Tolerance of Tobacco (*Nicotiana tabacum* and *N. rustica*) to High Rates of Lead Application to the Soil*

by

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SUMMARY

Eight genetically diverse tobacco types (Tsebelia of Agrinion, Samsun, Basma, Kabakulak, Burley, Virginia and Havana, belonging to *Nicotiana tabacum*, and Brasilia belonging to *N. rustica*) were grown in pots in the greenhouse where lead acetate had been added to the soil at the rates of 0, 0.1, 1.0 and 10.0 g Pb/kg soil. In the field, 20 g Pb/m² in two forms (nitrate and acetate) were applied to Burley and Kabakulak types. The greenhouse experiment showed that only the highest rate of lead addition to the soil reduced the leaf yield significantly. The higher yielding types (Havana and Samsun) were found to be the least tolerant, while Brasilia was the most tolerant. At all levels of lead addition to the soil the lead concentration in leaf was found to be highest in the first of the three harvests which were made. The relationship between log(lead level in the soil) and log(leaf lead concentration) seemed exponential. Although control treatments in the field and the greenhouse experiment showed comparable leaf lead concentrations, leaf lead concentrations at 20 g Pb/m² in the field experiment were found to be higher than those at 1.0 g/kg soil in the greenhouse experiment.

ZUSAMMENFASSUNG

Acht genetisch unterschiedliche Tabake (Agrinion-Tsebelia, Samsun, Basma, Kabakulak, Burley, Virginia und Havanna von der Species *Nicotiana tabacum* sowie Brasilia von der Species *N. rustica*) wurden im Ge-

wächshaus in Töpfen gezogen, wobei der Boden durch Zusetzen von Bleiacetat 0; 0,1; 1,0 bzw. 10,0 g Blei je kg enthielt. Bei Anzucht der Sorten Burley und Kabakulak im Freiland wurde dem Boden je m² 20 g Blei in Form des Nitrats bzw. des Acetats zugesetzt. Aus den Versuchen im Gewächshaus geht hervor, daß nur die höchste der applizierten Bleimengen eine signifikante Verminderung des Blattertrages bewirkte. Am empfindlichsten gegenüber der Einwirkung von Blei waren die Sorten mit gewöhnlich höherer Ertragsleistung (Havanna und Samsun), während die Widerstandsfähigkeit bei Brasilia am stärksten war. Bei allen Applikationsmengen war der Bleigehalt des Blattgutes am höchsten in den Proben der ersten der drei getätigten Ernten. Es bestand eine exponentielle Beziehung zwischen log(Bleiangebot im Boden) und log(Bleigehalt im Blattgut). Obwohl Kontrollversuche im Freiland und die Versuche im Gewächshaus zu vergleichbaren Ergebnissen hinsichtlich der im Blattgut enthaltenen Bleimenge führten, war diese im Feldversuch, wenn dem Boden je m² 20 g Blei zugesetzt worden war, höher als im Gewächshaus bei einem Bleigehalt von 1,0 g je kg Boden.

RESUME

Huit tabacs génétiquement différents (Agrinion-Tsebelia, Samsun, Basma, Kabakulak, Burley, Virginie et Havane appartenant à l'espèce *Nicotiana tabacum* ainsi que Brasilia appartenant à l'espèce *N. rustica*) ont été cultivés en serre dans des pots, le sol contenant 0; 0,1; 1,0 et 10,0 g de plomb par kg à la suite de l'addition d'acétate de plomb. En plein champ, pour la culture des types Burley et Kabakulak, le plomb a été additionné

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Table 1.
Total oven-dried leaf yields for the three harvests of the eight tobacco types grown in the green-house at four lead addition levels in the soil.

Harvest	Lead addition (g/kg soil)	Tobacco types								Mean
		Ts 49a	B 21	Hav Ilc	C 319	BX 101X125	S 53	KKP 14a	Brasilia	
— g/plant —										
1	control*	2.30	3.10	5.50	3.20	2.90	1.50	2.20	1.10	2.73
	0.1	1.63 ijk ⁺	2.97 de	5.50 a	2.90 ef	3.57 c	2.40 fg	3.13 cde	1.27 jkl	2.92 A
	1.0	1.80 efgh	2.90 ef	4.97 b	2.33 gh	3.10 cde	2.13 ghi	3.50 cd	0.73 mno	2.68 A
	10.0	0.45 no	0.43 no	1.07 klim	0.46 no	0.97 lmn	0.28 o	0.68 mno	0.73 mno	0.63 B
2	control	3.80	6.50	7.80	4.50	4.80	3.50	4.60	2.30	4.73
	0.1	2.73 efgh	6.30 abc	7.67 a	5.93 bcd	5.00 cd	4.93 cd	5.50 bcd	3.30 f	5.17 A
	1.0	3.20 ef	5.93 bcd	7.03 ab	5.20 cd	4.20 de	5.47 bcd	5.90 bcd	3.00 efg	4.99 A
	10.0	1.03 hi	1.33 ghi	1.83 fghi	1.53 fghi	2.03 fghi	0.83 i	1.78 fghi	2.33 fghi	1.60 B
3	control	6.80	8.90	16.50	8.80	7.10	12.80	11.60	13.50	10.75
	0.1	4.13 h	8.60 defgh	18.23 a	8.77 defgh	9.63 defg	16.97 ab	9.67 defg	11.53 cde	10.94 A
	1.0	5.37 fgh	7.23 efgh	15.97 abc	8.10 efgh	7.93 efgh	16.77 abc	10.27 def	11.67 cde	10.34 A
	10.0	4.13 h	4.80 gh	5.07 gh	6.07 fgh	5.40 fgh	4.72 gh	11.67 cde	13.20 bcd	6.48 B

* Control values were not included in the statistical analyses.

+ Means within a harvest followed by the same letter are not significantly different at $p=0.05$ according to Duncan's new multiple range test.

au sol à raison de 20 g par m² sous deux formes différentes (nitrate et acétate). Les expériences réalisées en serre ont montré que seule la plus concentrée des applications de plomb a conduit à une diminution significative du rendement en feuilles. Les types de tabac les plus sensibles à l'effet du plomb ont été ceux qui ont un rendement habituellement supérieur (Havane et Samsun), tandis que la résistance la plus grande a été observée dans le cas du Brasília. Pour toutes les quantités appliquées, la teneur en plomb la plus forte des feuilles a été relevée dans les échantillons de la première des trois récoltes effectuées. Il existe une relation de forme exponentielle entre log (teneur en plomb du sol) et log (teneur en plomb des feuilles). Bien que les essais de contrôle effectués en plein champ et les expériences en serre aient conduit à des résultats comparables en ce qui concerne la concentration du plomb dans les feuilles, celle-ci s'est avérée plus élevée en plein champ, pour 20 g de plomb par m² de sol, qu'en serre pour une teneur en plomb de 1,0 g par kg de sol.

INTRODUCTION

Lead is one of the heavy metals which suppresses plant growth by replacing those metals which act as catalysts or are found in enzymes. In tobacco research during the last twenty years, lead has not received as much attention as cadmium.

The increasing amounts of lead from human activities falling on cultivated land and the ability of plants to take up lead and tolerate concentrations several times higher than those normally found in the soil necessitate research into the response of tobacco plants to lead in the soil.

Lead enters tobacco plants mainly through the root. Research on lead translocation and distribution suggests that most of the lead taken up by the tobacco plant remains mainly in the root. The lead concentration of flue-cured tobaccos from several countries was found (10) to vary between 8 and 23 µg/g. The lead concentration decreased as plant size increased. According to RUICK and SCHMIDT (9) the highest lead concentrations were found in the root and the lower scrap leaf. In contrast to cadmium and zinc, which are readily translocated to the shoots, lead is much less mobile (1, 2). FRANK *et al.* (4), comparing the mean lead contents of fine sandy soils of Southern Ontario, Canada, with the mean concentrations in cured leaf from 1970 to 1975, found only one fifth as much in the leaves as in the soils.

Lead enters the human body mainly through inhalation. Absorbed and carried by the blood, it is accumulated in various organs. The amount of lead transferred from tobacco to mainstream particulate phase was found (10) to be generally less than 10%. Under average conditions, lead is taken in at approximate daily rates of 10 to 100 µg from the air (6). FRANZKE *et al.* (5) comparing the amount of heavy metals taken up by an average

smoker (at most 20 cigarettes per day) with the MAC values adopted in the Federal Republic of Germany convey the impression that cigarettes are an unimportant source of lead intake by humans.

Knowing that plant species respond differently to lead contents in the soil, and that there are varietal differences within each species, the work described in this paper had the objectives: to study the response and tolerance of several tobacco types to different concentrations of lead in the root medium, and lead concentrations in the leaf during the growing period.

MATERIALS AND METHODS

The study consisted of one greenhouse and one field experiment conducted at the Farm of the University of Thessaloniki, Greece. The greenhouse experiment was carried out during the winter under natural light conditions.

The following eight genetically diverse tobacco types were used: Tsebelia of Agrinion (Ts 49a), Samsun (S 53), Basma (BX 101×125), Kabakulak (KKP 14a), Burley (B 21), Virginia (C 319), Havana (Hav IIc) and Brasília. The first seven types belong to the species *Nicotiana tabacum*, while the eighth belongs to *N. rustica*. These types are described in detail by PAPAOKOSTA *et al.* (7).

Seeds were sown in seedbeds in soil, and 50-day old seedlings, uniform in size with three small leaves, were transplanted to pots (one seedling per pot) filled with 5.5 kg air-dried soil (< 2 mm) taken from the top 30 cm of the field in which the field experiment was carried out. Chemical and physical properties, on air-dry basis, of the soil used were: pH 7.6, organic matter 1.6%, CaCO₃ 1.5%, total nitrogen 810 ppm, Olsen P-PO₄ 13.3 ppm, clay 40%, silt 45%, C.E.C.* 31.5 milliequivalent/100 g, and lead extracted with 1% HNO₃ 0.9–1.1 ppm. Before transplanting, the lead was added to the soil of each pot as lead acetate trihydrate (A.R.) in amounts of 0.442, 4.42 and 44.2 g/pot to increase the lead content of the soil to about 0.1, 1.0 and 10.0 g/kg of soil on an oven-dry basis, respectively. The lead rates of 0.442 and 4.42 g/pot were applied after the lead acetate was dissolved in distilled water; the rate of 44.2 g/pot was applied as solid reagent. In all cases the soil was carefully mixed to obtain uniform distribution of lead in the soil. The experiment involved a total of 24 treatments (eight cultivars × three rates of lead). Three replications were used in a completely randomized design. Pots without any lead addition, one for each cultivar, were used as controls. The data obtained from these pots were not included in the statistical analyses of this study.

The seedlings were transplanted on 16th November 1983. During the growing period, disease and pest control was practised. No fertilizer was applied.

The leaves were harvested 94, 104 and 130 days after

* cation exchange capacity.

transplanting. Only mature leaves were cut in the first two harvests, but all the leaves in the third. After harvesting, the leaves were washed with distilled water, dried at 60 °C for 48 h, weighed, and ground. For the ground material the method of wet digestion with $\text{HNO}_3\text{-H}_2\text{O}_2$ of EDWARDS et al. (3) was used. The lead content of leaves was determined with an atomic absorption spectrophotometer (Perkin-Elmer 503).

In the field experiment, two tobacco (*N. tabacum*) types, B 21 and KKP 14a, were grown at two rates of lead addition, control and 20 g Pb/m². The latter rate was given in two forms, i.e. nitrate and acetate. The design was a randomized complete block with three replications.

Plots consisted of three 4 m long rows spaced 0.5 m apart for KKP 14a and 1.0 m apart for B 21. Fifty-day old seedlings were transplanted on 20th April 1984. The number of plants in the row was 16 for KKP 14a and 10 for B 21. Lead was applied three days after transplanting and followed immediately by a slight raking and application of 30 mm of irrigation water. No fertilizer was applied.

Weeds were hand controlled. The plants were kept free from pests and diseases. Soil moisture was kept at 50% of field capacity or above by irrigating when necessary. Only one leaf harvest was conducted on 31st July. Leaves were harvested only from the central row and from the 8 central plants for KKP 14a and from 6 plants for B 21. The procedures for leaf material preparation, digestion and lead measurement were the same as those used in the greenhouse experiment.

Five characteristics were measured in the greenhouse experiment: the time elapsed from transplanting to the appearance of the first flower on the individual plant, the total leaf yield, the leaf lead concentration for each harvest, the weighted mean leaf lead concentration, and the rate of leaf dry matter production. The weighted mean leaf lead concentration was calculated for each plant by dividing the total lead accumulated in the leaves by the total leaf yield. The mean rate of leaf dry matter production during the three growing periods, i.e. between transplanting and first harvest, between first and second, and between second and third harvests, was also calculated by dividing the weight of the

leaves produced during these periods by the time elapsed between the harvests, i.e. 94, 10 and 26 days.

Two characteristics were measured in the field experiment: the leaf lead concentration and the leaf area index (LAI) in each plot.

The data on total leaf yield (separately for each harvest), weighted mean leaf lead concentration, mean rate of leaf dry matter production, and LAI values were subjected to variance analysis. In all cases, except for total leaf yields and LAI values, the log transformation was used because the variances between lead levels were different.

RESULTS AND DISCUSSION

Greenhouse Experiment

The yield of leaves differed markedly among the different tobacco types (Table 1). We may divide the types into three groups according to the yield of oven-dried leaves of control and the low lead addition (i.e. 0.1 g Pb/kg soil) plants. The first group includes the higher yielding types Hav IIc and S 53; the second includes the types of intermediate productivity B 21, C 319, BX 101×125, KKP 14a and Brasilia; and the third, the least productive type Ts 49a.

The highest lead level in the soil reduced leaf yields (Table 1). In all harvests, the overall mean of total leaf yield at the highest lead level was significantly lower than those of the other two lead levels which did not differ from each other. Within each type, total yield of leaves at the highest lead level was significantly lower in the first two harvests for all types of *N. tabacum*, but for the higher yielding types Hav IIc and S 53 only in the third harvest. In the case of Brasilia there was no difference in lead levels in the total leaf yields at any harvest. These results show clearly the negative effect of the highest lead level on the growth of *N. tabacum* types up to the time of the second harvest. Only Brasilia tolerated the highest lead level; moreover, it was the only type that flowered at this level (Table 2). Conversely, the higher yielding types, i.e. Hav IIc and S 53, were the least tolerant. For each of the other types, a

Table 2.
Time elapsed from transplanting to the appearance of the first flower on individual plants for eight tobacco types grown in greenhouse at four lead addition levels in the soil.

Lead addition (g/kg soil)	Tobacco types								
	Ts 49a	B 21	Hav IIc	C 319	BX 101×125	S 53	KKP 14a	Brasilia	Mean
— days —									
control	83	87	87	80	77	93	87	83	84.6
0.1	81–87	77	87	77–80	80–82	84–93	85–90	80–87	83.4
1.0	80–87	77–82	88–93	77–81	83	93	89–90	80–87	86.0
10.0	—*	—	—	—	—	—	—	91–95	

* The sign (—) shows that plants did not flower during the time of experimentation.

Figure 1.

Change in the rate of leaf dry matter production with time for the group of the higher yielding tobacco types (—) and the group of the other types (---), grown in the greenhouse at the level of 0.1 (Δ , $\triangle$), 1.0 ($\bullet$, $\circ$) and 10.0 ($\blacksquare$, $\square$) g Pb/kg soil.

significant reduction in leaf yield was observed up to the time of the second harvest at the highest lead level. This could be due to the lead remaining available in the soil until this time. As is known, the lead availability in the soil solution, for soils with added lead, decreases with time as lead is immobilized by the solid phase. For pot soil treated at the highest lead level, lead availability decreased during the time between the second and the third harvests, to a level which, while still restraining the growth of types Hav Ilc and S 53, did not restrict the growth of the others. This is indicated in Fig. 1, in which the changes in the mean rates of leaf dry matter production with time are presented at the different lead levels for two groups of types; one group includes the higher yielding types Hav Ilc and S 53 and the second all the others. Within each group there was no difference with respect to the mean rate of leaf dry matter production between the lead level of 0.1 g/kg soil at any growing period; the mean rate of leaf dry matter production of 10.0 g/kg soil was significantly lower in the first two growing periods. In the third growing period it continued to be significantly lower for the high yielding group, but significantly higher for

Figure 2.

Relationship between leaf lead concentration and lead level in the soil for all tobacco types used in the greenhouse experiment. Control means are presented, although they were not included in the statistical analyses.

Least significant difference A is indicated for comparisons between the different lead levels at the same harvest or between the different harvests at the same lead level.

Least significant difference B is indicated for comparisons of the weighted means between the different lead levels.

the other group. The means of leaf lead concentration differed slightly between types at any lead level. The only significant difference was found at the highest lead level between the types which had the highest (Brasilia) and the lowest (S 53 and KKP 14a) values of means of leaf lead concentrations.

Leaf lead concentration increased, as expected, with increasing lead level in the soil (Fig. 2). The relationship between $\log(\text{lead level in the soil})$ and the $\log(\text{leaf lead concentration})$ seems to be exponential. The leaf lead concentration in the first harvest was significantly the highest at any lead level. It diminished from the first to the third harvest for the highest lead level, but at the other lead levels, leaf lead concentration in the third harvest was higher than that in the second harvest. The difference between these two harvests at the lead level of 1.0 g/kg soil was significant. The usual trend of leaf lead concentration is to decrease with time, because lead is immobilized in the soil and translocation dimin-

Table 3.
Leaf lead concentrations and leaf area index (LAI) values of two tobacco types grown in the field at three different lead addition levels in the soil.

Lead added		Leaf lead concentrations ($\mu\text{g/g}$)		LAI values	
g/m ²	form	tobacco type		tobacco type	
		B 21	KKP 14a	B 21	KKP 14a
0		31c*	36c	0.736ab	0.449c
20	acetate	132ab	164a	0.764a	0.482c
20	nitrate	169a	115b	0.629b	0.627b

* Any of the six means for each characteristic which is followed by the same letter is not significantly different at $p=0.05$ according to Duncan's new multiple range test.

ishes from the bottom to the upper leaves. Since it is known that the highest lead concentrations in tobacco plants are found in the root and the lower scrap leaf (9), it seems that the time of harvest and the location of harvested leaves are parameters which affect the subsequent stages of lead absorption and translocation to the remaining upper leaves.

Thus, the fact that the third harvest showed higher leaf lead concentrations than the second may be attributed to the longer period which elapsed from the second to the third harvest than from the first to the second. It seems that the longer the period between harvests the higher the amount of lead translocated to the top (upper leaves).

Field Experiment

Lead in the soil significantly increased the leaf lead concentration of both types B 21 and KKP 14a (Table 3). Control treatments in the field (Table 3) and the greenhouse experiment (Fig. 2) showed comparable leaf lead concentrations. Leaf lead concentrations at the 20 g Pb/m² level (Table 3) were much higher than those of the 1.0 g Pb/kg soil level (Fig. 2) in the greenhouse experiment although the 20 g Pb/m² level in the soil increased the lead content of the soil by less than 100 ppm. This must be due to the faster growth of tobacco plants in the field than in pots because the conditions in the field were more favorable for growth, i.e. longer daylight and higher temperature. Also, tobacco plants in the field exploited a larger soil volume.

Comparison of the sources of added lead in the field indicates that type KKP 14a absorbed more lead from the acetate than from the nitrate. The reverse occurred with the B 21 type (Table 3). The use of nitrate as the anion of the lead salt increases cation uptake. The final lead concentration of leaves is related to the total lead taken up by the roots and translocated to the leaves and to the total leaf dry matter. In the case of KKP 14a, which seems to be more tolerant to lead, the increase of leaf area possibly reduces the leaf lead concentration by causing dilution of the amount of lead per unit weight of tissue (8). For B 21, the higher rate of lead

uptake where Pb(NO₃)₂ was added, increased the leaf lead concentration to such a degree that its growth was delayed. This is shown in Table 1, although B 21 was not as productive under greenhouse conditions. This is clearly confirmed by the LAI values (Table 3) in which the lowest value for B 21 was found when B 21 was treated with lead nitrate.

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