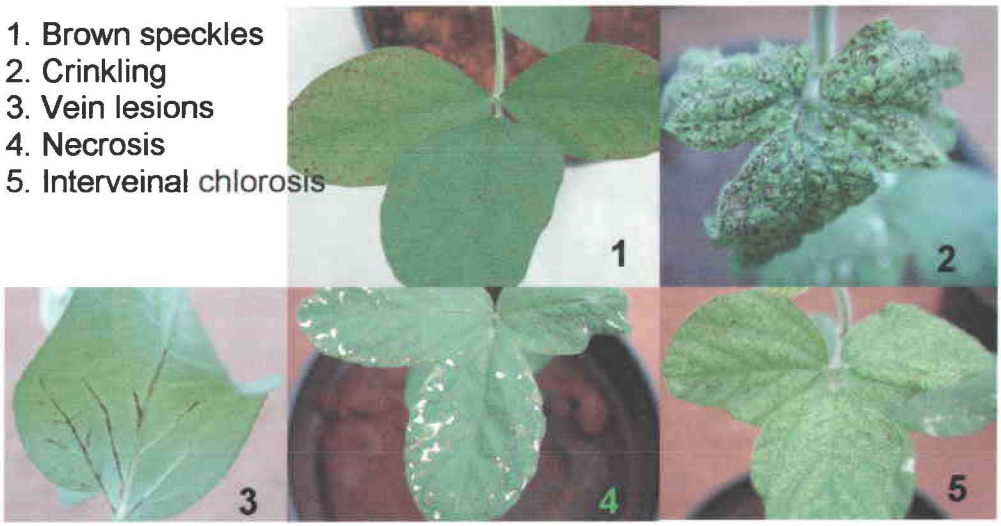


Fig. 5.1. Phytotoxic symptoms scored in the greenhouse experiment.

Growth analysis and calculations

Relative growth rate for the whole plant (RGR_{Plant}, $g\ g^{-1}\ d^{-1}$) or leaves only (RGR_{Leaf}) was calculated using the following equation from Hunt (1990):

$$RGR_{Plant/Leaf} = (lnBm_{T1} - lnBm_{T0}) / (T_1 - T_0) \quad \text{[Eqn. 5.1]}$$

Relative accumulation rate of Mn (RAR_{Mn}, $\mu g\ \mu g^{-1}\ d^{-1}$) was calculated using the following equation:

$$RAR_{Mn} = = \{ln(Bm_{T1} * C_{T1}) - ln(Bm_{T0} * C_{T0})\} / (T_1 - T_0) \quad \text{[Eqn. 5.2]}$$

where:

$RGR_{Plant/Leaf}$ is the mean plant/leaf relative growth rate over a given growth interval

RAR_{Mn} is the mean Mn relative accumulation rate over a given growth interval

Bm is the weight of biomass (whole plant, leaves) in g

C is the Leaf Mn concentration in $\mu g\ g^{-1}$

T_0 is a previous sampling date

T_1 is a later sampling date

The RGR was broken down into 2 components: net assimilation rate (NAR) and leaf area ratio (LAR). These parameters were calculated using the equations from Radford (1967) with an underlying assumption that over the period T_0 to T_1, leaf area and biomass are linearly related.

$$NAR = [(Bm_1-Bm_0)/(LA_1-LA_0)]\ [(ln LA_1-ln LA_0)/T_1-T_0] \quad \text{[Eqn. 5.3]}$$

$$LAR = [(LA_1-LA_0)/(Bm_1-Bm_0)]\ [(ln Bm_1-ln Bm_0)/ln LA_1-ln LA_0)] \quad \text{[Eqn. 5.4]}$$

where: LA is leaf area and other terms are as above.

Statistical analysis

Treatment effects on measured and calculated parameters were analyzed using the Analysis of Variance and treatment comparison procedures of the Statistical Analysis Systems (SAS, 1990). A probability of the 5% or less was considered significant. Treatment means were compared using Duncan's multiple range test likewise facilitated by SAS. The SAS regression procedure was used to determine linear equations, model probability and coefficients describing linear relationships between two parameters. To compare slopes of two regression lines, separate slopes from each replicate were calculated and treated as individual observations in the analysis of variance procedure. Time effect was analyzed as a split plot factor within each experiment unit.

Results

Soil pH and extractable Mn

Lime, growth conditions and their interaction significantly influenced soil pH and saturated paste-Mn (Table 5.3). Lime addition of 1.2 g kg^{-1} and 2.0 g kg^{-1} increased soil pH to within 0.3 units of the targeted pHs of 5.50 and 6.00, respectively (Table 5.4). These pH increases were measured after 37 days of incubating soil and lime, coinciding with 7 days after emergence (DAE) of the soybean plants. Soil pH levels were more or less maintained within 0.3 units of the targeted pH levels except in the unlimed soils where green manure was added. While P addition, shading and dry treatments did not influence soil pH, green manure addition in the unlimed soil significantly increased soil pH measured at 7 days after emergence from 4.78 to 5.48. This increase, however, was non-existent at 56 DAE (Table 5.4), indicating a transient increase in soil pH due to green manure decomposition.

Soil Mn extracted from the soil saturated paste ($SatPaste_{Mn}$) was extremely high at ~25 μg ml^{-1} in the unlimed soil, which decreased by ~20-fold with lime addition (Table 5.4). Green manure addition significantly increased $SatPaste_{Mn}$ by ~10 μg ml^{-1} in unlimed soil. Liming did not reduce the $SatPaste_{Mn}$ where green manure was added, in fact it was maintained at the level well above that in the unlimed control (*ibid*).

The log-transformed value of saturated paste-Mn was linearly correlated with soil pH (Fig. 5.2). The linear equation describing this relationship did not include the green manure treatments whose saturated paste-Mn was maintained at about 1.5 regardless of soil pH.

Plant Growth

Total above ground biomass of both soybean cultivars generally increased with time (Table 5.5, 5.6) from 14 DAE to 56 DAE at all soil pH levels and growth conditions imposed. Dry matter accumulation of unamended cv. Forrest tended to level off at 42 DAE and beyond (Fig. 5.3, 5.4). A similar increasing trend was observed for leaf area (Fig. 5.5, 5.6) although decreases after 42 DAE were common for both cultivars. Although maximum differences were observed at 42 DAE, we considered the ultimate treatment effect to be expressed at 56 DAE where maximum Mn phytotoxicity has occurred.

Table 5.3. Analysis of variance for factor effects on saturated paste-Mn and soil pH. Means used in the analysis are means across two soybean cultivars.

Effect	Sat paste- Mn	Soil pH (7 DAE)	Soil pH (56 DAE)
Lime (L)	*<0.0001*	*<0.0001*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*	*0.0012*
L x Tr	*<0.0001*	*<0.0001*	*0.0339*

Table 5.4. Growth condition by lime effect on soil pH at two sampling dates and saturated paste-Mn at 7 DAE.

Growth condition	Soil pH (7 DAE)					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	4.74 b	*0.02*	5.51 b	*0.01*	6.13 a	*0.02*
80-90% FC	4.75 b	*0.01*	5.53 b	*0.03*	6.20 a	*0.03*
40% Shade	4.86 b	*0.01*	5.62 ab	*0.03*	6.13 a	*0.003*
G. manure	5.48 a	*0.03*	5.69 a	*0.05*	6.14 a	*0.04*
P150	4.85 b	*0.10*	5.74 a	*0.06*	6.22 a	*0.11*
	Soil pH (56 DAE)					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	4.93 a	*0.08*	5.90 a	*0.06*	6.01 a	*0.10*
80-90% FC	4.90 a	*0.03*	5.56 bc	*0.08*	5.72 b	*0.04*
40% Shade	4.88 a	*0.14*	5.70 abc	*0.13*	5.61 b	*0.03*
G. manure	4.84 a	*0.05*	5.44 c	*0.05*	5.65 b	*0.06*
P150	4.74 a	*0.12*	5.80 ab	*0.10*	6.02 a	*0.03*
	Saturated paste-Mn, $\mu g\ ml^{-1}$ (7DAE)					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	24.66 b	*1.50*	1.56 b	*0.39*	1.47 b	*0.49*
80-90% FC	24.94 b	*1.73*	1.35 b	*0.33*	0.66 b	*0.07*
40% Shade	30.49 b	*0.88*	2.70 b	*0.48*	0.70 b	*0.21*
G. manure	35.48 a	*1.44*	30.54 a	*2.80*	32.31 a	*1.51*
P150	24.91 b	*1.59*	1.37 b	*0.58*	0.92 b	*0.19*

In a column, within each parameter or sampling date, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

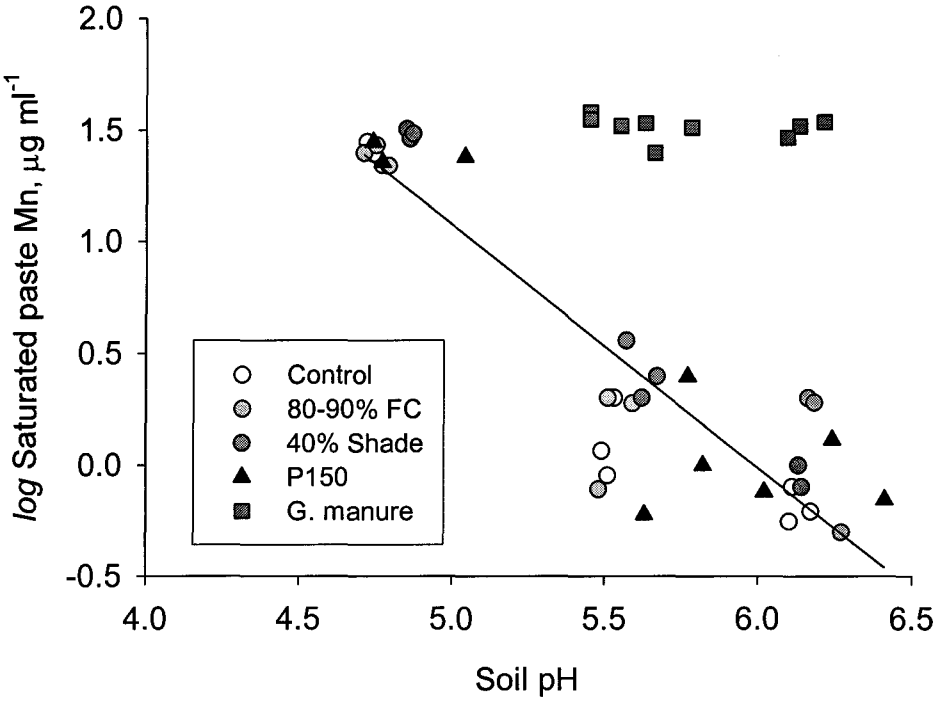


Fig. 5.2. Changes in saturated paste-Mn with soil pH over a range of growth conditions.

Table 5.5. Analysis of variance for the effects of time[a], lime and growth condition on selected plant measurement for soybean cv. Forrest.

Source	df	Leaf Biomass	Plant biomass	Leaf Area	E[b]	A[c]	Leaf Mn	Symptom[d]
Rep	2	*0.0966*	*0.0002*	*0.0198*	*0.9588*	*0.4926*	*0.9551*	*0.4773*
Growth condition (Tr)	4	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Lime (L)	2	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Tr x L	8	*<0.0001*	*<0.0001*	*<0.0001*	*0.044*	*0.0015*	*0.0010*	*<0.0001*
Time	3	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Time x L	6	*<0.0001*	*<0.0001*	*<0.0001*	*0.011*	*0.0078*	*<0.0001*	*0.2804*
Time x Tr	12	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0137*	*<0.0001*
Time (Lime x Tr)	24	*<0.0001*	*<0.0001*	*<0.0001*	*0.0046*	*0.1429*	*0.0002*	*0.0004*

[a] Time effect was analyzed as a subplot factor

[b]Transpiration rate; [c]CO_2 assimilation rate; [d]Cumulative symptom score

Table 5.6. Analysis of variance for the effects of time[a], lime and growth condition on selected plant measurement for soybean cv. Lee.

Source	df	Leaf Biomass	Plant biomass	Leaf Area	E[b]	A[c]	Leaf Mn	Symptom[d]
Rep	2	*0.0009*	*<0.0001*	*<0.0001*	*0.4410*	*0.4462*	*0.9449*	*0.0857*
Growth condition (Tr)	4	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Lime (L)	2	*<0.0001*	*<0.0001*	*<0.0001*	*0.0161*	*<0.0001*	*0.0001*	*<0.0001*
Tr x L	8	*<0.0001*	*<0.0001*	*<0.0001*	*0.0537*	*0.0531*	*<0.0001*	*0.0005*
Time	3	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Time x L	6	*<0.0001*	*<0.0001*	*<0.0001*	*0.0204*	*0.0021*	*0.1825*	*<0.0001*
Time x Tr	12	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0021*	*0.5153*	*<0.0001*
Time (Lime x Tr)	24	*<0.0001*	*<0.0001*	*<0.0001*	*0.0005*	*0.0007*	*0.0035*	*<0.0001*

[a] Time effect was analyzed as a subplot factor

[b]Transpiration rate; [c]CO_2 assimilation rate; [d]Cumulative symptom score

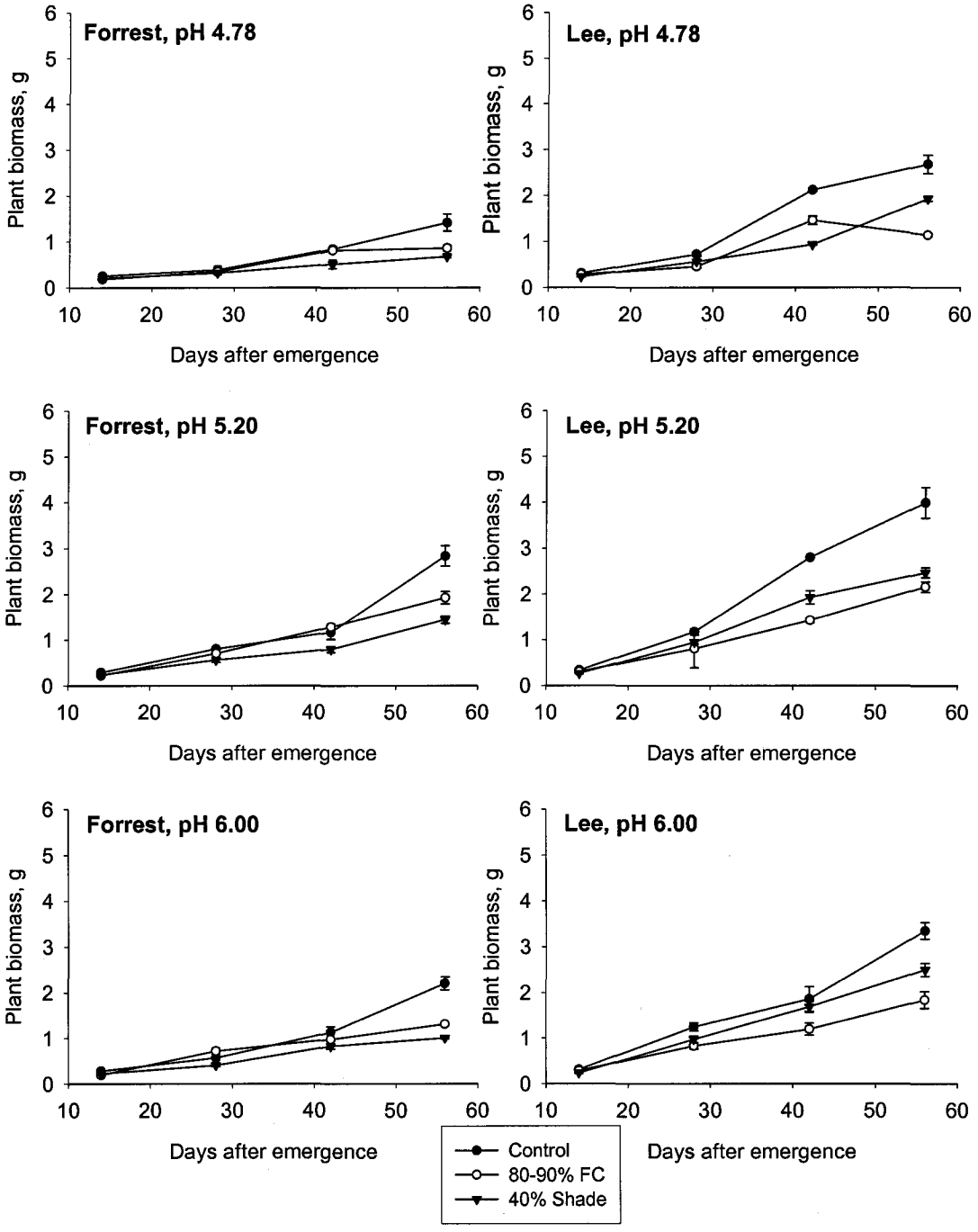


Fig. 5.3. Effect of shading and dry growth conditions on the dry matter accumulation of two soybean cultivars grown at varying soil pH.

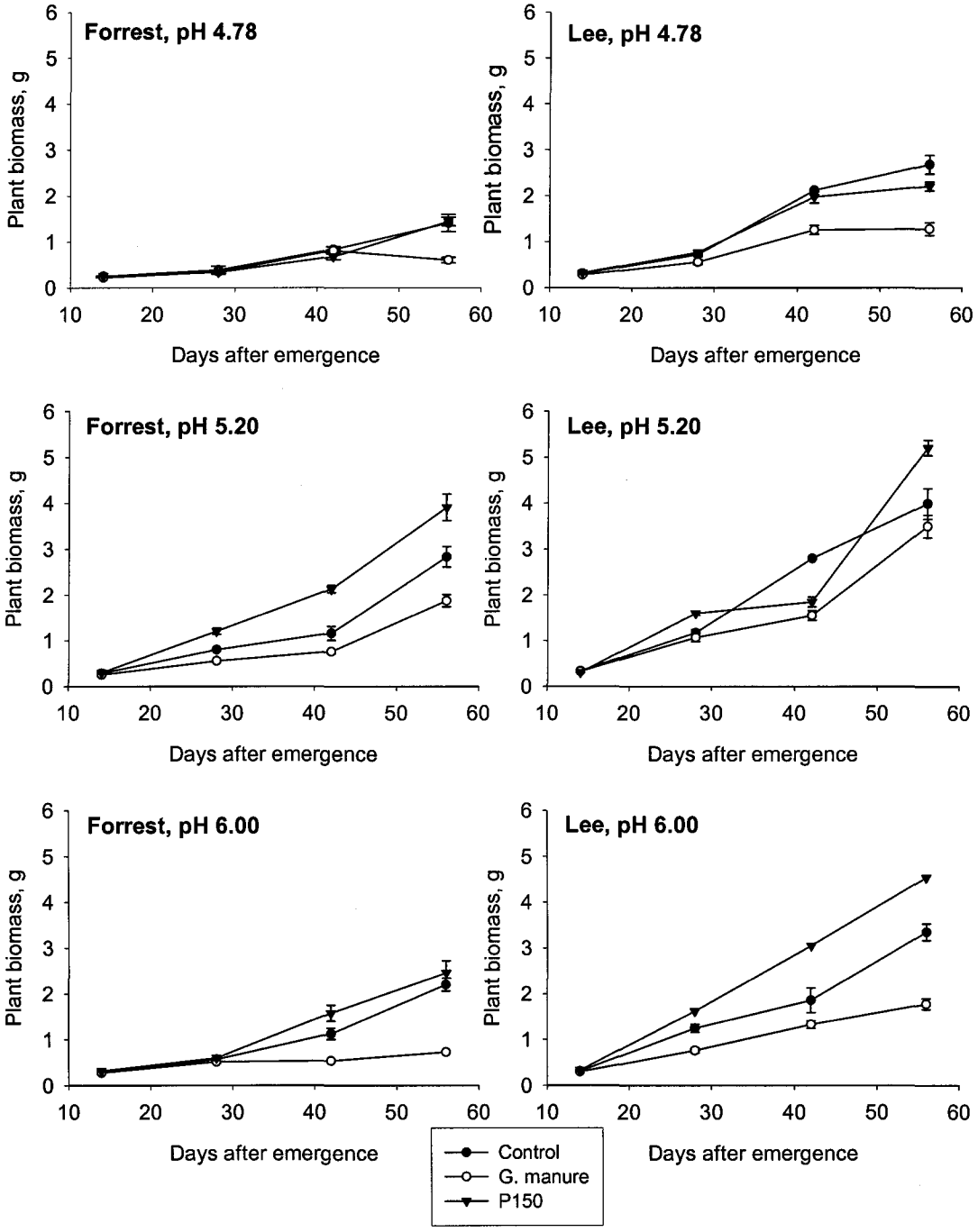


Fig. 5.4. Effect of P and green manure additions on the dry matter accumulation of two soybean cultivars grown at varying soil pH.

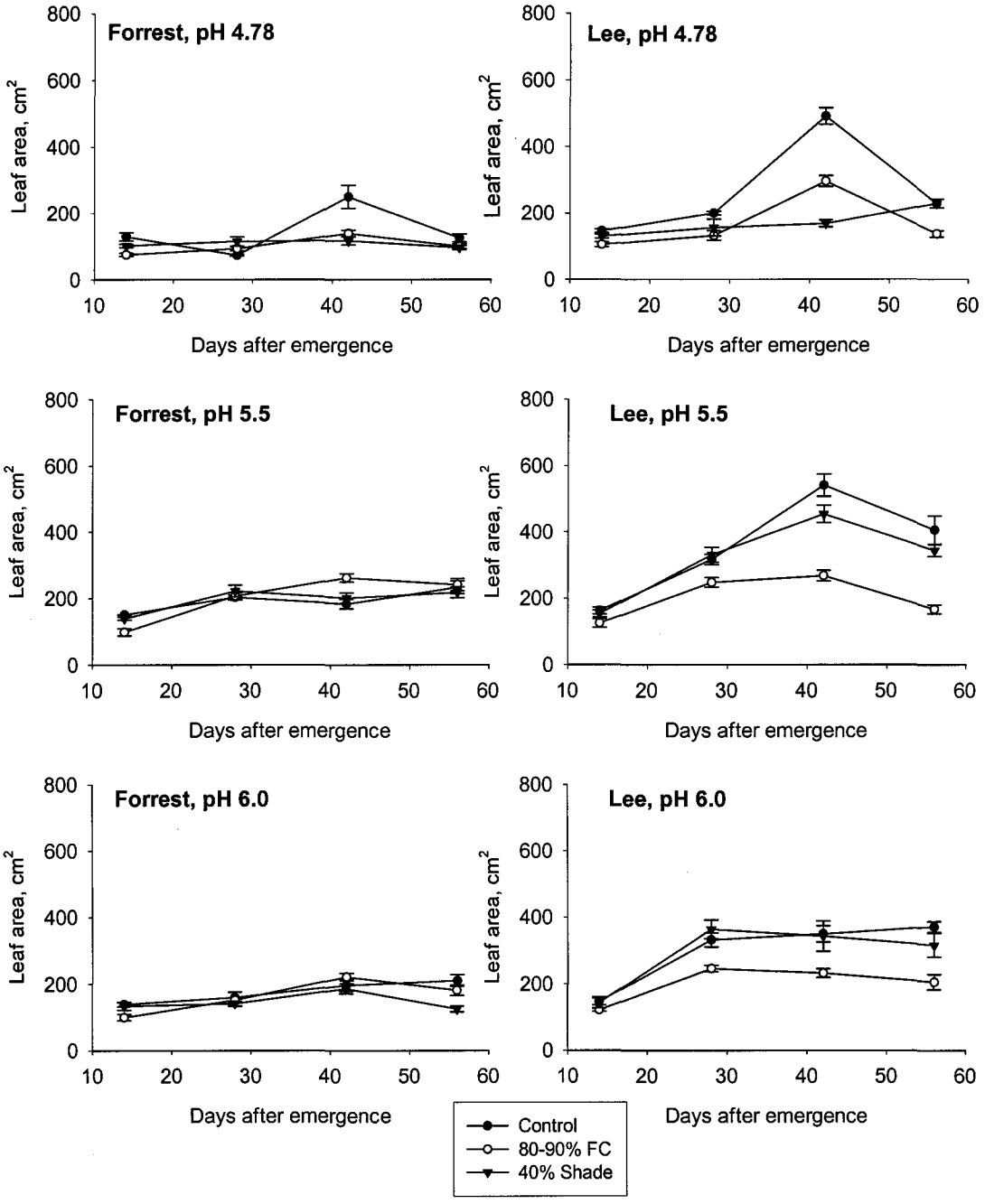


Fig. 5.5. Effect of shading and dry growth conditions on the leaf area accumulation of two soybean cultivars grown at varying soil pH.

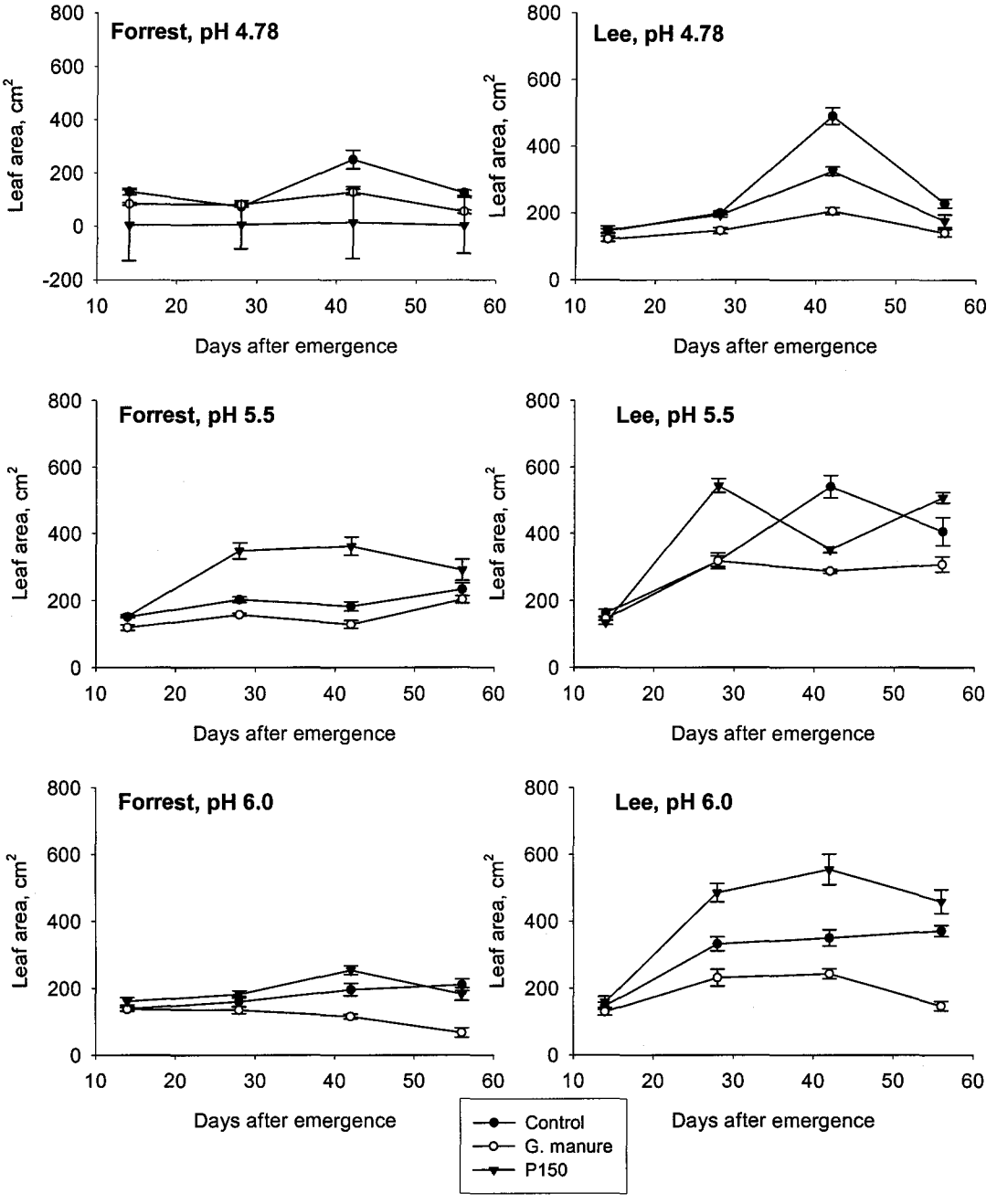


Fig. 5.6. Effect of P and green manure additions on the leaf area increase of two soybean cultivars grown at varying soil pH.

Leaf and total aboveground biomass was affected by cultivar, lime and growth conditions and their interaction (Table 5.7). Both leaf area and biomass accumulation (leaf and total aboveground) was generally higher for cv. Lee than cv. Forrest, and likewise higher at soil pH 5.5 than at pH 4.78 and 6.0. Phosphorus addition increased leaf biomass at pH 5.5 across cultivars (Table 5.8). Increases due to P at pH 4.78 and 6.00 were not significant. Dry growth conditions, shading and green manure addition decreased leaf biomass at all pH levels. However, the decreases in leaf biomass due to shading, green manure or dry growth conditions were not significant at pH 4.78, 5.50 and 6.00, respectively.

Total aboveground biomass was increased by P addition in Forrest at pH 5.5 and Lee at all pH levels (Table 5.9). Shading and dry growth conditions reduced biomass accumulation in both cultivars at all pH levels. Decrease in plant biomass due to green manure addition was significant at pH 5.50 and pH 6.00 in Forrest, and at pH 4.78 and pH 6.00 in Lee.

At all pH levels, the maximum leaf area and the maximum differences between treatment effects were observed at 42 DAE (Fig. 5.5, 5.6). This occurred despite an additional 14 days of growth. We considered the ultimate treatment effect to be expressed at 56 DAE, however. Across lime levels, green manure addition decreased leaf area in cv. Forrest but not cv. Lee (Table 5.10). Other treatments did not show significant effects. Across cultivars, P addition increased leaf area at pH 6.00, while all other treatments did not have a significant effect on leaf area at all pH levels.

Table 5.7. Analysis of variance for the effects of cultivar, lime and growth condition on plant biomass and leaf area.

Effect	Biomass (56 DAE)		Leaf area
	Leaf	Aboveground	(56 DAE)
Cultivar (V)	*<0.0001*	*<0.0001*	*<0.0001*
Lime	*<0.0001*	*<0.0001*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*	*<0.0001*
V x L	*0.1652*	*0.0406*	*0.0363*
V x Tr	*0.1477*	*<0.0001*	*0.0008*
L x Tr	*0.0041*	*<0.0001*	*0.0019*
V x L Tr	*0.0560*	*0.0455*	*0.0995*

Table 5.8. Lime x growth condition and cultivar effects on leaf biomass (g plant^{-1}) of soybeans at 56 DAE.

Growth Condition	Mean across Cultivar					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	0.662 ab	*0.084*	0.788 b	*0.100*	0.712 ab	*0.039*
80-90% FC	0.362 c	*0.021*	0.594 cd	*0.027*	0.490 bc	*0.055*
40% Shade	0.461 bc	*0.089*	0.497 d	*0.032*	0.453 c	*0.082*
G. manure	0.399 c	*0.084*	0.688 bc	*0.065*	0.328 c	*0.060*
P150	0.687 a	*0.042*	1.040 a	*0.061*	0.838 a	*0.130*
Cultivar	Mean across lime and growth condition					
Forrest	0.486 b	*0.033*				
Lee	0.714 a	*0.034*				

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.9. Cultivar x lime x growth condition effect on total aboveground biomass (g plant^{-1}) of soybeans at 56 DAE.

Growth Condition	Forrest					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	1.410 a	*0.184*	2.832 b	*0.213*	2.203 a	*0.140*
80-90% FC	0.858 b	*0.065*	1.922 c	*0.136*	1.311 b	*0.017*
40% Shade	0.672 b	*0.022*	1.443 c	*0.082*	1.007 bc	*0.022*
G. manure	0.605 a	*0.060*	1.878 c	*0.132*	0.728 c	*0.020*
P150	1.442 a	*0.089*	3.909 a	*0.290*	2.460 a	*0.261*
	Lee					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	2.680 a	*0.119*	3.987 b	*0.334*	3.353 b	*0.179*
80-90% FC	1.140 c	*0.026*	2.157 c	*0.114*	1.843 c	*0.182*
40% Shade	1.930 b	*0.054*	2.467 c	*0.108*	2.502 c	*0.142*
G. manure	1.280 c	*0.141*	3.495 b	*0.246*	1.779 c	*0.119*
P150	2.208 b	*0.096*	5.207 a	*0.166*	4.545 a	*0.473*

In a column, within each cultivar, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.10. Growth condition x cultivar and growth condition x lime effects on leaf area (cm^2) of soybeans at 56 DAE.

Growth Condition	Across lime level			
	Forrest		Lee	
Control	95 a	*9*	168 ab	*15*
80-90% FC	87 ab	*11*	113 b	*16*
40% Shade	73 ab	*10*	190 a	*18*
G. manure	54 b	*12*	130 ab	*23*
P150	96 a	*15*	191 a	*27*
	Across cultivar			
	Unlimed		Limed to pH 5.5	Limed to pH 6.0
Control	88 ab	*12*	160 ab *22*	145 a *19*
80-90% FC	59 a	*5*	128 b *11*	113 a *18*
40% Shade	119 a	*36*	141 ab *15*	135 a *38*
G. manure	49 b	*10*	151 ab *28*	76 a *21*
P150	70 ab	*11*	200 a *26*	160 a *33*

In a column, within each cultivar or lime level, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Symptom progression

A rating scale was developed that comprised scoring five Mn phytotoxicity symptoms separately and based on the percentage of leaves affected (Table 5.2, Fig. 5.1). This system allowed more detailed scoring of the overall severity of Mn phytotoxicity as it progressed over time. Interveinal chlorosis and crinkling of the young leaves as well as brown spots of the old leaves started to develop early in the growth period especially for plants grown at soil pH 4.78. In most cases, the leaves exhibited the symptoms before they were completely expanded. Without lime, cumulative symptom scores ranged from 0-6 at 7 DAE, reaching a

maximum of between 6 and 13 at 14 DAE and leveling off or slightly decreasing during the latter growth periods depending on the growth conditions (Fig. 5.7, 5.8).

During the early growth stage (28 DAE), cv. Lee tended to show less intense crinkling, brown spots and cumulative symptoms than Forrest (Fig. 5.9; Table 5.11, 5.12). Significant differences in cumulative symptom scores due to growth conditions were expressed only at pH 5.5 and 6.00 in cv. Forrest and at pH 4.7 in cv. Lee (Table 5.12). Cumulative symptom score in cv. Forrest was decreased by dry growth conditions, shading and P addition at pH 5.50. At pH 6.00, only dry conditions reduced the cumulative symptom scores. In the case of cv. Lee, shading decreased cumulative symptom score at pH 4.78.

The density of brown spots in cv. Forrest was decreased by dry growth conditions at pH 5.50 and pH 6.00 and by P addition at pH 5.50 (Table 5.13). In the case of cv. Lee, shading reduced brown spot scores at pH 5.5 while green manure increased brown spot scores at pH 5.50 and pH 6.00. Lime additions to increase soil pH decreased the severity of leaf crinkling in both soybean cultivars (Table 5.14). Dry and shaded growth conditions resulted in lower scores for leaf crinkling while green manure addition gave the opposite effect.

The chlorophyll reading using the SPAD meter was used to measure leaf greenness (yellowness). A low SPAD reading represents more yellow leaves and a higher reading indicates greener leaves. At 28 DAE, SPAD reading differed with lime and growth condition but was similar in both cultivar (Table

5.11). Lime addition significantly increased SPAD readings across cultivars and growth conditions (Table 5.15). Compared with the control, higher SPAD readings were obtained with dry growth conditions while similar SPAD readings were obtained from the green manure and shaded and P treatments in cv. Forrest. In cv. Lee, growth conditions did not affect SPAD readings across lime levels. SPAD readings tended to increase with time although declines were observed at later growth periods (Fig. 5.10, 5.11). The effect of growth conditions on SPAD readings at each growth period was not consistent over time for both cultivars Forrest and Lee (Fig. 5.10, 5.11). SPAD readings and cumulative symptom score did not seem to be well correlated in both soybean cultivars (Fig. 5.12), indicating that SPAD may not be a good tool in making semi-quantitative measurement of phytotoxic symptoms.

Uptake and concentration of manganese in the leaf tissue

The uptake of Mn generally increased with time, the amount of increase dependent on cultivar, soil pH and growth conditions (Fig. 5.13, 5.14). Mn uptake at 56 DAE was significantly influenced by cultivar, soil pH and growth conditions and their interactions (Table 5.16). The highest Mn uptake was observed in the P treatment for both cultivars at two pH levels (Table 5.17). One exception is for cv. Forrest at pH 6.00 where Mn uptake in the P treatment was significantly lower than that of the control. In the case of cv. Lee at pH 4.78, Mn uptake in the P treatment was similar with the control. A decrease in Mn uptake under dry growth condition was observed in Forrest at pH 6.00 and Lee at all pH

levels. Shading, on the other hand decreased Mn uptake of cv. Forrest at pH 4.78 and pH 6.00 and Lee at pH 5.50. A decreased Mn uptake due to green manure addition was likewise observed in Forrest at pH 4.78 and pH 6.00 and in Lee at pH 6.00.

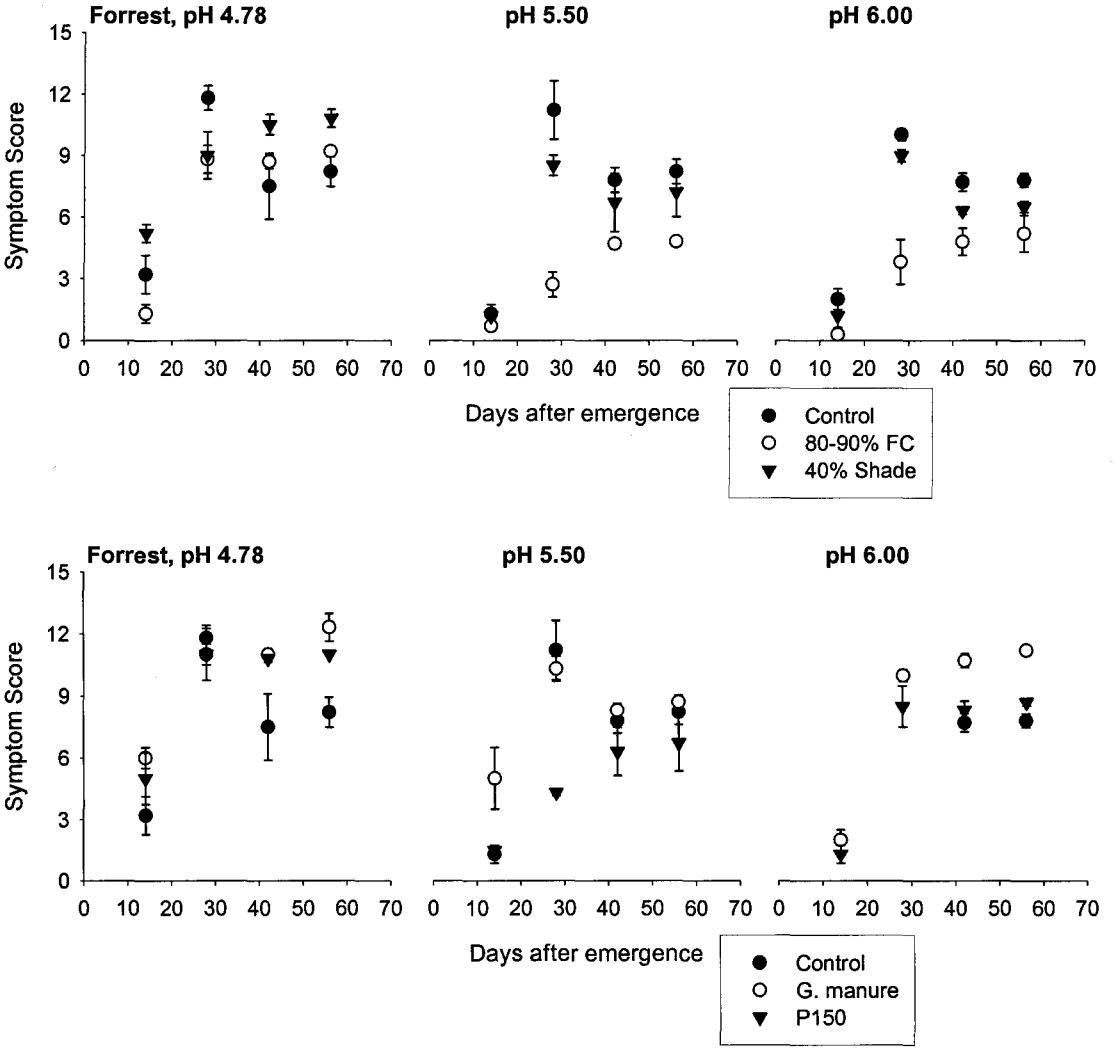


Fig. 5.7. Cumulative symptom scores of soybean cv. Forrest at three pH levels as influenced by dry and shaded growth conditions as well as P and manure additions.

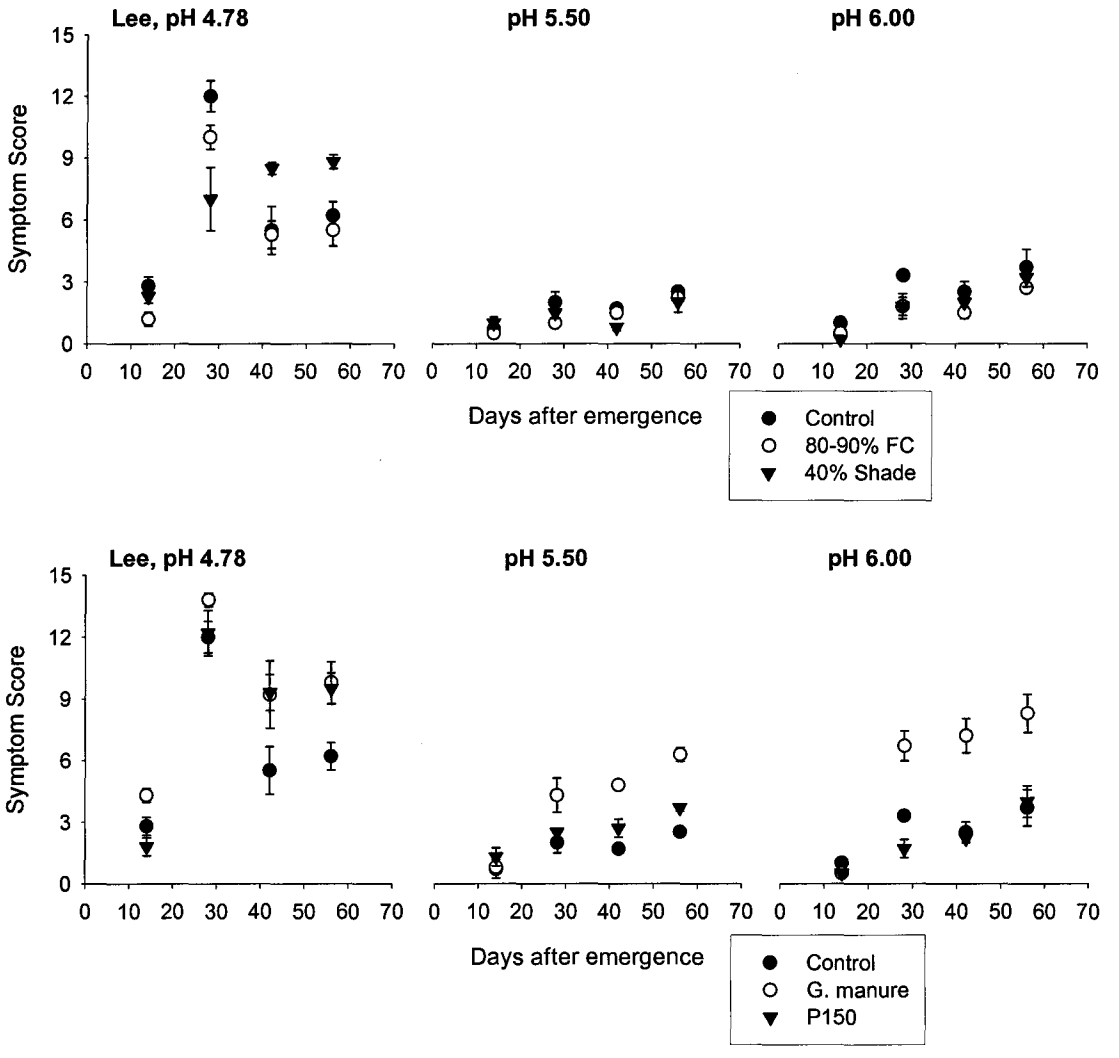


Fig. 5.8. Cumulative symptom scores of soybean cv. Lee at three pH levels as influenced by dry and shaded growth conditions as well as P and manure additions.

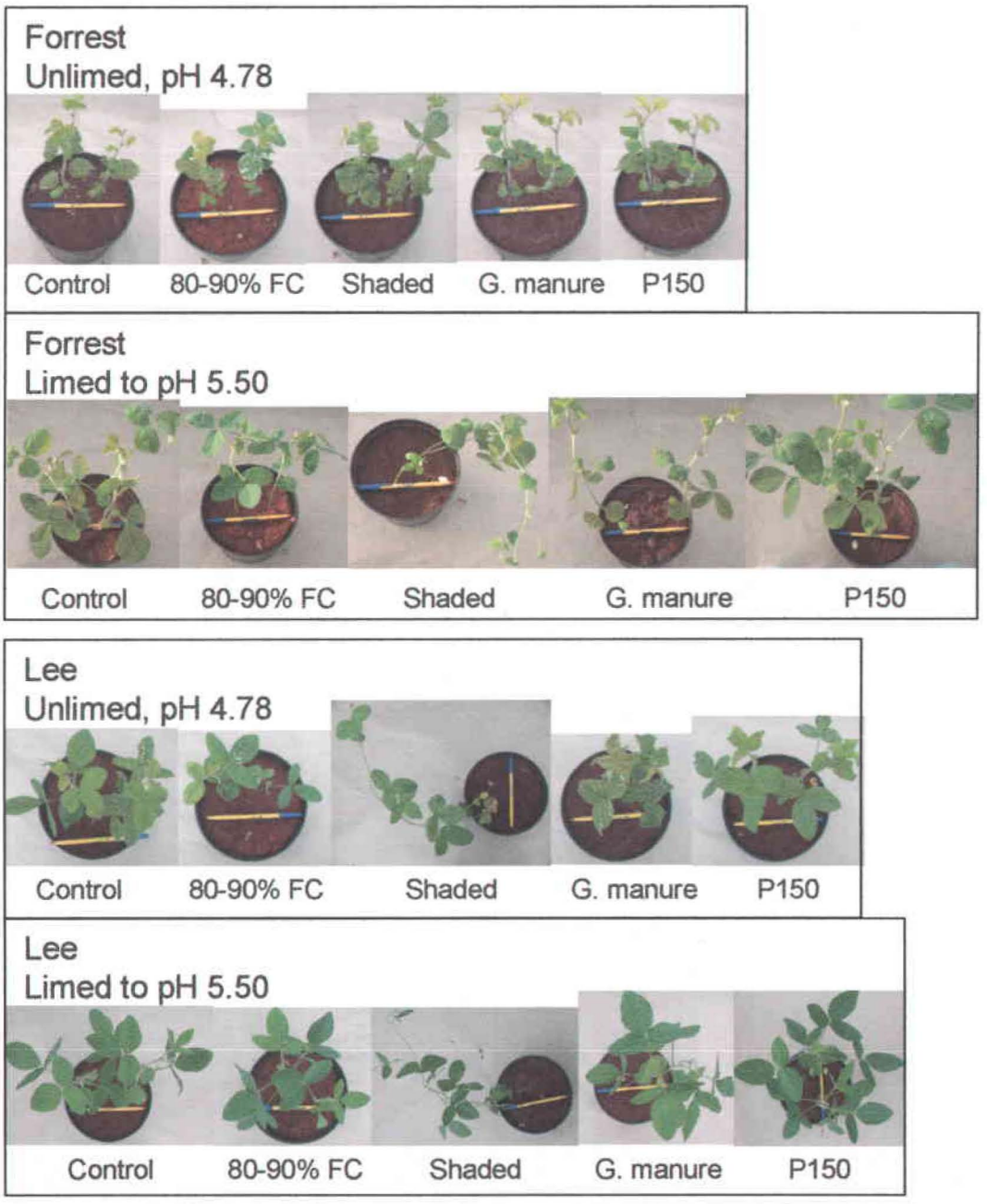


Fig. 5.9. Phytotoxic symptoms of Mn in two soybean cultivars at 28 days after emergence.

Table 5.11. Analysis of variance for the effects of cultivar, lime and growth condition on symptom scores and SPAD reading in soybean leaves.

Effect	Symptom score			SPAD Reading
	Brown spots	Crinkling	Cumulative	
Cultivar (V)	*<0.0001*	*<0.0001*	*<0.0001*	*0.4781*
Lime	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
V x L	*<0.0001*	*0.5536*	*<0.0001*	*0.0167*
V x Tr	*<0.0001*	*0.2096*	*<0.0001*	*0.0108*
L x Tr	*<0.0001*	*0.1533*	*0.0002*	*0.1697*
V x L Tr	*0.0007*	*0.3019*	*0.0024*	*0.6637*

Table 5.12. Cultivar x lime x growth condition effect on cumulative[a] phytotoxic symptoms of soybeans at 28 DAE.

Growth Condition	Forrest		
	Unlimed	Limed to pH 5.5	Limed to pH 6.0
Control	11.8 a *0.73*	11.2 a *0.60*	10.0 a *0.33*
80-90% FC	8.8 a *0.17*	2.7 c *0.17*	3.8 b *0.88*
40% Shade	9.0 a *0.44*	8.5 b *1.20*	9.0 a *0.28*
G. manure	11.0 a *0.67*	10.3 ab *0.33*	10.0 a *0.17*
P150	11.0 a *0.00*	4.3 c *1.36*	8.5 a *0.17*
	Lee		
	Unlimed	Limed to pH 5.5	Limed to pH 6.0
Control	12.0 ab *0.67*	2.0 a *0.00*	1.5 a *0.88*
80-90% FC	10.0 bc *0.76*	1.0 a *0.17*	0.7 a *0.17*
40% Shade	7.0 c *0.33*	1.5 a *0.50*	1.3 a *0.41*
G. manure	13.8 a *1.01*	4.3 a *0.33*	3.5 a *0.93*
P150	12.2 ab *0.76*	2.5 a *0.17*	1.5 a *0.76*

[a] Cumulative scores of five symptoms; Refer to Table 5.2 for rating scale

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.13. Cultivar x lime x growth condition effect on brown spot scores[a] of soybean leaves.

Growth Condition	Forrest					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	2.8 a	*0.17*	3.0 a	*0.00*	3.0 a	*0.00*
80-90% FC	2.5 a	*0.29*	1.0 b	*0.29*	1.7 b	*0.17*
40% Shade	2.8 a	*0.17*	3.0 a	*0.00*	3.0 a	*0.00*
G. manure	2.8 a	*0.17*	3.0 a	*0.00*	3.0 a	*0.00*
P150	2.8 a	*0.17*	1.3 b	*0.33*	2.7 a	*0.33*
	Lee					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	2.8 a	*0.17*	0.8 b	*0.17*	1.0 b	*0.00*
80-90% FC	2.7 a	*0.17*	0.7 bc	*0.17*	0.5 b	*0.29*
40% Shade	2.3 a	*0.33*	0.2 c	*0.17*	0.5 b	*0.29*
G. manure	3.0 a	*0.00*	1.7 a	*0.17*	2.5 a	*0.29*
P150	2.8 a	*0.17*	0.7 bc	*0.17*	0.7 b	*0.17*

[a]Refer to Table 5.2 for rating scale

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.14. Single factor effects of cultivar, lime and growth condition on leaf crinkling at 28 DAE.

Factor	Effect	Crinkling score	
Cultivar	Forrest	1.6 a	*0.12*
	Lee	0.6 b	*0.11*
Lime	Unlimed	1.8 a	*0.16*
	Limed to pH 5.50	0.7 b	*0.13*
	Limed to pH 6.00	0.8 b	*0.14*
Growth condition	Control	1.4 b	*0.22*
	80-90% FC	0.7 c	*0.19*
	40% Shade	0.7 c	*0.20*
	G. manure	1.6 a	*0.19*
	P150	1.1 b	*0.22*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.15. Cultivar x growth condition and cultivar x lime effects on SPAD readings of soybean leaves.

Growth condition	Mean across lime level			
	Forrest		Lee	
Control	31.42	*1.28*	32.62	*1.15*
80-90% FC	38.08	*0.98*	35.38	*1.77*
40% Shade	29.92	*1.30*	34.18	*1.25*
G. manure	33.61	*1.15*	32.34	*1.17*
P150	34.32	*1.06*	35.05	*1.43*
Lime level	Mean across growth condition			
	Forrest		Lee	
Unlimed	31.60	*1.06*	29.85	*1.01*
Limed to pH 5.5	35.45	*0.98*	35.77	*0.66*
Limed to pH 6.0	33.35	*1.16*	36.12	*0.59*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

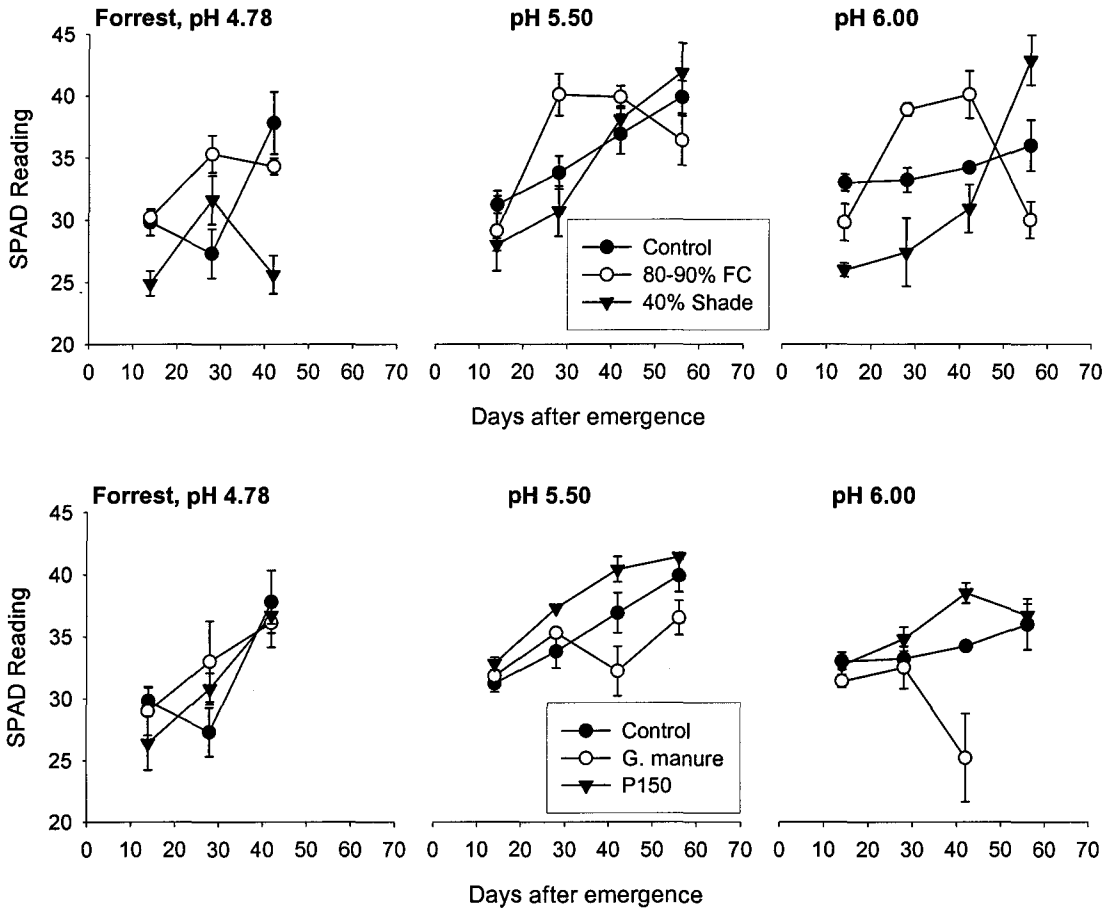


Fig. 5.10. SPAD readings of soybean cv. Forrest at three pH levels as influenced by dry and shaded growth conditions as well as P and manure additions.

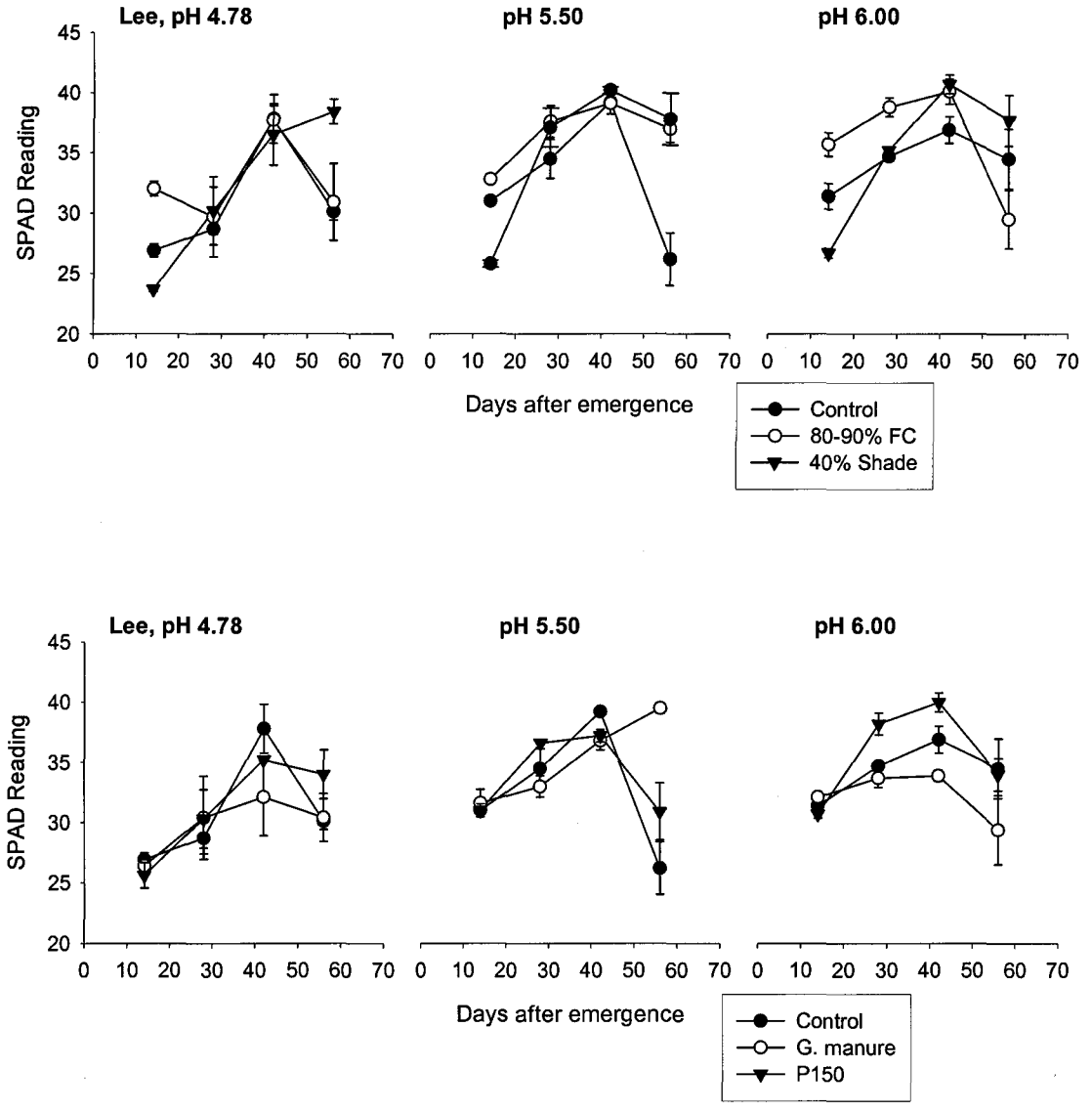


Fig. 5.11. SPAD readings of soybean cv. Lee at three pH levels as influenced by dry and shaded growth conditions as well as P and manure additions.

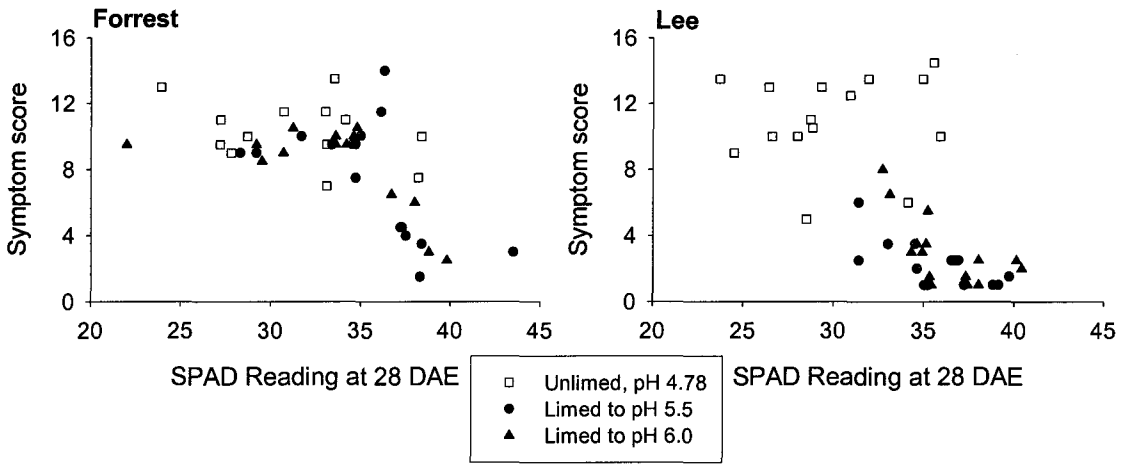


Fig. 5.12. Relation of SPAD reading to cumulative symptom score in two soybean cultivars grown over a range of growth conditions and soil pH.

The concentration of Mn in young and old leaves generally increased with time for both cultivars (Table 5.5, 5.6, Fig.5.15-5.20). The old leaves tended to have higher concentrations of Mn compared with the young leaves (Fig.5.15-5.20). The gap between young and old leaf Mn was widest during the middle growth periods of 28 DAE and 42 DAE. This gap tended to be smallest during the later growth period (56 DAE). Both cultivars grown under dry conditions tended to have the small differences between young and old leaf Mn under most pH levels. The control treatment in both cultivars showed a smaller difference between young leaf Mn and old leaf Mn at pH 5.5 compared with the lower and higher pH levels. In both cultivars, the dry growth condition showed somewhat constant differences over time in Mn concentration between young and old leaves up to 42 DAE . At 56 DAE, Mn concentrations between young and old leaves were similar. Shading led to similar young and old leaf Mn concentrations

at 28 DAE in unlimed Forrest and at 42 DAE in unlimed Lee. The differences between young and old leaf Mn was mostly maintained in the green manure treatment except under pH 4.78 where similar concentrations were observed in cv. Forrest at 14-28 DAE and in cv. Lee at 42 DAE. A similar trend was observed in the case of P treatment, where old leaf Mn was mostly higher than young leaf Mn except in the early plant growth stage at pH 4.78 and at later growth stage for the higher pH levels.

The mean leaf Mn (mean of old and new leaf) concentrations at four sampling dates were significantly affected by growth conditions, soil pH, cultivar and their interactions (Table 5.18). Soybean cv. Lee tended to have higher leaf Mn than cv. Forrest (Table 5.19). The decline in leaf Mn due to lime addition seemed to be more pronounced in cv. Forrest than cv. Lee. Dry growth conditions significantly decreased leaf Mn of cv. Lee where lime was added (Table 5.19). This effect was not observed in cv. Forrest. On the other hand, green manure increased leaf Mn of cv. Lee only at pH 6.0 and Forrest at pH 5.5. Shading and P addition had no significant influence on leaf Mn of both cultivars at all pH levels (Table 5.19).

Concentration of other nutrients in the leaves

The nutrients Ca, Mg and P were expected to interact with plant Mn uptake. Interaction can be positive, enhancing Mn uptake or negative, reducing Mn uptake. The concentration of Ca, Mg and P in the leaves was influenced by cultivar, lime (soil pH) and growth conditions and their interactions. The influence

of factor interaction was different for each nutrient (Table 5.20). The effect of lime on leaf Ca, Mg and P differed with cultivar. The effect of growth condition on leaf Ca differed with lime levels while the effect of growth condition on leaf P differed with cultivar. Mean leaf Ca, Mg and P ranged from 1.47-2.39%, 0.59-0.72%, and 0.14-0.21% in soybean cultivars Forrest and Lee across growth conditions.

Leaf Ca of cv. Forrest increased where the soil was limed to pH 5.50 (Table 5.21). There was no additional increase with further adjustment to pH 6.00. For both cultivars, leaf Ca decreased with shading at pH 5.50 and increased with green manure at pH 5.50 and 6.00. Leaf Mg, on the other hand, was increased at pH 5.50 in Forrest. A decrease in leaf Mg was observed in Lee where soil was limed to pH 5.50 and pH 6.00 (Table 5.22). Leaf P decreased with lime addition to adjust soil pH to 5.50 in both cultivars. No further decrease was observed with further adjustment to pH 6.00. Growth conditions did not affect leaf P concentrations in cv. Lee (Table 5.20). Leaf P of cv. Forrest, on the other hand, decreased under dry growth conditions and with green manure addition (Table 5.23). A significant increase in leaf P was observed with the higher amount of P added.

Rates of CO_2 assimilation and transpiration

The processes of CO_2 assimilation (photosynthesis) and transpiration are both important to plant growth and are reflected in how much plant tissue is accumulated over a given growth period. The phytotoxicity of Mn is expected to

result in the reduction in biomass growth, which would be expressed initially as reduced rates of CO_2 assimilation and transpiration.

The rates of CO_2 assimilation generally declined with time in most growth conditions and pH levels while that of transpiration showed fluctuations with time (Table 5.5, 5.6; Fig. 5.21, 5.22, 5.23, 5.24). The decline in assimilation rate differed with growth conditions and pH levels. The fluctuation in transpiration rate, however, seemed to be similar among growth conditions, pH levels and cultivar. The decline in assimilation rate of cv. Forrest under dry growth conditions was more apparent than those in the control and shaded conditions (Fig. 5.21). Likewise, a more apparent decline in assimilation rate of Forrest was observed where green manure was added, as compared with the control and P treatments (Fig. 5.21). Where no lime and green manure was added in the soils, the leaves of cv. Forrest were severely damaged (crinkled, chlorotic, with many brown spots), there was no measurement of CO_2 assimilation and transpiration at 56 DAE. In cv. Lee, a more apparent decline in assimilation rate was observed in the dry and shaded conditions compared with the control treatment (Fig. 5.22). Likewise, the decline in assimilation rate was more apparent in the green manure treatment at pH 4.78 and in the P treatment at pH 6.00 (Fig. 5.22).

Due to a similar trends among growth condition effects with time, mean CO_2 assimilation and transpiration were averaged over the four sampling dates to determine differences among growth condition, lime and cultivar effects. Mean

CO_2 assimilation rates and transpiration rates over four sampling dates significantly differed with cultivar, soil pH and growth conditions and their interaction (Table 5.24). Soybean cv. Lee showed higher rates of both CO_2 assimilation and transpiration (Table 5.25, 5.26). Higher rates of CO_2 assimilation and transpiration were observed at pH 5.5 compared to pH 4.7 and 6.0 in both cultivars. A decline in assimilation rate due to dry growth conditions was observed in Forrest at pH 4.78 and pH 5.50 and in Lee at pH 5.50 and pH 6.00. A similar decline in the rate of CO_2 assimilation due to shaded growth conditions was observed in cv. Forrest at pH 4.78 and pH 5.50 and in cv. Lee at pH 6.00. Green manure addition decreased assimilation rates of cv. Forrest at all pH levels and cv. Lee at pH 6.00. Rates of CO_2 assimilation in the P treatment were similar with the control. Similarly, rates of transpiration of cv. Forrest were significantly decreased under dry and shaded growth conditions and with green manure addition at all pH levels except at pH 6.00 where transpiration rate under shaded condition was similar with the control (Table 5.26). In cv. Lee, dry growth conditions decreased transpiration rates at all pH levels while green manure addition decreased transpiration rate only at pH 6.00. On the other hand, P treatment gave similar rates of transpiration as the control.

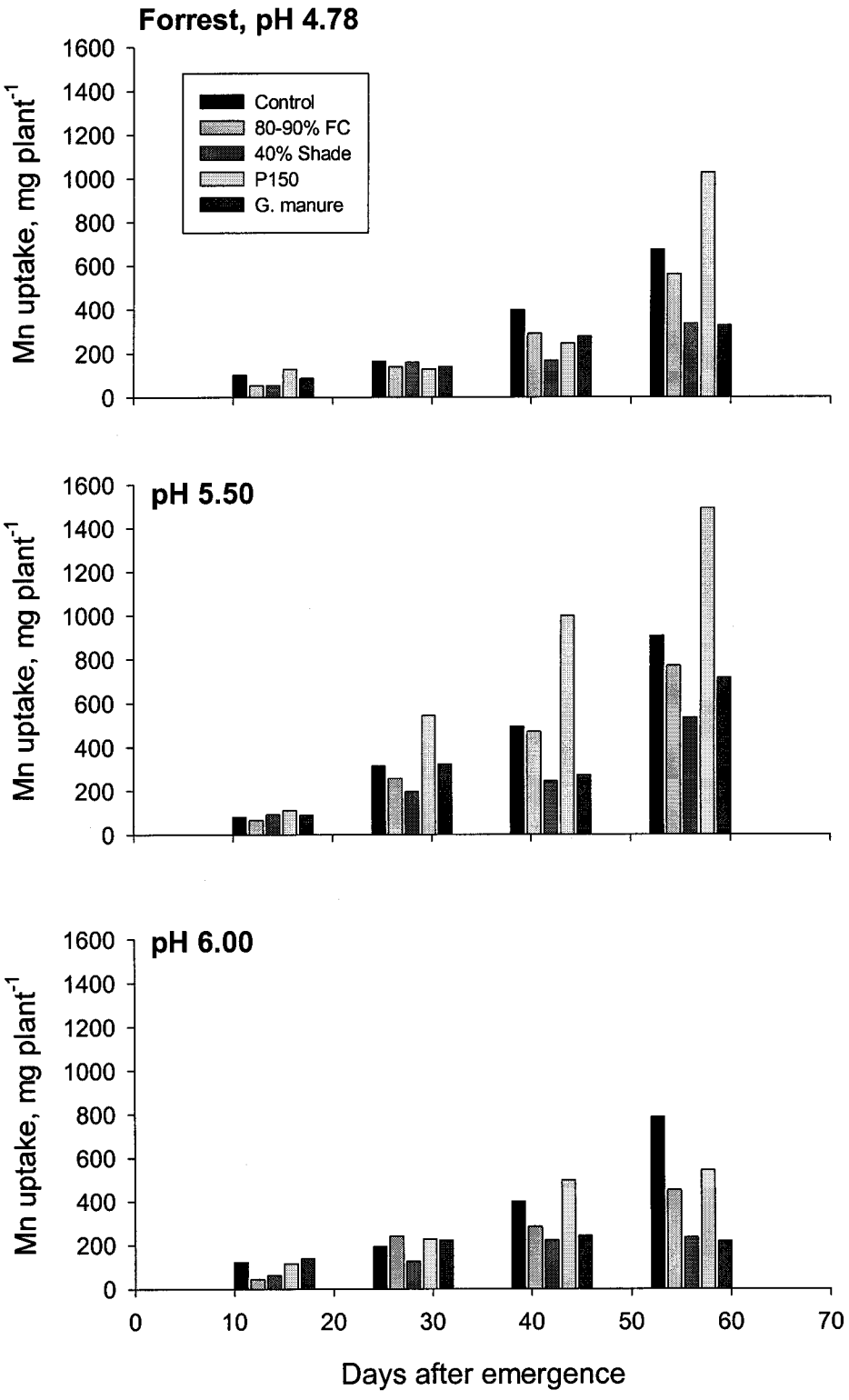


Fig. 5.13. Accumulation of Mn in the leaves of soybean cv. Forrest grown at varying soil pH and growth conditions.

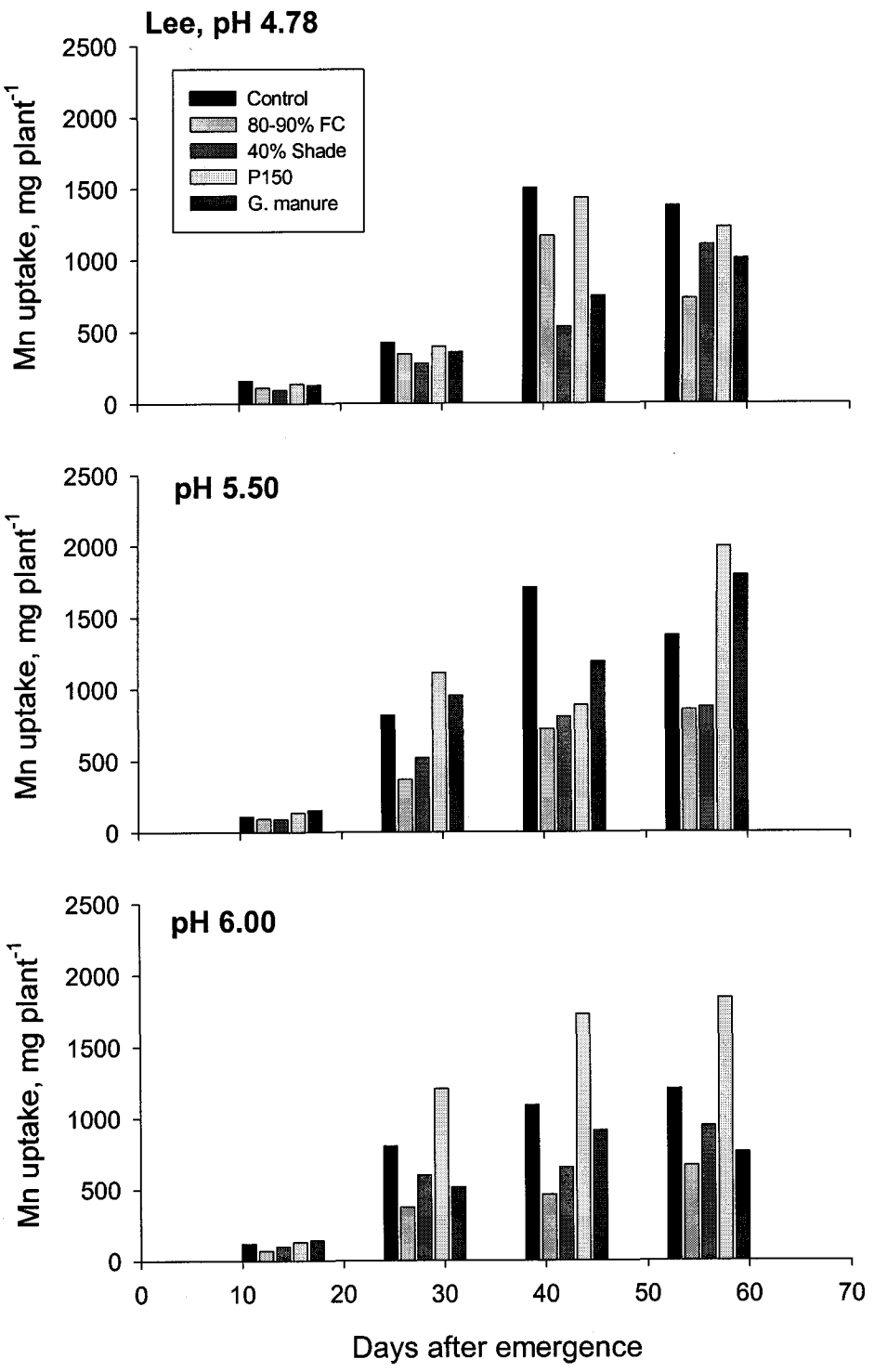


Fig. 5.14. Accumulation of Mn in the leaves of soybean cv. Lee grown at varying soil pH and growth conditions.

Table 5.16. Analysis of variance for the effects of cultivar, lime and growth condition on soybean Mn uptake (56 DAE) and estimated water use across four sampling dates.

Effect	Mn uptake (56 DAE)	Water use
Cultivar (V)	*<0.0001*	*<0.0001*
Lime (L)	*<0.0001*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*
V x L	*0.3428*	*0.0462*
V x Tr	*0.0005*	*<0.0001*
L x Tr	*0.0007*	*0.0002*
V x L Tr	*0.0008*	*<0.0001*

Table 5.17. Lime x cultivar x growth condition effects on Mn uptake (mg plant^{-1}) at 56 DAE.

Growth Condition	Forrest					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	674 b	*96*	906 b	*171*	786 a	*8*
80-90% FC	562 bc	*83*	770 b	*60*	452 b	*24*
40% Shade	336 c	*47*	535 b	*55*	236 c	*20*
G. manure	328 c	*40*	715 b	*17*	217 c	*27*
P150	1027 a	*74*	1492 a	*258*	544 b	*51*
	Lee					
	Unlimed		Limed to pH 5.5		Limed to pH 6.0	
Control	1377 a	*236*	1372 b	*42*	1202 b	*204*
80-90% FC	731 b	*25*	853 c	*146*	665 c	*82*
40% Shade	1105 ab	*164*	873 c	*64*	943 bc	*25*
G. manure	1009 ab	*171*	1795 ab	*25*	759 c	*49*
P150	1230 ab	*17*	1993 a	*173*	1840 a	*268*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

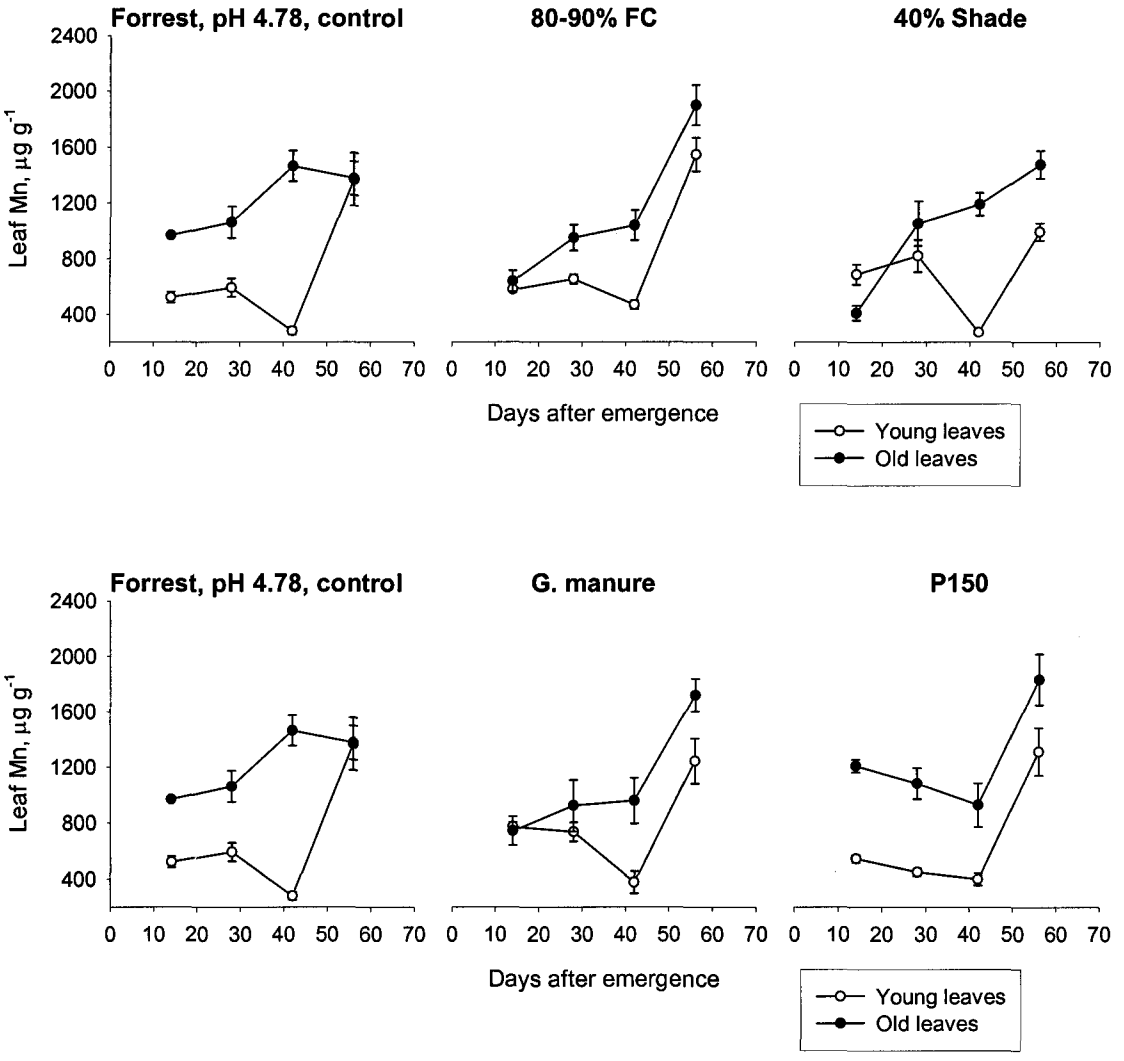


Fig. 5.15. Changes in leaf Mn concentration in soybean cv. Forrest grown in unamended soil and varying growth conditions.

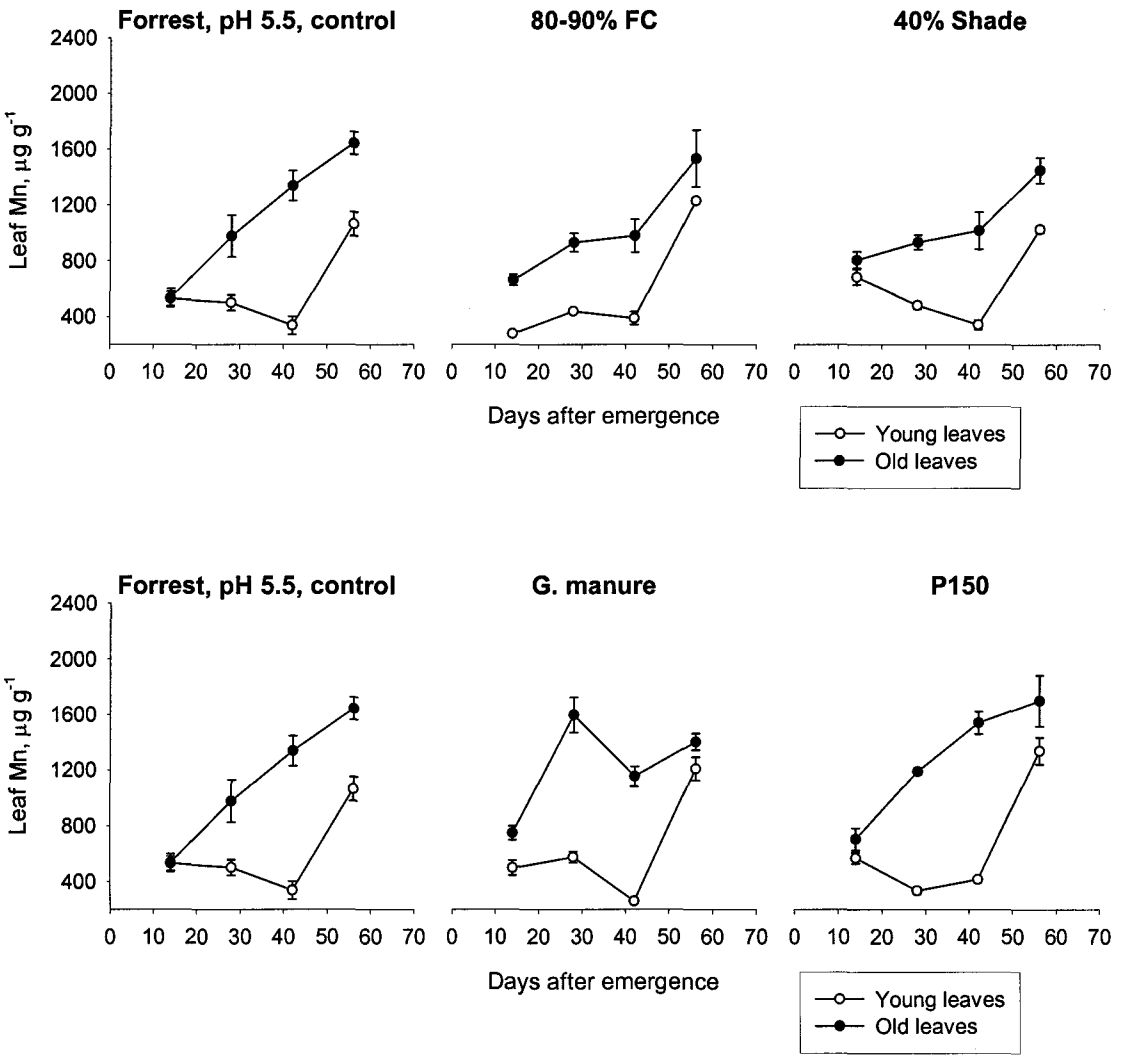


Fig. 5.16. Changes in leaf Mn concentration in soybean cv. Forrest grown at soil pH 5.5 and varying growth conditions.

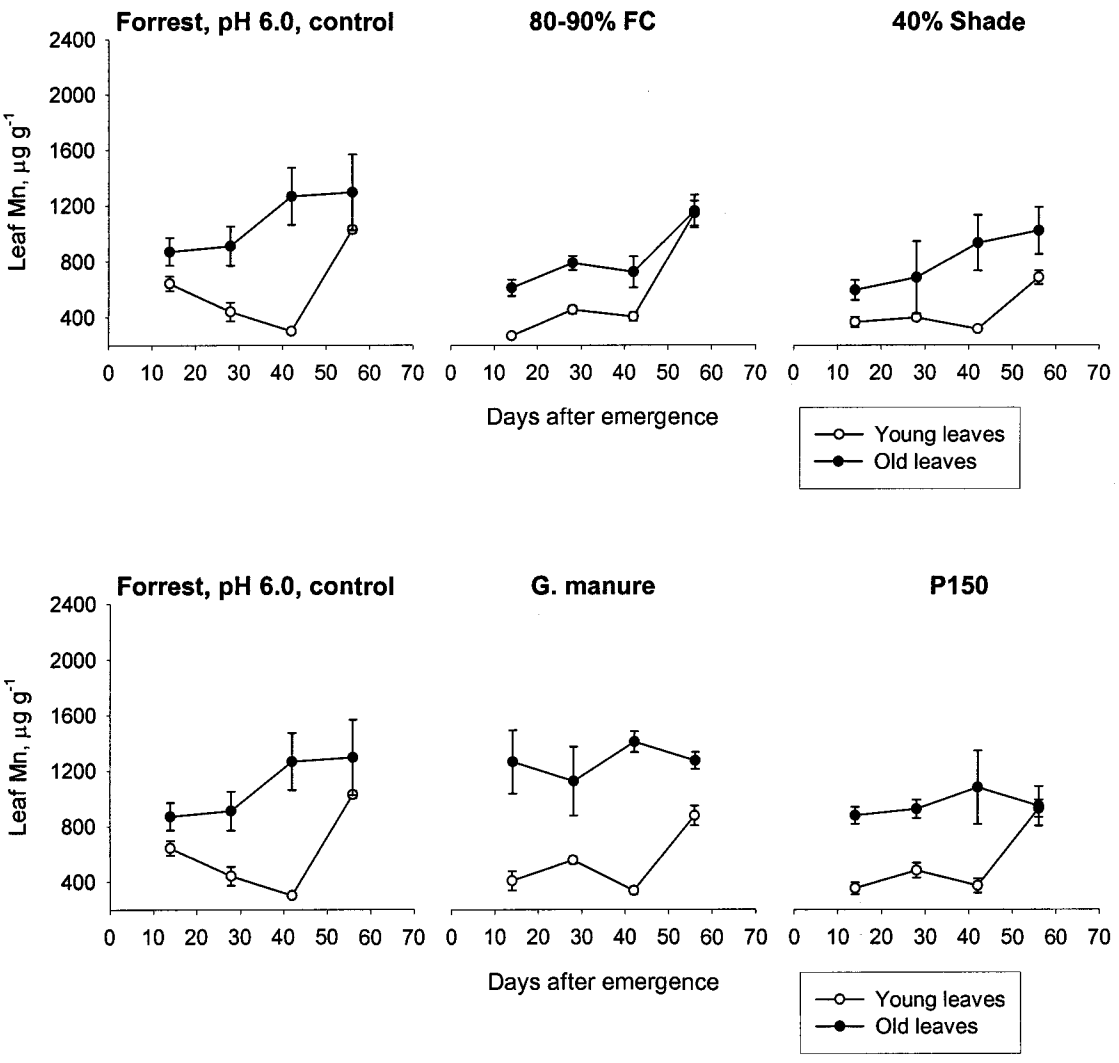


Fig. 5.17. Changes in leaf Mn concentration in soybean cv. Forrest grown at soil pH 6.0 and varying growth conditions.

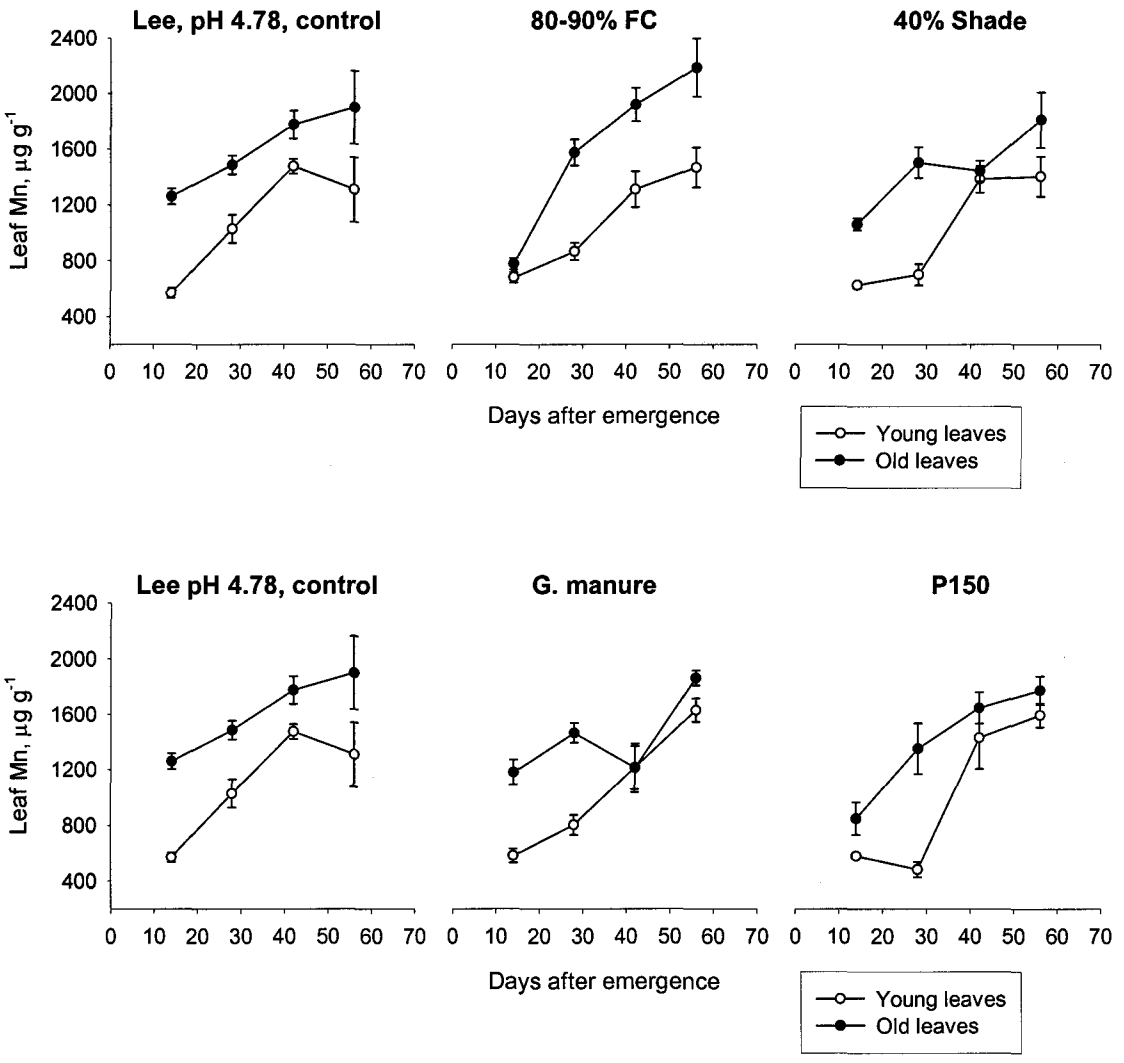


Fig. 5.18. Changes in leaf Mn concentration in soybean cv. Lee grown in unamended soil and varying growth conditions.

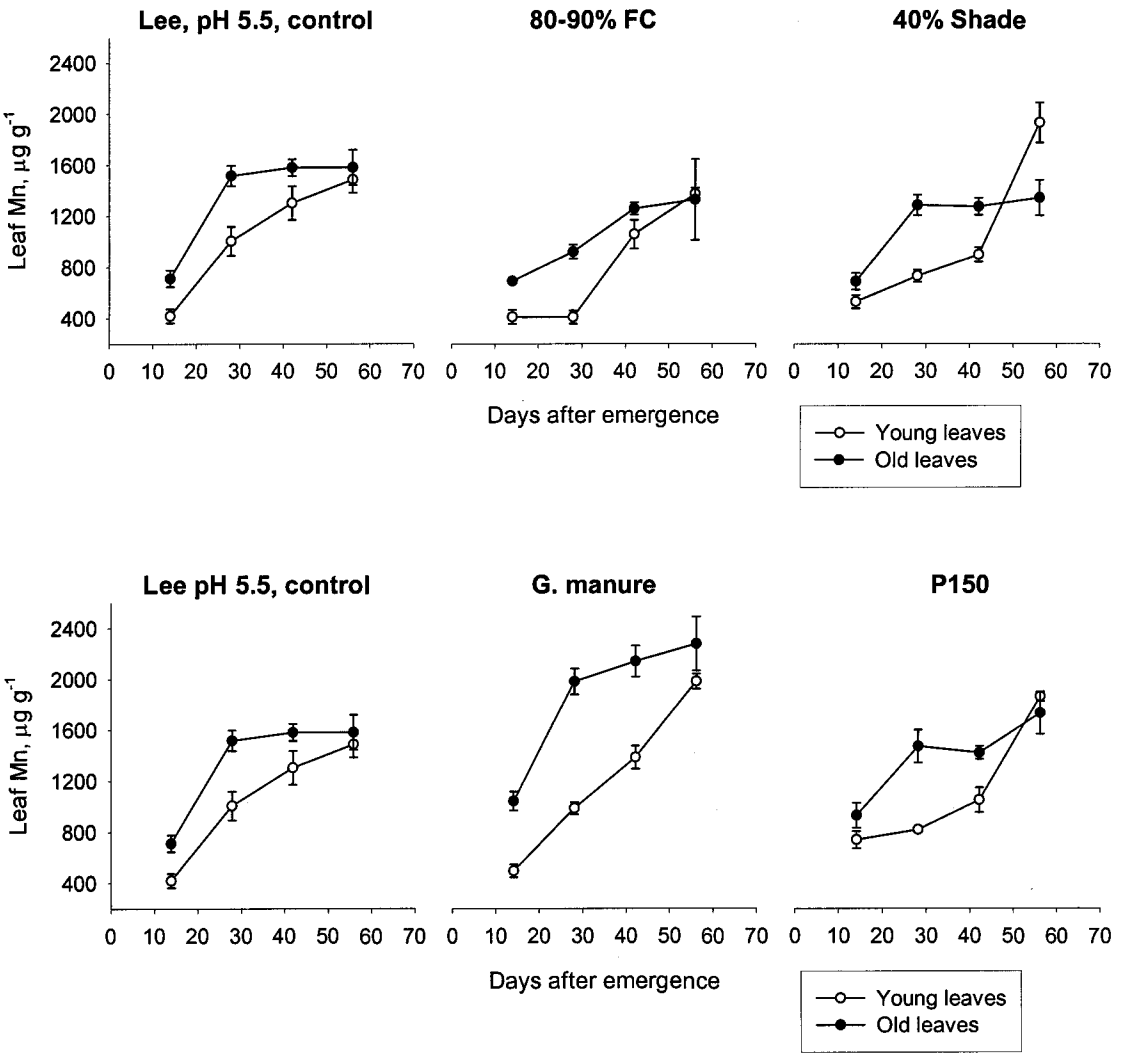


Fig. 5.19. Changes in leaf Mn concentration in soybean cv. Lee grown at soil pH 5.5 and varying growth conditions.

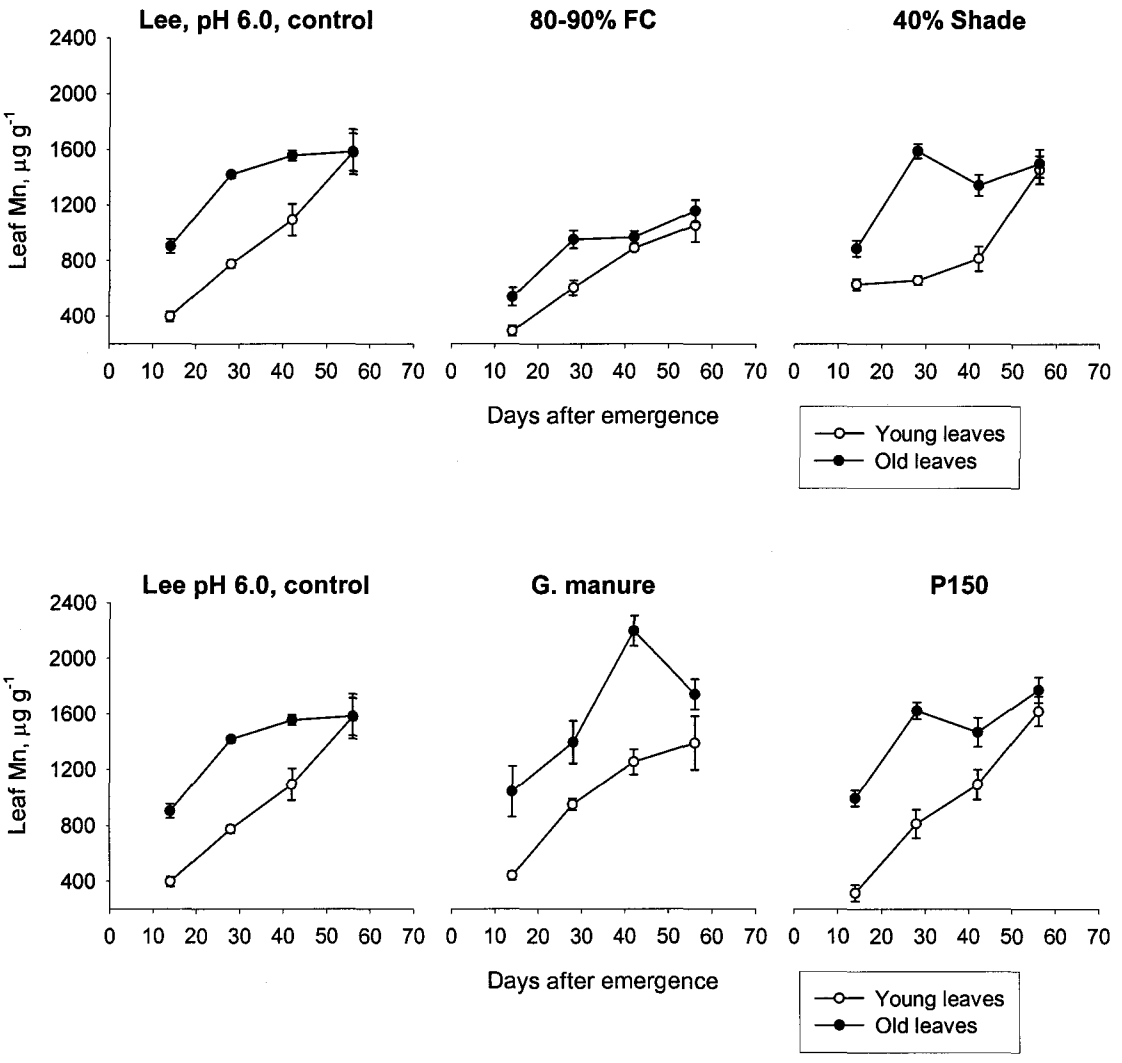


Fig. 5.20. Changes in leaf Mn concentration in soybean cultivar Lee grown at soil pH 6.0 and varying growth conditions.

Table 5.18. Analysis of variance for the effects of cultivar, lime and growth condition on leaf Mn concentrations at four sampling dates.

Effect	Leaf Mn			
	14 DAE	28 DAE	42 DAE	56 DAE
Cultivar (V)	*0.0051*	*<0.0001*	*<0.0001*	*<0.0001*
Lime	*0.0001*	*0.0230*	*0.0086*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*	*<0.0001*	*0.0111*
V x L	*0.7573*	*0.3980*	*0.0868*	*0.1975*
V x Tr	*0.3057*	*0.1516*	*0.1220*	*0.0007*
L x Tr	*0.0052*	*<0.0001*	*<0.0001*	*0.0326*
V x L Tr	*0.0007*	*0.0195*	*0.0019*	*0.1352*

Table 5.19. Lime x cultivar x growth condition effects on leaf Mn ($\mu g\ g^{-1}$) at 28 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	859 a	*71*	788 b	*52*	729 ab	*38*
80-90% FC	842 a	*78*	735 b	*7*	665 b	*57*
40% Shade	985 a	*95*	783 b	*7*	783 b	*43*
G. manure	862 a	*32*	1196 a	*116*	885 a	*74*
P150	779 a	*82*	850 b	*85*	781 ab	*40*
	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	1287 a	*39*	1210 a	*114*	1344 b	*77*
80-90% FC	1354 a	*35*	815 b	*54*	821 c	*64*
40% Shade	1224 a	*165*	1301 a	*49*	1128 b	*131*
G. manure	1238 a	*160*	1257 a	*75*	1706 a	*129*
P150	984 a	*63*	1351 a	*31*	1247 b	*79*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.20. Analysis of variance for the effects of cultivar, lime and growth condition on leaf nutrient concentrations at 28 DAE.

Effect	Leaf Nutrient		
	Ca	Mg	P
Cultivar (V)	*0.3592*	*0.0049*	*<0.0001*
Lime	*<0.0001*	*0.2045*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*	*<0.0001*
V x L	*0.0083*	*<0.0001*	*0.0003*
V x Tr	*0.3481*	*0.7595*	*<0.0001*
L x Tr	*0.0018*	*0.6692*	*0.7778*
V x L Tr	*0.3947*	*0.3335*	*0.7400*

Table 5.21. Cultivar x lime and lime x growth condition effects on leaf Ca concentration at 28 DAE.

Lime	Cultivars			
	Forrest		Lee	
Unlimed	1.47 c	*0.042*	1.59 c	*0.036*
Limed to pH 5.50	2.01 b	*0.088*	1.97 b	*0.078*
Limed to pH 6.00	2.39 a	*0.082*	2.19 a	*0.063*

	Lime levels					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	1.60 ab	*0.062*	1.99 b	*0.074*	2.21 b	*0.065*
80-90% FC	1.50 b	*0.079*	1.86 bc	*0.033*	2.06 b	*0.075*
40% Shade	1.52 ab	*0.045*	1.75 c	*0.050*	2.31 ab	*0.115*
G. manure	1.65 a	*0.056*	2.52 a	*0.104*	2.56 a	*0.163*
P150	1.44 b	*0.072*	1.82 bc	*0.019*	2.30 ab	*0.094*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.22. Cultivar x lime effect on leaf Mg concentration at 28 DAE.

Lime	Cultivars			
	Forrest		Lee	
Unlimed	0.59 b	*0.022*	0.72 a	*0.017*
Limed to pH 5.50	0.65 a	*0.017*	0.64 b	*0.016*
Limed to pH 6.00	0.64 ab	*0.016*	0.62 b	*0.013*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.23. Cultivar x lime and cultivar x growth condition effects on leaf P concentration at 28 DAE.

Lime	Cultivars			
	Forrest		Lee	
Unlimed	0.31 a	*0.019*	0.18 a	*0.010*
Limed to pH 5.50	0.21 b	*0.016*	0.14 b	*0.008*
Limed to pH 6.00	0.21 b	*0.014*	0.14 b	*0.006*
Growth condition				
Control	0.25 b	*0.021*	0.15 ab	*0.013*
80-90% FC	0.18 c	*0.016*	0.14 b	*0.009*
40% Shade	0.27 ab	*0.015*	0.18 a	*0.015*
G. manure	0.19 c	*0.018*	0.145 ab	*0.013*
P150	0.32 a	*0.029*	0.18 a	*0.004*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

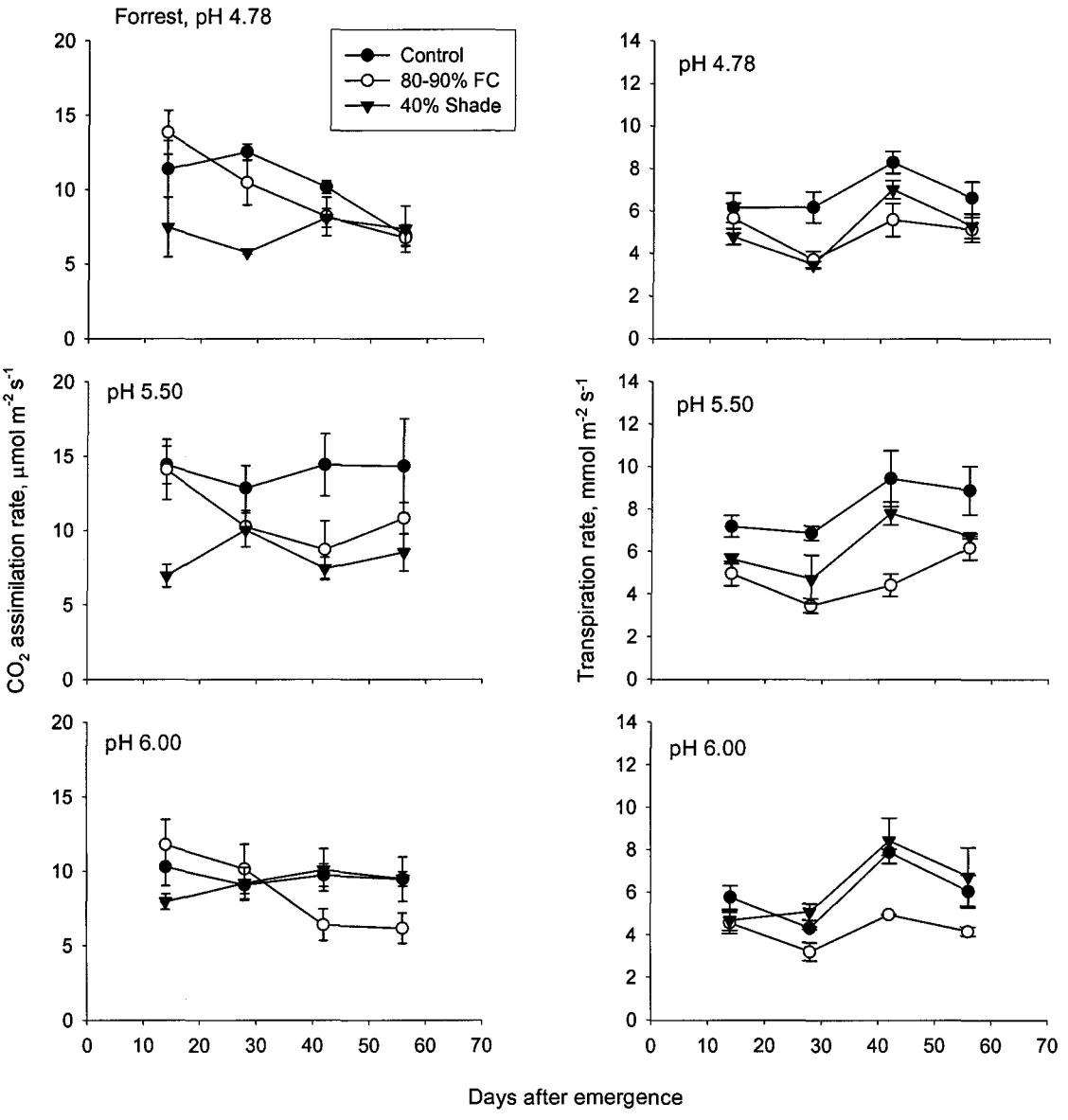


Fig. 5.21. Effect of dry and shaded growth conditions on CO_2 assimilation and transpiration of soybean cv. Forrest grown at varying soil pH.

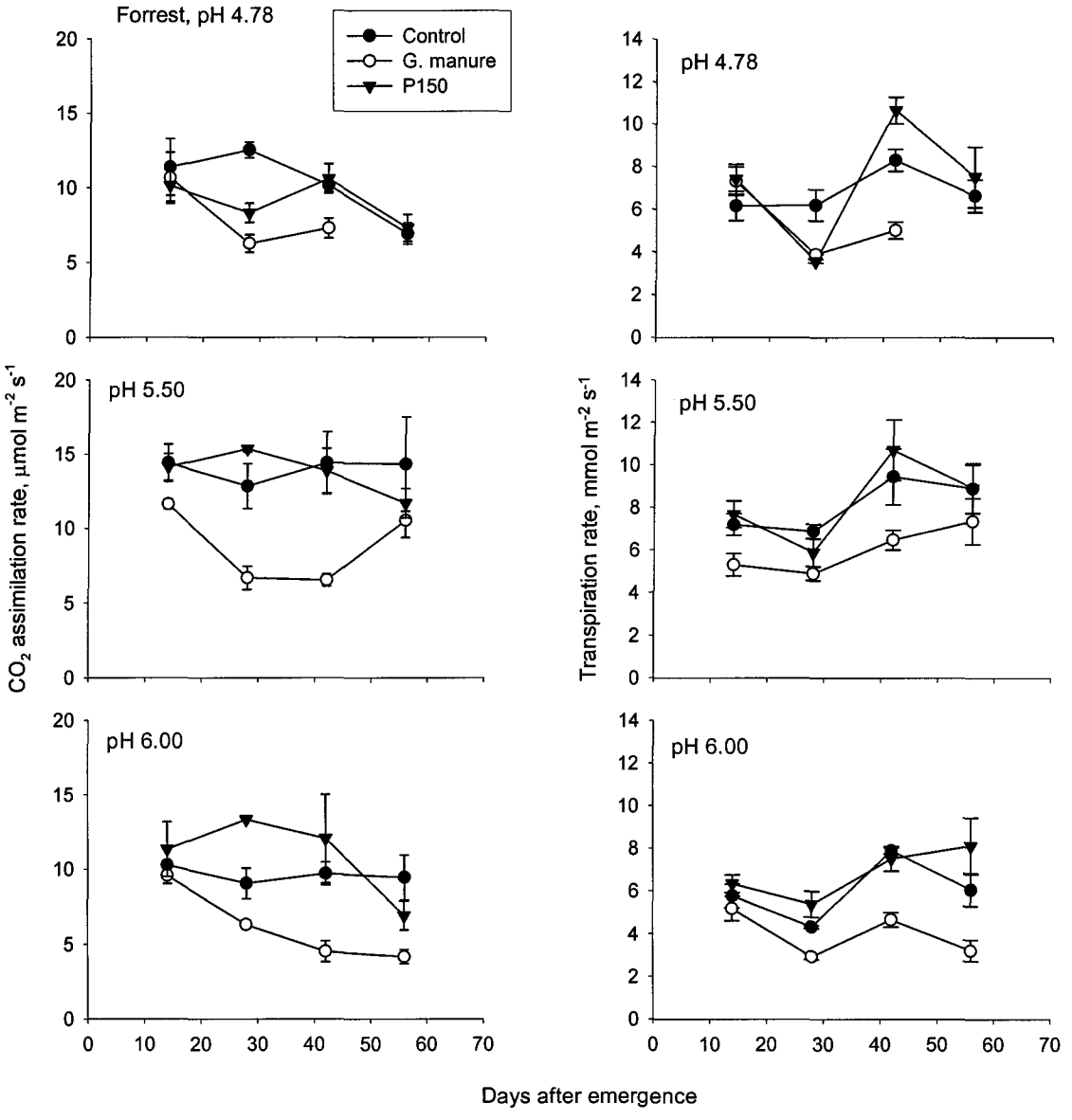


Fig. 5.22. Effect of P and green manure additions on CO_2 assimilation and transpiration of soybean cv. Forrest grown at varying soil pH. Leaves of unlimed Forrest with green manure were severely crinkled, hence no measurement was obtained at 56 DAE.

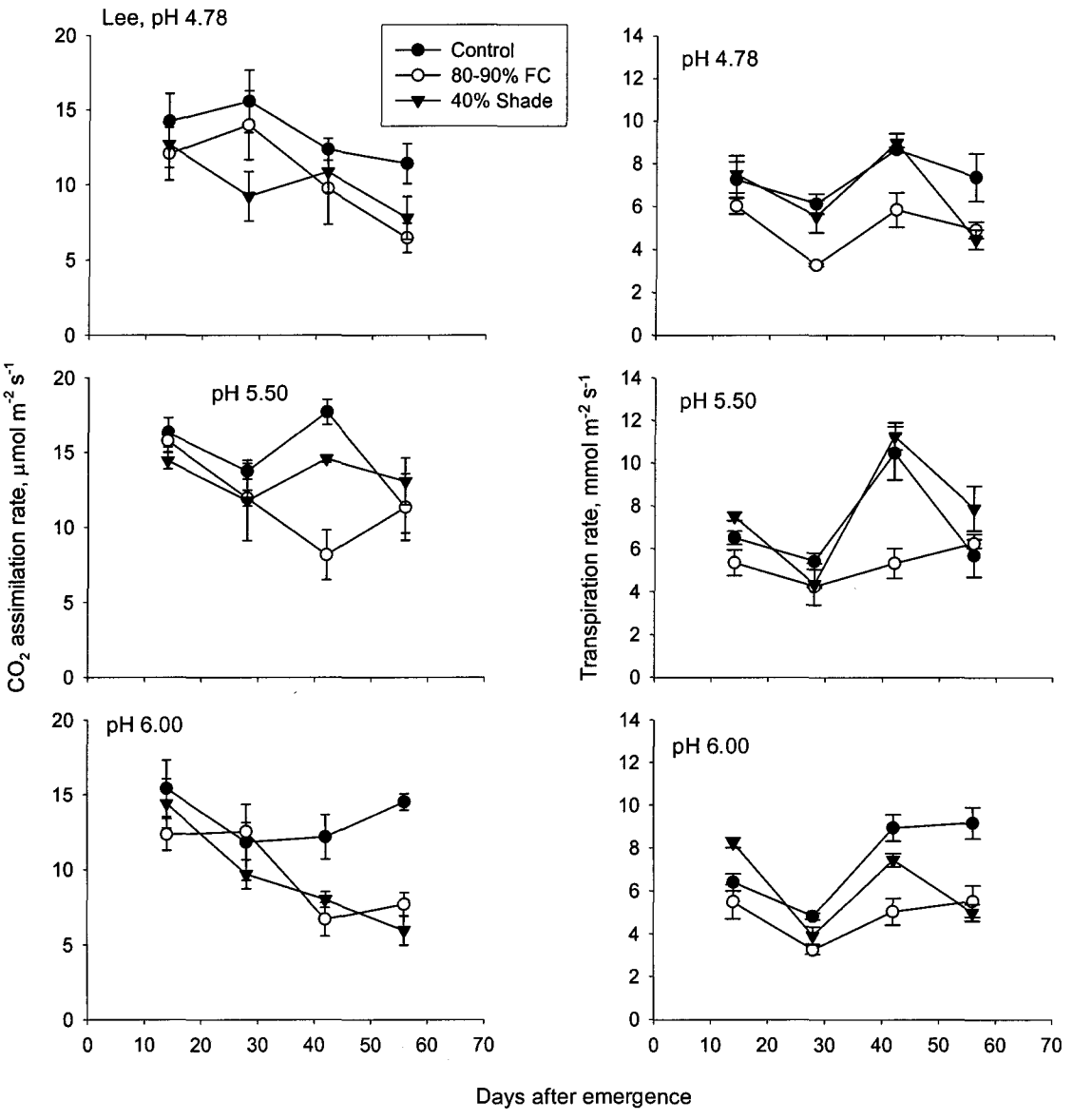


Fig. 5.23. Effect dry and shaded growth conditions on CO_2 assimilation and transpiration of soybean cv. Lee grown at varying soil pH.

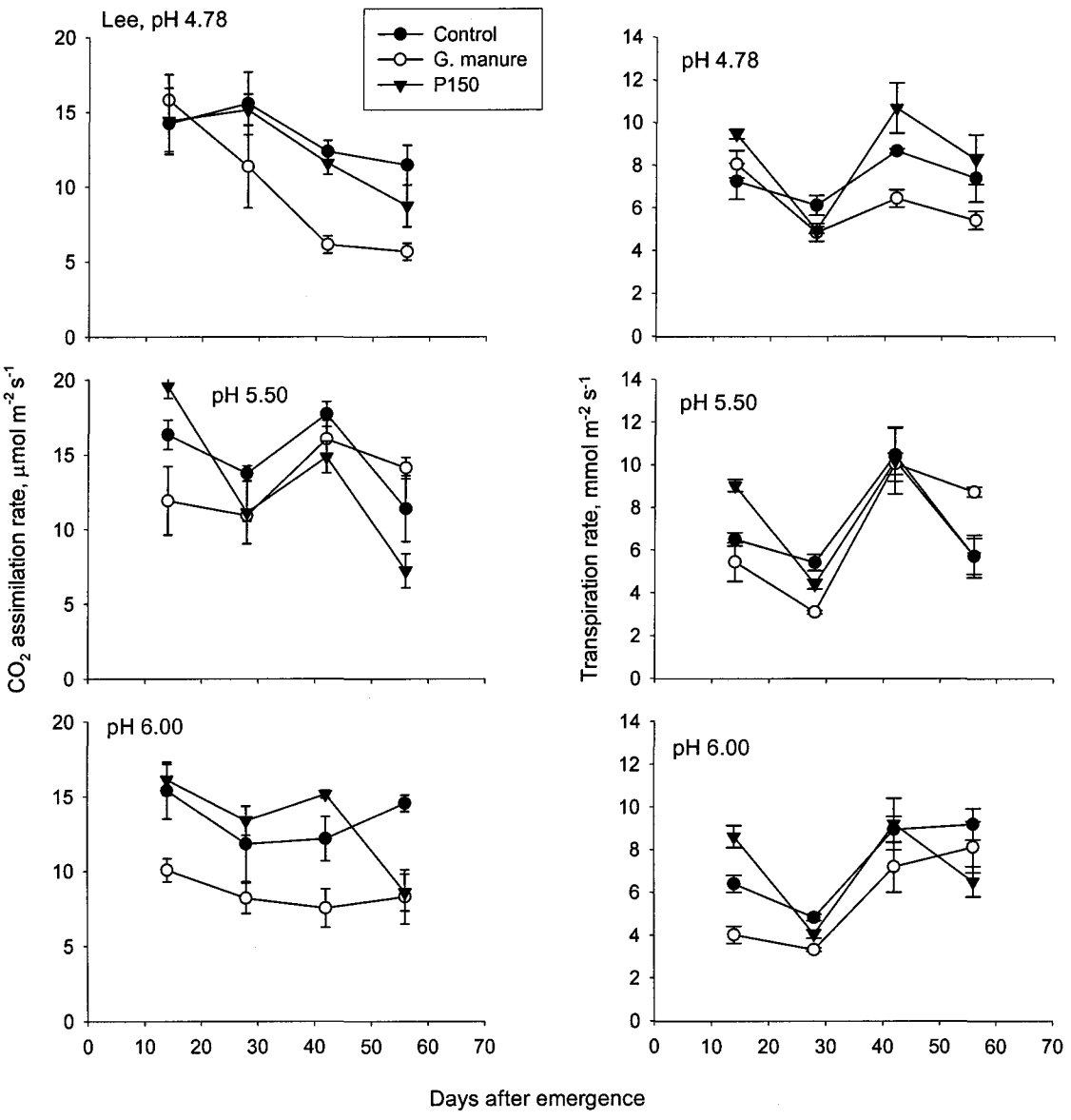


Fig. 5.24. Effect P and green manure additions on CO_2 assimilation and transpiration of soybean cv. Lee grown at varying soil pH.

Table 5.24. Analysis of variance for the effects of cultivar, lime and growth condition on CO_2 assimilation and transpiration rates of soybeans averaged across four sampling dates.

Effect	CO_2 assimilation	Transpiration
Cultivar (V)	*<0.0001*	*0.0003*
Lime	*<0.0001*	*<0.0001*
Growth condition (Tr)	*<0.0001*	*<0.0001*
V x L	*0.8839*	*0.1695*
V x Tr	*0.1914*	*0.1428*
L x Tr	*0.2302*	*0.2674*
V x L Tr	*0.0107*	*0.0234*

Table 5.25. Cultivar x lime x growth condition effect on CO_2 assimilation rate (μmol m^{-2} s^{-1}) averaged across four sampling dates (14, 28, 42, 56 DAE).

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	10.24 a	*0.762*	13.99 a	*0.917*	9.62 a	*0.744*
80-90% FC	9.81 b	*0.308*	10.95 b	*0.917*	8.61 a	*0.431*
40% Shade	7.16 c	*0.899*	8.22 b	*0.323*	9.17 a	*0.655*
G. manure	8.07 bc	*0.325*	8.85 b	*0.577*	6.14 b	*0.368*
P150	9.09 abc	*0.434*	13.76 a	*1.322*	10.91 a	*1.212*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	13.40 a	*0.744*	14.78 a	*0.656*	13.48 a	*0.894*
80-90% FC	10.57 a	*0.973*	11.80 b	*0.899*	9.82 b	*0.466*
40% Shade	10.15 a	*1.289*	13.46 ab	*0.826*	9.51 b	*0.097*
G. manure	9.72 a	*1.316*	13.23 ab	*0.753*	8.52 b	*0.659*
P150	12.46 a	*1.309*	13.18 ab	*0.793*	13.30 a	*0.357*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.26. Cultivar x lime x growth condition effect on transpiration rates (mmol H_2O m^{-2} s^{-1}) averaged across four sampling dates (14, 28, 42, 56 DAE).

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	6.81 a	*0.282*	8.10 a	*0.423*	6.01 a	*0.163*
80-90% FC	5.01 b	*0.316*	4.75 b	*0.215*	4.23 b	*0.083*
40% Shade	5.14 b	*0.271*	6.22 b	*0.330*	6.24 a	*0.532*
G. manure	5.40 b	*0.325*	6.00 b	*0.080*	4.00 b	*0.166*
P150	7.27 a	*0.663*	8.29 a	*0.827*	6.83 a	*0.449*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	7.35 ab	*0.385*	7.02 a	*0.439*	7.34 a	*0.475*
80-90% FC	5.02 c	*0.230*	5.29 b	*0.187*	4.83 c	*0.496*
40% Shade	6.62 b	*0.568*	7.75 a	*0.455*	6.15 ab	*0.111*
G. manure	6.18 bc	*0.416*	6.83 a	*0.176*	5.67 bc	*0.355*
P150	8.33 a	*0.614*	7.34 a	*0.370*	7.10 a	*0.429*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Water Use

The amount of water that goes through the plant was suspected to be strongly related to the amount of manganese accumulated in the plant tissue. Plant water use was estimated from transpiration rate and total leaf area at each sampling date. The assumption made is that the youngest fully mature trifoliolate represents the transpiration capacity of the whole plant. The average water use over four sampling dates was used in the statistical analysis shown in Table 5.16. Average water use of soybean was significantly influenced by cultivar, lime and

growth conditions and their interactions (Table 5.16). In cv. Forrest, a decrease in water use was observed under dry growth conditions at pH 5.5 and pH 6.00 (Table 5.27). Shading decreased water use at pH 4.78 and pH 5.50. A similar effect of green manure was observed at all pH levels. On the other hand, an increase in water use due to P addition was observed at pH 5.50 and pH 6.00 while an increase was observed at pH 4.78. In cv. Lee, dry growth condition and green manure addition decreased water use at all pH levels. Shading decreased water use only at pH 4.78. The addition of P increased water use only at pH 6.00.

Table 5.27. Cultivar x lime x growth condition effect on estimated water use (mmol s^{-1} $plant^{-1}$; average of four sampling dates: 14, 28, 42, 56 DAE).

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.124 a	*0.011*	0.156 b	*0.005*	0.107 b	*0.004*
80-90% FC	0.052 c	*0.005*	0.095 c	*0.003*	0.070 c	*0.002*
40% Shade	0.056 c	*0.005*	0.121 bc	*0.008*	0.094 b	*0.009*
G. manure	0.039 c	*0.002*	0.093 c	*0.003*	0.047 d	*0.003*
P150	0.086 b	*0.004*	0.241 a	*0.023*	0.132 a	*0.026*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.206 a	*0.014*	0.269 a	*0.029*	0.227 b	*0.016*
80-90% FC	0.088 b	*0.005*	0.105 c	*0.007*	0.096 d	*0.015*
40% Shade	0.109 b	*0.008*	0.261 a	*0.020*	0.169 c	*0.005*
G. manure	0.095 b	*0.010*	0.184 b	*0.002*	0.105 d	*0.009*
P150	0.181 a	*0.012*	0.252 a	*0.011*	0.284 a	*0.008*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Relative rates of plant growth and manganese accumulation

A quantitative measure of how fast the plants grew and how fast the plant accumulated Mn is given by relative growth rate (RGR_{Plant}) and relative accumulation rates (RAR_{Mn}). The relative rates of plant growth (RGR_{Plant}), leaf growth (RGR_{Leaf}), and Mn accumulation (RAR_{Mn}) and the difference between RGR_{Leaf} and RAR_{Mn} ($RGRRAR_{Diff}$) averaged across two growth intervals (14-28 DAE, 28-42 DAE) were significantly affected by cultivar, lime and growth conditions and their interactions (Table 5.28).

Factor and factorial interaction effects tended to be similar for the relative growth rate of the leaves and of the whole plant (Table 5.28). While increases in RGR_{Plant} were observed due to P addition, decreases due to shading and green manure addition were observed in both cultivars (Table 5.29). An increase in RGR_{Plant} due to dry conditions was observed in cv. Forrest while a decline was observed in cv. Lee. Soybean cv. Lee gave higher $RGR_{Leaf/Plant}$ than cv. Forrest (Table 5.29-5.30). Lime addition generally increased $RGR_{Leaf/Plant}$ across cultivars and growth conditions. Dry growth condition increased RGR_{Leaf} of cv. Forrest at pH 5.50 and pH 6.00 and decreased RGR_{Leaf} of cv. Lee at pH 5.5 (Table 5.30). Shading decreased RGR_{Leaf} only at pH 4.78 while green manure addition decreased RGR_{Leaf} at pH 5.50 and pH 6.00. Phosphorus addition, on the other hand, increased RGR_{Leaf} at pH 5.50. In cv. Lee, decreases in RGR_{Leaf} due to dry conditions and shading were observed at pH 5.50 and pH 4.78,

respectively. Green manure addition decreased RGR_{Leaf} where soil pH were adjusted to 5.50 and 6.00. RGR_{Leaf} was increased by P addition at pH 6.00.

The rate of Mn accumulation was generally higher in cv. Lee than cv. Forrest (Table 5.28, 5.31). An increase in Mn accumulation rate due to dry condition while decreases due to shading (pH 4.78), green manure (pH 5.50 and 6.00) and P additions (pH 4.78) were noted in cv. Forrest at pH 6.0 (Table 5.31). Dry growth conditions, shading, green manure and P additions decreased the rate of Mn accumulation in cv. Lee at pH 5.50. At the lower and higher pH levels, there was no effect of growth conditions on the rate of Mn accumulation.

A decrease in $RGRRAR_{Diff}$ occurred under dry conditions while decreases due to shading and green manure (both at pH 5.50) and P addition (pH 4.78) were noted in cv. Forrest. An increase in $RGRRAR_{Diff}$ was noted in cv. Lee where P was added at pH 5.50. All other growth conditions did not affect this parameter at three pH levels.

Net assimilation rate and Leaf area ratio

The parameter $RGR_{PLant/Leaf}$ has two important components: net assimilation rate and leaf area ratio. At any instant in time, an increase in $RGR_{Plant/Leaf}$ can be due to an increase in net assimilation rate or an increase in leaf area ratio. Increases in net assimilation rate (NAR) were observed in cv. Forrest subjected to dry condition (pH 4.78) or where green manure was added (pH 6.00) while an increase was observed where P was added at pH 5.50.

Soybean cv. Lee, on the other hand, showed slight but non-significant increase in NAR due to P addition. Significant decreases in NAR were observed under dry conditions (pH 5.50 and 6.00), shading (all pH levels) and green manure addition (pH 5.50 and 6.00).

A decrease in LAR due to dry conditions while decreases due to green manure and P additions were observed in both cultivars Forrest and Lee at pH 4.78. Shading, on the other hand, increased LAR of Forrest at pH 5.50 and 6.00 and Lee at pH 6.00.

Plant biomass and Leaf Mn determinant

An attempt to diagnose phytotoxicity of Mn in plants initially involves a search for a measurement in plant or soil that is well correlated with a measure of phytotoxicity, like biomass for example. A measure of available soil Mn from the saturated paste extract was poorly correlated with plant biomass (Fig. 5.25). Likewise, symptom scores and leaf Mn did not predict plant biomass production of both soybean cultivars (Fig. 5.26). Nutrient ratios such as Ca:Mn, Mg:Mn or P:Mn were also poor predictors of plant biomass (Fig. 5.27). Leaf area at 28 DAE, on the other hand, was correlated with plant biomass at 56 DAE (Fig. 5.28). However, leaf area was not related to leaf Mn concentration (Fig. 5.28).

The absolute difference between relative growth rate and Mn accumulation rate, which reflects a change in leaf Mn concentration over time likewise failed to predict biomass production of both soybean cultivars (Fig. 5.29).

Relations between Relative growth rates (Plant/Leaf) and Relative accumulation rate of manganese

The rate by which biomass accumulates in relation to how fast Mn accumulates inside the biomass shows a different picture compared to the relation between the final biomass production and leaf Mn concentration at an earlier stage of the soybean plant.

The rates of leaf growth of cv. Forrest and cv. Lee were exceeded by rates of Mn accumulation in the leaves (Fig. 5.30). Both parameters can be considered unitless although the values were derived from units of different magnitude; i.e. g leaf biomass, μg Mn uptake, so that their absolute values can be directly compared. A higher rate of Mn accumulation compared to biomass production is reflected in an increasing leaf Mn concentration with time (Fig. 5.31). An increase in leaf growth rate seemed to accompany an increase in relative accumulation rate. Plotting these parameters showed a significant positive correlation between leaf growth rate and Mn accumulation rate described by a linear equation in both cultivars Forrest and Lee (Fig. 5.32). These rates were averaged across the three growth intervals 14-28, 28-42 and 42-56 DAE. Correlation of current RGR_{Leaf} and RAR_{Mn} at each of these growth intervals also showed significant positive correlation (Fig. 5.33). We chose to relate only leaf growth rate to Mn accumulation rate based on the observation that the relative growth rate of the leaves of the leaves was strongly and positively correlated with relative growth rate of the whole aboveground biomass at all growth intervals considered (Fig. 5.34).

Transpiration, water use and rates of leaf growth and Mn accumulation

Plant water use is the most likely process that links leaf growth and uptake of Mn. Plant growth and Mn uptake are likely to depend on amount of water that goes through the plant during its lifetime. Although transpiration rate appeared to be correlated to Mn uptake and rates of leaf growth and Mn accumulation, the correlation coefficients were somewhat weak (Fig. 5.35). The correlation coefficients were considerably improved by using estimated water use instead of transpiration rates (Fig. 5.36). This is because plant water use takes into account the total amount of transpiring leaves that contribute to the overall plant water uptake by the plant. An underlying assumption, however, is that all leaves are equally capable of transpiring water, which may not be true especially where Mn phytotoxicity caused different amount of damage to younger and older leaves.

Interaction between current RGR_{Leaf} and future RAR_{Mn} and current RAR_{Mn} and future RGR_{Leaf}

Relating leaf growth rate at a current growth interval ($cRGR_{Leaf}$) to Mn accumulation rate at a future growth interval ($fRAR_{Mn}$) and *vice versa* allowed testing of our hypothesis on the feedback interaction between these rate parameters. In cv. Forrest, this expected interaction was not observed. RGR_{Leaf} at growth interval 14-28 DAE did not correlate well with RAR_{Mn} at growth interval 28-42 DAE (Fig. 5.37). Similarly, RAR_{Mn} at growth interval 14-28 DAE did not correlate well with RGR_{Leaf} at growth interval 28-42 DAE. This observation was

true for the succeeding growth intervals considered. In cv. Lee, a negative interaction between $c\text{RGR}_{\text{Leaf}}$ and $f\text{RAR}_{\text{Mn}}$ and between $c\text{RAR}_{\text{Mn}}$ and a $f\text{RGR}_{\text{Leaf}}$ were observed continuously over the growth interval considered (Fig. 5.38). The relation between $c\text{RGR}_{\text{Leaf}}$ and $f\text{RAR}_{\text{Mn}}$ and the corresponding relation between $c\text{RAR}_{\text{Mn}}$ and a $f\text{RGR}_{\text{Leaf}}$ was described by linear function with a negative slope and significant correlation coefficients.

Table 5.28. Analysis of variance for the effects of cultivar, lime and growth condition on relative rates of leaf area expansion, plant growth, and Mn accumulation of soybeans (average of growth intervals 14-28 and 28-42 DAE) and the difference between plant growth rate and Mn accumulation rate.

Effect	$\text{RGR}_{\text{Plant}}$	RGR_{Leaf}	RAR_{Mn}	RGR_{Leaf}-RAR_{Mn}
Cultivar (V)	<0.0001	<0.0001	<0.0001	<0.0001
Lime	<0.0001	0.0234	0.0023	0.0314
Growth condition (Tr)	<0.0001	<0.0001	<0.0001	0.0023
V x L	0.1207	0.2574	0.0902	0.2729
V x Tr	<0.0001	<0.0001	0.0043	0.5266
L x Tr	<0.0001	<0.0001	0.0145	0.0167
V x L Tr	<0.0001	0.0005	<0.0001	0.0030

Table 5.29. Cultivar x lime x growth condition effects on plant relative growth rate ($g\ g^{-1}\ d^{-1}$) averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0427 abc	*0.0016*	0.0461 b	*0.0041*	0.0489 ab	*0.0048*
80-90% FC	0.0529 a	*0.0026*	0.0626 a	*0.0010*	0.0579 a	*0.0033*
40% Shade	0.0318 c	*0.0071*	0.0425 bc	*0.0037*	0.0466 b	*0.0009*
G. manure	0.0460 ab	*0.0011*	0.0390 c	*0.0030*	0.0238 c	*0.0016*
P150	0.0342 bc	*0.0042*	0.0700 a	*0.0018*	0.0567 a	*0.0025*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0685 a	*0.0006*	0.0756 a	*0.0038*	0.0636 bc	*0.0070*
80-90% FC	0.0588 ab	*0.0046*	0.0550 c	*0.0011*	0.0504 c	*0.0037*
40% Shade	0.0500 b	*0.0011*	0.0709 ab	*0.0031*	0.0702 ab	*0.0011*
G. manure	0.0538 b	*0.0038*	0.0553 c	*0.0024*	0.0537 c	*0.0034*
P150	0.0647 a	*0.0036*	0.0643 bc	*0.0034*	0.0793 a	*0.0043*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.30. Cultivar x lime x growth condition effects on leaf relative growth rate ($g\ g^{-1}\ d^{-1}$) averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0399 ab	*0.0027*	0.0426 bc	*0.0041*	0.0406 bc	*0.0036*
80-90% FC	0.0480 a	*0.0040*	0.0546 ab	*0.0025*	0.0513 a	*0.0046*
40% Shade	0.0245 c	*0.0043*	0.0329 cd	*0.0055*	0.0373 c	*0.0014*
G. manure	0.0409 ab	*0.0041*	0.0295 d	*0.0039*	0.0126 d	*0.0007*
P150	0.0351 bc	*0.0034*	0.0615 a	*0.0027*	0.0488 ab	*0.0007*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0596 a	*0.0017*	0.0571 a	*0.0047*	0.0532 b	*0.0047*
80-90% FC	0.0534 ab	*0.0058*	0.0460 b	*0.0005*	0.0417 bc	*0.0022*
40% Shade	0.0417 b	*0.0039*	0.0580 a	*0.0028*	0.0540 b	*0.0021*
G. manure	0.0508 ab	*0.0034*	0.0453 b	*0.0018*	0.0402 c	*0.0033*
P150	0.0575 a	*0.0057*	0.0532 ab	*0.0026*	0.0687 a	*0.0066*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.31. Cultivar x lime x growth condition effects on Mn accumulation rate ($\mu g\ \mu g^{-1}\ d^{-1}$) averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0493 a	*0.0063*	0.0646 a	*0.0056*	0.0416 b	*0.0042*
80-90% FC	0.0594 a	*0.0069*	0.0699 a	*0.0017*	0.0666 a	*0.0059*
40% Shade	0.0400 ab	*0.0074*	0.0350 b	*0.0074*	0.0455 b	*0.0031*
G. manure	0.0411 ab	*0.0032*	0.0386 b	*0.0050*	0.0202 c	*0.0020*
P150	0.0230 b	*0.0042*	0.0786 a	*0.0022*	0.0536 ab	*0.0063*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	0.0805 a	*0.0088*	0.0985 a	*0.0050*	0.0794 a	*0.0074*
80-90% FC	0.0848 a	*0.0033*	0.0730 b	*0.0030*	0.0693 a	*0.0040*
40% Shade	0.0609 a	*0.0058*	0.0783 b	*0.0029*	0.0684 a	*0.0061*
G. manure	0.0630 a	*0.0057*	0.0730 b	*0.0020*	0.0684 a	*0.0080*
P150	0.0830 a	*0.0113*	0.0676 b	*0.0041*	0.0908 a	*0.0118*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.32. Cultivar x lime x growth condition effects on the difference between leaf growth rate and Mn accumulation rate averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	-0.0094 bc	*0.0037*	-0.0220 c	*0.0057*	-0.0010 a	*0.0016*
80-90% FC	-0.0114 bc	*0.0031*	-0.0153 bc	*0.0008*	-0.0153 b	*0.0019*
40% Shade	-0.0154 c	*0.0036*	-0.0022 a	*0.0024*	-0.0082 ab	*0.0042*
G. manure	-0.0002 ab	*0.0073*	-0.0091 ab	*0.0031*	-0.0076 ab	*0.0015*
P150	0.0121 a	*0.0009*	-0.0171 bc	*0.0028*	-0.0048 ab	*0.0058*
	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	-0.0209 a	*0.0076*	-0.0314 b	*0.0011*	-0.0250 a	*0.0029*
80-90% FC	-0.0313 a	*0.0029*	-0.0270 b	*0.0025*	-0.0275 a	*0.0038*
40% Shade	-0.0192 a	*0.0036*	-0.0203 ab	*0.0042*	-0.0145 a	*0.0041*
G. manure	-0.0122 a	*0.0077*	-0.0277 b	*0.0038*	-0.0281 a	*0.0064*
P150	-0.0256 a	*0.0079*	-0.0144 a	*0.0048*	-0.0221 a	*0.0054*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.33. Analysis of variance for the effects of cultivar, lime and growth condition on net assimilation rate and leaf area ratio of soybeans.

Effect	Net assimilation rate	Leaf Area Ratio
Cultivar (V)	*<0.0001*	*0.0782*
Lime	*0.0095*	*0.006*
Growth condition (Tr)	*<0.0001*	*<0.0001*
V x L	*0.0184*	*0.2343*
V x Tr	*0.0006*	*0.8076*
L x Tr	*<00001*	*0.0032*
V x L Tr	*0.0002*	*0.0354*

Table 5.34. Cultivar x lime x growth condition effects on net assimilation rate[a] averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	11.10 bc	*0.884*	12.65 bc	*0.299*	14.35 ab	*0.182*
80-90% FC	16.89 a	*0.769*	18.25 ab	*0.118*	18.23 a	*0.244*
40% Shade	6.51 c	*0.100*	7.05 c	*0.120*	10.32 b	*0.379*
G. manure	8.41 bc	*0.180*	9.35 c	*0.164*	3.66 c	*0.404*
P150	13.97 ab	*0.226*	20.36 a	*0.177*	18.00 a	*0.968*
	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	19.27 a	*0.930*	19.99 a	*0.187*	18.20 a	*0.147*
80-90% FC	21.23 1	*0.201*	15.56 b	*0.456*	13.51 b	*0.970*
40% Shade	12.31 b	*0.148*	14.74 b	*0.558*	12.12 b	*0.445*
G. manure	20.91 a	*0.127*	14.26 b	*0.128*	13.15 b	*0.677*
P150	23.63 a	*0.273*	16.82 ab	*0.718*	22.37 a	*0.257*

[a]Mean and standard error of the mean ($x10^{-5}$)

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

Table 5.35. Cultivar x lime x growth condition effects on leaf area ratio averaged across growth intervals 14-28 DAE and 28-42 DAE.

Growth Condition	Forrest					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	358 a	*8*	322 b	*5*	300 b	*5*
80-90% FC	292 b	*14*	294 b	*12*	264 b	*12*
40% Shade	366 a	*28*	440 a	*11*	372 a	*11*
G. manure	249 b	*10*	295 b	*11*	296 b	*11*
P150	297 b	*11*	282 b	*9*	305 b	*9*
Growth Condition	Lee					
	Unlimed, pH 4.78		Limed to pH 5.50		Limed to pH 6.00	
Control	322 a	*6*	331 ab	*32*	272 b	*6*
80-90% FC	264 b	*14*	281 b	*7*	283 b	*5*
40% Shade	348 a	*24*	378 a	*7*	406 a	*1*
G. manure	261 b	*3*	293 b	*10*	291 b	*26*
P150	255 b	*9*	312 b	*6*	286 b	*5*

In a column, means followed by a common letter are not significantly different at the 5% level by Duncan's Multiple Range Test.

Numbers in italics are standard error of the mean.

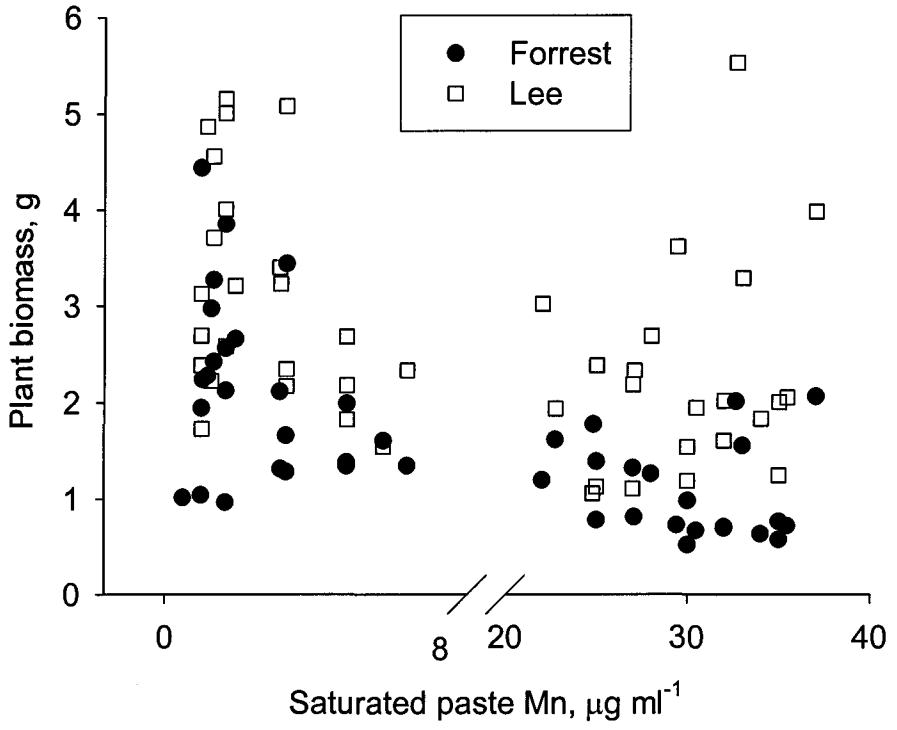


Fig. 5.25. Relation of saturated paste-Mn to plant biomass of two soybean cultivars.

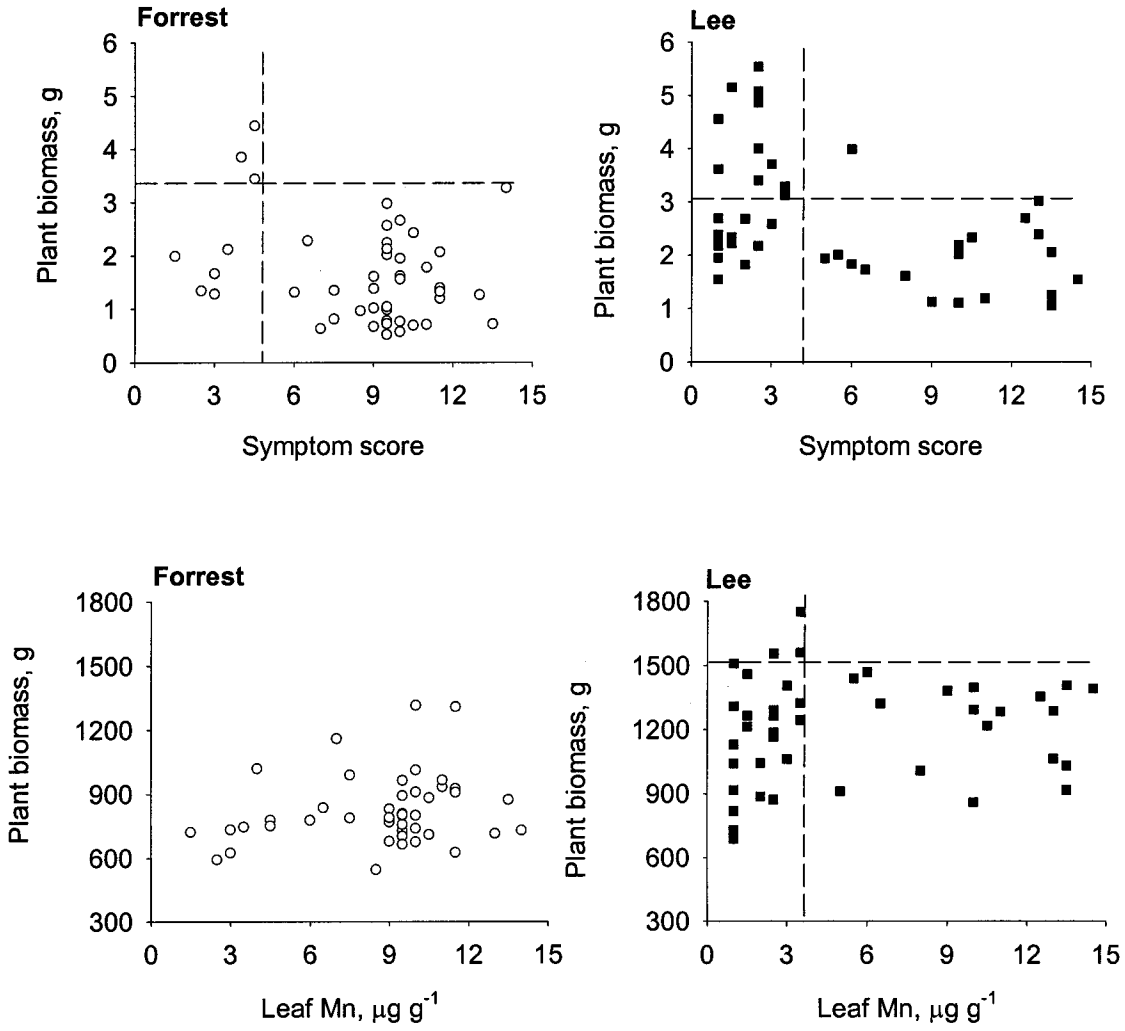


Fig. 5.26. Cumulative symptom score and leaf Mn as predictors of plant biomass production of two soybean cultivars grown over a range of soil pH and growth conditions.

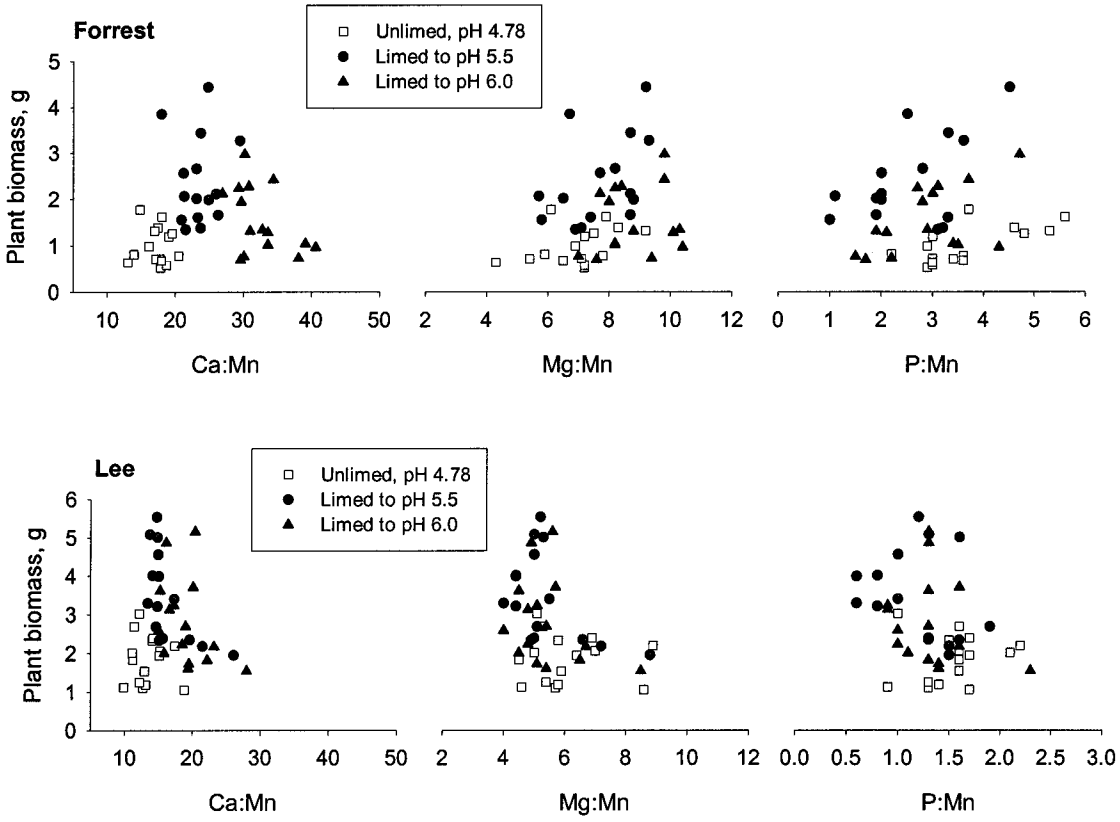


Fig. 5.27. Nutrient to Mn ratios as predictors of plant biomass production of two soybean cultivars grown over a range of soil pH and growth conditions.

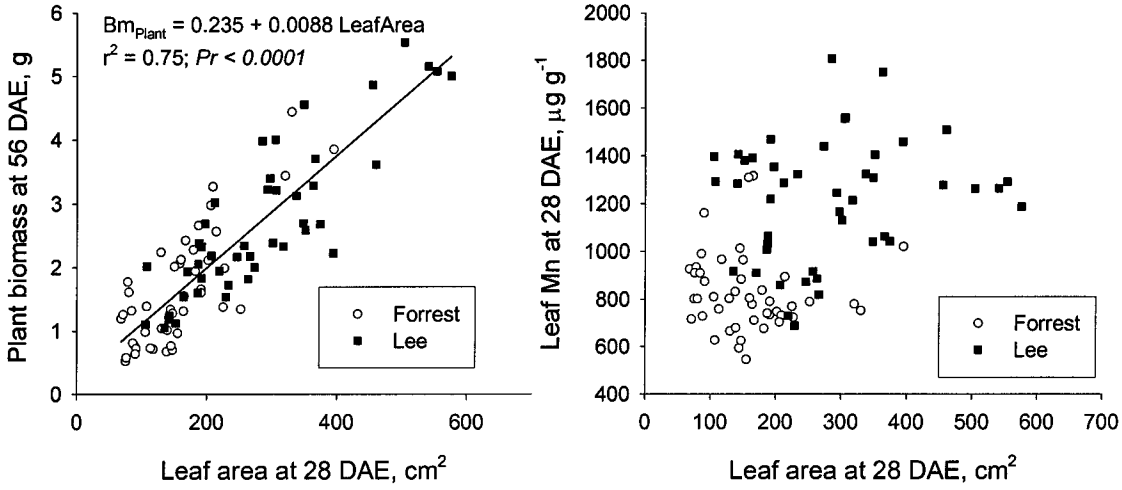


Fig. 5.28. Leaf area as a predictor of plant biomass and leaf Mn of two soybean cultivars grown over a range of soil pH and growth conditions.

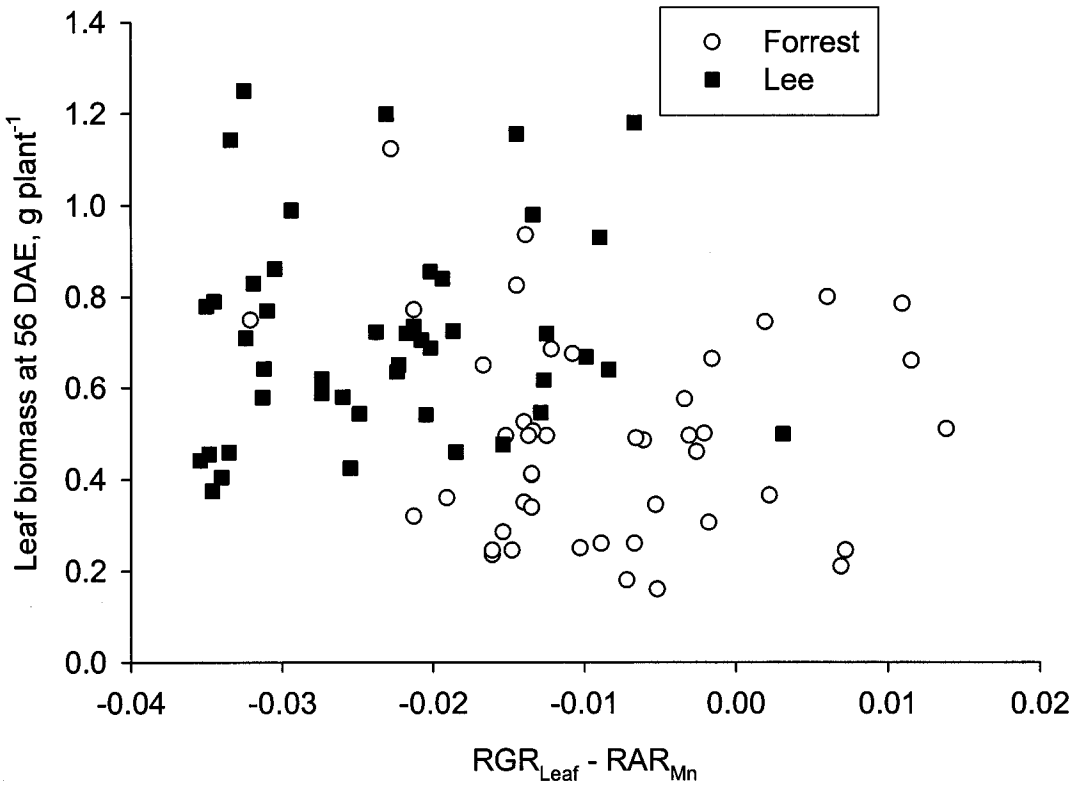


Fig. 5.29. Relation of the difference between leaf growth rate and Mn accumulation rate to leaf biomass production of soybean.

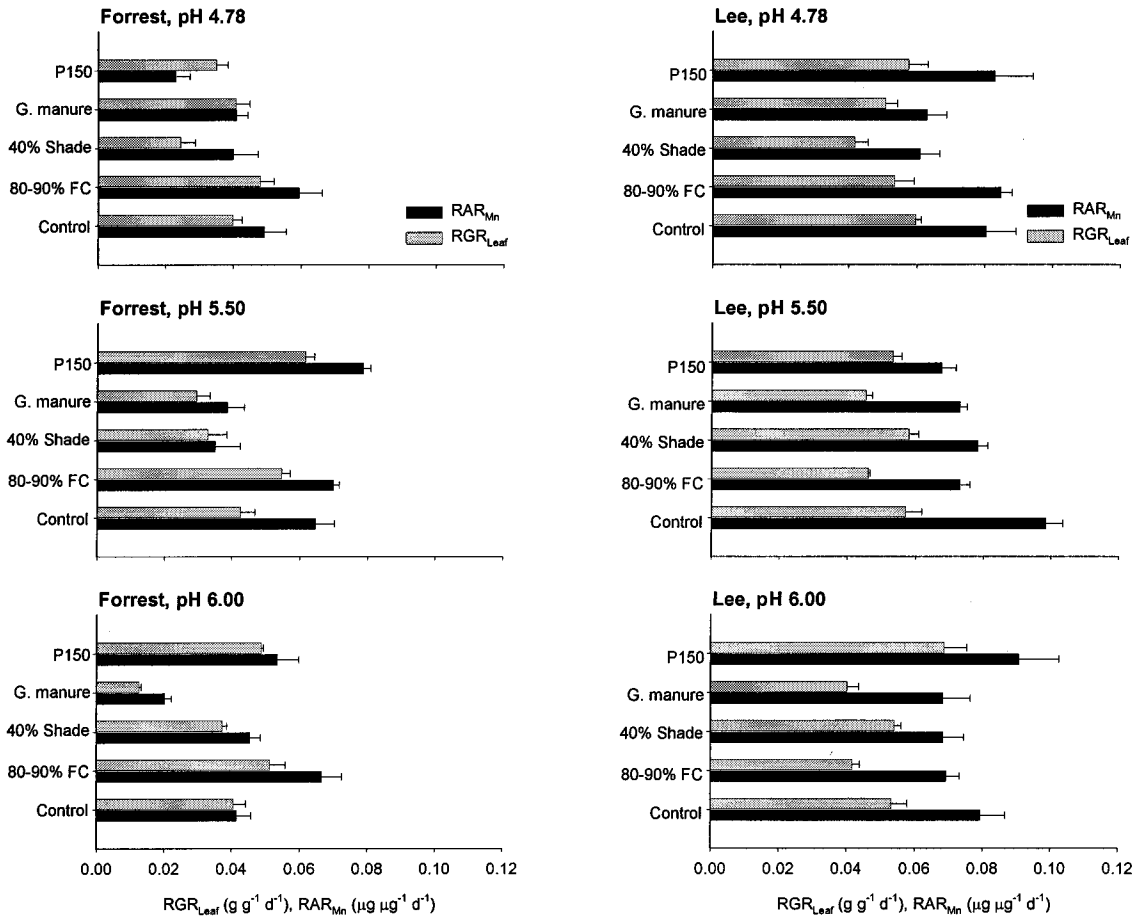


Fig. 5.30. Comparison between RGR_{Leaf} and RAR_{Mn} of two soybean cultivars grown at varying soil pH and growth conditions.

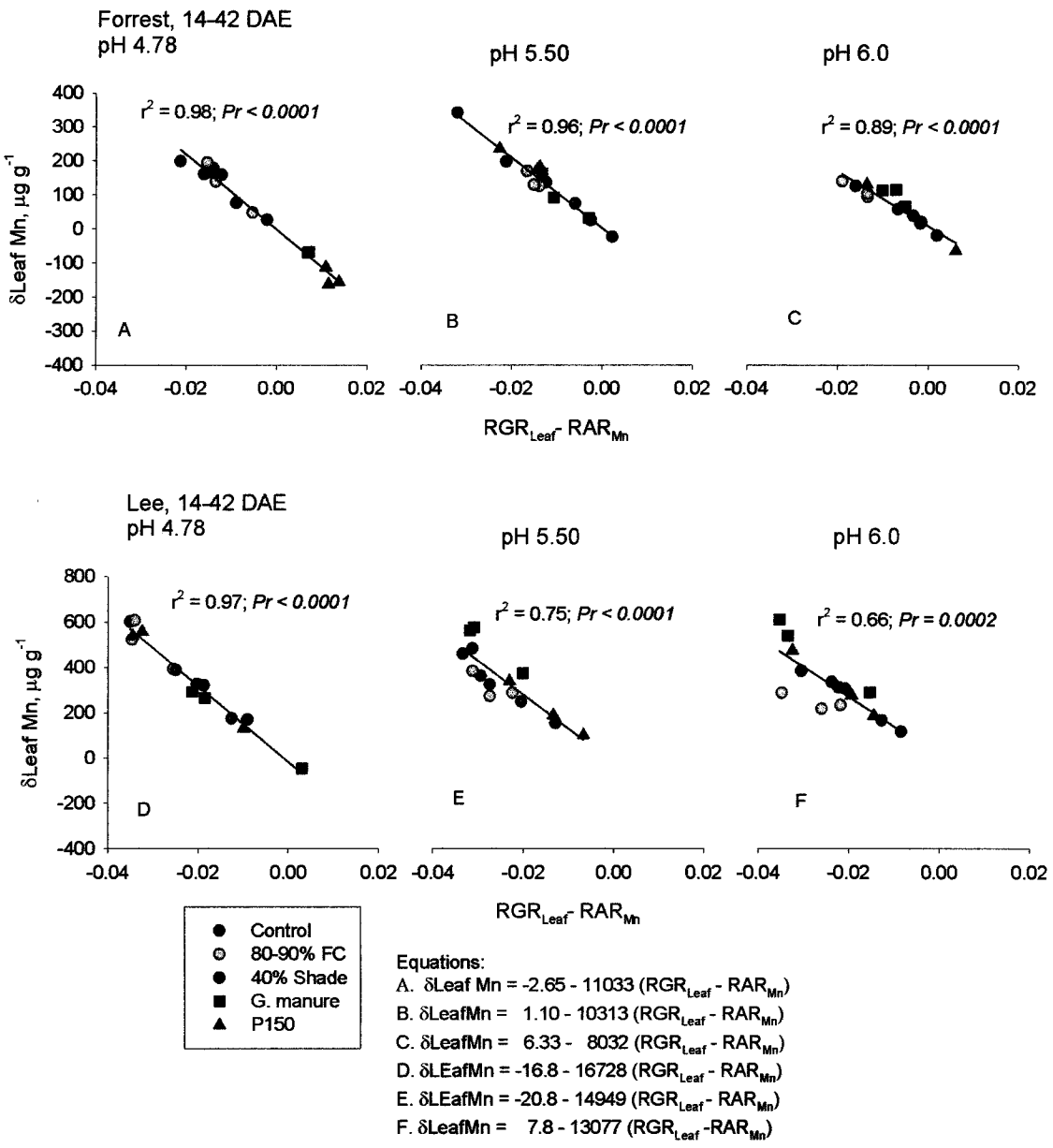


Fig. 5.31. The relation of the absolute difference between RGR_{Leaf} and RAR_{Mn} to the change in leaf Mn concentration over 56 days of soybean growth.

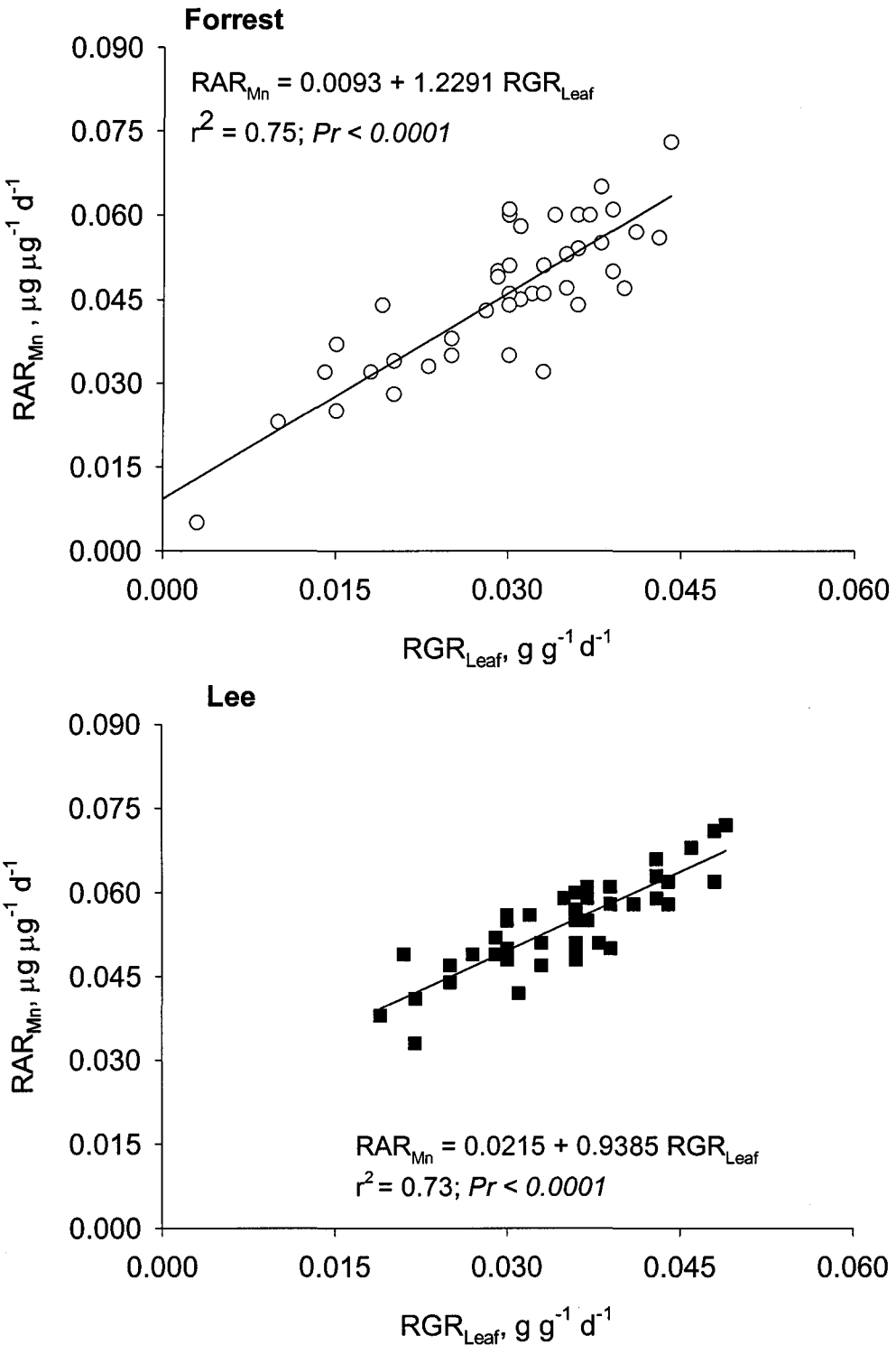


Fig. 5.32. Correlation between RGR_{Leaf} and RAR_{Mn} (mean of three growth intervals) of two soybean cultivars grown over a range of soil pH and growth conditions.

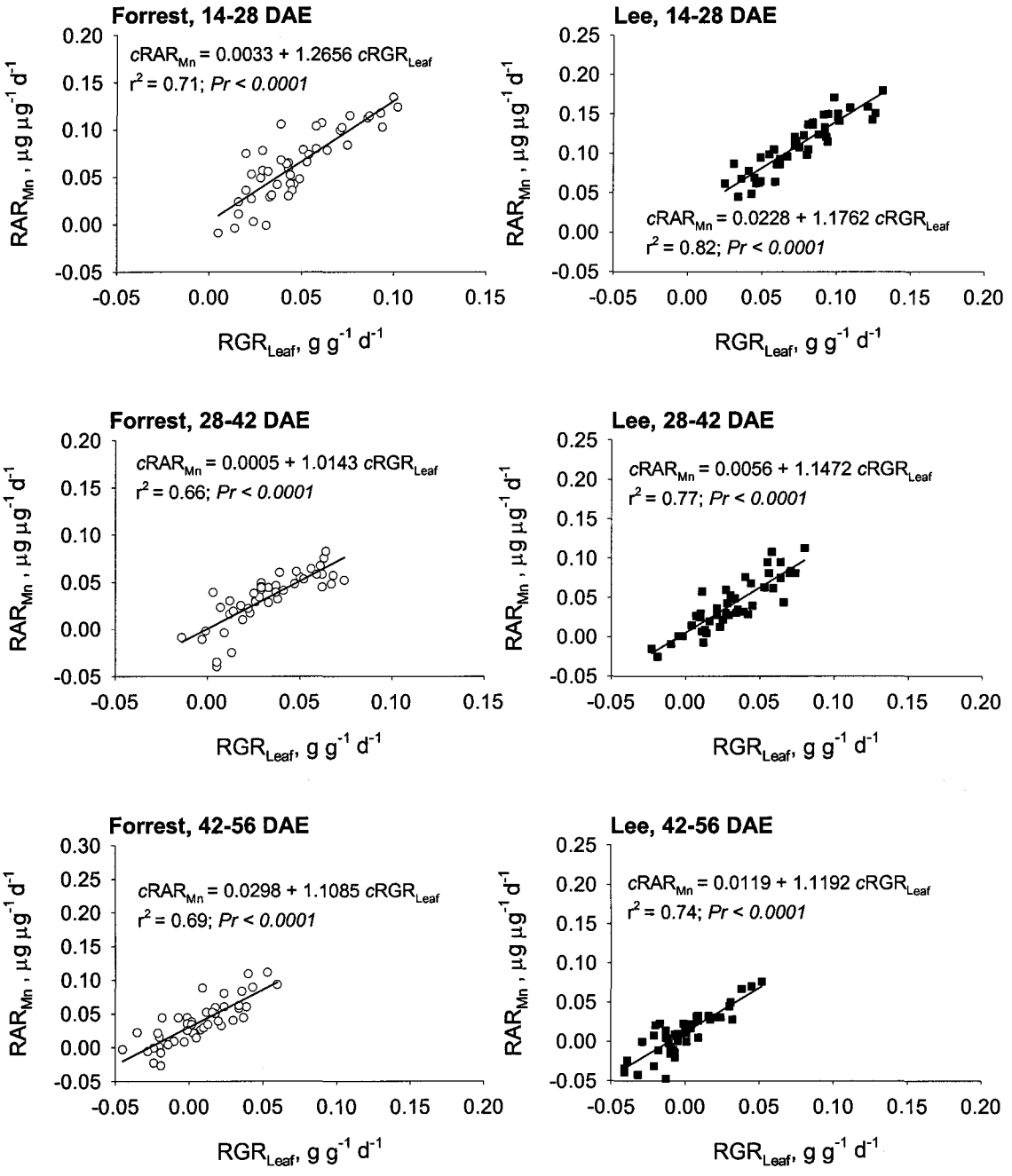


Fig. 5.33. Correlation between RGR_{Leaf} and RAR_{Mn} at three growth intervals of two soybean cultivars grown over a range of soil pH and growth conditions.

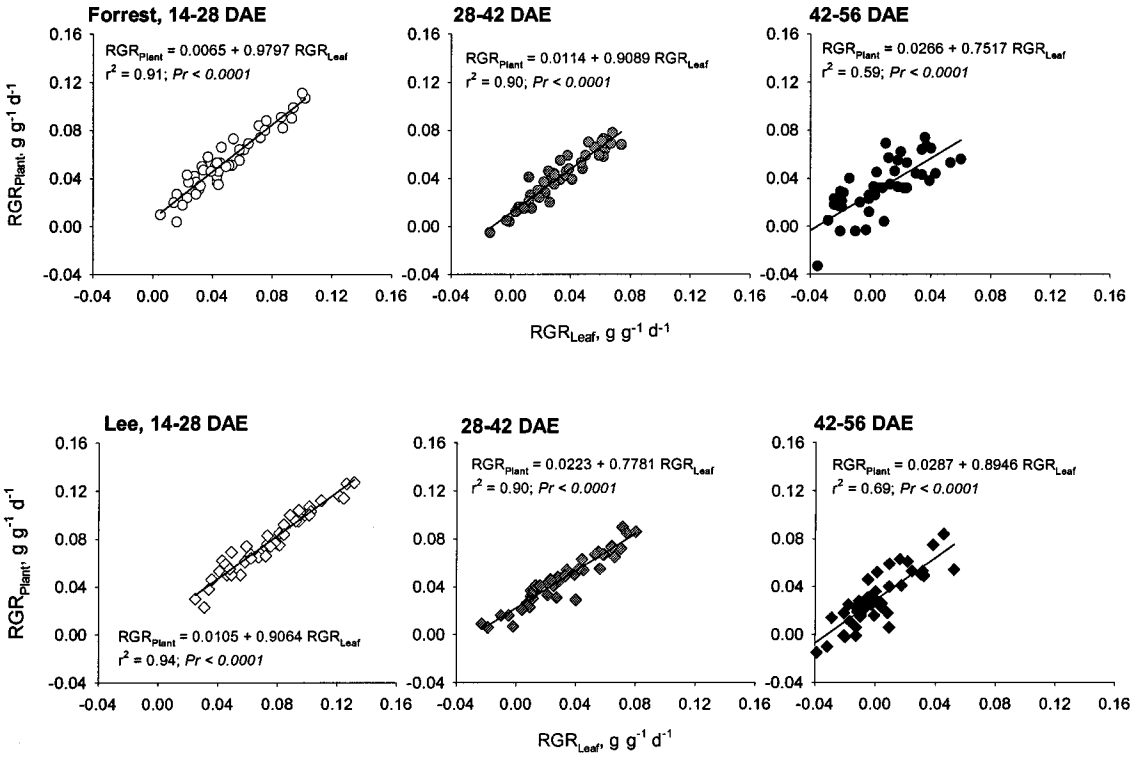


Fig. 5.34. Correlation between RGR_{Plant} and RGR_{Leaf} of two soybean cultivars at three successive growth intervals.

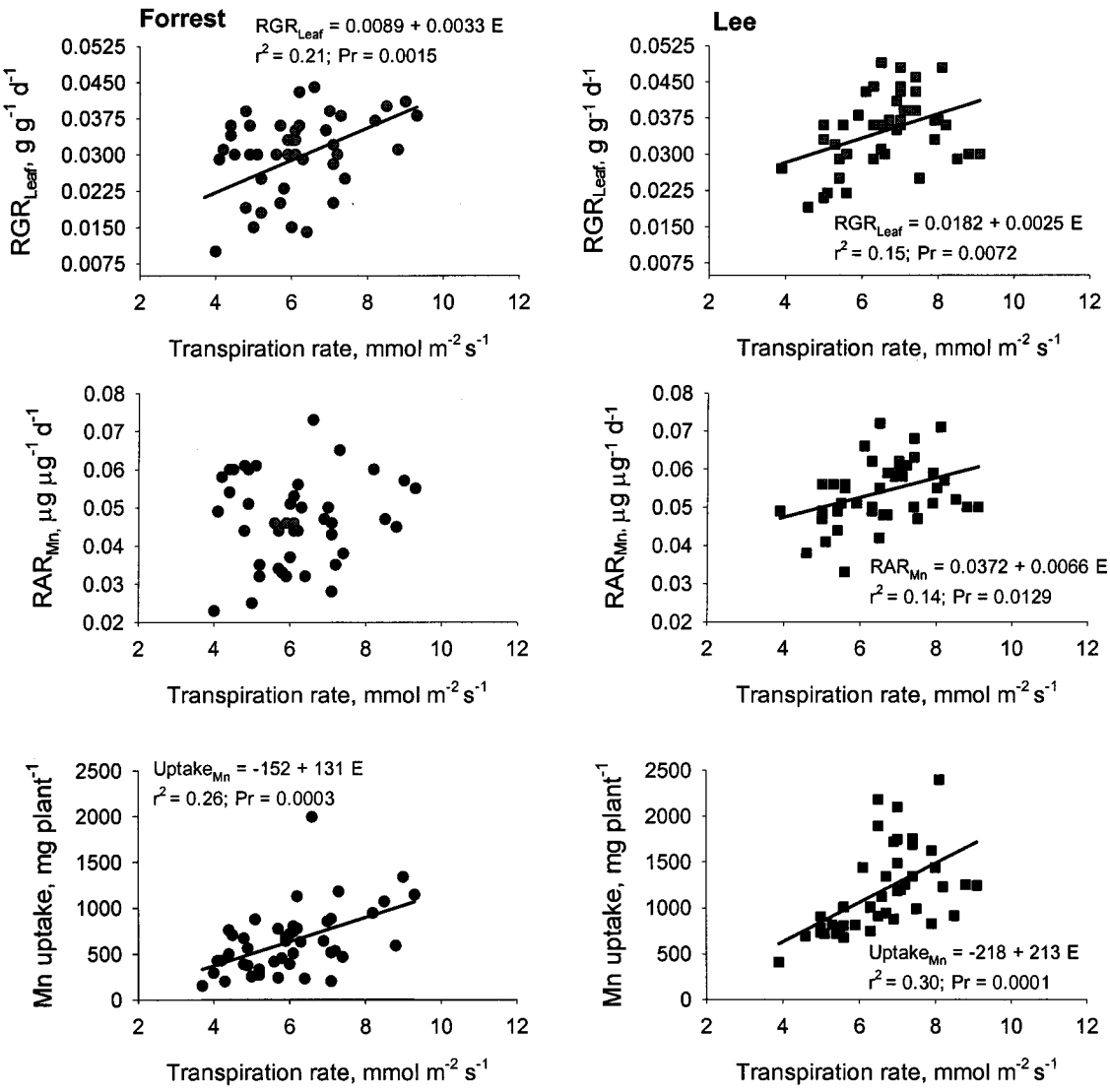


Fig. 5.35. Correlation of transpiration rate to RGR_{Leaf}, RAR_{Mn} and Mn uptake of two soybean cultivars grown at varying soil pH and growth conditions.

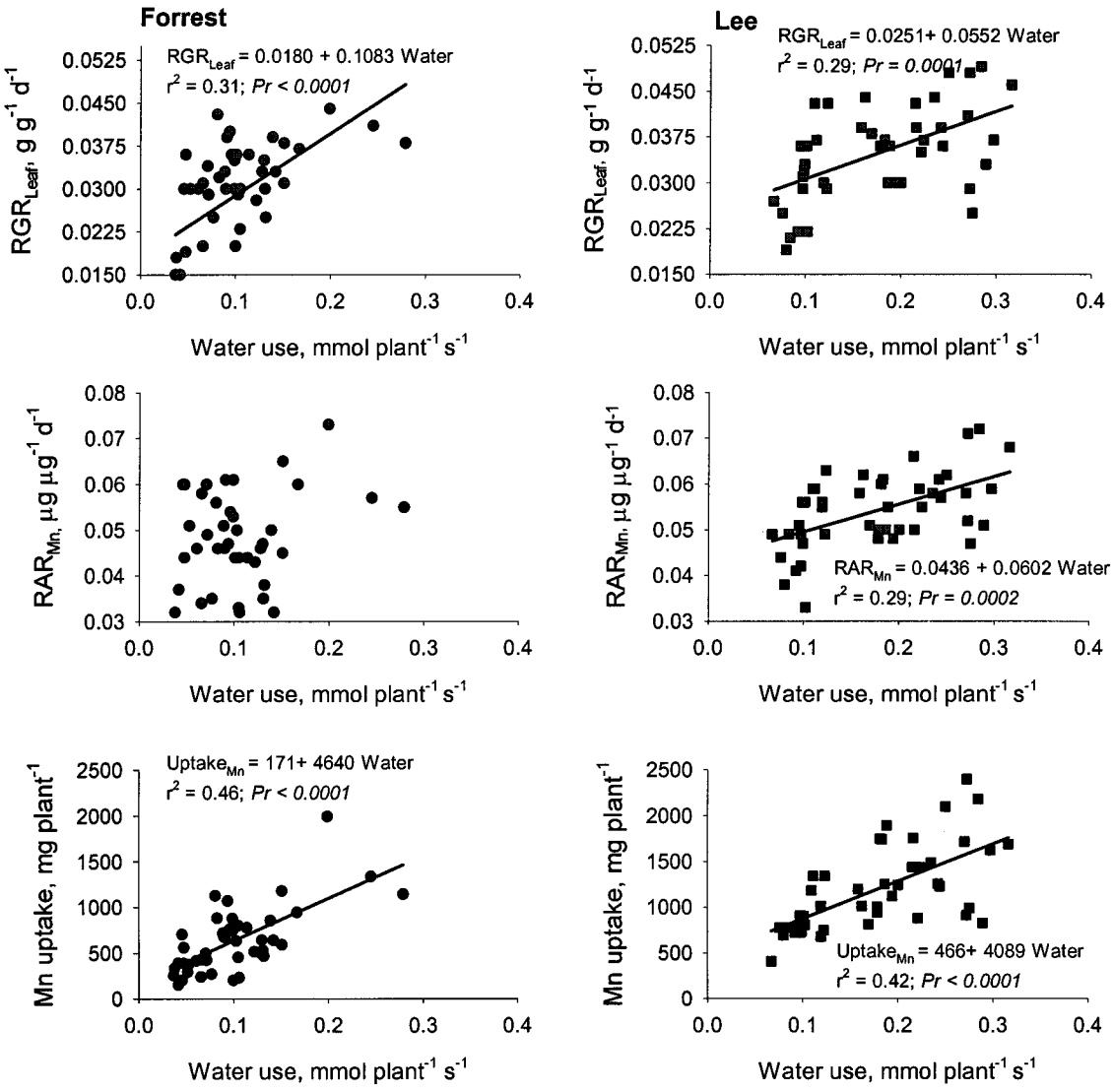


Fig. 5.36. Correlation of water use to RGR_{Leaf}, RAR_{Mn} and Mn uptake of two soybean cultivars grown at varying soil pH and growth conditions.

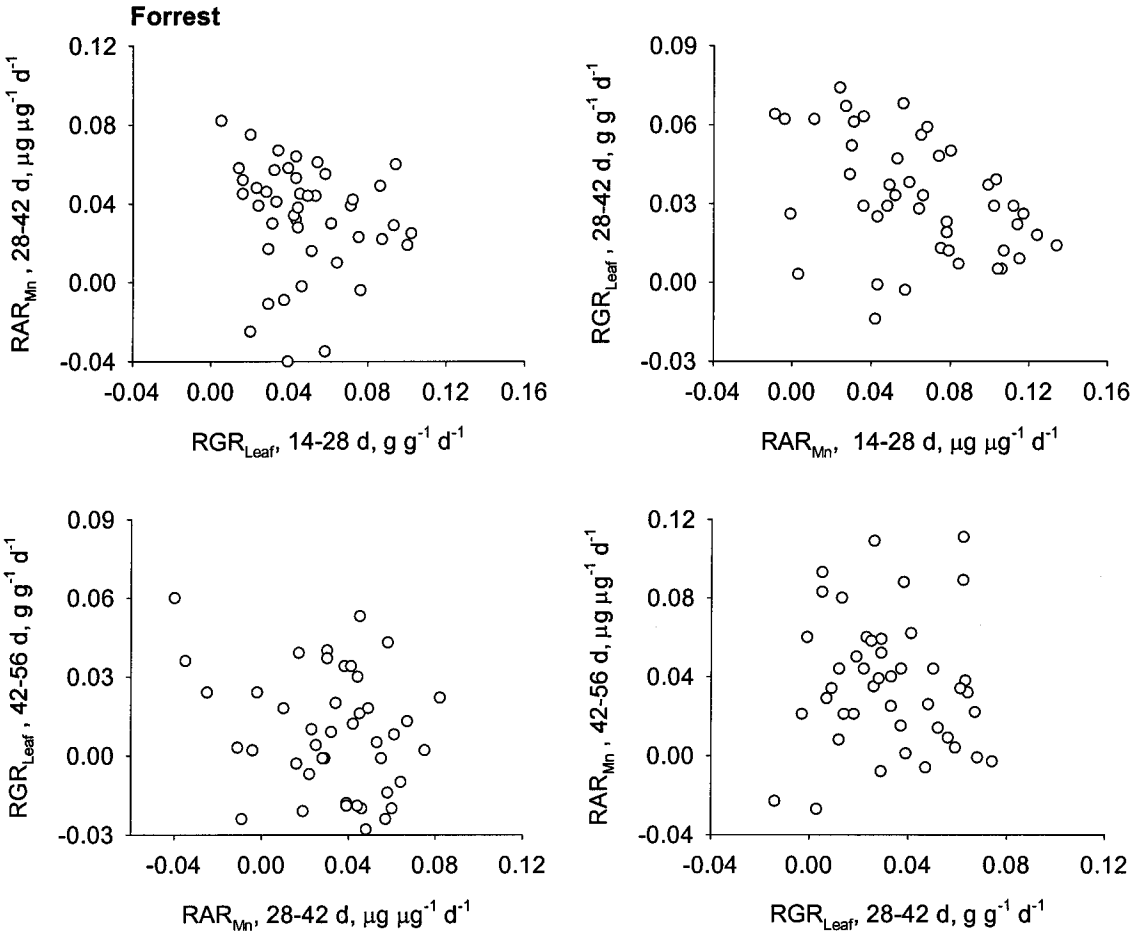


Fig. 5.37. Feedback effect of current RGR_{Leaf} on future RAR_{Mn} and feedback effect of current RAR_{Mn} on future RGR_{Leaf} of Mn-susceptible Forrest.

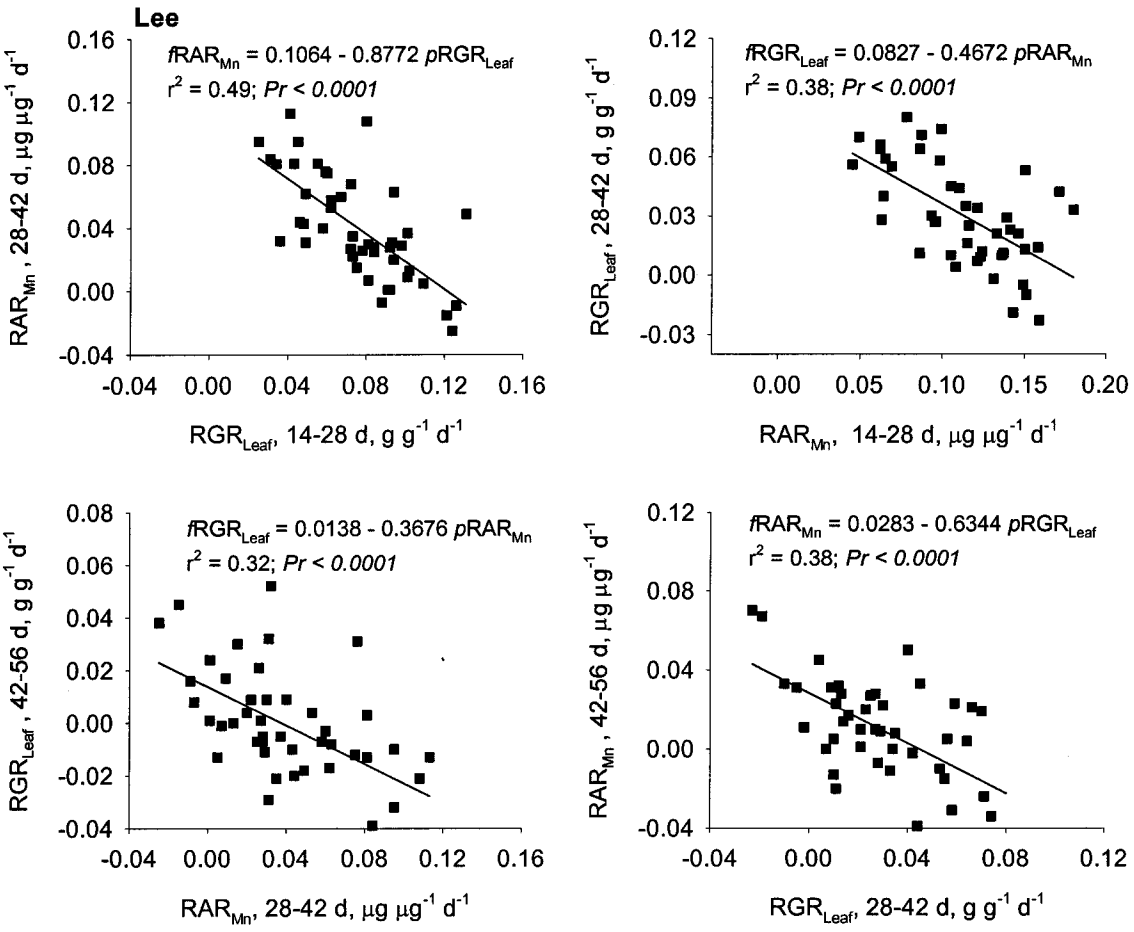


Fig. 5.38. Feedback effect of current RGR_{Leaf} on future RAR_{Mn} and feedback effect of current RAR_{Mn} on future RGR_{Leaf} of Mn-tolerant Lee.

Discussion

Predictors of the severity of manganese phytotoxicity

Several of the early studies on the phytotoxicity of Mn^{2+} have shown a significant relation between leaf Mn and total aboveground biomass accumulation, expressed either as total plant tissue produced or relative to a control (no toxicity) (Vega *et al.*, 1992; Le Bot *et al.*, 1990; Bethlenfalvay and Franson, 1989; Horiguchi, 1987; Ohki, 1976). In these studies, the attempt was to use leaf Mn as an indicator or predictor of Mn phytotoxicity. Most of these studies used one particular cultivar and grew the plants over a range of Mn concentration in a nutrient solution or one soil. In our study, we grew 2 cultivars of soybean in one soil over a range of soil pH and growth conditions including dry (80-90) % field capacity, 40% shaded, added 75 mg kg^{-1} P, and added 10 mg kg^{-1} *Leucaena leucocephala* as green manure. We considered soil pH representing levels of available and potentially toxic soil Mn, and growth conditions representing levels of potential growth rate. The expectation was that P addition would provide high growth rate potential because P enhances early plant growth. On the other hand, dry conditions and shading would restrict dry matter accumulation due to water stress and lack of sunlight, respectively. These growth conditions were expected to set different plant growth potentials without affecting soil Mn level. Green manure addition, on the other hand was expected to aggravate Mn phytotoxicity by increasing soil Mn levels regardless of soil pH. The results in Table 5.4 coincide with this expectation. Dry growth conditions, shading and P addition did not affect the amount of soil solution Mn

from the saturated paste extract. On the other hand, green manure addition significantly increased saturated paste-Mn at all pH levels, in fact nullifying the effect of soil pH adjustment on soil Mn. The expected further decrease in saturated paste-Mn with increasing pH up to 6.0 was not attained. In fact saturated paste-Mn at pH 5.5 was similar with that at pH 6.0.

Over the range of soil pH and growth conditions tested, we did not find any clear relation between saturated paste-Mn, leaf Mn or toxicity scores on aboveground biomass production. For example, plotting aboveground biomass *versus* leaf Mn concentrations showed a scatter of points, indicating no significant relationships. Symptom scores indicate a range of biomass possible at symptom scores below 6 and another range in biomass accumulation possible when symptom scores are above 6. However, there is no clear relation between symptom scores and biomass growth. Leaf area at an earlier growth stage, on the other hand was correlated with plant biomass at a later growth stage, which indicates the possibility of using leaf area in predicting the severity of Mn phytotoxicity. Changes in leaf area, however, is not specific the presence or severity of Mn phytotoxicity. As our results indicate, leaf area was decreased by reduced availability of water and increased by reduced intensity of sunlight.

The lack of correlation between total accumulated biomass and leaf Mn concentrations can be interpreted as a lack of control of Mn concentration on biomass production. This could mean that factors or processes other than the leaf Mn concentration are affecting biomass production. The processes of CO_2

assimilation and transpiration are possible candidates. We observed that assimilation rate but not transpiration rate declined with time, and that these plant processes varied with growth conditions. The effect of P was to increase assimilation rate and transpiration rate while that of dry conditions was to decrease both assimilation rates and transpiration rates. Shading on the other hand, decreased assimilation and had no significant effect on transpiration rates. At the onset of toxicity, we would expect CO_2 assimilation to decrease but not transpiration rate. This means that the plant stops producing biomass but transpiration continues so that the existing biomass can be maintained. This would mean that Mn would be taken up even when biomass production has stopped. As the toxicity progresses and the leaves become more severely damaged, then the transpiration rate is likely to decrease. It is the rate by which the plant succumb to the toxicity that will determine its final biomass and leaf Mn concentration. A susceptible cultivar would tend to have less Mn in its leaves because the leaves become severely damaged and transpiration and water use decline rapidly and so would the uptake of Mn.

Lack of control of leaf Mn on biomass production can also mean that total Mn concentration may not accurately represent the Mn that elicit phytotoxic response from the plant. Only a fraction of the total Mn (the 'active' Mn pool) may actually be toxic to plant cells. One of the proposed mechanism of tolerance to excess Mn is sequestration in the vacuole. Rufty *et al.* (1979) suggested that leaves that grow fast have the opportunity to produce larger vacuoles, which can sequester more Mn. Once in the vacuole, Mn is considered inactive and would

not injure plant cells. It has been proposed as for the detoxification of other metals, that Mn is transported as Mn-malate complex through the cytoplasm, and accepted and stored as Mn-oxalate complex inside the vacuole (Memon and Yatazawa, 1984). The complex between Mn and polyphenolics in the leaves has been shown to enhance tolerance of horticultural trees (Aoba, 1986).

Several authors showed significant relationship between plant biomass production and nutrient ratios, for example, Ca:Mn or Mg:Mn (Le Bot, *et al.*, 1990; Hue *et al.*, 2001). In our experiment, however, we did not find a high correlation between the ratios of Mn to Ca, Mg and P to biomass production. The range of leaf Ca, Mg and P concentrations in the two cultivars Forrest and Lee were 1.47-2.39%, 0.59-0.72 %, and 0.14-0.21%, respectively. Deficient to marginal range of Ca concentration in soybean leaves was found to be 0.40-0.90% at 21 days after sowing (Reuter *et al.*, 1997). For Mg, the deficient to marginal concentration range was 0.19-0.2the 5% at flowering while for P, it was 0.29-0.34% at 31 days after sowing. While Ca and Mg concentrations in the test plants exceeded the suggested marginal concentration of these nutrients for soybeans, the P concentrations were below the suggested marginal P concentration. The test plants possibly suffered from P deficiency in addition to excess Mn in the soil.

Symptom scores also did not correlate well with biomass production. An additional evidence of this is the slower symptom progression and generally lower symptom score accompanied by a slower biomass growth under dry

growth conditions. On the other end, higher biomass production with P addition was obtained regardless of the more severe symptoms than those observed under dry growth conditions. Shading also resulted in a slow progression of symptoms as well as a slower biomass growth.

The concentration of Mn in the soil solution measured by saturated paste extract was likewise a poor predictor of biomass production. This suggests that soil Mn have poor control over the phytotoxic response of the plant via biomass accumulation. Soil solution Mn represents potentially toxic amounts in the soil. The final phytotoxic response, however is determined by the level of plant tolerance to the potentially toxic amounts. This tolerance level can be further modified by environmental and nutritional conditions of the plant.

Dual feedback effects between RAR_{Mn} and RGR_{Plant}

The relation between RGR_{Leaf} and RAR_{Mn} was described by a linear function and significant for both soybean cultivars over a range of pH levels and growth conditions. This indicates the possibility of predicting RAR_{Mn} from RGR_{Leaf} of both Mn-susceptible and Mn-tolerant soybean cultivars. Data from six experiments with different levels of CO_2 and irradiance in chrysanthemum allowed Willis *et al.* (1992) to predict RAR_{Mn} from RGR_{Plant} using a linear model. We used only RGR_{Leaf} in this prediction because it was highly correlated with RGR_{Plant}, hence, relating both to RAR_{Mn} was unnecessary. The correlation between RGR_{Leaf} and RAR_{Mn} may indicate that the uptake of Mn is driven by plant growth and that RAR_{Mn} can be predicted *via* RGR_{Plant}. The reverse situation

where Mn uptake drives plant growth appears to be illogical where Mn is in excess in the growth medium. Between relative growth rate and relative accumulation rate, relative growth rate is perhaps more easily predicted from environmental conditions.

The significant correlation between RAR_{Mn} and RGR_{Leaf} can be explained by the correlation of RAR_{Mn} and RGR_{Leaf} to transpiration rates or plant water use. RGR_{Leaf} is likely driven by water intake. After cell multiplication, growth is mainly an effect of cell expansion facilitated by water inside the cell that makes it turgid. Due to the poorly regulated nature of Mn uptake, RAR_{Mn} may also be correlated with water intake. Kitao *et al.* (2001) argued that since Mn is transported from roots to shoot freely along the transpiration stream without remobilization to other organs after reaching the leaves, then the Mn concentration in the leaves should reflect the cumulative amount of transpiration. This also explains why older leaves tend to have higher Mn concentrations. Plant water use estimated from transpiration rate and total leaf area somewhat correlated with RAR_{Mn} and Mn uptake of Forrest. A more significant correlation was observed between water uptake and RAR_{Mn}, RGR_{Leaf} and Mn uptake for Lee.

It is clear from our results that concurrent RAR_{Mn} and RGR_{Leaf} are highly correlated and that this correlation tends to depreciate with time. This indicates that the uptake of Mn is initially driven by growth and at the same time, growth is influenced by Mn uptake. However, as toxicity progresses, one may exert more impact over the other so that the correlation of concurrent RGR_{Leaf} and RAR_{Mn}

becomes weaker with time. We hypothesized that a current RGR_{Plant} may have an impact on the future RAR_{Mn} and the current RAR_{Mn} may have an impact on the future RGR_{Plant}. With time the impact of one over the other becomes more important so that the concurrent correlation becomes weak as the toxicity progresses.

The central hypothesis of this research work is that the phytotoxic response of plants to excess Mn in the growth media is governed by water use and may be exhibited as a dual feedback interaction between RAR_{Mn} and RGR_{leaf}. The role of water use both in growth and Mn uptake and the observed correlation of estimated water use to RGR_{Leaf}, RAR_{Mn} and Mn uptake supports this hypothesis. A negative effect of current RGR_{Leaf} on future RAR_{Mn} and the simultaneous negative effect of current RAR_{Mn} on future RGR_{Leaf}, referred to as "dual feedback effect" was exhibited by Mn-tolerant Lee. The feedback effects were described by a linear function with negative slopes and r^2 ranging from 0.32 to 0.49. Soybean cv. Forest, however, did not show this kind of dual feedback effect. The cv. Forrest has been tested very susceptible to excess Mn in the growth media (Carter *et al.*, 1975, 1976)). This susceptibility may have caused the severe impact of RAR_{Mn} on RGR_{Leaf} and that this impact occurred during the early growth period. Since we used a 2 week- growth interval as observation window for RAR_{Mn} and RGR_{Leaf}, we may have failed to capture this expected feedback effects in the cv. Forrest.

Based on the observed relationships between RAR_{Mn} and RGR_{Leaf}, we propose a dynamic model of Mn phytotoxicity (Fig. 5.39). For a particular plant growing in a medium with excess Mn, there is a particular biomass and Mn concentrations associated with each growth stage designated by Time 1 to n. For each growth interval T_1-T_2, there is a corresponding RGR_{1-2} to produce $Biomass_2$ from $Biomass_1$. Also, there exists an RAR_{1-2} that explains the change from $Conc_1$ to $Conc_2$. Our model suggests that there is no direct connection between biomass and leaf Mn concentration, and that each of these parameters are controlled by underlying growth rate and accumulation rate processes which affect each other continuously and negatively to produce the future biomass and leaf Mn concentration.

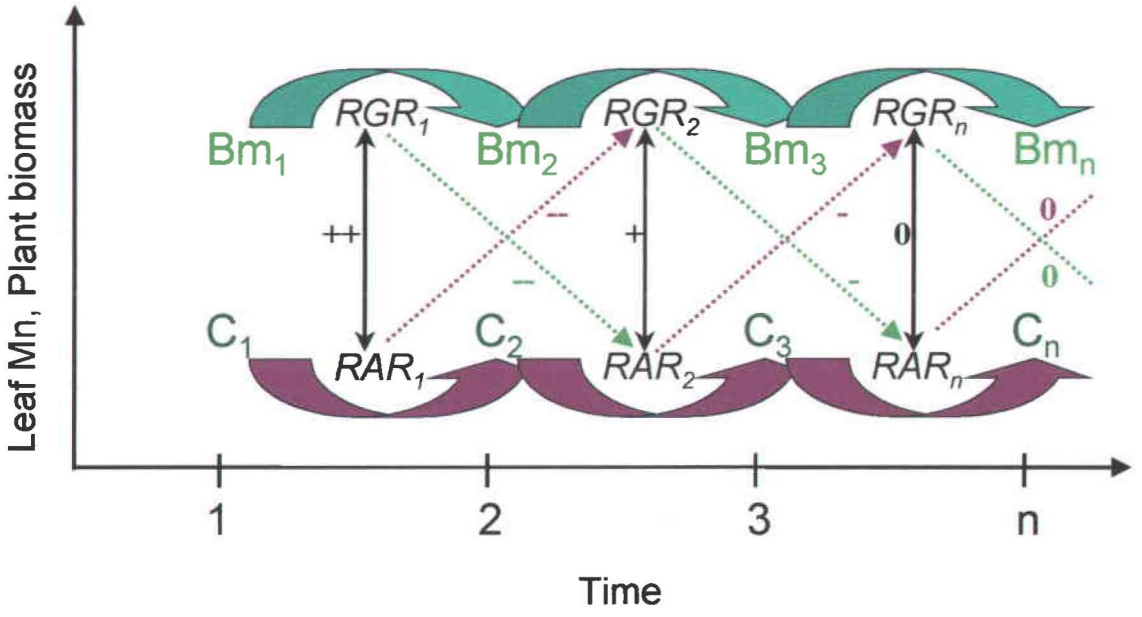


Fig. 5.39. A proposed dynamic model of Mn phytotoxicity showing dual feedback interaction between plant growth rate and Mn accumulation rate.

Note: + indicates positive correlation, - indicates negative correlation and 0 indicates no correlation.

Conclusions

It is clear from our study that the use of soil or leaf Mn concentration to diagnose the degree of Mn phytotoxicity has limited use particularly over a range of growth conditions affecting both plant growth rate and Mn uptake rate. The role of saturated paste-Mn was only to indicate potential toxicity; the actual phytotoxicity tends to depend on the capability of the plant to tolerate high tissue Mn, as indicated by less severe toxicity symptoms in Mn-tolerant Lee regardless of higher tissue Mn concentration in comparison with Mn-susceptible Forrest. The saturated paste-Mn, however, appears to be an accurate soil test to determine potential toxicity. Since Mn uptake is poorly regulated and appears to accompany the plant's demand for water, it would mean that the concentration of

Mn in the soil solution would have a significant impact on Mn uptake rate. In fact, Sadana and Claasen, 2000) predicted that Mn uptake would be most sensitive to Mn in the soil solution.

We conclude based on the results of this study that growth conditions such as water stress, shading and the addition of P and green manure affect the phytotoxic response of plants grown under excess Mn occur *via* the effect of such conditions on plant growth rate and Mn accumulation rate. The effect of P addition is to increase plant growth rate and decrease Mn accumulation rate while that of shading and green manure addition is to decrease both plant growth rate and Mn accumulation rate. Dry growth conditions, on the other hand, increased plant growth rate and increased Mn accumulation rate of Mn-susceptible cv. Forrest. The opposite effect was observed in Mn-tolerant cv. Lee These effects were observed mostly where soil pH was adjusted from pH 4.78 to pH 5.5, leading to a 20-fold decrease in saturated paste-Mn.

At the same point in time, Mn uptake rate and leaf growth rate were highly correlated especially during early plant growth. This is consistent with the poorly regulated nature of Mn uptake, being driven by growth and linked to water uptake. In fact, both relative growth rate and relative accumulation rate of Mn as well as total uptake of Mn were significantly correlated with the estimated water use by the plant.

The lack of correlation between total biomass accumulation and leaf Mn concentrations may be due to the apparent lack of direct control between

biomass growth and Mn uptake. We explain this *via* a dynamic model of Mn phytotoxicity based on the dual feedback effect of RGR_{Leaf} on RAR_{Mn} observed to be significant for Mn-tolerant Lee. While current RGR_{Leaf} and RAR_{Mn} are positively correlated, the current RGR_{Leaf} exerts a continuous and negative effect on future RAR_{Mn}. At the same time, the current RAR_{Mn} exerts a continuous negative effect on future RGR_{Leaf}. This dual feedback interaction between RGR_{Plant} and RAR_{Mn} results in a final biomass, which is not necessarily related to a current or past leaf Mn concentration. Due to the susceptibility of the cv. Forrest to excess Mn, this dual feedback effect was likely to occur during the early growth period before our measurements and thus we were not able to capture this effect.

Implications for Diagnosis and Management

A dynamic model of phytotoxic response to excess Mn (Fig. 5.39) highlights the importance of the rates of plant growth and the corresponding uptake of Mn and the interaction with time between these two events. Diagnosing potential Mn toxicity in the soil, therefore, may need to focus on soil tests that will relate closely to the potential for Mn uptake rate by the plant. Likewise, the main goal for managing excess Mn in acid soils may be set to either or simultaneously, increase growth rate or decrease Mn uptake rate. Management strategies that would either increase plant growth rate or decrease Mn uptake rate are expected to enhance tolerance to excess Mn in the growth medium. Our experimental results demonstrate that P application can increase early plant growth rate, and

decrease uptake rate of Mn, with the net result of enhanced tolerance to tissue damage caused by excess Mn. Interestingly, dry growth condition had opposite effects on plant growth rate and Mn accumulation rate of Mn-susceptible and Mn-tolerant cultivars. The growth rate of Mn-susceptible variety was increased while that of the Mn-tolerant cultivar was decreased by dry growth conditions. An increase in growth rate due to dry growth condition was however accompanied by increased Mn accumulation rate. Although dry growth conditions increased accumulation rate of Mn in the Mn-susceptible cultivar, it also delayed the progression of toxicity symptoms. A decrease in plant growth rate associated with dry growth conditions is an obvious disadvantage for yield production. However, it may be possible to breed or select for drought tolerant plant that would require less water for the same growth potential, and therefore absorb less Mn and perform well under conditions of excessive Mn in the soil. Shading decreased both relative growth rate and Mn accumulation rate but slowed down the progression of phytotoxic symptoms. This growth condition might be relevant in situations where growing seasons are associated with the frequency of rains and intensity of sunlight. Rainy season with less intense sunlight could offer an advantage to the crop grown in well-drained, high-Mn soil. The effect of green manure is to intensify the potential toxicity by increasing Mn concentration in the soil solution. It might be possible, however, to select organic materials that will supply nutrients to plants without increasing Mn level in the soil. In field situation, the growth rate of the plant is inherently higher than in the greenhouse so that the effect of increased potential toxicity of available soil Mn might be less.

Chapter 6

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

It is imperative for today's world to tap every available land resource to produce food for the booming population in every nation. One such resource, untapped for its full potential are the acid uplands, which are difficult yet fragile ecosystems that need urgent research attention if we are to use them in the future. Acid soils comprise a significant proportion of the land area in Asia (30%), Africa (39%) and America (55%) (Sanchez and Salinas, 1981). The acidity in these soils associates with deficiencies of nutrients such as N, P, K, Ca, Mg and Mo and toxic amounts of Al and Mn.

The occurrence of excess Mn in soils can be the effects of residual Mn oxides, acid soil pH and reduced soil status. Parent rock of basaltic origin, through weathering, results in residual accumulation of Mn minerals in the soil. Temperature and rainfall in the tropics facilitate fast weathering and leaching of mobile bases, hence acidifying the soil. The addition of fresh organic materials and flooding provides electrons for reduction processes and restricts O_2 presence in the soil, hence providing for reduced soil status. These processes lead to Mn^{2+} concentrations in excess of crop requirement, eliciting phytotoxic response by plants exposed in this environment.

Although excess Mn in the soil can be measured by soil tests such as saturated paste extract, water, Mehlich 3, DTPA and other chemical extractants, the resulting value can be only interpreted as potential Mn toxicity. Manganese phytotoxicity can be measured, in principle, through growth reduction or leaf Mn concentration, the critical value of which can vary between species or between cultivars of the same species. To complicate matters, nutrients (Si, Ca, Mg, P, Fe) and environmental conditions (sunlight, temperature, moisture) further modify this response. Therefore, potential toxicity measured as available Mn in the soil is not always equivalent to phytotoxicity expressed as symptoms or growth and yield reductions. Herein lies the difficulty of using soil tests or plant tests alone to diagnose the presence and predict the severity of Mn phytotoxicity.

A series of experiments were designed to fulfill three main objectives: 1) diagnose the presence and predict the severity of potential Mn toxicity and phytotoxicity in acid soils using indicators, soil and plant analyses, and symptoms; 2) investigate the dynamics of Mn phytotoxicity through measurement of the interaction between plant growth rate and Mn accumulation rate over a range of growth conditions; and 3) evaluate the effectiveness of lime, P, manure, mulch and cultivars in alleviating Mn phytotoxicity by using rates of plant growth and Mn accumulation as the main criteria. Related to objective 1, we hypothesized that plant analysis, soil analysis or symptom scores may not accurately diagnose the presence or predict the severity of Mn phytotoxicity over a range of growth conditions that modify growth rate. Our central hypothesis to fulfill objective 2 is that Mn phytotoxicity is a dynamic process governed by water

use and exhibited as continuous negative interactions between plant growth and Mn accumulation. The tendency of most plant species to absorb Mn beyond their requirement for normal growth and functioning, suggests a poorly regulated nature of Mn uptake. A continuous accumulation of Mn in the leaves, leading to a substantially higher concentrations in older (actively transpiring for a longer period of time) compared with younger leaves further suggests the relation of water use by the leaves to Mn uptake into the tissue. Enhanced tolerance to excess Mn by manipulating environmental factors and nutrient levels in the growth medium can be explained by an increase in potential plant growth rate set by the given growth conditions. High growth rate will tend to accompany high accumulation rate of Mn through water use so that during the progression of Mn phytotoxicity, plant growth rate and Mn accumulation rate will interact continuously and negatively, with the final phytotoxic response dependent on these interactions. A consequence of this dynamics is that the resulting biomass or yield may or may not be related to the concentration of Mn in the leaves. If this dynamics truly occur, then it is possible to manage excess Mn by devising management strategies that maximizes growth rate and minimizes Mn accumulation rate, which is what objective 3 relates to. We propose that this dynamics be exploited in the identification of options for managing excess Mn in acid soils. Because high plant growth rate is to an extent equivalent to high biomass production or yield, the management goal would be to maximize growth rate. Part of this growth rate maximization would involve minimizing

accumulation rate of Mn, thus avoiding the negative impact of Mn accumulation on plant growth.

A fundamental technique used in testing our hypotheses is growth analysis, with relative growth rate as one of its basic components. Relative growth rate was calculated as a measure of how fast an existing biomass accumulates additional biomass within specific growth intervals. Likewise, relative accumulation rates gave a measure of how fast plants with an existing amount of Mn in the leaves accumulate additional Mn. Relative growth rate was further broken down into two components: net assimilation rate and leaf area ratio.

Field Experiments

The experiment described in Chapter 3 is preliminary to confirm suspected Mn toxicity in an acid soil in *Barangay* San Antonio, a small village in Isabela, in northern Philippines. The soil is Rugao series, classified as *fine, isohyperthermic Typic Kandiudalf* (US Soil Taxonomy), very acid, low in exchangeable bases and high in exchangeable Al and saturated paste-Mn. Diagnostic tools such as soil indicators, soil and plant analysis, toxicity symptoms and plant growth and yield measurements were used to assess the potential toxicity and phytotoxicity of Mn in soybeans. We used soybean cultivars (PSB Sy2 and PSB Sy6), lime and green manure as means to modify the phytotoxic response of soybeans to excess Mn in the soil. Manganese was highly likely present in excess amounts as indicated by the presence of Fe-Mn concretions

and extremely acid pH of 4.3 combined with the possibility of excess soil moisture during the typhoon seasons. Extractable Mn by water and KCl were in excess of a suggested critical value of 2.5 mg kg^{-1}. The high soil test for Mn was associated with slower growth, reduced biomass production and grain yields, and leaf Mn concentrations exceeding a critical value of 500 mg kg^{-1} for soybean. Liming reduced the concentration of leaf Mn as well as extractable Mn in the soil. These reductions were accompanied by increased biomass production and grain yield. Peanut green manure had similar but less pronounced effects on leaf Mn, biomass production and grain yield although there was no change in extractable soil Mn. The cultivar PSB Sy2, with greater biomass production and grain yields than PSB Sy6 highlights the effectiveness cultivar selection in alleviating Mn phytotoxicity in an acid soil.

Chapter 4 describes another field experiment conducted at the same site in *Barangay* San Antonio, where options for managing excess Mn in acid soils were tested using the same soybean cultivars as the test crop. Cultivar, liming, addition of P, water (through mulching) and manures (chicken and green manure) were expected to provide a range of growth rate conditions that will enhance tolerance to excess Mn in the soil. It was hypothesized that the most successful management was one or combinations of these options that will maximize growth rate and minimize Mn accumulation rate. Liming at 2 t ha^{-1} neutralized considerable amounts of exchangeable Al, but not saturated paste-Mn. Mulching and P addition had no effects on soil Mn while manure addition caused a two- to three-fold increase in saturated paste-Mn. Soybean cv. PSB

Sy6 was more responsive to management treatments in terms of leaf growth rate and Mn accumulation rate. Manure and P application generally increased leaf growth rate of both cultivars. Leaf growth rate was higher in manure treatment compared to P and higher in chicken manure compared to green manure only in PSB Sy6. Accumulation rate of Mn was increased only by green manure application in PSB Sy2. In PSB Sy6, green manure and P application increased Mn accumulation rate. Between P and manure, the latter gave higher Mn accumulation rate. Between chicken manure and green manure, the former gave higher accumulation rate in PSB Sy6.

Results indicate that the increase in growth rate tends to be accompanied by an increase in accumulation rate of Mn and that this increase in growth rate translates to increased yields even when levels of soil solution Mn were increased as in the case of manure addition. Biomass production and grain yield in the manure treatment far exceeded those in the P and higher lime treatment. Between green manure and chicken manure, the latter was more effective in increasing biomass and grain yield. The difference in manure effect was attributed to the higher nutrient content, and therefore, higher nutrient supplying capacity of chicken manure compared to green manure.

Greenhouse Experiment

The dynamics of plant growth, Mn uptake and phytotoxicity was investigated in an acid manganiferous Oxisol in Hawaii using soybean cultivars Forrest and Lee as the test plants. The soil is Wahiawa series, a clayey,

kaolinitic Isohyperthermic, Rhodic Eutrustox, very acid (pH 4.78) and known to contain about 17 g kg^{-1} total Mn (Hue, 2001). We hypothesized that the dynamics of the phytotoxic response to excess Mn is governed by plant water uptake and expressed as continuous and negative interaction between relative growth rate and relative accumulation rate of Mn, which determines the final biomass and leaf Mn concentration that may or may not be related to each other. Lime levels to adjust soil pH represented levels of potential Mn toxicity while P, dry, shade and manure treatments represented levels of potential growth rate. Lee represented a Mn-tolerant soybean variety while Forrest represented a Mn-susceptible variety, which enabled comparison of the dynamics of Mn phytotoxicity between tolerant and susceptible varieties.

Liming reduced saturated paste-Mn by 20-fold. Green manure addition increased saturated paste-Mn from about 25 to 35 $\mu g\ ml^{-1}$ in un-amended soil, maintaining this level even where pH was adjusted to 5.50 and 6.00. Lee tended to have higher leaf Mn but also higher rates of leaf/plant growth and Mn accumulation than Forrest. Phosphorus addition generally increased while green manure addition and shading generally decreased rates of leaf growth rate of both cultivars. Dry growth conditions, on the other hand increased leaf growth rate in cultivar Forrest and decreased leaf growth rate in cv. Lee. The effect of growth conditions on Mn accumulation rate in cv. Forrest tended to differ with pH. The addition of P decreased Mn accumulation rate in unlimed soil while shading and green manure decreased Mn accumulation rate at pH 5.50. At pH 6.00, dry growth conditions increased while green manure decreased Mn accumulation

rate. In cv. Lee, all growth conditions decreased Mn accumulation rate only at pH 5.50.

Diagnosis and Prediction of Mn Phytotoxicity

The potential toxicity of Mn in the acid soil of San Antonio was diagnosed with soil indicators, soil analysis (water-extractable Mn, using 2.5 mg kg^{-1} as critical value)) and plant analysis (leaf Mn, using 500 mg kg^{-1} as a critical value). Soil extractable Mn, leaf Mn or leaf nutrient ratios with Mn, and symptom scores were inaccurate predictors of the severity of Mn phytotoxicity in terms of plant biomass or grain yield particularly in the second field experiment and in the greenhouse experiment where growth conditions were expected to establish considerably wide range of growth rates. The poor correlation between soil Mn or leaf Mn with biomass or grain yield reflects anomalous response of the soybean cultivars to the different growth conditions. For example, manure application increased soil extractable Mn and leaf Mn but also increased plant growth rates, biomass production and yield in the field experiment. Water stress, on the other hand, reduced leaf Mn, but also reduced phytotoxic symptoms, plant growth rate and biomass production in cv. Lee.

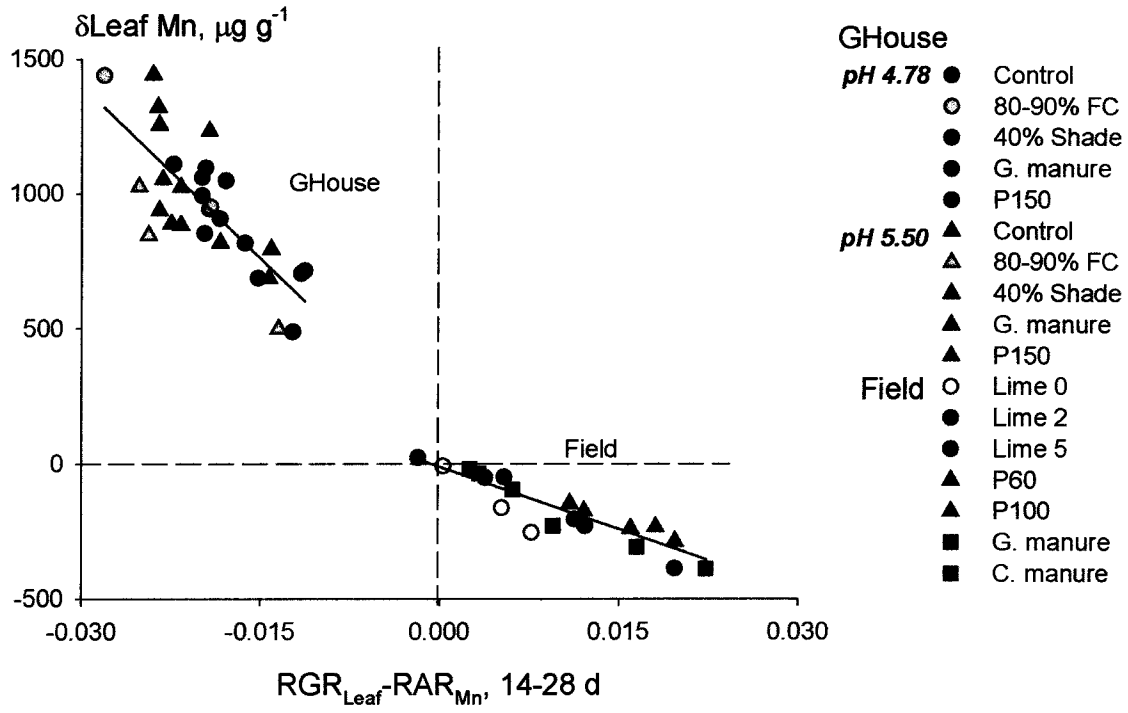
In the first field experiment where treatments essentially include lime (with or without), green manure (with or without) and two cultivars, leaf Mn and symptom scores were correlated with grain yield. This is probably because the range of growth rate established by the treatments was very narrow.

Dynamics of Mn Phytotoxicity

The lack of correlation between leaf Mn and biomass is probably due to the lack of direct control between biomass growth and Mn uptake. The lack of correlation can be explained by a proposed dynamic model of Mn phytotoxicity response based on a continuous negative interaction between relative growth rate and relative accumulation rate of Mn. This model predicts that concurrent growth rate and accumulation rates are positively correlated possibly due to a more fundamental linkage of growth and Mn uptake through water uptake. However, current growth rate has a negative effect on future accumulation rate and at the same time, current accumulation rate has a negative impact on future growth rate. This dynamics, called the 'dual feedback effect' between rates of relative growth of the plant and accumulation of Mn was observed in soybean cv. Lee but not cv. Forrest, apparently because of the susceptibility of cv. Forrest to excess Mn. This dynamics could have occurred fast and early in the plant growth and was not captured in the 2-week growth interval that was used in the growth analysis.

In the field experiment, current leaf growth rate influenced future Mn accumulation rate negatively and significantly. Current Mn accumulation rate had similar effects on future relative growth rate. A major difference between the greenhouse and field situation is the level of soil extractable Mn, which was about 20 times as much in Wahiawa than in Rugao soil series. Liming the soil to pH 5.5 reduced soil Mn similar to that of soil Mn in un-amended Rugao. A consequence of high extractable Mn in Wahiawa is that soybeans exhibited Mn

accumulation rates that were much higher than leaf growth rates, and therefore a continually increasing leaf Mn concentration with time (Fig. 6.1). In the field, leaf growth rates exceeded Mn accumulation rates, consistent with decreasing leaf Mn concentration with time (Fig. 6.1). Here we have two fundamentally different situations: a field situation where leaf growth rate exceeded Mn accumulation rate and a greenhouse situation where Mn accumulation rate exceeded relative growth rate. The saturated paste-Mn level in the soil did not explain this difference, however (Fig. 6.2). In the field situation, the crop has a better chance of growing out of the toxicity while in the greenhouse situation, and the soybean plants experienced chronic phytotoxicity. Tissue damage can be expected even in tolerant cultivars and subsequent death may just be a matter of time where phytotoxicity is chronic.



Equations:

GHouse $\delta Mn = 118 - 42753\ (RGR_{Leaf}\text{-}RAR_{Mn})$ $r^2 = 0.61$; *Pr < 0.0001*

Field $\delta Mn = -12 - 15373\ (RGR_{Leaf}\text{-}RAR_{Mn})$ $r^2 = 0.78$; *Pr < 0.0001*

Fig. 6.1. Change in leaf Mn concentration determined by the absolute difference between leaf growth rate and Mn accumulation rate.

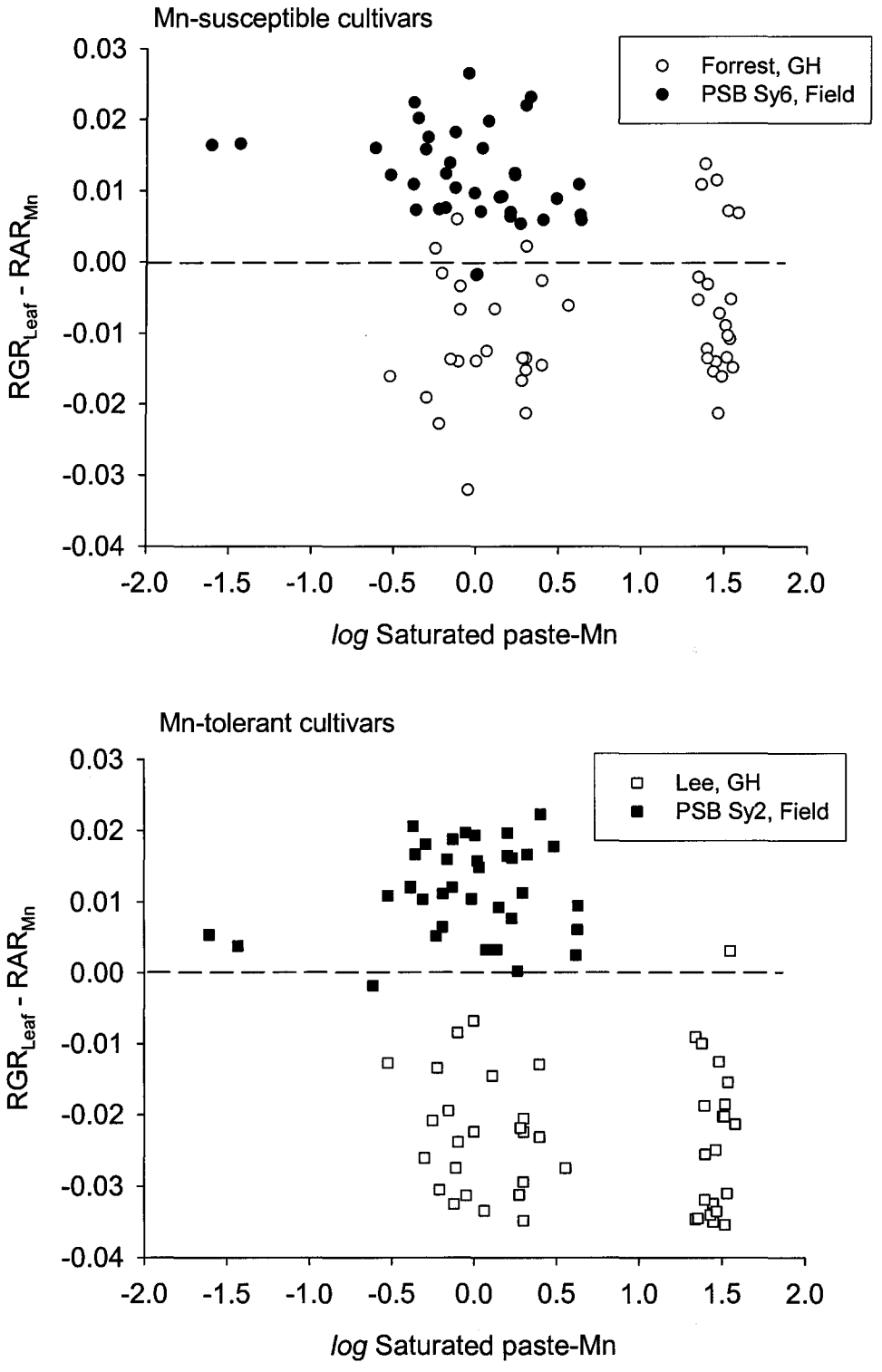


Fig. 6.2. Relation of soil Mn to the absolute difference between plant growth rate and Mn accumulation rate.

Management of Excess Mn in Acid Soils

Various growth conditions tested resulted in varying plant growth rates and Mn accumulation rates. Our main findings is that the phytotoxic response to excess Mn in the soil can be modified by P, manure, water and light management; that growth reductions set by excess Mn in the growth medium can be counteracted upon by higher growth potentials set by a management strategy. Whether the ultimate effect is enhanced tolerance or enhanced phytotoxicity is dependent on the effect of the growth conditions on the plant relative growth rate and relative accumulation rate of Mn. The effect of P addition in both field and greenhouse situations was increased rates of plant growth and Mn accumulation. Water stress in the greenhouse reduced rates of leaf growth in Mn-tolerant cv. Lee and increased leaf growth rate of Mn-susceptible cv. Forrest. Shading, on the other hand, resulted in decreased leaf growth rate as well as decreased accumulation rate of Mn without affecting soil Mn. In the greenhouse, green manure increased soil Mn and decreased rates of plant growth and Mn accumulation. It is logical to expect increased soil Mn to aggravate Mn phytotoxicity and therefore reduce plant growth. Manure effect in the field experiment was most surprising: increased soil Mn accompanied increased plant growth rate, Mn accumulation rate, and yield. Apparently, manures provided nutrients such as P, Ca, Mg and K, which were beneficial to overall plant growth despite an increase in soil extractable Mn. Management treatments in the field such as P and manure application that increased leaf growth rate also increased Mn accumulation rate such that the absolute difference between the two rates

were smaller compared with the control. This suggests that in this environment, an increase in plant growth rate may be more important that an increase in Mn accumulation rate. In the greenhouse, where P was found to increase the absolute difference between leaf growth rate and accumulation rate at some pH levels, a decrease in Mn accumulation rate may be more important, and more attainable than trying to increase plant growth rate.

Conclusions

Three major conclusions can be made from the evidences presented. The first conclusion relates to the diagnostic or predictive criteria for Mn phytotoxicity. Plant analysis measuring total leaf Mn, soil analysis measuring saturated paste-Mn, or Mehlich-Mn, and phytotoxic symptom scores were not accurate predictors of the degree of Mn phytotoxicity based on growth and yield reduction. An evidence of this is the poor correlation between test values and biomass or yield over the range of growth conditions established by water, P, manure and sunlight in the field or in the greenhouse. Plant and soil tests as well as toxicity scores, therefore, must be used with caution in diagnosing the presence and predicting the severity of Mn phytotoxicity.

The second conclusion relates to the dynamics of Mn phytotoxicity in soybeans, which seems to be governed by plant water use and exhibited as continuous negative interaction between rates of plant growth and Mn accumulation. Current relative growth rates were positively correlated with current Mn accumulation rate, the correlation linked by water use. However, we

found negative correlation between current relative growth rate and future Mn accumulation rate as well as a similar correlation between current Mn accumulation rate and future relative growth rate. The continuous negative interaction between growth rates and Mn accumulation rates describe out proposed dynamic model of Mn phytotoxicity. This model suggests that the regulation of water use and plant growth is a key strategy in managing excess Mn in soils. Plant growth rate and Mn accumulation rate are connected by water uptake, however, thus the need for a strategy to disconnect these two rate processes through selection or breeding for cultivars with high water use efficiency.

The third conclusion relates to the management strategies to alleviate phytotoxic response to excess Mn in acid soils. The management treatments tested allowed maximization of growth rates, which ultimately led to maximized biomass production or grain yields even when soil Mn is in excess. Where plant growth rate exceeded Mn accumulation rate (field), an increase in growth rate accompanied by an increase in Mn accumulation rate, due to manure and P additions, led to increased yield. Where Mn accumulation rate exceeded plant growth rate (greenhouse), P addition which decreased Mn accumulation rate, barely increased plant growth rate and was therefore less effective in increasing biomass production. A decrease in Mn accumulation rate due to shading, green manure, and dry conditions were not accompanied by an increase in growth rate, and therefore did not increase biomass production. The difference between plant growth rate and Mn accumulation rate, which reflects maximized plant growth

and minimized Mn accumulation, seemed to be an important criteria in evaluating a management strategy. However, maximization of plant growth may be more important than minimization of Mn accumulation where plant growth rate exceeds Mn accumulation rate. Where Mn accumulation rate exceeds plant growth rate, minimization of Mn accumulation rate may be more important to attain the highest possible absolute difference between the two rates. These findings imply that the evaluation of a management strategy for alleviating Mn phytotoxicity is a three-step process: first, it must establish a condition where growth rate exceeds accumulation rate; second, it must maximize plant growth rate; and third, it must maximize the difference between plant growth rate and Mn accumulation rate.

Recommendation

This research work provide evidence that given a level of excess Mn in the soil, growth rate can be modified by manipulation of light, water and nutrients and that this manipulation can confer enhanced tolerance to excess Mn in the growth medium. By maximizing growth rate to an extent, we can ensure faster plant biomass accumulation, and consequently, higher yields. We also presented evidence that the phytotoxic response to Mn is related to water use by the plant and expressed as a continuous and negative interaction between current plant growth rate and future Mn accumulation rate and at the same time a similar effect of current Mn accumulation rate on future relative growth rate. From this

dynamics, we have established a "growth rate approach" to managing excess Mn in acid soils with 'moderate' potential Mn toxicity.

The proposed model of Mn phytotoxicity based on the dual feedback effect of relative growth rate on relative Mn accumulation rate can be used in identifying management options to alleviate phytotoxic effects of excess Mn in acid soils. The overall goal is to maximize plant growth rate and minimize Mn accumulation rate. An essential step in achieving this goal is to reduce soil extractable Mn to a "moderate level", defined as a level of soil Mn, which under the given growth conditions would allow plant growth rate to exceed Mn accumulation rate. When soil Mn is 'moderate', the focus of management is shifted from eliminating excess Mn in the soil to maximizing plant growth rate and minimizing relative accumulation rate of Mn. Maximum growth rate can be achieved by maintaining sufficient water supply, addition of P and manures to boost early vegetative growth. Unfortunately, these two options result necessarily in increased Mn uptake rate. This is because plant growth rate and Mn uptake rate is linked by water uptake. The only possible way to simultaneously maximize growth rate and minimize Mn uptake rate is to select or breed for cultivars with high plant growth rate and water use efficiency. In this way, minimal water uptake will ensure maximum plant growth rate.

To be able to successfully implement these goals in real field situation, there are a few basic tools that are still unavailable for our use. First is a diagnostic tool to classify soils according to whether they have slight, moderate or severe potential toxicity. Saturated paste-Mn is a likely successful soil test for

this purpose because of the correlation between Mn uptake and water uptake. Is there a critical level for saturated paste-Mn that would allow us to estimate under a given growth condition that plant growth rate will exceed Mn accumulation rate? Comparing the greenhouse and field experiment results, it seems like saturated paste-Mn does not simply dictate whether relative growth rate will exceed Mn accumulation rate (Fig. 6.2). We need to be able to use this test so that it would predict potential toxicity over a given growth period of the target crop. This means that if a soil test indicates 0.5 $\mu g\ ml^{-1}$, the next question we need to ask is whether we would expect this level to be expressed in the soil over the entire growth period or only at certain times and when? Because of the sensitivity of Mn oxide chemistry to moisture or temperature, for example, the timing and method of soil sample collection and processing for analysis is critical.

The second question that we need to address is whether a species or cultivar with high relative growth rate are necessarily tolerant of excess Mn and whether high growth rate and high water use efficiency are independent plant characters so that one can actually combine them in a cultivar. Our first guess is that tolerance may not be directly related to high relative growth rate at species or cultivar level. Tolerance is usually conferred by an existing detoxification mechanism in the plant and may not be due to high relative growth rates at species or cultivar level so that selecting for high relative growth rate rather than Mn tolerance itself may fail to identify tolerant cultivars. This brings us to one important research question that we did not address in this work. This is the question of what is the underlying mechanism for increased tolerance conferred

by increased growth rate due to environmental or nutrient manipulations. At this point, we can only speculate, as other investigators have done before: that increased growth rate may have produced in bigger cells, with bigger vacuoles that can sequester more Mn, or that increased growth rate may have increased the levels of compounds such as malate, oxalate, ascorbate or citrate which have been identified to be associated with 'inactive' Mn inside plant cells. These important questions need to be considered in any future experiments on Mn phytotoxicity.

REFERENCES

1. Adams, F. 1981. Nutritional imbalances and constraints to plant growth on acid soils. J. Plant Nutr. 4:81-87.

2. Alam, S., M.H. Rahman, S. Kamei and S. Kawai. 2002. Alleviation of manganese toxicity and manganese-induced iron deficiency in barley by additional potassium supply in nutrient solution. Soil Sci. Plant Nutr. 48:387-392.

3. Anderson, I. and H.J. Evans. 1956. Effect of manganese and certain other metal cations on isocitric dehydrogenase and malic enzyme activities in *Phaseolus vulgaris*. Plant Physiol. 31: 21-28.

4. Aoba, K. 1986. Excess manganese disorder in fruit trees. Japan Agric. Res. Quarterly. 20:38-47.

5. Bartlett, R. and B. James. 1980. Studying dried, stored soil samples-some pitfalls. Soil Sci. Soc. Am. J. 44:721-724.

6. Beadle, C.L. 1985. Plant growth analysis. pp. 20-25. In *Techniques in Bioproductivity and Photosynthesis*. 2nd ed. J. Coombs, D.O. Hall, S.P Long and J.M.O, Scurlock (eds). Pergamon Press, Inc., Elmsford, NY.

7. Bekker, A.W., N.V. Hue, L.G.G. Yapa and R.G. Chase. 1994. Peanut growth as affected by liming, Ca-Mn interactions, and Cu plus Zn applications to oxidic Samoan soils. Plant Soil 164:203-211.

8. Benac, R. 1976. Response of a sensitive (*Arachis hypogea*) and a tolerant (*Zea mays*) species to different concentrations of manganese in the environment. Ser. Biol. 11:43-51.

9. Bertsch, P.M. and P.R. Bloom. 1996. Aluminum. pp. 517-550. In *Methods of Soil Analysis Part 3. Chemical Methods*. Soil Science Society of America, Madison, WI.

10. Bethlenfalvay, G.J. and R.L. Franson. 1989. Manganese toxicity alleviated by mycorrhizae in soybean. J. Plant Nutr. 12:953-970.

11. Bortner, C.E. 1935. Manganese toxicity in tobacco. Soil Sci. 39:15-33.

12. Boyle, F.W. Jr., and W.L. Lindsay. 1986. Manganese phosphate equilibrium relationships in soils. Soil Sci. Soc. Am. J. 50:588-593.

13. Bueno, P. and A. Piqueras. 2002. Effect of transition metal on stress, lipid peroxidation and antioxidant enzyme activities in tobacco cell cultures. Plant Growth Regulation 36:161-167.

14. Bureau of Soils and Water Management Special Report. Survey of representative soil profiles in *Barangay* San Antonio, Philippines. 1999. A special survey conducted in connection with the establishment of the core experiment of the SM-CRSP Project in the Philippines. *Unpub.*

15. Campbell, L.C. and R.S. Nable. 1988. Physiological functions of manganese in plants. In *Manganese in Soils and Plants.* pp. 87-95. R.D. Graham, R.J. Hannam and N.C. Uren (eds.). Kluwer Academic Publishers. Dordrecht, The Netherlands.

16. Carneiro, J.P., A. de Verennes and H. Amante. 2001. Manganese toxicity in three species of annual medics. J. Plant Nutr. 24:1957-1964.

17. Carter, O.G., I.A. Rose and P.F. Reading. 1975. Variation in susceptibility to manganese toxicity in 30 soybean genotypes.

18. Cheng, B.T. and G.J. Ouelette. 1972. Manganese toxicity in potatoes as affected by various P sources. Canad. J. Soil Sci. 52:274-276.

19. Chesworth, W. 1991. Geochemistry of micronutrients. pp. 96-99. In *Micronutrients in Agriculture.* J.J. Mortvedt, F.R. Cox, L.M. Shuman and R.M. Wech (eds.) Soil Science Society of America, Madison, WI.

20. Clairmont, K.B., W.G. Hagar and E.A. Davis. 1986. Manganese toxicity to chlorophyll synthesis in tobacco callus. Plant Physiol. 80:291-293.

21. Clarkson, D.T. 1988. The uptake and translocation of Mn by plant roots. pp. 101-111. In *Manganese in Soils and Plants.* R.D. Graham, R.J. Hannam and N.C. Uren (eds.). Kluwer Academic Publishers. Dordrecht, The Netherlands.

22. Corton, T., R.S. Yost, T. George and J.B. Lasquite. 1999. Core Experiment in Isabela, Philippines. Soil Management Collaborative Research Support Program Annual Report. 1999.

23. Corton, T.M.C. and T. George. 2000. Philippine Report: Characteristics of the Upland Rice Production System. Paper presented at the Soil Management Collaborative Research Support Program Workshop, held at PhilRice, Nueva Ecija, Philippines on October, 2000.

24. Corton, T.M.C., T. George and J.B. Friday. 1998. Exploratory rapid rural appraisal of Barangay Centro San Antonio, Ilagan, Isabela, Philippines. A report submitted to the Soil Management Collaborative Research Support Program, USAID.

25. Cotter, D.J. and U.N. Mishra. 1968. The role of organic matter in soil manganese equilibrium. Plant Soil 29:439-448.

26. Crawford, T.W. Jr., J.L Stroehlein and R.O. Kuehl. 1989. Manganese and rates of growth and mineral accumulation in cucumber. J. Am. Soc. Hort. Sci. 114:300-306.

27. De Marco, D.G., C.B. Li and P.J. Randall. 1995. Manganese toxicity in *Trifolium balansae, T. resupinatum, T. subterraneum, Medicago murex, M. polymorpha, M. sativa, Lotus pedunculatus,* and *Ornithus compressus:* relative tolerance and critical toxicity concentrations. Austr. J. Expt. Agr. 35:367-374.

28. Edreva, A. and E. Apostolova. 1989. Manganese toxicity in tobacco: a biochemical investigation. Agrochimica 33:441-451.

29. Elamin, O.M. and G.E. Wilcox. 1986. Manganese toxicity development in muskmelons as influenced by nitrogen form. J. Amer. Soc. Hort. Sci. 111:323-327.

30. El-Jaoual, T. and D. A. Cox. 1998. Manganese toxicity in plants. J. Plant Nutr. 21:353-386.

31. Foy, C.D and T.A. Campbell. 1984. Differential tolerances of amaranthus strains to high levels of aluminum and manganese in acid soils. J. Plant Nutr. 7:1365-1388.

32. Foy, C.D., H.W. Webb and J.E. Jones. 1981. Adaptation of cotton genotypes to an acid, manganese toxic soil. Agron. J. 73:107-111.

33. Foy, C.D., R.L. Chaney and M.C. White. 1978. The physiology of metal toxicity in plants. Ann. Rev. Plant Physiol. 29:511-567.

34. Foy, C.D., R.R. Weil and C.A. Coradetti. 1995. Differential manganese tolerances of cotton genotypes in nutrient solution. J. Plant Nutr. 18:685-706.

35. Foy, C.D. 1988. Physiological effects of hydrogen, aluminum and manganese toxicities in acid soil. pp. 57-86. In *Soil Acidity and Liming*. Adams, F. (ed.). Agronomy Monograph No. 12. ASA, CSSA and SSSA, Inc. Madison, WI.

36. Fuller, W.H. and A.W. Warrick. 1985. Soils in waste treatment and utilization. Volume I. Land treatment. CRC Press Inc., Boca Raton, FL.

37. Galvez, L., R.B. Clark, L.M. Gourley and J.W. Maranville. 1989. Effects of silicon on mineral composition of sorghum grown with excess manganese. J. Plant Nutr. 12:547-561.

38. Gambrell, R. P. Manganese. pp. 665-683. In *Methods of Soil Analysis Part 3. Chemical Methods*. SSSA Book series no. 5. Soil Science Society of America and American Society of Agronomy, Madison, WI.

39. Gerretsen, F.C. 1950. Manganese in relation to photosynthesis. III. Uptake of oxygen by illuminated crude chloroplast suspensions. Plant soil 2:323-342.

40. Gilkes, R.J. and R.M. McKenzie. 1988. Geochemistry and mineralogy of manganese in soils. pp. 23-35. In *Manganese in Soils and Plants*. R.D. Graham, R.J. Hannam and N.C. Uren (eds.). Kluwer Acad. Pub. Norwell, MA.

41. Giller, K.E., F. Amijee, S.J. Brodric, S.P. McGrath, C. Mushil, O.T. Fdje and J.B. Smithson. 1992. Toxic concentrations of iron and manganese in leaves of *Phaseolus vulgaris* L. growing on freely drained soils of pH 6.5 in Northern Tanzania. Commun. Soil Sci. Plant Anal. 23:787-792.

42. Godo, G.H. and H.M. Reisenauer. 1980. Plant effects on soil manganese availability. Soil Sci. Soc. Am. J. 44:993-995.

43. Goldberg, S.P. and K.A. Smith. 1984. Soil manganese: E values, distribution of manganese-54 among soil fractions, and effects of drying. Soil Sci. Soc. Am. J. 48:559-564.

44. Gomez, K. A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. 2nd ed. John Wiley and Sons. p. 205.

45. Gonzalez, A., K.L. Steffen and J.P. Lynch. 1998. Light and excess manganese. Implications for oxidative stress in common bean. Plant Physiol. 118:493-504.

46. Graham, R.D. and J.P. Quirk. 1988. An Historical Preface. In *Manganese in Soils and Plants*. pp. 1-5. R.D. Graham, R.J. Hannam and N.C. Uren (eds.). Kluwer Academic Publishers. Dordrecht, The Netherlands.

47. Gupta, U., E.W. Chipman, and D.C. Mackay. 1970. Influence of manganese and pH on chemical composition, bronzing of leaves, and yields of carrots grown on acid sphagnum peat soil. Soil Sci. Soc. Amer. 34:762-764.

48. Harrison, H.C. and E.L. Bergman. 1981. Calcium, magnesium and potassium interrelationships affecting cabbage production. J. Am. Soc. Hort. Sci. 106:500-503.

49. Heenan, D.P and D.G. Carter. 1975. Response of two soybean cultivars to manganese toxicity as affected by pH and calcium levels. Austr. J. Agric. Res. 26:967-974.

50. Heenan, D.P. and L.C. Campbell. 1990. The influence of temperature on the accumulation and distribution of manganese in two cultivars of soybean (*Glycine max* L Merr). Austr. J. Agric. Res. 1990.

51. Heenan, D.P. and O.G. Carter. 1976. Tolerance of soybean cultivars to manganese toxicity. Crop Sci. 16:389-391.

52. Heenan, D.P. and O.G. Carter. 1977. Influence of temperature on the expression of manganese toxicity by two soybean varieties. Plant Soil 47:219-227.

53. Heintze, J.G. 1968. Manganese phosphate reactions in aqueous systems and the effects of application of monocalcioum phosphate on the availability of manganese to oats in alkaline soils. Plant Soil 24:407-423.

54. Horiguchi, T. 1987. Mechanism of manganese toxicity and tolerance of plants. II. Deposition of oxidized manganese in plant tissues. Soil Sci. Plant Nutr. 33:595-606.

55. Horiguchi, T. 1988. Mechanism of manganese toxicity and tolerance of plants. VII. Effect of light intensity on manganese induced chlorosis. J. Plant Nutr. 11:235-246.

56. Horst, W. J. 1988. The physiology of Mn toxicity. pp. 175-188. In *Manganese in Soils and Plants*. R.D. Graham, R.J. Hannam and N.C. Uren (eds.). Kluwer Academic Publishers, Dordrecht, The Netherlands.

57. Horst, W.J. and H. Marshcner. 1990. Mineral nutrition of higher plants. Academic Press. Boston, MA.

58. Horst, W.J., M. Fecth, A. Naumann, A.H. Wissemeier and P. Maier. 1999. Physiology of manganese toxicity tolerance in *Vigna unguiculata* (L.) Walp. J. Plant Nutr. 162:263-274.

59. Houtz, R.L., R.O. Nable and G.M. Cheniae. 1988. Evidence for effects on the in vivo activity of ribulose biphosphate carboxylase/oxygenase during development of Mn toxicity in tobacco. Plant Physiol 86:1143-1149.

60. Hue, N.V. 1988. A possible mechanism for manganese toxicity in Hawaii soils amended with a low-Mn sewage sludge. J. Environ. Qual. 17:473-479.

61. Hue, N.V. 1999. Report on trip to the Philippines. 17-23 Jan. 1999. USAID Grant no. LAG-G-00-97-0002-00. SM-CRSP IntDSS Project. Available for download from http:/intdss.soil.ncsu.edu/smcrsp/Download/Download.htm (verified 04 December 2002.

62. Hue, N.V., J.A. Silva, G. Uehara, R.T. Hamasaki, R. Uchida and P. Bunn. 1998. Manganese toxicity in acid soils in Hawaii. P. 238. In Agronomy Abstracts. ASA, CSSA and ASA, Madison, WI.

63. Hue, N.V., S. Vega and J. A. Silva. 2001. Manganese toxicity in a Hawaiian Oxisol affected by soil pH and organic amendments. Soil Sci. Soc. Amer. J. 65(1): 153-160.

64. Hue, N.V. and Y. Mai. 2002. Manganese toxicity in watermelon as affected by lime and compost amended to a Hawaiian Oxisol. Hort. Sci. 37:656-661.

65. Hughes, N.P. and R.J.P. Williams. 1988. An introduction to manganese biological chemistry. pp. 7-19. In *Manganese in soils and plants*. R.D. graham, R. J. Hannam and C. Uren (eds.) Kluwer Academic publishers, Dordrecht, the Netherlands.

66. Hunt, R. 1978. Plant Growth Analysis. The Camelot Press Ltd. Southhampton, Great Britain. pp. 8-25.

67. Hunt, R. 1990. Basic Growth Analysis. Plant growth analysis for beginners. Unwin Hyman, Ltd. , Winchester, MA. pp. 25-42.

68. Hutton, E.M., W.T. Williams and C.S. Andrew. 1978. Differential tolerance to manganese in introduced and bred lines of *Macroptilium atropurpureum*. Austr. J. Agric. Res. 29:67-79.

69. Iwasaki, K. and A. Matsumura. 1999. Effect of silicon on alleviation of manganese toxicity in pumpkin (*Cucurbita moschata Duch cv.* Shintosa). Soil Sci. Plant Nutr. 45:909-920.

70. Johnson, M.O. 1924. Manganese chlorosis of pineapple. Its cause and control. Hawaii Agricultural Experiment Station Bulletin No. 52. Government Printing Office, Washington. 38 p.

71. Jones, J.P. and R.L. Fox. 1978. Phosphorus nutrition of plants influenced by manganese and aluminum uptake from an Oxisol. Soil Sci. 126:230-236.

72. Jones, R.H. and R.C. Menary. 1974. The toxic effect of manganese on nitrate reductase. In Mechanisms of Regulation of Plant Growth. R.L. Bieleski, A.R. Ferguson and M.M. Creswell. Royal Society N.Z., Wellington. pp. 67-70.

73. Jucker, E.I., C.D. Foy, J.C. Paula and J.A. Centeno. 1999. Electron paramagnetic resonance studies of manganese toxicity, tolerance and amelioration with silicon in snapbean. J. Plant Nutr. 22:769-782.

74. Kamprath, E.J. 1984. Crop response to lime on soils in the tropics. pp. 349-366. In *Soil Acidity and Liming*. F. Adams (ed.). Agronomy Monograph No. 12. ASA, CSSA, SSSA, Madison, WI.

75. Kamprath, E.J. and C.D. Foy, 1971. Lime-fertilizer-plant interactions in acid soils. pp. 105-141. In *Fertilizer Technology and Use*. R. A. Olson, T.

J. Army, J. , J. Hanway and V.J. Kilmer (eds.). Soil Science Society of America, Madison, WI.

76. Kang, B.T. and R.L. Fox. 1980. A methodology for evaluating the manganese tolerance of cowpea (*Vigna unguiculata*) and some preliminary results of field trials. Field Crops Res. 3:199-210.

77. Kelley, W.P. 1909. The influence of manganese on the growth of pineapples. Hawaii Agricultural Experiment Station Bulletin No. 23. Government Printing Office, Washington. 14 p.

78. Kelley, W.P. 1912. The function and distribution of manganese in plants and soils. Hawaii Agricultural Experiment Station Bulletin No. 26. Government Printing Office, Washington. 56 p.

79. Kennedy, C.W. and J.E. Jones. 1991. Evaluating quantitative screening methods for manganese toxicity in cotton genotypes. J. Plant Nutr. 14:1331-1339.

80. Kitao, M., T.T. Lei, T. Nakamura and T. Koike. 2001. Manganese toxicity as indicated by visible foliar symptoms of Japanese white birch *(Betulla platyphylla var. japonica*). Environ. Pollution 111:89-94.

81. Kohno, Y. and C.D. Foy. 1983. Differential tolerance of bush bean cultivars to excess manganese in solution and sand culture. J. Plant Nutr. 6:877-893.

82. Le Bot, J., E.A. Kirkby and M.L. Beusichem. 1990. Manganese toxicity in tomato plants: effects on cation uptake and distribution. J. Plant Nutr. 13:513-525.

83. Le Bot, J., M.J. Goss, M.J.G.P.R. Carvalho, M.L. van Beustchem and E.A. Kirkby. 1990. The significance of the manganese to magnesium ratio in plant tissues for growth and alleviation of manganese toxicity in tomato (*Lycopersicon esculentum)* and wheat (*Triticum aestevum*) plants. Plant Soil.

84. Le Mare, P.H. 1977. Experiments on effects of phosphorus on the manganese nutrition of plants. I. Effects of monocalcium phosphate and its hydrolysis derivatives on manganese in ryegrass grown in two Buganda soils. Plant Soil 47:593-605.

85. Leinweber, P., L. Haumaier and W. Zech. 1997. Sequential extractions and 31P NMR spectroscopy of phosphorus forms in animal manures, whole soils and particle size separates from a densely populated livestock area in northwest Germany. Biol. Fertil. Soils 25:89-94.

86. Lindsay, W. L., 1991. Inorganic equilibria affecting micronutrients in soils. pp. 96-99. In *Micronutrients in Agriculture*. 2nd ed. J.J. Mordvedt, F.R. Cox, L.M. Shuman and R.M. Welch, eds. pp. 297-328. SSSA Book Series No. 4. Madison, WI, USA.

87. Lindsay, W.L. 2001. Chemical Equilibria in Soils. The Blackburn Press, New Jersey. p. 25.

88. Lytle, C.M., F.W. Lytle and B.N Smith. 1996. Use of XAS to determine the chemical speciation of bio-accumulated manganese in *Potamogeton pectinatus*. J. Environ. Qual. 25: 311-316.

89. Maas, E.V., D.P. Moore and B.J. Mason. 1969. Influence of calcium and magnesium on manganese absorption. Plant Physiol. 44:796-800.

90. Marschner, H. 1995. Mineral Nutrition of Higher Plants. 2nd ed. Academic Press, San Diego, CA.

91. Marsh, K.B., L.A. Peterson and B.H. McCown. 1989. A microculture method for assessing micronutrient uptake. II. The effect of temperature on manganese uptake and toxicity in potato shoots. J. Plant Nutr. 12:219-232.

92. Marsh, K.B., L.A. Peterson and B.H. McCown. 1987. A microculture method for assessing nutrient uptake: The effect of phosphate on manganese uptake and toxicity. J. Plant Nutr. 10:1457-1469.

93. Marshner, H. 1991. Mechanism of adaptation of plants to acid soils. pp. 683-702. In: *Plant-Soil Interactions at Low pH*. Proceedings of the second International Symposium on Plant-Soil Interactions at Low pH. 24-29 June 1990. West Virginia, USA. R.J. Wright, V.C. Baligar and R.P. Murrmann (eds.) Kluwer Acad. Publ. Netherlands.

94. McDaniel, K.L and F.R. Toman. 1994. Short-term effects of manganese toxicity on ribulose 1,5 bisphosphate carboxylase in tobacco chloroplasts. J. Plant Nutr. 17:523-536.

95. Mehlich, A. 1984. Mehlich 3 soil test extractant, a modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409-1416.

96. Memon, A.R. and M. Yatazawa. 1984. Nature of manganese complexes in manganese accumulator plant *Acanthopanax sciadophylloides*. J. Plant Nutr. 7:961-974.

97. Mgema, W.G. and R.B. Clark. 1995. Sorghum genotypic differences in tolerance to manganese. J. Plant Nutr. 18:983-993.

98. Misra, A. 1996. Genotypic variation of manganese toxicity and tolerance of Japanese mint. J. Herbs Spices Medic. Plants 4:3-13.

99. Moraghan, J. T. and T. J. Freeman. 1978. Influence of FeEDDHA on growth and manganese accumulation in flax. J. Soil Sci. Soc. Am. 42:455-459.

100. Moraghan, J.T. 1979. Manganese toxicity of flax growing on certain calcareous soils low in available iron. Soil Sci. Soc. Am. J. 43:1177-1180.

101. Morgan, P.W., D.M Taylor and H.E. Joham. 1976. Manipulations of IAA-oxidase activity and auxin deficiency symptoms in intact cotton plants with manganese nutrition. Physiol Plant. 37:149-156.

102. Morris, F. D. and W.H. Pierre. 1949. Minimum concentrations of manganese necessary for injury to various legumes in culture solutions. Agron. J. 41:107-112.

103. Morris, H.D. 1948. The soluble manganese content of acid soils and its relation to the growth and manganese content of sweet clover and lespedeza. Soil Sci. Soc. Am. Proc. 13:362-371.

104. Morris, H.D. and W.H. Pierre. 1947. The effect of calcium, phosphorus and iron on the tolerance of lespedeza to manganese toxicity in culture solutions. Proc. Soil Sci. Soc. Am. 12:382-386.

105. Mortley, D.G. 1993. Manganese toxicity and tolerance in sweetpotato. HortScience 28:812-813.

106. Nable, R.O., R.L. Houtz and G.M. Cheniae. 1988. Early inhibition of photosynthesis during development of manganese toxicity in tobacco.

107. National Land Use Committee. 1985. Guide to the National and Regional Thematic Maps. NLUC, National Economic Development Authority. Manila, Philippines.

108. Nkana, J.C.V, F.M.G. Tack and M.G. Verloo. 1998. Paper pulp as an amendment to a tropical acid soil: Effects on growth of ryegrass. Commun. Soil Sci. Plant Anal. 29:1329-1340.

109. Ohki, K. 1976. Manganese deficiency and toxicity levels for 'Bragg' soybeans. Agron. J. 68:861-864.

110. Ohki, K. 1981. Manganese critical levels for soybean growth and physiological processes. J. Plant Nutr. 3:271-284.

111. Ohki, K. 1985. Manganese deficiency and toxicity effects on photosynthesis, chlorophyll and transpiration in wheat. Crop Sci. 25:187-191.

112. Panda, S., M.K. Raval, and U.C. Biswal. 1987. Manganese-induced peroxidation of thylakoid lipids and changes in chlorophyll a fluorescence during aging of cell-free chloroplasts in light. PhotoChem. 26:3217-3219.

113. Parker, M.B., H.B. Harris, H.D. Morris and H.F. Perkins. 1969. Manganese toxicity of soybeans as related to soil and fertility treatments. Agron J. 61:515-518.

114. Pfeffer, P.E., S. Tu and W.V. Gerasimowicz. 1986. In vivo ^{31}P MR studies of corn root tissue and its uptake of toxic metals. Plant Physiol. 80:77-84.

115. Ponnamperuma, F.N., T.A. Loy and E.M. Tianco. 1969. Redox equilibria in flooded soils: II. The manganese oxide systems. Soil Sci. 108:48-57.

116. Porter, G.S., J.B. Bajita-Locke and N.V. Hue. *In press.* Manganese solubility and phytotoxicity affected by soil moisture, oxygen levels, and green manure additions.

117. Radford, P.J. 1967. Growth analysis formulae-their use and abuse. Crop Science 7,171-175.

118. Raper, C.D., Jr. 1977. Relative growth and nutrient accumulation rates for tobacco. Plant Soil 46:473-486.

119. Rayment, G.E. and K.A. Verral. 1980. Soil manganese tests and the comparative tolerance of kikuyu and white clover to manganese toxicity. Trop. Grassl. 14:105-104.

120. Recel, M.R., G.I.P. Urriza and L.T. Evangelista. 1990. Management and utilization of selected acid soils in the Philippines. Soils and Water technical Bull. VII-1. BWSM, Manila, Philippines.

121. Reuter, D.J., J.B. Robinson and C. Dutkiewics (eds.). 1997. Plant Analysis: An Interpretation Manual. CSIRO Publication. Collingwood, Vic., Australia. p. 128.

122. Robson, A.D. and J.F. Loneragan. 1970. Sensitivity of annual Medicago species to Mn toxicity as affected by calcium and pH. Part III. Austr. J. Agron. Res. 21:223-232.

123. Romheld, V. and H. Marschner. 1991. Functions of micronutrients in plants. In *Micronutrients in Agriculture*. 2nd ed. J.J. Mordvedt, F.R. Cox,

L.M. Shuman and R.M. Welch, eds. pp. 297-328. SSSA Book Series No. 4. Madison, WI, USA.

124. Rufty, T.W., G.S. Miner, and C.D. Raper, Jr. 1979. Temperature effects on growth and manganese tolerance in tobacco. Agron. J. 71:638-644.

125. Sadana, U.S. and N. Claasen. 2000. Manganese dynamics in the rhizosphere and manganese uptake evaluated by a mechanistic model. Plant Soil 218:233-238.

126. Sanchez, P.A. and J.G. Salinas. 1981. Low-input technology for managing Oxisols and Ultisols in tropical America. pp. 280-486. In *Advances in Agronomy*. N.C. Brady (ed.) Academic press, New York, NY

127. Schuman, L.M and O.E. Anderson. 1976. Interactions of manganese with other ions in wheat and soybeans. Commun, Soil Sci. Plant Anal. 7:547-555.

128. Shuman, L. M. 1988. Effect of organic matter on the distribution of manganese copper, iron, and zinc in soil fractions. Soil Sci 146:192-198.

129. Shuman, L.M. 1991. Chemical forms of Micronutrients in soils. pp. 133-134. In *Micronutrients in Agriculture*. 2nd ed. J.J. Mortvedt, F.R. Cox, L.M. Shuman and R.M. Wech (eds.) Soil Science Society of America, Madison, WI.

130. Sirkar, S. And J.V. Amin. 1974. The manganese toxicity of cotton. Plant Physiol. 54:539-543.

131. Sparrow, L.A. and N.C. Uren. 1987. Oxidation and reduction of manganese in acidic soils: Effect of temperature and soil pH. Soil Biol. Biochem. 19:143-148.

132. Statistical Analysis Systems (SAS). 1990. User's Guide: Statistics. SAS Institute, Inc. Cary, NC.

133. Subrahmanyan, D. and V.S. Rathore. 2000. Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. Photosynthetica 38:449-453.

134. Sumner, M.E., M.V. Fey and A.D. Noble. 1991. Nutrient status and toxicity problems in acid soils. pp. 167-173. In *Soil Acidity*. B. Ulrich and M.E. Sumner (eds.). Springer-Verlag Berlin Heidelberg.

135. Unni, P.N., G. Santhakumar and S.R. Nair. 1995. Metal toxicity in acid soils of Kerala- Effect of manganese on growth and physiology of rice (*Oryza sativa* L.) cv. Jaya. Intern. J. Environ. Studies 47:151-158.

136. Van der Vorm, P.D.J. and A. Van Diest. 1979. Aspect of the F and Mn nutrition of rice plants. I. Iron and manganese uptake by rice plants grown under aerobic and anaerobic conditions. Plant Soil 51: 233-246.

137. Vega, S., M. Calisay and N. V. Hue. 1992. Manganese toxicity in cowpea (*Vigna unguiculata*) as affected by soil pH and sewage sludge amendments. J. Plant Nutr. 15:219-232.

138. Vlamis, J. and D. E. Williams. 1967. Manganese and silicon interaction in the *Gramineae*. Plant Soil 27: 131-140.

139. Wang, J., B.P. Evangelou and M.T. Nielsen. 1992. Surface chemical properties of purified root cell walls from two tobacco genotypes exhibiting different tolerance to manganese toxicity. Plant Physiol. 100:496-501.

140. Wang, J., M.T. Nielsen and B.P. Evangelou. 1994. A solution culture study of manganese-tolerant and –sensitive tobacco genotypes. J. Plant Nutr. 17:1079-1093.

141. Welch, R.M., W.H. Allaway, W.A. House and J. Kubota. 1991. Geographic distribution of trace element problems. pp. 36-51. In *Micronutrients in Agriculture*. J.J. Mordvedt, F.R. Cox, L.M. Shuman and R.M. Welch, eds. pp. 297-328. SSSA Book Series No. 4. Madison, WI, USA.

142. Willits, D.H., P.V. Nelson, M.M. Peet, M.A. Depa and J.S. Kuehny. 1992. Modeling nutrient uptake in chrysanthemum as a function of growth rate. J. Am. Soc. Hort. Sci. 117:769-774.

143. Wissemeier, A.H. and W.J. Horst. 1992. Effect of light intensity on manganese toxicity symptoms and callose formation in cowpea (*Vigna unguiculata* (L.) Walp.). Plant Soil 143:299-309.

144. Wissemier, A.H. and W.J. Horst. 1987. Callose deposition in leaves of cowpea (*Vigna unguiculata* [L.] Walp as a sensitive response to high Mn supply. Plant and Soil 142: 283-286.

145. Woolhouse, H.W. 1983. Toxicity and tolerance in the responses of plants to metals. pp. 245-300. In: *Encyclopedia of Plant Physiology*. O.L. Lange, R.S. Nobel, C.B. Osmond and H. Zeigler (eds.). New Series Vol. 12C. Springer Verlag, Berlin.

146. Wu, S. 1994. Effect of manganese excess on the soybean plant cultivated under various growth conditions. J. Plant Nutr. 17:991-1003.