Lipophilic Quinones in Mineral-Deficient Maize Leaves[1]

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Abstract

Lipophilic chloroplast quinone types—plastoquinones A, B and C, vitamin K_1, and tocopherolquinones—were determined in mineral-deficient maize plants grown in nutrient culture. The lack of each of the major mineral elements—nitrogen, phosphorus, potassium, sulphur, magnesium and calcium—affected the chloroplast quinone content in distinct ways. Ca deficiency showed a high PQ A and PQ C content, N deficiency reduced PQ A, B and C content; P and S deficiencies reduced only the amount of PQ A present, K deficiency resulted in low PQ A but an accumulation of PQ B and PQ C types, Mg deficiency gave low PQ A and PQ B but high PQ C values. On the basis of this, a scheme for the participation of each major mineral element in the biosynthesis and the interconversion of plastoquinone types is proposed. The correlation between the size of osmiophilic globules and lipophilic quinone content is also discussed. In contrast to senescent plants where an increased PQ content results in increased osmiophilic globule size, in mineral deficient plants no correlation between PQ content and osmiophilic globule size can be established.

Many previous studies describe mineral deficiencies of higher plants (6, 7, 8, 9, 11, 12, 13, 15) and try to correlate the fine structure of the chloroplasts of such plants with photosynthetic reactions (9). However, no single study undertakes to examine the lipophilic chloroplast quinone content of the same species in nutrient culture under conditions of all six major mineral element deficiencies—S, Mg, Ca, N, P and K. This is a determination of how the various major mineral element deficiencies affect the plasto- and tocopherolquinone content of maize chloroplasts. In a related study by our group the fine structure of chloroplasts from the same plants is correlated with each major mineral element deficiency.

Materials and Methods

Maize seed (H 55 X C 103 Rf) was obtained from the Agricultural Alumni Seed Association, Lafayette, Indiana, "Krum" for growing plants in nutrient culture from the Silbrico Corp., 6300 River Rd., Hodgkins, Illinois 60525.

All solvents for the extraction of lipids from leaves were reagent grade. Heptane from the Phillips Petroleum Co. was redistilled before use.

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Seeds were germinated in "Krum" in flats in the greenhouse and watered with deionized water until the seedlings showed 2-4 leaflets (4-7 days). At this stage, the remains of the kernel were pinched off from each seedling, and the seedlings were transplanted in groups of 4-6 into glazed earthenware culture flasks filled with "Krum." After this, each set of deficient plants (S, Mg, Ca, N, P, and K) was watered with nutrient solutions recommended by Hoagland and Arnon (4) to induce the various mineral deficiencies. The control plants received a complete nutrient solution at all times.

Since plants suffering from N, P and K deficiencies were severely stunted, another set of plants was grown allowing normal development to proceed for 4 weeks before inducing these 3 deficiencies. Thus more leaf material became available for study.

Leaf samples for lipophilic quinone extraction were collected from 8-week-old plants in case of S, Mg, and Ca deficiencies and 12-week-old plants in case of N, P and K deficiencies.

Chloroplast quinones were extracted from 30-60 g of fresh leaves by the methods of Barr, Henninger and Crane (2). Quinones were separated and purified on an alumina column (Merck's acid-washed alumina treated with 6% water) with 0.2 -35% diethyl ether in petroleum ether as eluants for various fractions. Amounts of each quinone were assayed spectrophotometrically after reduction with sodium borohydride (2). The identity of each quinone was double-checked by spotting on thin layer plates with appropriate standards.

Absorption spectra of whole leaves were measured with a Unicam SP. 800 spectrophotometer. Leaf sections (1 x 3 cm) were cut with a razor blade and taped to pieces of thin glass with Scotch tape before insertion in a cuvette holder in the space provided for samples. Spectra were taken against an opaque glass strip inserted in the cuvette holder in place of a standard. The opaque glass was used to minimize scattering effects.

Results

The macroscopic symptoms of the six major mineral deficiencies studied in maize leaves—S, Mg, Ca, N, P, and K—are depicted in Figure 3. S deficiency is expressed as severe chlorosis, Mg deficiency as replacement of chlorophyll by red anthocyanin pigments, especially in the tips of older leaves; Ca deficiency by curling and distortion of the younger leaves, P deficiency by the darkening of green areas and the appearance of red pigments along the leaf tips and margins of the leaf; K deficiency by yellowing and death of bottom leaves and lighter-green appearance of top leaves. N, P and K deficiencies are also accompanied by severe stunting unless treated as described in the Methods section.

The absorption spectra of these leaves are shown in Figures 1 and 2. It can be seen that all deficient leaves except Ca have a relatively lower absorbance in the chlorophyll a region (660-680 nm). The red

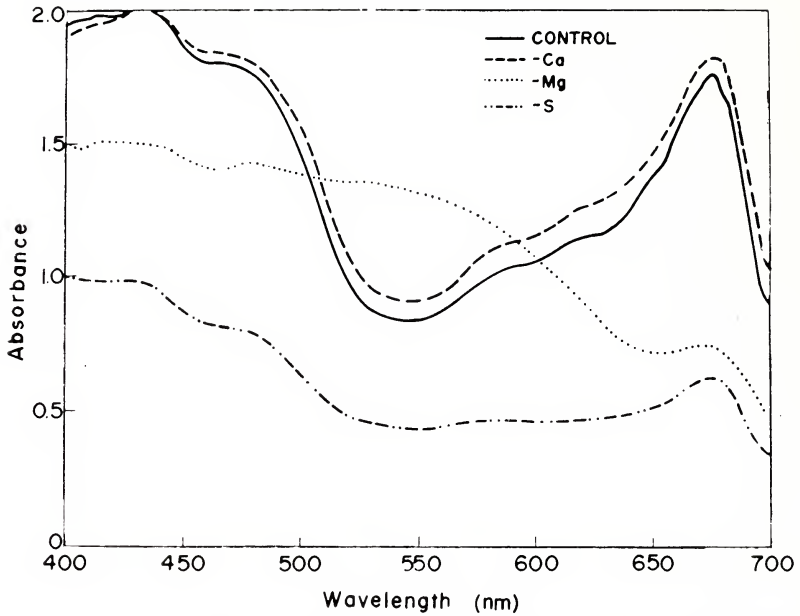


FIGURE 1. *The absorption spectra of whole S-, Mg- and Ca-deficient maize leaves compared to the spectrum of a normal maize leaf. Spectra recorded against opaque glass.*

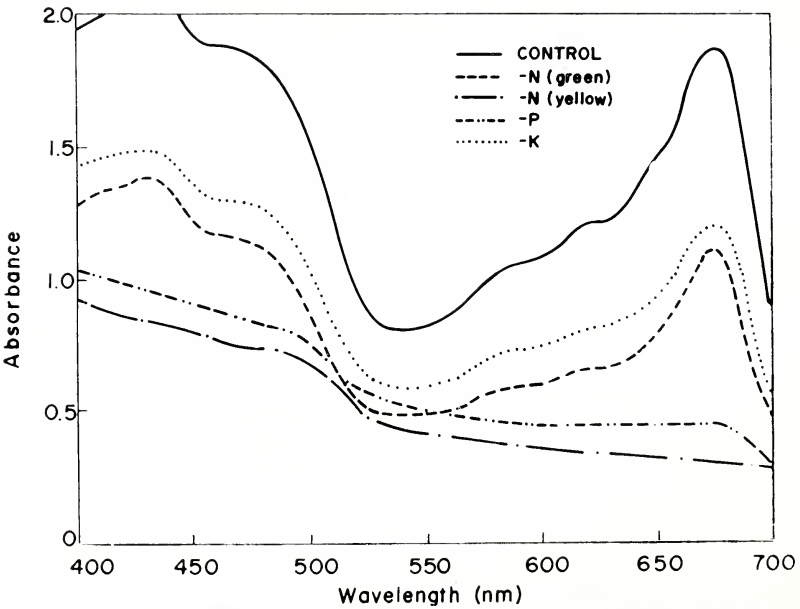


FIGURE 2. *The absorption spectra of whole N-, P- and K-deficient maize leaves compared to the spectrum of a normal maize leaf. Spectra recorded against opaque glass.*

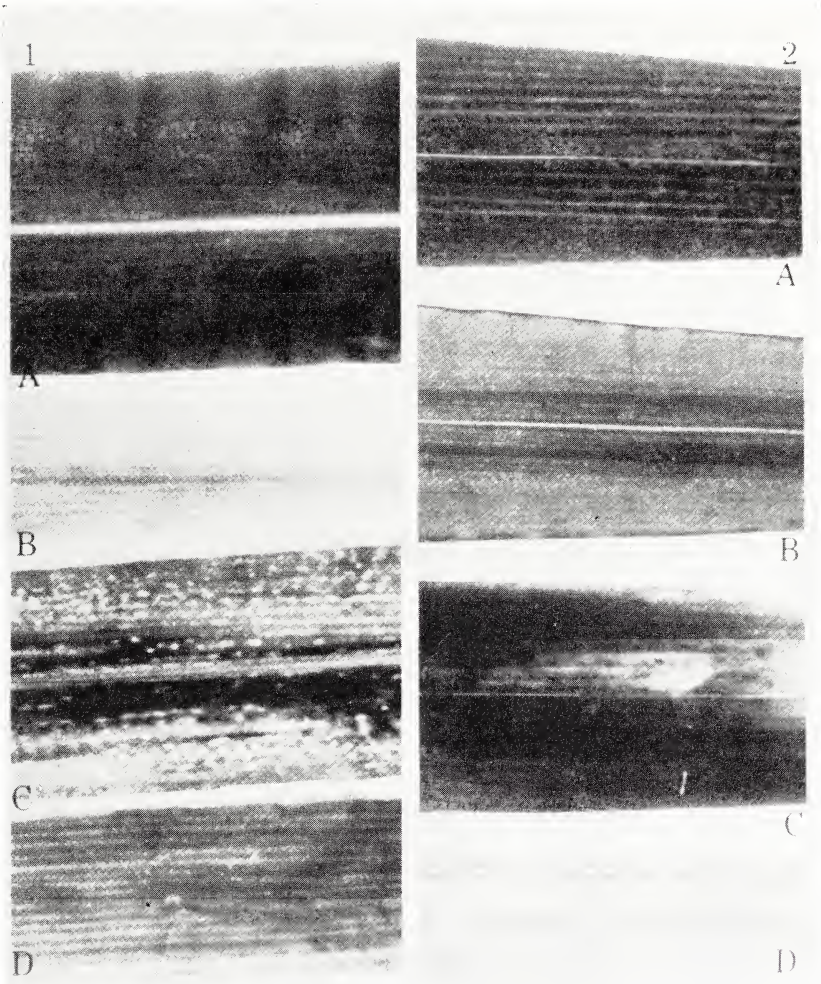


FIGURE 3. *The macroscopic differences between six types of mineral-deficient maize leaves compared to a normal maize leaf. 1A-control; 1B-S deficient; 1C-Mg deficient; 1D-Ca deficient; 2A-control; 2B-N deficient; 2C-P deficient; 2D-K deficient.*

anthocyanin pigments found in Mg deficient leaves show increased absorbance in the 530-550 nm region.

Table 1 shows the severity of each deficiency by comparing a mineral element analysis of each type of deficient leaf to mineral elements of a normal leaf.

Table 2 compares the dry weights (as % of wet weight) and chlorophyll a to chlorophyll b ratios in six different types of mineral-

TABLE 1. *A summary of mineral element analysis of deficient maize leaves compared to normal maize leaves.*[1]

Designation	Per Cent of Dry Weight				Ratio deficient/control
	N	P	K	Mg	
Control	2.2	0.26	1.88	0.49	—
— N	1.1	0.14	2.08	0.24	50%
— P	1.8	0.07	1.80	0.38	27%
— K	2.2	0.34	0.31	1.04	17%
— Mg (red)	2.8	0.62	5.26	0.04	8.2%

[1] Mineral element analysis performed by International Minerals Corporation, Growth Science Center, Libertyville, Illinois 60048

deficient maize leaves. It can be seen that by percent the dry weights of the 12-week-old N, P, and K deficient plant series are higher than the 8-week-old S, Mg and Ca deficients, with the exception of the reddish tips of Mg deficient leaves which were drier on the plant than the green leaves.

According to Table 2, the amounts of chlorophyll per g dry weight vary greatly (1.54-15.43) while the ratios of chlorophyll a to chlorophyll b are more constant throughout the whole series except in N deficiency.

Table 3 shows the types of lipophilic quinones that occur in each of the deficient maize leaves examined and the amounts per unit dry weight. Since variations in the quinone content occur within the same plant (1), a change of less than 50% in quinone content is arbitrarily disregarded as probably not significant. By such standards, the PQ A content is low in the green parts of Mg deficient plants and in N, P, and K deficiencies. Thin layer chromatography of the various PQ A fractions substantiates this (Fig. 4, 1), PQ B is substantially higher in K deficient leaves (*ibid*; spots below PQ A but above Q_{10} standards are PQ B). The lowest vitamin K content is found in S deficiency and the red parts of Mg deficiencies (Fig. 4, 2). PQ C is higher in the green parts of Mg, Ca, and K deficiencies (Fig. 4, 3). The α-TQ content is high in the affected parts of Ca deficiency but low in N and K deficiencies (Fig. 4, 4).

Discussion

Several species of mineral deficient higher plants have been used to study photosynthesis by means of the Hill reaction (9). Deficient plants generally yielded lower rates than normal plants. Since plastoquinone A has been implicated as a member of the electron transport chain between Photosystems I and II (3), the question arose whether the lower Hill reaction rates encountered in deficient plants were caused by lower levels of PQ or whether they were also affected by the presence of other lipophilic chloroplast quinones, such as members of the PQ B or PQ C series and tocopherolquinones. For this reason we analysed maize leaves deficient in all the major mineral elements—S,

TABLE 2. *A summary of weight and chlorophyll data for six major mineral deficiencies of maize compared to normal maize leaves.*

Designation	Wet wt. (g)	Dry wt. (g)	Dry wt./wet wt. (%)	Chlorophyll (mg)	M g chlorophyll/ g dry wt.	Ratio chlorophyll a/ chlorophyll b
Control	100.0	14.17	14.2	82.49	5.82	2.90
— S	26.8	2.99	11.2	11.68	3.91	2.45
— Mg (red parts)	31.7	8.21	25.9	12.63	1.54	2.55
— Mg (green parts)	47.0	5.58	11.9	49.48	8.87	2.86
— Ca (affected parts)	16.0	2.09	13.1	28.40	13.59	2.63
— Ca (green parts)	72.0	9.57	13.3	124.30	12.98	2.78
Control	53.0	13.58	25.6	198.38	14.61	2.58
— N	37.5	9.91	26.4	24.46	2.47	2.01
— P	43.0	8.70	20.2	134.28	15.43	2.83
— K	60.0	12.04	20.1	108.90	9.04	2.67

TABLE 3. *A summary of lipophilic plastid quinone types from six major mineral deficiencies of maize compared to quinones in normal maize leaves.*

Designation	Micromoles Quinone/g Dry Weight							
	PQ A	PQ B	PQ C_{1-6}	Total PQ	Vit. K_1	UQ_9	α-TQ	Ť-TQ
Control	0.184	0.035	0.124	0.343	0.078	0.102	0.194	<0.001
— S	0.067	0.027	0.080	0.174	0.013	0.047	0.161	<0.001
— Mg (red parts)	0.073	0.007	0.037	0.117	0.019	0.037	0.071	<0.001
— Mg (green parts)	0.018	<0.001	0.261	0.280	0.059	0.125	0.104	<0.001
— Ca (affected parts)	0.163	0.019	0.077	0.259	0.077	0.079	0.402	<0.001
— Ca (green parts)	0.214	0.052	0.235	0.501	0.099	0.110	0.345	<0.001
Control	0.160	0.029	0.122	0.309	0.047	0.147	0.239	0.037
— N	0.045	0.015	0.051	0.111	0.045	0.115	0.051	0.065
— P	0.006	0.075	0.135	0.216	0.063	0.094	0.292	<0.001
— K	0.004	0.253	0.205	0.462	0.047	0.062	0.056	0.021

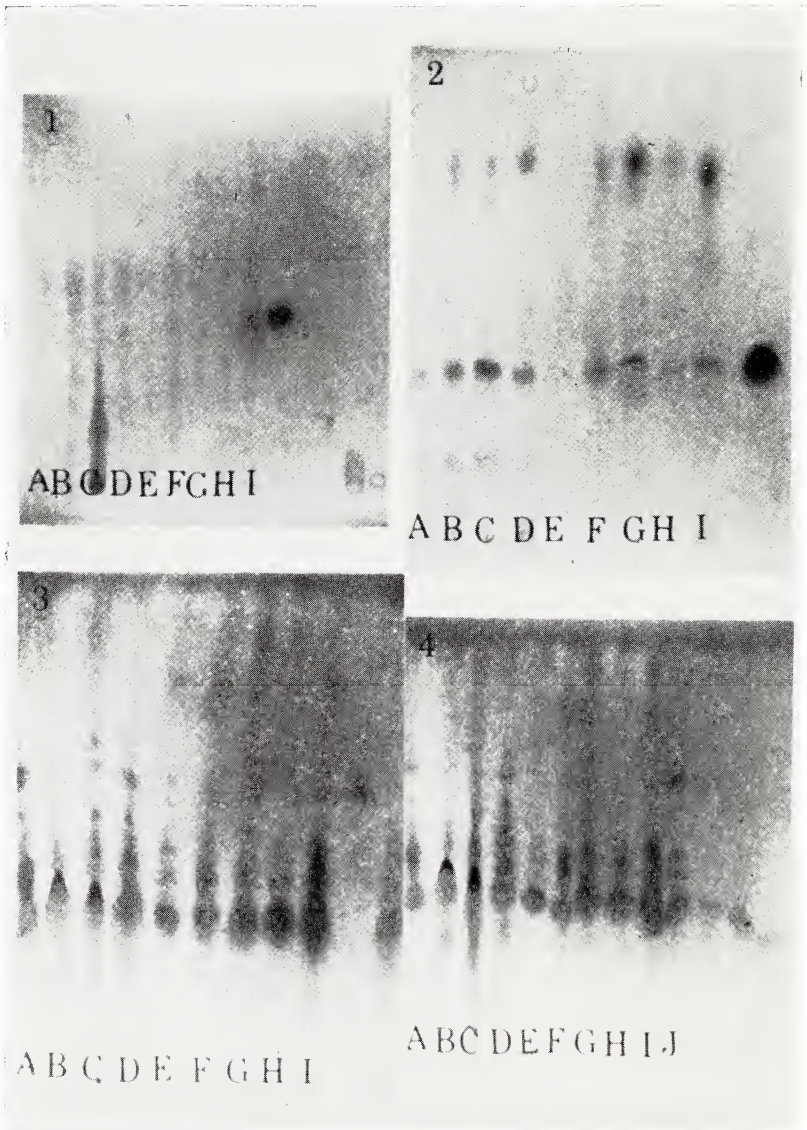


FIGURE 4. *Thin-layer chromatography of quinone types of six different major mineral-deficient maize leaves compared to quinones of normal maize chloroplasts. A-J refer to deficiencies in the following order: -N, -P, -K, control; -S, -Mg(red), -Mg(green), -Ca(affected), -Ca(green) and control. Silica gel GHR plates developed in varying concentrations of chloroform-heptane (80:20 to 50:50), sprayed with reduced Nile blue spray and photographed under ultraviolet light (350 nm). 1) Plastoquinone A series against standard vitamin K_1, PQ A, UQ_{10}, PQ C_{5-6} and α-TQ spots in the order indicated from left to right. 2) Vitamin K_1 series against standard vitamin K_1 spot. 3) Plastoquinone C_{1-6} series against standard UQ_{10} and PQ C_{5-6} spots. 4) α-Tocopheryl-quinone series against standard α-, β-, Υ and -tocopherylquinone spots in the order indicated from left to right.*

Mg, Ca, N, P, and K—for their chloroplast quinone content and if possible, to correlate the quinone content to the fine structure of each type of chloroplast.

An elemental analysis of N, P, K and Mg deficient leaf types (Table 1) shows that our deficiencies had a lower mineral content. A corresponding lipophilic quinone analysis (Table 3) could theoretically provide an answer to the following question: 1) Does the lack of a certain quinone type under a particular deficiency suggest the need for this element in the biosynthesis of this quinone? 2) Does overabundance of a particular kind of quinone imply that a certain mineral element is involved in the interconversion of lipophilic chloroplast quinones and does the lack of this element result in the accumulation of quinones previous to this block? 3) Does a low total PQ (PQ A, PQ B and PQ C combined) imply that a certain mineral element affects early precursors in plastoquinone biosynthesis?

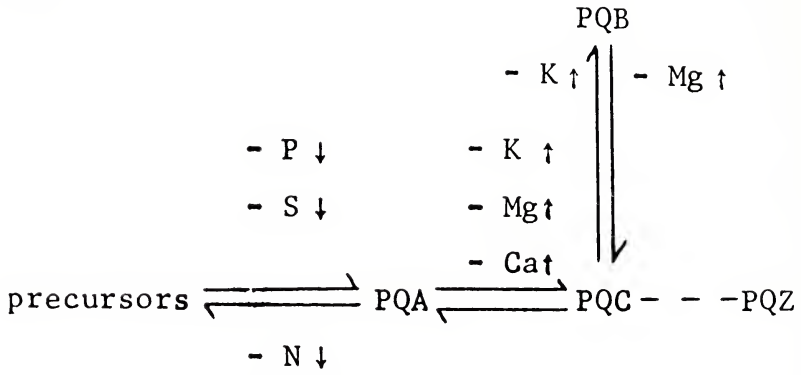
Examining the data obtained in this study, the most significant points are as follows: a low total PQ content in S, N and P deficiencies (Table 3). The lack of these elements thus seems to affect PQ precursors in the mevalonate pathway. It is known that lack of S results in decreased coenzyme A levels which prevent mevalonate biosynthesis and thus results in decreased prenyl sidechain availability for PQ biosynthesis. Likewise, lack of P could affect the isopentanyl pyrophosphate pathway of prenyl sidechain synthesis. Nitrogen deficiency is expected to increase the levels of non-nitrogenous substances, such as starch and possibly plastoquinones, but that is not the case with PQ, as our data show. Similar results were obtained by Tendille, Gervais and Gaborit (10) who found a decrease of PQ A in N and S deficient maize plants.

A low PQ A content in the green parts of Mg deficiency and a higher but still suboptimal amount in the red parts of Mg deficiency can be correlated to the development of the monocot leaf in which the tip part is the oldest and, therefore, accumulated as much PQ A as it could before severe deficiency set in at later stages. In the green Mg deficient parts there is also an increased amount of PQ C present implying that lack of Mg stimulates the conversion of PQ A to PQ C. A virtual absence of PQ B in the green parts of Mg deficient leaves may also imply that Mg is required in the conversion of PQ C to PQ B or that hydrolysis of PQ B is stimulated in absence of Mg.

In K deficiency, low PQ A is associated with a slightly increased amount of PQ C but an abundance of PQ B. Thus in the absence of K, most PQ remains esterified and the part that is converted to PQ C still cannot be dehydroxylated to yield PQ A. Thus, Mg, K and Ca seem to affect reactions involved in the interconversion of PQ types.

In summary, on the basis of total PQ values or the predominance of one type of quinone over another, the following scheme for mineral

element participation in the biosynthesis of plastoquinones is postulated:



This scheme is based on Wallwork and Crane (14) where PQ A gives rise to PQ C, a quinone which differs from PQ A by the presence of a hydroxyl group in the isoprenoid sidechain, and where PQ C can be esterified to yield PQ B or further peroxidation products, such as PQ Z.

The number and size of osmiophilic globules seen in chloroplasts by electron microscopy may be influenced by the amount of lipophilic quinones present. Lichtenthaler (5) found a positive correlation between the size of plastoglobuli and the amount of plastid quinones and carotenoids. Large globules with an increased content of PQ and α-tocopherol are especially prominent during senescence.

An examination of osmiophilic globules from mineral deficient maize plants by Hall *et al.* (unpublished data) leads to a tentative conclusion that in S, Mg and N deficiencies where a reduced number of chloroplasts are detected in the mesophyll cells of the leaf and where the total plastoquinone content is lower compared to control plants, the size or number of osmiophilic globules is not necessarily reduced. Conversely, in the green parts of Ca deficient and K-deficient plants where the total plastoquinone content is higher, the number and size of plastoglobuli is not always increased. Thus, conclusions about a correlation between osmiophilic globules and lipophilic chloroplast quinone content in mineral deficient plants are only tentative because three or more factors may be responsible for increased globule dimensions: 1) an increase in lipophilic quinones directly, 2) an accumulation of lipophilic quinone precursors in globules, 3) an accumulation of unrelated substances. Which of the above factors are operating requires further study but it is clear that osmiophilic globule size can not be directly correlated to the plastoquinone content of mineral deficient plants in contrast to senescent plants (5).

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