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Stunting Syndrome in Peanuts and Agronomic Approaches for its Release

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Abstract

Stunting of peanuts (*Arachis hypogaea* L.) was observed in newly cultivated areas of commercial fields, or following soil fumigation. Application of Vapam (metham sodium) in three commercial peanut fields eliminated the native VAM infection between soil surface and a 20 cm soil depth when 60% reduction in VAM colonization was found at a soil layer of 20–60 cm with no significant changes observed at deeper soil layers. Screenhouse and field experiments were performed to test new approaches to repress this syndrome. Various mineral treatments involving additional applications of Zn, P or N to the plants or inoculation of peanuts with vesicular-arbuscular mycorrhizal (VAM) fungus were studied. The screenhouse experiment revealed that application of the VAM fungus *Glomus macrocarpum* curtailed the stunting effect at three different N levels (0, 4 and 12 mM KNO_3). The addition of Zn or micronutrients soil fumigation with Vapam elicited the same response as with the VAM fungus. An increase in the shoot dry weight, albeit to a much lesser extent than that obtained by the addition of Zn or VAM was achieved by enhancing P availability. The stunting syndrome was also reduced under field conditions by the same treatments.

Keywords: peanuts, VA-mycorrhiza, *Glomus macrocarpum*, stunting syndrome, N_2-fixation, zinc deficiency

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1. Introduction

"Stunting syndrome" is characterized by poor plant growth and the appearance of small chlorotic leaves at early stages of peanut growth. The phenomenon was observed in non-fertile or fumigated soils. In general, growth inhibition lasted from a few days to several weeks, with plants subsequently resuming normal growth.

It is generally accepted that chemical or physical soil infestation results in a reduction of plant pathogens and normally will improve plant growth and crop yield. However, some reports document growth retardation following such treatments (Martin et al., 1963; Menge, 1983). Both partial and complete sterilization of soils increase the availability of soluble salts as well as the amount of extractable macro- and micro-nutrient elements, including P, Mn, Cu and Zn (Aldrich and Martin, 1952). While the release of such elements in some soils is injurious to crops, it was demonstrated that they are not the cause of the stunting syndrome (Martin, et al., 1963).

In agronomic systems requiring soil fumigation, it is important to understand the stunting mechanism manifested in field crops. The stunting syndrome observed following soil fumigation was attributed to a poor distribution of vesicular-arbuscular mycorrhizae (VAM) fungi (Menge, 1983). A symbiosis between VAM fungi and their host plants results in improved mineral nutrition of the plants due to an extended growth of mycorrhizal hyphae beyond the root-hair zone (Cooper, 1986). Where concentrations of micronutrients in the soil is low, the presence of VAM fungi is essential for the plant to absorb sufficient amounts of immobile elements such as P, Cu, and Zn (Menge et al., 1978).

Biocide applications to control soil-borne pests frequently eliminate root colonization by VAM fungi (Rhodes and Gerdemann, 1975). Thus, for many crops growing in fumigated soils, the addition of VAM fungi is necessary to improve plant growth and yield. This has been demonstrated for fruit trees (Kleinschmidt and Gerdemann, 1972; Menge et al., 1977; Lambert et al., 1973), field crops (Jensen, 1982; Menge, 1983; Krishna and Daft, 1984) and some vegetable crops (Marschner, 1986). However, large-scale methods for direct application of VAM inocula to field crops have not yet been devised. In this study, the stunting syndrome was investigated in peanuts (*Arachis hypogaea* L.) under controlled conditions and several agronomic treatments for its prevention were evaluated. These treatments were shown to have agronomic potential in field experiments as well.

2. Materials and Methods

Plant materials

Screenhouse experiments were conducted on peanut plants (*Arachis hypogaea* L.) cv. Shulamit, and in the filed experiment on *A. hypogaea* L. cv. Hanoch. Both cultivars were obtained from Hazera Co., Haifa, Israel.

Screenhouse experiment

In 1988, 25 L containers were filled with sandy dune soil that had been mixed with superphosphate ($Ca(H_2PO_4)_2$) and potassium sulphate K_2SO_4 (0.15 g/kg). No viable VAM-fungal propagules were found in this sandy soil determined by the MPN technique (Haas and Krikun, 1985). Peanut seeds were sown at the beginning of May. Four seeds per container were sown and plants were thinned to two following emergence. Irrigation water contained one-fifth of the Hoagland solution (Hoagland and Arnon, 1938) and one of three levels of KNO_3: 0, 4 or 12 mM. Once a week, the containers were flushed with tap water to avoid the accumulation of minerals.

The experimental design was a randomized block, 2×3, with 4 replications and VAM and N levels as factors. The experiment was repeated in 1989 using the same experimental design, except that the containers were buried in the soil to prevent over-heating of the root zone. Six replications were used. The experiment was performed in a sandy soil (taken from non-fumigated fields near Kibbutz Kerem-Shalom), 3.4% total carbonates, 25.4% S.P., EC = 0.19 dS/m, 0.53 meq/l Na, 1.98 meq/l Ca + Mg, 14 ppm NO_3, 1.9 ppm K and 22 ppm P. The soil was amended with superphosphate and potassium sulfate as above and also treated with Vapam (metham sodium) at a rate of 25 ml/m^2 soil surface. After the Vapam evaporated (two weeks), the containers filled with Vapam-treated or Vapam-free soils, were moved to a trial pit (45 cm deep) where they were placed on a 25 cm deep gravel layer above polyethylene sheets. The sheets were used to isolate the containers from the soil below, while providing proper drainage. Before planting, each container was washed with 20 L tap water and then allowed to dry. The treatments imposed in this experiment were: Mn – a weekly application of micronutrients as in the Hoagland solution; Zn – a manual application of $ZnSO_4 \cdot 7H_2O$ (1.2 g/l) one and 4 weeks after emergence; P – a manual application of K_2HPO_4 (0.6 g/l) at the same time intervals.

Plants were harvested after 60 days. At harvest, shoots and roots were separated, dried at 70° C for 72 hr, and weighed individually. Prior to root drying, nodules were counted. Phosphorus content was determined by the ammonium molybdate method (Olsen and Dean, 1965), N content by the micro

Kjeldhal analysis (Stubblefield and de Turk, 1960) and Zn content by atomic absorption spectrophotometry. Leaf CO_2 fixation was determined 55 days after emergence, using a Li-COR 6000 leaf gas exchange apparatus. Photosynthesis was measured on fully expanded leaves attached to the plants under the same light conditions as plant growth.

Field experiment

The field trial was conducted in the northern Negev at Kibbutz Sufa on a sandy soil that had been treated with Vapam, at a concentration of 50 ml/m^2. Peanut seeds were sown in two rows, on 1.83 m seedbed width each, on April 2nd, 1990. The seedbed was prepared and maintained according to recommended agronomic practice in this area. The distribution of mycorrhizal fungi in Vapam-treated soils was measured in samples taken at 3 different locations in the western Negev. For each location, treated and untreated fields were sampled at 3 depths: 0 to 20, 20 to 60 and 60 to 90 cm below the soil surface. Viability of native mycorrhiza in each sample was determined by the MPN (most probable number) technique (Haas and Krikun, 1985).

The experiment consisted of 10 treatments in 6 randomized blocks (Table 1), plot size was 1.8×12 m. Plants were sampled three times during the growth season to assess VAM root colonization on the plant roots. Plant development throughout the season (April to September) was scored visually, thus providing an estimate of a "foliage bed cover index" (where full bed cover was 130 plants, while full row cover was 100 plants).

Table 1. Treatment of combinations and element levels of Zn, P and N, incorporated, spread or sprayed on the soil surface and application of VAM fungus in a field experiment

Treatment	Application	
	Method	Time
1. Control	–	
2. Zn (9.1 g/m^2 $ZnSO_4$)	Incorporated (IN)	Before sowing (BS)
3. P (57 g/m^2 superphosphate)	"	"
4. Zn + P	"	"
5. Zn	Spread on surface (SS)	Before emergence (BE)
6. P	"	"
7. Zn + P	"	"
8. Avason* (5% Zn + 15% N) 3%	Sprayed (SP)	31 d after emergence
9. Avason* (5% Zn + 15% N) 1%	"	31 & 46 d after emergence
10. VAM (200 g/m at 2.7 prop/g)	Incorporated	4 d before planting

* Avason = a commercial product of fertilizers and chemical compounds, Ltd.

Inoculation procedure

Inoculation with VAM fungi and *Rhizobium* bacteria was performed at planting. In both experiments, the inoculation of plants with a mixture of *Bradyrhizobium* strains was carried out by applying 0.1 g commercial peanut inoculant (5×10^9 cells/g, Bio-Lab, Jerusalem) per seed. In addition, an isolate of *G. macrocarpum* (provided by J.H. Haas, Dept. of Plant Pathology, Agricultural Research Organization, Bet Dagan) was also applied in all experiments as described previously (Patterson et al., 1990). The occurrence of VAM colonization was evaluated according to Phillips and Hayman (1970).

Statistical analysis

An analysis of variance was performed for all experiments, with significance at the $P\leq 0.05$ level. Data were analyzed further using Duncan's Multiple Range Test ($P\leq 0.05$) to assess significant treatment effects.

3. Results and Discussion

Unfertilized, nonmycorrhizal plants showed symptoms of stunting or deficiency, that were followed by growth retardation. Leaves were small and turned pale green or yellowish-green. These symptoms were often accompanied by the formation of short internodes and necrotic spots at the tips or edges of some leaves. It is suggested that growth inhibition was caused by the same mechanism that triggers stunting syndrome in field-grown peanuts.

Screenhouse experiments

Inoculation of plants with VAM fungi in greenhouse experiments resulted in a significant increase in peanut plant growth (Patterson, 1990), as well as enhanced nodule activity. These observations suggested that fertilization and VAM inoculation of peanuts could repress the stunting syndrome. Indeed, N fertilization increased plant growth significantly in non VAM plants, especially when irrigated with a solution of 4 mM KNO_3 (Table 2). Growth inhibition was relieved by VAM colonization, even at 0 mM KNO_3.

Plants inoculated with VAM fungi yielded more shoots and had greater pod weights, by 60 and 200%, respectively, without N addition. In the presence of VAM, the stunting syndrome was avoided at all KNO_3 concentrations. Fertilization with high levels of N also prevented this phenomenon, although untreated plants still showed a 15% inhibition in plant growth. This inhibition was reflected mainly in pod yield that was significantly lower in non VAM than

Table 2. The effect of N fertilization and VAM fungi on growth of peanut plants. Application of VAM fungi and *Bradyrhizobium* was at sowing. Plants were sampled 60 days after emergence. Values are the means of 4 replications and are not significantly different when followed by the same letter by Duncan's multiple range test at $P \leq 0.05$.

Treatment		Shoot weight	Nodule number	Gynophores	Pod yield	1000 seed weight
KNO_3	VAM	g/plant	#/plant	#/plant	g/plant	kg
0 mM	+	87ab	220a	187ab	117b	1.6ab
	−	54c	69bc	105b	58c	1.3b
4 mM	+	94a	200a	147a	119b	1.8ab
	−	74b	113b	130ab	123ab	1.6ab
12 mM	+	92a	32c	156a	139a	2.2a
	−	80ab	6c	117b	113b	1.7ab

in VAM plants. The VAM treatments enhanced nodulation, especially when no N was applied. This implied that factors besides nutrition were involved. Thus, in poor soils, and in the absence of VAM fungi, N-nutrition may compensate for the delay in plant development.

The specific activity of N_2 fixation by *Rhizobium* in the presence of VAM was more efficient (Patterson, 1990; Patterson et al., 1990). Similar results were obtained with other legume plants (Subba Rao and Krishna, 1988). VAM colonization enhanced nodule formation (Table 2), and was suggested as a specific contribution to the *Rhizobium*-legume symbiosis, probably by an increased P uptake (Barea et al., 1992). A similar increase in nodule formation was obtained when P was applied to the peanut plants (Table 3). In nonfumigated soils, addition of microelements increased the shoot dry weight by 30%, as compared with untreated controls. Inoculation of these soils with VAM fungi resulted in an increase of 40% in shoot dry weight. This was further emphasized where soil fumigation with Vapam eliminated the microbiota. The addition of VAM fungi or microelements to these soils enhanced shoot growth by 83% and 65%, respectively (Table 3). Thus, it can be concluded that Mn application may substitute for the VAM fungal treatment. This can be attributed to a increased photosynthetic activity as a result of the treatments. Although non-treated soils contain low inoculum levels of native VAM fungi with low infection rates (Table 3), they were not sufficient to affect significantly plant growth (compare Vapam-treated and untreated controls).

Application of Vapam in commercial peanut fields eliminated the native VAM infection at the three test locations (data not shown). Using the MPN technique we found that maximum reduction of VAM colonization was obtained between soil surface and a 20 cm soil depth. A 60% reduction in VAM colonization was found at a soil layer of 20–60 cm with no significant changes

Table 3. The effect of VAM fungi, micronutrients, (Mn, Zn or P) on shoot growth, CO_2 fixation and mineral content in peanuts as related to soil fumigation. Harvest was 60 days after emergence. Values are means of 4 replicates and are not significantly different when followed by the same letter by Duncan's multiple range test at $P \leq 0.05$.

Treatment	Shoot weight (g/plant)	Nodule number (#/plant)	VAM (%)	CO_2 fixation (mg $CO_2/dm^2 \cdot h$)	Mineral content (g/plant)		
					N	P	Zn
Untreated soil							
Control	69c	165ab	17	15b	1.97b	0.21a	1.03b
VAM	97a	120b	60	20ab	2.12ab	0.31a	3.16a
Mn	90a	180a	23	18ab	2.52a	0.27a	3.15a
Vapam treated soil							
Control	52c	108b	0	12b	1.56b	0.12b	0.73c
VAM	95a	130b	65	22a	2.38a	0.38a	4.27a
Mn	86a	160ab	0	18ab	2.14a	0.25a	2.58ab
Zn	87a	140ab	0	19ab	2.00ab	0.24a	4.78a
P	65b	190a	0	15b	1.82ab	0.26a	1.30b

observed at deeper soil layers (data not shown). Menge (1983) suggested that the application of many soil fungicides may reduce or delay native VAM infections. It is possible that as a result of soil fumigation, plant growth at its early stages suffered from a lack of VAM root colonization, leading to a deficiency in essential elements. As deeper soil layers were not affected, this deficiency was transient, and was relieved as roots reached the lower soil profiles. This lag period depended on soil conditions, such as fertility, humidity and structure.

Stunting symptoms observed in our work were similar to those shown in commercial peanut fields (D. Sadan, personal communication). According to his observations, stunted plants were associated with a low Zn content in the leaves, as was previously reported for other crops (Menge, 1983). On the other hand, VAM infection increased the concentration of Zn in some agronomic crops (Marschner, 1986). Inoculation of soil with VAM fungi increased the Zn content more than 3-fold, confirming that a Zn deficiency is one of the main factors responsible for the stunting syndrome (Table 3). Control plants contained a very low amount of P and Zn and the addition of VAM fungi or micronutrients alleviated symptoms of this deficiency. It is now well-established that VAM plants absorb P more efficiently than do non-mycorrhizal plants (Mosse and Hayman, 1971; Bethlenfalvay, 1992). Mycorrhizal hyphae are able to absorb and translocate P from remote areas,

that would otherwise be non-accessible to plant roots (Rhodes and Gerdemann, 1975; Sylvia, 1992). Nevertheless, although plant growth was increased by 25% following P application, it was still significantly lower than in plants grown after applications of Mn, Zn or VAM fungi.

Zn functions in plants, either as a metal component of enzymes, or as a functional, structural or regulatory co-factor of a large number of enzymes (Marschner, 1986). Thus, changes in plant metabolism occur as a result of Zn deficiency. It is very common to find Zn deficiency in plants growing in calcareous soils (Trehan and Sekhon, 1977). Kothari et al. (1990) showed that 48% of the total Zn uptake by maize (*Zea mays* L.) plants was due to VAM hyphal transport, when a VAM-fungal species (*G. macrocarpum*) was used. Since Zn is essential for normal plant development, proper Zn nutrition may prevent the stunting syndrome.

Field experiments

In order to validate these important conclusions, a field experiment was conducted involving an additional application of Zn, P or microelements, as well as the inoculation of soils with a VAM fungus. It was found that the application of Zn to the plants, 31 and 46 days after sowing (treatments 8–9) or VAM befor sowing (treatment 10), significantly affected plant growth (Table 4). While 73% of the control plants showed signs of damage after 50 days of growth, during the same time period, only 3, 6 and 30% of the plants from treatments 9,

Table 4. Effect of the different treatments on the number of plants damaged by stunting per 10 m length row. Total number of plants per 10 m was 100. Values are means of 6 replications and are not significantly different when followed by the same letter by Duncan's multiple range test at $P \leq 0.05$.

	Treatment		Days from planting		
			39	44	50
1.	Control		33ab	46ab	73a
2.	Zn	INBS	19bc	32bc	60ab
3.	P	INBS	41a	58a	80a
4.	Zn+P	INBS	15c	31bcd	47ab
5.	Zn	SSBE	16c	25bcd	47ab
6.	P	SSBE	31ab	49ab	64ab
7.	Zn+P	SSBE	21bc	36abc	48ab
8.	Avason	3%	11c	11cd	6c
9.	Avason	1%	8c	6d	3d
10.	VAM		16c	26bcd	10bc

INBS, SSBE – see Table 1

8 and 10, respectively, showed any deleterious effects. Inasmuch as germination takes place soon after sowing, the number of days from planting can be used as a convenient measure of growth.

Deleterious effects were even more pronounced when ground cover was determined. A linear regression between the percentage of stunted plants and the estimated ground cover index was found ($y = -0.2X + 44.8$)). Thus, the only treatments that yielded significant results were treatments 8–10, in which soil coverage was increased by 35 to 60% 50 days after planting (Table 5). In other words, better plant growth was obtained and the stunting syndrome was avoided by the application of Zn after emergence or by VAM colonization. It is important to mention that, as in the screenhouse experiments, application of P was not equally effective, but more P levels should be tested in this experimental system. Despite the fact that stunting syndrome finally abated in all treatments, initial inhibition of growth persisted throughout the growth period up to the final sampling. Therefore, the significant increase in pod yield was made possible only in treatments 7 to 10, that were able to prevent the development of the stunting syndrome (Table 6). Here, the increase in pod yield, and the increase in pod number per m^2, was from 25 to 39%, as compared with control plants. Average pod and kernel weight remained unchanged. The discrepancy between the results obtained in this treatment to treatments 2, 4 and 5 can be attributed to both time and mode of application. This observation suggests that utilization, concentration and plant age are important factors in

Table 5. Estimated ground covered area as a function of the different treatments. Visual estimation was carried out on 6 rows based on the following criteria: 130 = full cover of the entire area; 100 = full cover of the row only. Values are means of 6 replications and are not significant ($P \leq 0.05$) when followed by the same letter.

	Treatment		Days after planting				
			50	66	79	92	107
1.	Control		28c	45b	60b	73b	93b
2.	Zn	INBS	32bc	44b	65ab	80ab	102ab
3.	P	INBS	30bc	43b	63b	79ab	95b
4.	Zn+P	INBS	33bc	50ab	68ab	85ab	101ab
5.	Zn	SSBE	33bc	57ab	77ab	91ab	110ab
6.	P	SSBE	30bc	56ab	77ab	88ab	105ab
7.	Zn+P	SSBE	35bc	60ab	76ab	88ab	110ab
8.	Avason	3%	44a	65ab	85ab	97ab	103ab
9.	Avason	1%	45a	68a	83ab	97ab	108ab
10.	VAM		38ab	71a	96a	111a	128a

INBS, SSBE – see Table 1

Table 6. The effect of Zn, P or VAM fungi application on shoot and pod yield, pod number and average pod and grain weight. Harvest was on day 107 after emergence. Values are means of 6 replications and are not significantly different ($P \leq 0.05$) when followed by the same letter.

	Treatment		Total pod yield ton/ha	Average pod weight g	Pods/m^2 #	Average grain weight g	Shoot yield tons/ha
1.	Control		5.36c	3.03a	175c	1.52a	4.19b
2.	Zn	INBS	5.65bc	3.06a	185bc	1.56a	4.13b
3.	P	INBS	5.48bc	3.06a	177c	1.57a	4.40ab
4.	Zn+P	INBS	5.92bc	3.13a	188bc	1.56a	4.88ab
5.	Zn	SSBE	6.61bc	3.22a	204bc	1.61a	5.61a
6.	P	SSBE	5.98bc	3.16a	188bc	1.53a	4.58ab
7.	Zn+P	SSBE	6.70ab	3.12a	214ab	1.55a	5.28ab
8.	Avason	3%	7.47a	3.21a	234a	1.57a	5.49a
9.	Avason	1%	6.71ab	3.13a	213ab	1.60a	5.02ab
10.	VAM		7.42a	3.32a	223ab	1.63a	4.91ab

INBS, SSBE – see Table 1

utilization of Zn as a restoration factor to the stunting syndrome and should be studied further.

Results obtained in the field experiment confirm and reinforce those derived from the screenhouse study: the application of P and N relieve plant growth retardation only partially, while a much better response is obtained with Zn and VAM application under both controlled and field conditions. In fact, early soil inoculation with VAM fungi, or proper application of Zn and micronutrients, are capable of preventing the stunting syndrome in peanuts and better yield can be achieved with these treatments.

The potential of using VAM fungi as "biofertilizers" (Menge, 1983) in large-scale agricultural production is intriguing. Unfortunately, at present, this is unattainable, since large quantities of VAM inocula are not available.

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