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THE EFFECT OF SILICON ON YIELD AND MANGANESE-54 UPTAKE AND DISTRIBUTION IN THE LEAVES OF BARLEY PLANTS GROWN IN CULTURE SOLUTIONS[1]

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Silicon in the form of its compounds occupies 87 % of the earth's crust and constitutes a large percentage of the ash of some plants grown in soil. The addition of silicon to culture solutions has been shown by a number of workers (2, 3, 5) to result in an increase in plant yield. In spite of its beneficial character, and the large amounts absorbed by plants, this element has not been shown to be essential to higher plants in the strict sense of the word. The beneficial effects obtained by the addition of silicon to culture solutions may well be due to secondary causes rather than to an essentiality on the part of silicon.

Previous work in this department by A. Ulrich (unpublished data) concerning the effect of silicon on yield and on the necrotic symptoms observed on the leaves of barley plants grown in culture solutions,

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would also seem to suggest that silicon is an essential element. Ulrich found that the appearance of the dark brown spots observed on leaves of barley plants could be delayed by the addition of soluble silicates to the culture solution with a resulting marked increase in yield. This effect was also observed by the authors (6, 7) in Hoagland culture solutions in which the macro-salt was maintained at both one-fifth and full strength concentrations. That this effect was not peculiar to silicon was shown when the necrosis was eliminated by increasing the macro-salt to full or double strength or by decreasing the micro-salt (particularly Mn) concentrations of the solutions.

Experimental

The culture solutions used in the following experiments were, with one exception (footnote table II), that of 20 % Hoagland Solution, #2. The salts used consisted of CP or reagent grade chemicals dissolved in distilled water. The resulting solutions, adjusted to pH 5.5 with $Ca(OH_2)$ or H_2SO_4, were kept well aerated. The following table gives the salts and concentrations used in preparing the 20 % solutions. The full strength solution was prepared by increasing the macro-salt concentrations to 5 times the value given while keeping the micro-salt concentrations as shown.

Macro-salt	Concentration, millimoles	Micro-salt	Concentration,* ppm
$NH_4H_2PO_4$	0.2	H_3BO_3	0.025
KNO_3	1.2	$MnCl_2$	0.5 or 5.0
$Ca(NO_3)_2$	0.8	$ZnSO_4$	0.05
$MgSO_4$	0.4	$CuSO_4$	0.02
		H_2MoO_4	0.01
		$FeSO_4$**	0.10

* Concentration refers to the element italicized in the micro-salt.

** Fe was added twice weekly to each solution at 0.10 ppm.

An experiment was conducted from April 29 to June 10, 1954 to determine what effect the addition of silicon to a 20 % Hoagland solution #2 (1) would have upon the growth of Atlas Barley (*Hordeum vulgare* L.). Five one-week-old barley seedlings were placed in 45 liters of culture solution containing Mn at a concentration of 0.5 ppm. The plants were grown in the presence and absence of 10 ppm of silicon which was added to the solutions as sodium silicate (adjusted to pH 5.5 with H_2SO_4). Plants were harvested at 4 and 6 weeks, oven dried at 70° C and analyzed colorimetrically for Mn following a HNO_3 digestion and the development of the $KMnO_4$ color with periodate in the presence of phosphoric acid. The concentration of Mn found in the plants as well as yield data are shown in table I.

A second experiment was carried out during the period November 10 to December 20, 1955 in which individual barley seedlings were placed in Pyrex beakers containing 2 liters of one-fifth strength Hoagland solution. The initial Mn concentration in all beakers was 5.0 ppm and silicon as sodium silicate was added to one-half of the solutions at a concentration of 30 ppm. The plants were harvested at 6 weeks, oven dried, and weighed. Yield data for this experiment is given in table II.

Table I

The Effect of Silicon on the Manganese Content of Leaves and on the Yields of Barley Plants Grown in 20 % Hoagland Solution with 0.5 ppm Mn

Treatment	Yield (gm plant)		Mn in plant (ppm)			Necrosis in old leaves
	Top	Root	New leaves	Old leaves	Tops	
			A. 4-wks growth			
+Si	4.0	2.0	61	303	171	None
−Si	1.6	0.9	104	305	204	Moderate
			B. 6-wks growth			
+Si	14.4	7.2	53	412	192	None
−Si	11.2	3.8	37	410	184	Severe

It is evident from the data in tables I and II that the addition of silicon had a marked beneficial effect on both root and top growth. This increase in yield is in agreement with the data reported by other workers (2, 3, 5). A probable reason for the increase in yield shown here may be ascertained from the last columns of tables I and II. These columns show the amount of necrosis, reported as visible symptoms of Mn toxicity as observed on the plants. Necrotic symptoms were entirely absent in plants grown in culture solutions containing silicon whereas very heavy spotting was observed at 6 weeks in plants grown in the absence of silicon. This necrosis, occurring on all of the older leaves, effectively reduced the photosynthetic capacity of the plants as compared with the healthy green plants grown in the presence of silicon. An increase in yield with a decrease in necrosis was

Table II

The Effect of Silicon on Yield and Necrotic Symptoms of Barley Grown in 20 % Hoagland Solution with 5 ppm Mn

	Yield of tops gms/plant *		Yield of roots gms/plant		Mn toxicity symptoms	
	+Si	−Si	+Si	−Si	+Si	−Si
	1.41	0.46	0.43	0.13	None	Heavy spotting
	1.31	0.52	0.38	0.13	None	"
	1.46	0.51	0.52	0.14	None	"
	1.56	0.53	0.45	0.16	None	"
	1.51	0.61	0.41	0.16	None	"
Av	1.45	0.53	0.44	0.14		

* Plants grown in full strength Hoagland solution containing 5 ppm Mn in the absence of silicon had a top wt of 1.24 gm, a root wt of 0.3 gm, and were slightly spotted.

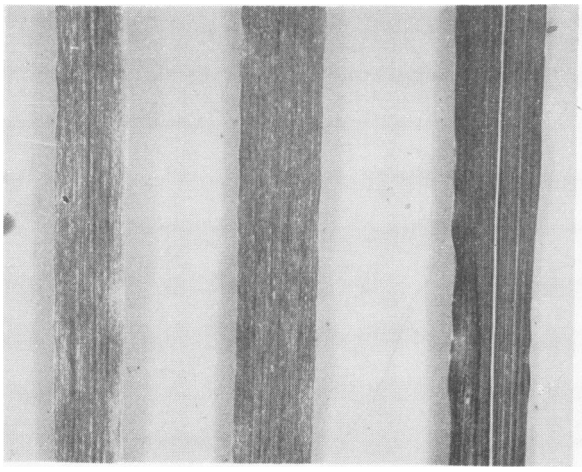

FIG. 1. Photograph of the 4th, 5th, and 6th leaves of barley plants grown in Hoagland solution in the presence of 5.0 ppm Mn and 30.0 ppm Si.

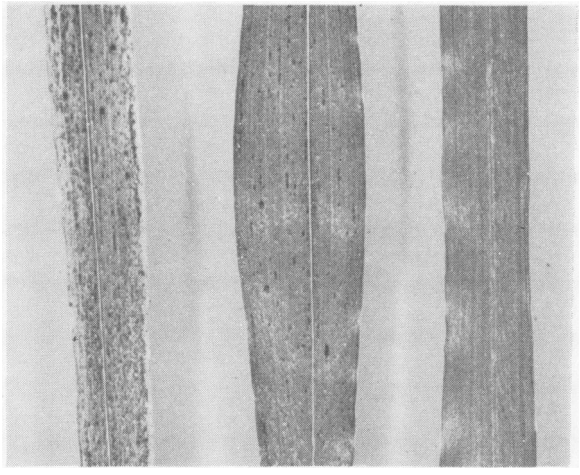

FIG. 3. Photograph of the 4th, 5th, and 6th leaves of barley plants grown in Hoagland solution in the presence of 5.0 ppm Mn and in the absence of Si.

also obtained by increasing the macro-salt concentration from one-fifth to full Hoagland solution (table II). A top weight of 1.24 gm and a root weight of 0.3 gm was obtained with full strength Hoagland solution in the absence of silicon. These values are nearly as great as the 1.45 gm tops and 0.5 gm roots obtained with one-fifth strength solutions in the presence of silicon and are considerably greater than the 0.44 gm tops and 0.14 gm roots obtained with one-fifth strength solutions in the absence of silicon. The barley plants in the full strength Hoagland solutions were only slightly spotted while those in the one-fifth strength solutions were heavily spotted when silicon was absent. Thus, the higher macro-salt concentration as well as the addition of silicon reduced necrosis and produced a greater yield than was obtained for plants grown in one-fifth strength solutions in the absence of silicon.

Although silicon had a marked effect on yield it is clear from table I that it had no apparent effect on the Mn content of the old leaf tissue. The concentration of Mn in the leaves at 4 weeks was 300 ppm and at 6 weeks 400 ppm regardless of whether silicon had been added to the culture solutions or not. The Mn content of the roots and new leaves were quite variable. The similarity in the Mn content of old leaves in the presence or absence of silicon is markedly different from the effect exhibited by an increase in the macro-salt concentration of the solution. In the latter case the added salt is antagonistic to Mn and represses the uptake of Mn by the plant (7) thus decreasing the Mn concentration in the leaves. If the necrotic spots observed in the absence of silicon are a result of an increase in the Mn content of the tissue to a toxic level, then the plants grown in the presence of silicon, having the same level of Mn in the older leaves might have a different distribution of Mn within the leaves.

To investigate this possibility an experiment was carried out using radioactive Mn^{54}. The experimental

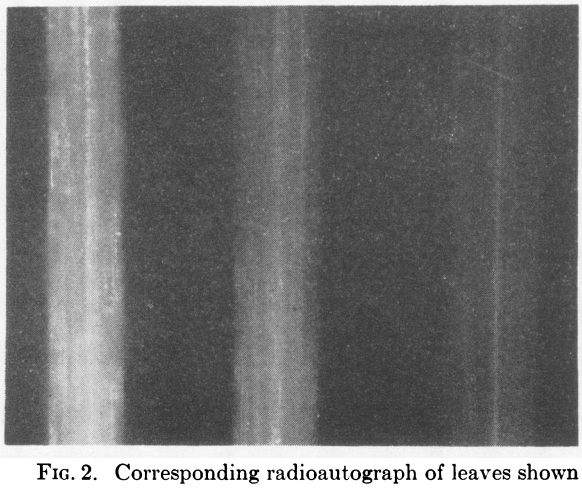

FIG. 2. Corresponding radioautograph of leaves shown in figure 1.

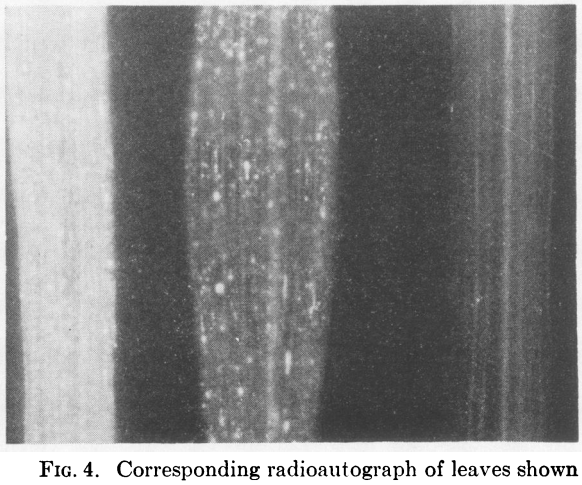

FIG. 4. Corresponding radioautograph of leaves shown in figure 3.

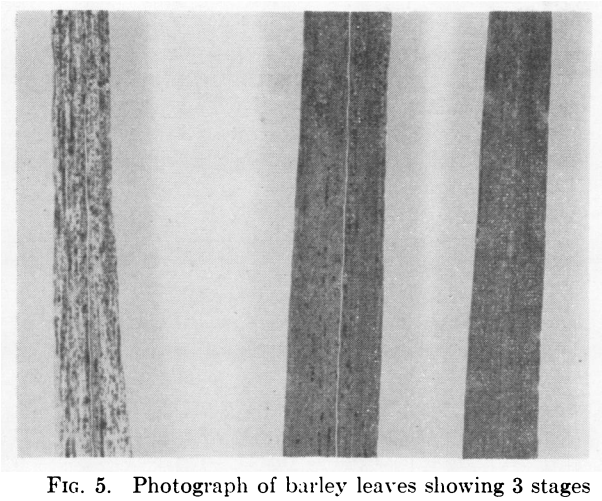

FIG. 5. Photograph of barley leaves showing 3 stages of necrosis.

set up was the same as that described above for the second experiment with Mn at 5.0 ppm and Si at 30 ppm. Twelve microcuries of Mn^{54} were added to each of 4 beakers at the beginning of the experiment and to an equal number of beakers following three weeks of growth. In each instance two of the beakers contained 30 ppm Si and 2 of the beakers contained no Si. The plants were harvested at 6 weeks and the 4th, 5th, and 6th leaves of plants which had been growing in the presence of Mn^{54} from the beginning of the experiment were selected for radioautographs. These leaves were chosen in order to examine autographs of heavily, moderately, and lightly spotted leaves. The leaves were pressed between blotters and oven dried at 70° C prior to exposure to film. Enlarged sections of the radioautographs are shown in figures 2, 4, and 6. Photographs of the same leaf areas are shown in figures 1, 3, and 5.

A distinct difference is observed in the distribution of Mn in the leaf tissue of the silicon and non-silicon treatments (figs 2 and 4). The leaves from the silicon treatment show a decidedly more even distribution of Mn^{54} than is observed in the leaves of the non-silicon treatment. In both cases an increase in the intensity of radioactivity is observed in going from the youngest to the oldest leaf. Radioactive islands or spots are observed in the same location on the leaf autograph as are the necrotic spots on the photographs (figs 1 and 3). This fact is most noticeable in the moderately spotted, center, leaf in figures 3 and 4. Both the radioautograph (fig 2) and the photograph (fig 1) of leaves from the silicon treatment show a complete absence of spotting due to radioactive islands or necrotic tissue. This absence of localized areas of concentrated radioactivity shows the unique effect of silicon in changing the Mn distribution in the plant tissue so that a toxic quantity does not accumulate as is found in its absence. A comparison of the youngest leaf from the non-silicon treatment in figures 5 and 6 shows the presence of radioactive islands where no visible necroses have developed. It would thus appear that the Mn concentration while higher in these areas has not yet increased to a toxic level resulting in visible injury to the tissue. A greater quantity of radioactive islands in the absence of necrosis might have been observed if the ratio of Mn^{54} to total Mn had been greater.

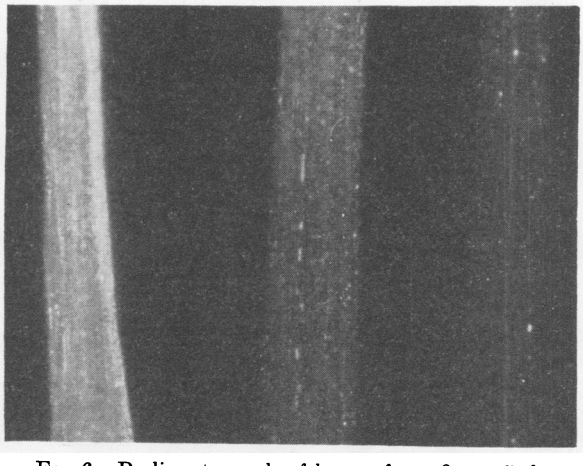

FIG. 6. Radioautograph of leaves from figure 5 showing radioactivity in areas where no visible spotting has appeared as yet.

TABLE III

DISTRIBUTION OF Mn^{54} IN BARLEY LEAVES WHEN ADDED AT THE BEGINNING OR THE THIRD WEEK OF EXPERIMENT

Si	Cotyledon	Leaves		
		1st	2nd	3rd
	A. Mn^{54} added at beginning of the expt			
+	.... *	3790	2260	2120
+	....	3640	2720	2380
−	....	1800	1850	1920
−	....	1955	2270	2135
	B. Mn^{54} added at 3rd wk of the expt			
+	1900	1700	1770	1900
+	1800	1460	1490	....
−	320 **	460 **	1120	1260
−	230 **	330 **	1200	1500

* Cotyledons were used for radioautograph studies.
** Counts were so close to background that they have no significance.
Hoagland solution, 20 %; Mn conc, 5 ppm. Data reported as cpm/gm.

Radioactivity of barley leaves in counts per minute per gram of oven dried leaf tissue is reported in table III. The counts were made on the cotyledons and the 1st, 2nd, and 3rd leaves of the plants grown in the previous experiments. The cotyledons and the 4th, 5th, and 6th leaves of plants grown in Mn^{54} from the beginning of the experiment were not counted for Mn^{54} as they had been used in the prep-

aration of radioautographs. The dried leaves were cut into sections and placed in a small dish under the counting tube of the Geiger counter. While this does not give a precise count of all of the radioactivity, it allows a comparative evaluation between treatments. The data show a slightly higher concentration of Mn in the 1st leaf of the Si treated plants than in the 2nd and 3rd leaves. This is in line with previous data (table I) which showed older leaves accumulating more Mn than younger leaves. The 1st leaf of plants grown in the presence of silicon contained more Mn than a similar leaf from plants grown in the absence of silicon. This may have been due to an early development of necrosis on the leaves from plants grown in the non-silicon treatments producing a rapid desiccation and early death of the leaf as compared to the silicon treated plants whose leaves were still green and healthy at the time of harvest. There was no noticeable difference in the Mn content of the 2nd and 3rd leaves between treatments or within a treatment. These data indicate that plants were able to continue supplying Mn even to old leaves as long as the tissues were healthy.

In section B of the table the counts are given on leaves from plants to which Mn^{54} was added 3 weeks after the seedlings were placed in the culture solutions. In all treatments 5 leaves were present on the original stock and at least 1 or 2 suckers had developed at the time the Mn^{54} was added to the solutions. The leaves on the Si treated plants were all green and healthy at the time of radioactivity addition whereas in the non-silicon plants the cotyledons and the 1st leaf were heavily spotted, the 2nd leaf was moderately to heavily spotted, and the 3rd leaf had spots 1 to 2 inches down the leaf from the tip. As may be observed in the table, Mn moved into the cotyledon and the 1st and 2nd leaves of the silicon treated plants in much greater quantity than went into the leaves from the non-silicon treated plants which had been heavily spotted at the time the Mn^{54} was added to the solutions. The less heavily spotted leaves took up more Mn than did those which had been heavily spotted. At the time of harvest the cotyledons and the first 4 leaves of the original stock of the plants grown in the absence of Si were dried up and dead whereas all of the leaves from the plants grown in the presence of silicon were green and healthy. The data for the counts on the cotyledon and 1st leaf of the non-silicon treatment are questionable in as much as the count was only slightly greater than observed for background.

Discussion and Summary

Mn was found to be toxic to barley plants when grown in Hoagland culture solutions at concentrations from 0.5 to 5.0 ppm. The necrotic symptoms which develop are small dark brown spots which first appear at the tips of the older leaves and spread down the leaf until the entire blade is covered. This necrosis may be alleviated by increasing the macro-salt concentration, decreasing the micro-salt concentration, or by the addition of soluble silicates to the culture solutions. In all cases a decrease in the amount of necrosis is associated with an increase in yield. Unlike Ca, Mg, and K which eliminate Mn toxicity by repressing the amount of Mn moving into the leaf, silicon has no effect on the content of Mn in the leaf tissue.

Radioautographs of the leaves of soybean, tomato, and alfalfa removed from plants grown in Mn^{54} were made by Romney and Toth (4). Small islands of radioactivity were observed in the older leaves with decreasing amounts in younger leaves. Similar autographs were made by the authors on the leaves of barley plants grown in culture solutions containing Mn^{54} in the presence and absence of Si. A comparison of the radioautographs with photographs of the same leaf areas showed a direct correlation between the presence of radioactive islands and the visible necrotic spots. Radioautographs of leaves from plants grown in the presence of Si showed no islands of concentrated radioactivity as were observed in its absence. The radioactivity in barley leaves from plants grown in the presence of silicon was shown to be present in a uniform distribution throughout the leaf.

When Mn^{54} was added to the plants after they had been growing in culture solutions for 3 weeks it was noted that the radioactivity moved freely into the green healthy leaves but not into leaves which were necrotic and nearly dead at the time of addition of the radioactive Mn.

The beneficial effect of silicon on the yield of barley plants grown in standard Hoagland solutions is due to a secondary effect and is not a result of the overcoming of a deficiency of silicon. Silicon alters the distribution of Mn in the leaf tissue rather than excluding it from the plant as occurs in the presence of high concentrations of macro-salt. The unique effect of Si in preventing the accumulation of Mn in localized toxic concentrations is of great value in preventing necrosis due to Mn toxicity. However, the exact mechanism by which Si accomplishes this phenomenon is as yet unknown. These experiments, of course, do not preclude the essentiality of silicon for higher plants. They demonstrate alternative ways of achieving the beneficial effect obtained by the addition of silicon in the reduction of manganese toxicity in barley leaves.

The authors are indebted to John B. Capindale of the Department of Soils and Plant Nutrition for the preparation of the radioautographs used in this paper.

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KREBS CYCLE ACTIVITY OF MITOCHONDRIA FROM ENDOSPERM OF SUGAR PINE SEED (PINUS LAMBERTIANA DOUGL.)[1]

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Previous studies on the enzyme activities associated with plant particulate fractions have been limited to germinating seedlings or to storage structures derived from Angiosperm type plants (5, 10). The preceding publication described some properties of mitochondria from seedlings and embryos of *Pinus lambertiana,* a Gymnosperm (15). In this paper these studies have been extended to pine endosperm, a type of tissue not yet investigated.

Although the endosperm of Gymnosperm seeds is functionally analogous to the endosperm of Angiosperm seeds, it is of different origin and genetic constitution (3). Therefore, only the desire to maintain a simplified nomenclature justifies the use of the term endosperm. Pine endosperm is haploid female gametophyte tissue, and does not result from a fusion between two or more nuclei as is the case in Angiosperms. This difference should be borne in mind in attempting to compare the results reported here to those from other plant tissues.

Metabolic and radioactive tracer studies were employed to obtain evidence for the Krebs cycle enzyme system in mitochondria isolated from homogenates of pine endosperm. Ungerminated and germinating sugar pine seeds were used for these studies. Some variations in the oxidative enzyme activity associated with these particles during the first ten days of germination are presented.

Materials and Methods

The majority of the studies were carried out on seeds harvested in September, 1954 and 1956, at Pino Grande, near Placerville, California. However, the seeds used in the initial experiments establishing the presence of the Krebs cycle were harvested in 1953 in the same vicinity. The cones were dried in a circulating air drying oven at 70° C until the cone scales had reflexed and the seeds had dropped out or could be shaken out. Seeds were then dewinged, cleaned by hand, and placed in a sealed glass jar which contained $CaCl_2$ as a drying agent. The seeds were stored at 5° C until used.

In most of the experiments reported here, mitochondria were isolated from the endosperm of ungerminated seeds. The seeds had been soaked for six hours in aerated water at 5° C before the embryo and endosperm were separated. In a few experiments (fig 1) the endosperm was obtained from germinating seeds at daily intervals. In those experiments the

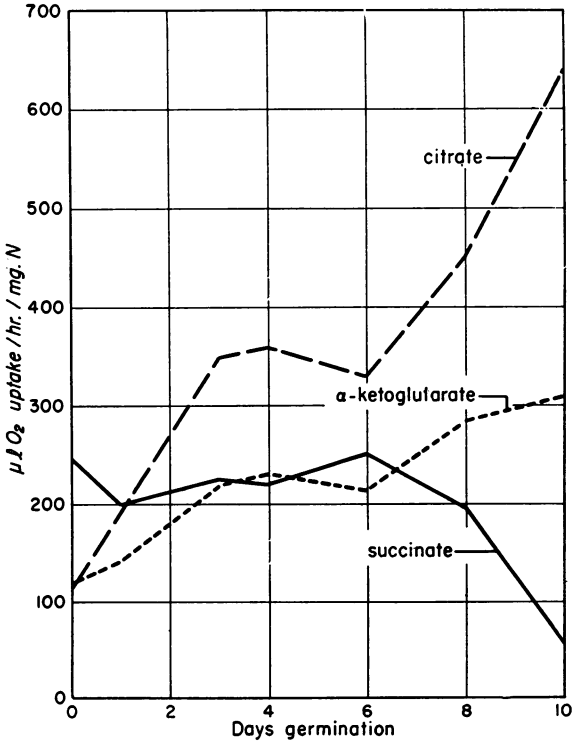

Fig. 1. Changes in the rate of substrate oxidation by endosperm mitochondria. Conditions were those employed in table I.

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