STUDIES ON THE NITROGEN METABOLISM OF PLANTS*

VIII. UTILIZATION OF α-OXIMINOCARBOXYLIC ACIDS BY OAT PLANTS

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Summary

Oat plants were grown in culture solutions containing hydroxylamine, oximinopropionic acid, *trans*-oximinosuccinic acid and *cis*- and *trans*-oximinoglutaric acids in different concentrations at pH 7. Morphological changes and nitrogen contents, including protein-N contents, of these plants are described. It is shown that all these oximes can be utilized as a source of nitrogen for protein synthesis; optimum concentrations for protein synthesis in oats is about 1 x 10^{-5}M for hydroxylamine, 1 x 10^{-4}M for oximinopropionate and 1 x 10^{-3}M for the oximino-dicarboxylic acids.

The amount of protein synthesis was small in plants grown in solutions of hydroxylamine and oximinopropionic acid which also caused marked depression in dry weight production, especially in roots. In solutions of oximino-dicarboxylic acids plants were normal and the amount of protein synthesis was relatively large, though less than that in plants grown in nitrate solutions of the same molarity.

The possibility that oximinosuccinate and oximinoglutarate are intermediaries in the formation of proteins from nitrate is briefly discussed.

I. Introduction

Vickery *et al.* (1940) have shown that when ammonium salts containing N^{15} are supplied to plants the isotope quickly appears in amino-acids, amides, and proteins within the plant. Data have accumulated which show that, so far as ammonium salts are concerned, mechanisms are present in plants whereby amino-acids and amides may be produced from ammonia and keto-acids—especially oxalacetic and α-ketoglutaric acids.

It has been generally held that nitrates are reduced in the leaf ultimately to ammonia. Recently Burström (1945) has claimed that in wheat leaves nitrate reduction is dependent on light intensity and suggested from his evidence that reduction of nitrate to ammonia did not occur, but that a direct carbon-nitrogen assimilate was formed which was probably an oxime; in this respect leaves differ from roots where nitrate reduction is not dependent on light. This view gains some support from Endres' (1935) isolation of a carboxime from *Azotobacter* and from Virtanen's (1939) claim that he had isolated β-oximinosuccinic

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acid from nodules of *Rhizobium*. Whether an oxime is an intermediary in the metabolism of these organisms has been the subject of controversy (Burris and Wilson 1945; Virtanen 1947) and Virtanen (1947) has recently suggested that in *Rhizobium* much of the nitrate supplied might be reduced to ammonia and that the oxime is formed in relatively small amounts. Wood *et al.* (1948) investigated the toxicity to *Azotobacter* of a number of oximino-derivatives and showed, *inter alia*, that α-oximinosuccinic and α-oximinoglutaric acids are relatively non-toxic and furthermore that *Azotobacter* could utilize these compounds as a source of nitrogen when deprived of all other forms of that element. In this paper we have investigated the utilization by oat plants of hydroxylamine, oximinopropionic acid, oximinosuccinic acid, and oximinoglutaric acids.

II. Methods

(*a*) *Cultural and Analytical*

A pure line of oats (*Avena sativa* var. Mulga) was used. Seeds were germinated on waxed mosquito netting stretched over porcelain dishes filled with distilled water. After 10-14 days the seedlings were transferred to a basal culture solution in glass museum jars of 2.5 litres capacity which were coated externally with black paint and internally with paraffin wax. Six even seedlings were placed in each jar; they were inserted through holes in a wooden cover-block which, in each experiment, was freshly coated with paraffin wax; the seedlings were held in place by means of paraffined cotton wool. Glass distilled water was used throughout and the solutions were aerated three times daily.

The basal nutrient solution had the following composition in g. per litre of distilled water, all salts being of A.R. grade: KCl, 0.5 g.; KH_2PO_4, 0.25 g.; K_2HPO_4, 0.25 g.; NaCl, 0.1 g.; $CaSO_4.H_2O$, 0.5 g.; $MgSO_4.7H_2O$, 0.5 g.; Fe_2Cl_6, 0.02 g.; H_3BO_3, 0.5 mg.; 0.5 mg. Mn as $MnSO_4.4H_2O$; 0.2 mg. Zn as $ZnSO_4$; 0.1 mg. Cu as $CuSO_4.5H_2O$; 0.25 mg. Mo as $NaMoO_4.2H_2O$. The pH value of the solution was usually pH 7.0.

Growth of seedlings was continued in the basal solution until N-deficient symptoms appeared, i.e. yellowing of tips of first-formed leaves. At this stage appropriate treatments of the different nitrogen compounds were added; the oximino-acids were dissolved in water neutralized with NaOH and made up to convenient concentration before addition to the basal solution.

Oximinopropionic acid, *trans*-oximinosuccinic acid, and *cis*- and *trans*-oximinoglutaric acids were synthesized and purified by the methods described by Wood *et al.* (1948).

In order to avoid possible contamination and also decomposition, the nutrient solutions (including the basal) were changed frequently—usually every two or three days.

The plants were grown in a glasshouse until harvest when a typical plant from each treatment was carefully drawn to scale; the remainder were harvested, roots and shoots separated and dried quickly under forced draught at 90°C.

Protein-N and soluble-N were determined by micro-Kjeldahl after extraction of dried material and precipitation of protein with trichloracetic acid at pH 4.5 according to the method described by Hanson, Barrien, and Wood (1941).

(b) The Problem of Absorption of Oximino-Acids by Plants

It is difficult to show that the oximino-acids have been absorbed as such by plants. Endres (1935) and Lemoigne, Monguillon, and Desveaux (1936) have described methods for estimation of free and "combined" hydroxylamine (oxime), based on oxidation to nitrite of freed hydroxylamine, by heating the latter with iodine in glacial acetic acid and subsequent diazotization and coupling with sulphanilic acid and α-naphthylamine (Griess reagent). Steward and Street (1947) have referred to some difficulties in applying the method to plant tissue, but give a table indicating the presence of both free and "combined" hydroxylamine in leaves of legumes. We have not estimated oxime-nitrogen in the plants because we do not believe that the above methods are capable of measuring oxime-nitrogen in the dicarboxylic acids we have employed. It is probable that the methods are applicable with α (*cis*)-oximinoglutaric acid which is relatively stable in slightly acid as well as in alkaline solution and can be recovered from boiling aqueous solution. However, like Cramer (1891), we have found that β (*trans*)-oximinosuccinic acid is quite stable in neutral or alkaline solutions but is extremely labile in acid solution; even at room temperatures an aqueous solution of the pure acid decomposes rapidly, losing carbon dioxide and water and forming cyanoacetic acid which can be recovered as red crystals; in alkaline solution cyanoacetic acid readily forms malonic acid and ammonia. In neutral or alkaline solutions β (*trans*)-oximinoglutaric acid is stable, but is labile in acid solution; even on gentle warming a pure aqueous solution of the acid loses carbon dioxide and is converted into the half-amide of succinic acid. It is obvious, therefore, that the methods described above will not free hydroxylamine from these acids which can then be oxidized to nitrite.

The culture solutions were approximately neutral and were changed frequently in order to prevent occurence of the changes outlined above and also to minimize possible bacterial contamination. Investigations were carried out from time to time on the culture solutions. Large volumes have been distilled under reduced pressure and have failed to yield any ammonia. The possibility that the oxime group of the oximino-acids might be oxidized to nitrite or nitrate (e.g. by ferric chloride present) has also been investigated. Using Rider and Mellon's (1946) quantitative modification of the Griess reagent, it has not been possible to detect the presence of nitrite or of nitrate in our culture solutions which were up to three days old. With *cis*-oximinoglutaric acid, however, small amounts of nitrite were detected in solutions of pH 6.8, and containing 1.0×10^{-3}M or more of the oximino-acid, which were four days old; the concentration of nitrite on the

fifth day was 2 x 10^{-8}M, on the seventh day the concentration was 2 x 10^{-7}M, and on the eleventh day 1 x 10^{-6}M. These amounts are too small to provide detectable changes in nitrogen contents of the plants. For example, in Experiment 6, described below, the solutions, in contrast to all other experiments, were changed only weekly, since over the whole experimental time, breakdown of the oxime only provided 0.05 mg. N as nitrite in the culture solutions and available to the plants. It is considered reasonably certain, therefore, that hydrolytic or oxidative changes in the solutions under the conditions described were negligible and that the changes in nitrogen fractions within the plant were brought about following absorption of the oximino-acids.

III. Results

(*a*) *Growth of Oats on Hydroxylamine and Oximinopropionate*

Experiment 1.—A preliminary experiment was carried out in order to determine the morphological effects and toxicity of different concentrations of oximino-derivatives. Oat seedlings were grown as described until they were N-deficient, when hydroxylamine, oximinopropionic acid, and *trans*-oximinosuccinic acid were added to the basal solution each in quantities to give final molar concentrations of 1 x 10^{-6}, 5 x 10^{-6}, 1 x 10^{-5}, 5 x 10^{-5}, and 1 x 10^{-4}M. Control pots containing no added oxime and also others containing 1 x 10^{-4}M potassium nitrate were prepared at the same time. The pH of the culture solution in each case was adjusted to pH 7 with NaOH solution and each culture solution was completely renewed each day for 30 days: at the end of 30 days the results outlined below were observed.

Over the range of concentrations investigated *trans*-oximinosuccinic acid produced no toxic symptoms; development of roots and shoots was normal although plants were obviously N-deficient at the lower concentrations.

With hydroxylamine and oximinopropionate toxic symptoms in plants were similar. At all concentrations stunting of the root system occurred, even when compared with control plants grown on solutions with no added nitrogen; at concentrations higher than 1 x 10^{-5}M with hydroxylamine and 5 x 10^{-5}M with oximinopropionate the roots were brown in colour and no lateral roots developed; at the concentrations mentioned the roots were still brownish in colour with few laterals; at lower concentrations roots were not discoloured and development of laterals occurred.

The effect on the shoot system was less marked. At the higher concentrations leaf development and tillering were suppressd; incipient tillers developed on plants in 1 x 10^{-5}M hydroxylamine and in 5 x 10^{-5}M oximinopropionate and at slightly lower concentrations tillering occurred even though the root system remained stunted.

The concentrations of hydroxylamine and oximinopropionate at which tillering and lateral root development were suppressed were approximately the same as those found toxic to *Azotobacter* by Wood *et al.* (1948).

Experiment 2.—Duplicate sets of oat seedlings were grown as described previously in basal solutions containing oximinopropionate, potassium nitrate, and no added nitrogen, at the concentrations and under conditions set out in Table 1. The solutions were adjusted to pH 7 and completely renewed every two days. One set was harvested after plants had grown for 20 days in the oximinopropionate solutions and the other set after 34 days. Results of analyses for dry weight, protein-N and soluble-N are given in Table 1 and typical plants from each treatment after 34 days are illustrated in Figure 1.

TABLE 1

DRY WEIGHTS (G./5 PLANTS), PROTEIN-N, SOLUBLE-N, AND TOTAL-N (MG./5 PLANTS) OF OAT PLANTS GROWN IN NUTRIENT SOLUTIONS AT pH 7 WITH α-OXIMINOPROPIONIC ACID AS SOLE SOURCE OF NITROGEN

Molarity	Shoots				Roots		Total Plant	
	D.W. (g.)	Prot.-N (mg.)	Sol.-N (mg.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)
*Series A**								
Nil (Control)	0.145	1.80	0.84	2.64	0.132	0.30	0.277	2.94
7.0×10^{-5}M Oxime	0.190	3.22	1.28	4.50	0.091	0.31	0.281	4.81
1.4×10^{-4}M Oxime	0.184	2.37	1.62	3.99	0.090	0.26	0.279	4.25
3.5×10^{-4}M Oxime	0.166	1.93	0.77	2.70	0.074	0.23	0.240	2.93
7.0×10^{-4}M Oxime	0.173	2.13	0.82	2.95	0.069	0.10	0.242	3.05
3.5×10^{-4}M KNO_3	0.261	10.14	3.00	13.10	0.139	0.51	0.400	13.61
Series B†								
Nil (Control)	0.233	2.42	0.27	2.69	0.178	0.34	0.401	3.03
7.0×10^{-5}M Oxime	0.263	3.47	1.53	5.00	0.094	0.29	0.357	5.29
1.4×10^{-4}M Oxime	0.284	4.33	1.17	5.55	0.101	0.31	0.385	5.86
3.5×10^{-4}M Oxime	0.226	2.28	1.17	3.45	0.075	0.23	0.301	3.68
7.0×10^{-4}M Oxime	0.214	2.49	1.13	3.62	0.078	0.16	0.292	3.78
3.5×10^{-4}M KNO_3	0.892	35.10	9.68	44.80	0.278	1.90	1.170	46.70

* Plants 20 days in oxime solutions. All solutions changed every two days.

† Plants 34 days in oxime solutions.

Experiment commenced 3.v.46.

In appearance the plants were similar to those described in Experiment 1. Root development was poor even when compared with the control receiving no extra N; incipient tillering and lateral root development occurred at 1.4×10^{-4}M and both were obvious at 7×10^{-5}M. The analyses show that, compared with the *minus*-N control, the shoots increased in dry weight in all treatments, being greatest at 1×10^{-4}M and 7×10^{-5}M. However, root weight increment was suppressed; root dry weights of plants exposed to oximinopropionate were less than that of the *minus*-N control; also no change in dry weight of the roots of these plants occurred between the 20th and 34th days.

Compared with the *minus*-N control the protein-N of the shoots of treated plants increased in amount in 7×10^{-5}M and 1.4×10^{-4}M solutions of oximinopropionate, being greatest in the former concentration after 20 days and in the

latter after 34 days; at other toxic concentrations no protein synthesis occurred. In roots the total-N was less in treated plants than in the control and no change in N-content occurred between the 20th and 34th days.

The evidence suggests, therefore, that at concentrations of about 1 x 10^{-4}M shoots but not roots of oat plants can utilize oximinopropionic acid to synthesize proteins, though the extent of such synthesis is small compared with plants grown on nitrate solutions.

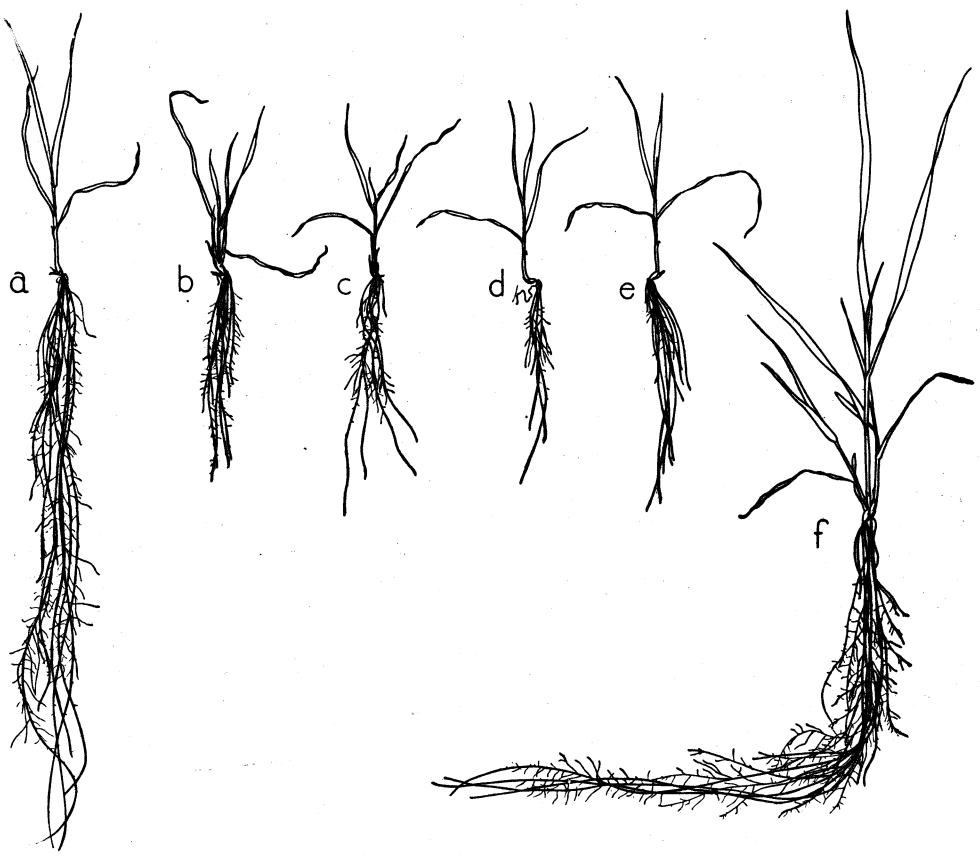

Fig. 1.—Appearance of typical plants grown in basal solution containing the following nitrogenous treatments: (a) no added nitrogen; (b) 7.0 x 10^{-5}M α-oximinopropionate; (c) 1.4 x 10^{-4}M α-oximinopropionate; (d) 3.5 x 10^{-4}M α-oximinopropionate; (e) 7.0 x 10^{-4}M α-oximinopropionate; (f) 3.5 x 10^{-4}M potassium nitrate. (One-sixth natural size.)

Experiment 3.—The technique employed was as described previously. Three different treatments with hydroxylamine were supplied and also oximinopropionic acid, alanine, ammonium sulphate, and potassium nitrate in solutions of equal molarity. Details of concentration and treatment are shown in Table 2. Typical plants after 38 days in the culture solutions are illustrated in Figure 2 and results of analyses are shown in Table 2.

TABLE 2

DRY WEIGHTS (G./6 PLANTS), PROTEIN-N, SOLUBLE-N, AND TOTAL-N (MG./6 PLANTS) OF OAT PLANTS* GROWN IN NUTRIENT SOLUTIONS AT pH 6.8 WITH HYDROXYLAMINE AND OTHER FORMS OF NITROGEN

Molarity	Shoots				Roots		Total Plant	
	D.W. (g.)	Prot.-N (mg.)	Sol.-N (mg.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)
Nil (Control)	0.277	3.27	1.22	4.49	0.231	1.96	0.508	6.45
7.0×10^{-5}M Hydroxylamine†	0.241	3.24	1.88	4.12	0.133	2.03	0.374	6.15
3.5×10^{-5}M Hydroxylamine†	0.242	6.75	2.71	9.46	0.117	2.34	0.359	11.80
3.5×10^{-4}M Hydroxylamine	0.239	1.27	1.18	2.45	0.111	1.15	0.350	3.60
3.5×10^{-4}M Oximinopropionic	0.231	2.31	1.06	3.37	0.102	1.30	0.333	3.67
3.5×10^{-4}M Alanine	0.768	26.50	11.40	37.90	0.293	13.20	1.061	51.10
3.5×10^{-4}M $(NH_4)_2SO_4$	0.564	25.50	13.20	38.70	0.238	13.10	0.802	51.80
3.5×10^{-4}M KNO_3	1.274	18.90	7.20	26.10	0.498	14.90	1.772	41.00

* Plants 38 days in nitrogenous solutions.

† Solutions changed daily; remainder every three days.

Experiment commenced 4.vi.46.

From these it appears that hydroxylamine in 3.5×10^{-5}M concentration can be utilized by shoots of oat plants to form protein-N, the amount of synthesis being small, possibly owing to the dilution. At this concentration plants produce tillers but there is suppression of dry weight increase and no nitrogen synthesis is apparent in roots. The behaviour of hydroxylamine, therefore, is apparently similar to that of oximinopropionic acid but is toxic at lower concentrations.

TABLE 3

DRY WEIGHTS (G./6 PLANTS), PROTEIN-N, SOLUBLE-N, AND TOTAL-N (MG./6 PLANTS) OF OAT PLANTS* GROWN IN NUTRIENT SOLUTIONS AT pH 7 WITH *TRANS*-OXIMINOSUCCINIC ACID AS SOLE SOURCE OF NITROGEN

Molarity	Shoots				Roots		Total Plant	
	D.W. (g.)	Prot.-N (mg.)	Sol.-N (mg.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)
Nil (Control)	0.193	2.69	0.99	3.78	0.180	1.82	0.373	5.60
3.5×10^{-5}M Acid†	0.223	4.25	1.62	5.87	0.200	2.88	0.423	8.75
7.0×10^{-5}M Acid	0.235	4.63	2.00	6.63	0.207	3.21	0.442	9.84
3.5×10^{-4}M Acid	0.240	4.83	2.99	7.82	0.206	3.54	0.446	11.36
7.0×10^{-4}M Acid	0.319	9.62	4.28	13.90	0.204	5.77	0.523	19.67
3.5×10^{-4}M KNO_3	0.490	13.62	4.14	17.76	0.490	5.50	0.980	23.26

* Plants 36 days in nitrogenous solutions.

† Solutions changed daily; remainder every three days.

Experiment commenced 5.vii.46.

Other treatments in this series (Fig. 2 and Table 2) emphasize the differences when plants were grown in solutions of equal molarity of oximinopropionic acid, alanine (the amino-acid corresponding to oximinopropionic acid), ammonium sulphate, and potassium nitrate.

Fig. 2.—Appearance of typical plants grown in basal solution containing the following nitrogenous treatments: (a) no added nitrogen; (b) 7.0 x 10^{-5}M hydroxylamine; (c) 3.5 x 10^{-5}M hydroxylamine; (d) 3.5 x 10^{-4}M α-oximinopropionate; (e) 3.5 x 10^{-4}M alanine; (f) 3.5 x 10^{-4}M potassium nitrate. (One-sixth natural size.)

(*b*) *Growth of Oats on* β (trans)-*Oximinosuccinate*

Experiment 4.—Concentrations of oximinosuccinate and potassium nitrate used and experimental conditions employed are shown in Table 3. Typical plants from each treatment after 36 days are illustrated in Figure 3 and results of analyses of plant material are shown in Table 3.

In appearance the plants were strikingly different from those grown in hydroxylamine and oximinopropionate, and described previously; they confirm the results obtained in Experiment 1. The root systems in all cases were normal although at all oxime concentrations the dry weights of the roots were probably not significantly greater than that of the control; however, increase in dry weight of the shoots occurred with increased oxime concentration.

Protein synthesis occurred in both shoots and roots, and in all cases the protein content of the plants increased as the concentration of oximinosuccinate increased. Plants grown in 3.5 x 10^{-4}M oximinosuccinate contained less (about half) nitrogen than those grown in potassium nitrate at the same concentration, but plants grown on 7 x 10^{-4}M oximinosuccinate were comparable, so far as N-content was concerned, with those grown in nitrate at the lower concentration.

It appears, therefore, that oat plants utilize oximinosuccinate readily as a source of nitrogen.

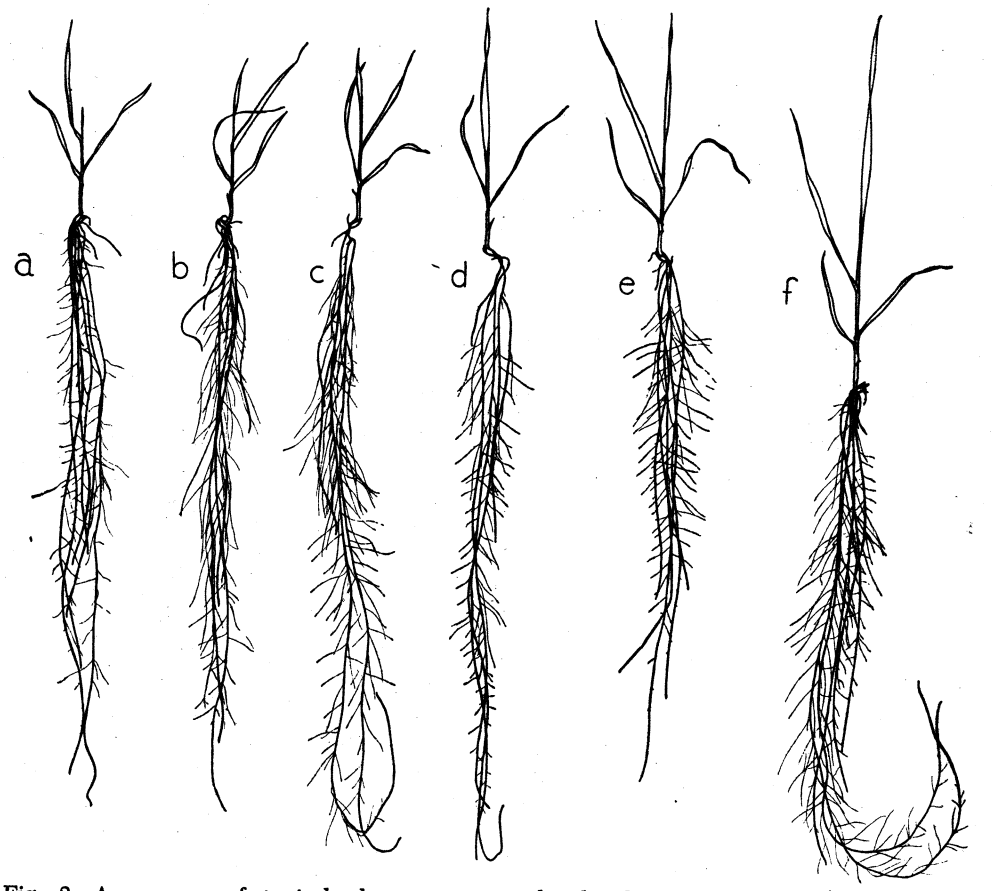

Fig. 3.—Appearance of typical plants grown in basal solution containing the following nitrogenous treatments: (a) no added nitrogen; (b) 3.5 x 10^{-5}M *trans*-oximinosuccinate; (c) 7.0 x 10^{-5}M oximinosuccinate; (d) 3.5 x 10^{-4}M oximinosuccinate; (e) 7.0 x 10^{-4}M oximinosuccinate; (f) 3.5 x 10^{-4}M potassium nitrate. (One-sixth natural size.)

(*c*) *Growth of Oats on* cis- *and* trans-*Oximinoglutarate*

Experiment 5.—Three treatments, including *trans*-oximinoglutaric acid, were applied as shown in Table 4 where conditions are also set out. Typical plants from each treatment are illustrated in Figure 4 and results of analyses are given in Table 4.

The results were similar to those described above for plants grown in oximinosuccinate. Roots and shoots were normal in appearance and it is apparent from Table 4 that *trans*-oximinoglutarate is utilized for protein synthesis by oats.

Experiment 6.—Oat plants were grown in solutions containing *cis*-oximinoglutarate and potassium nitrate at the concentrations and under conditions shown in Table 5.

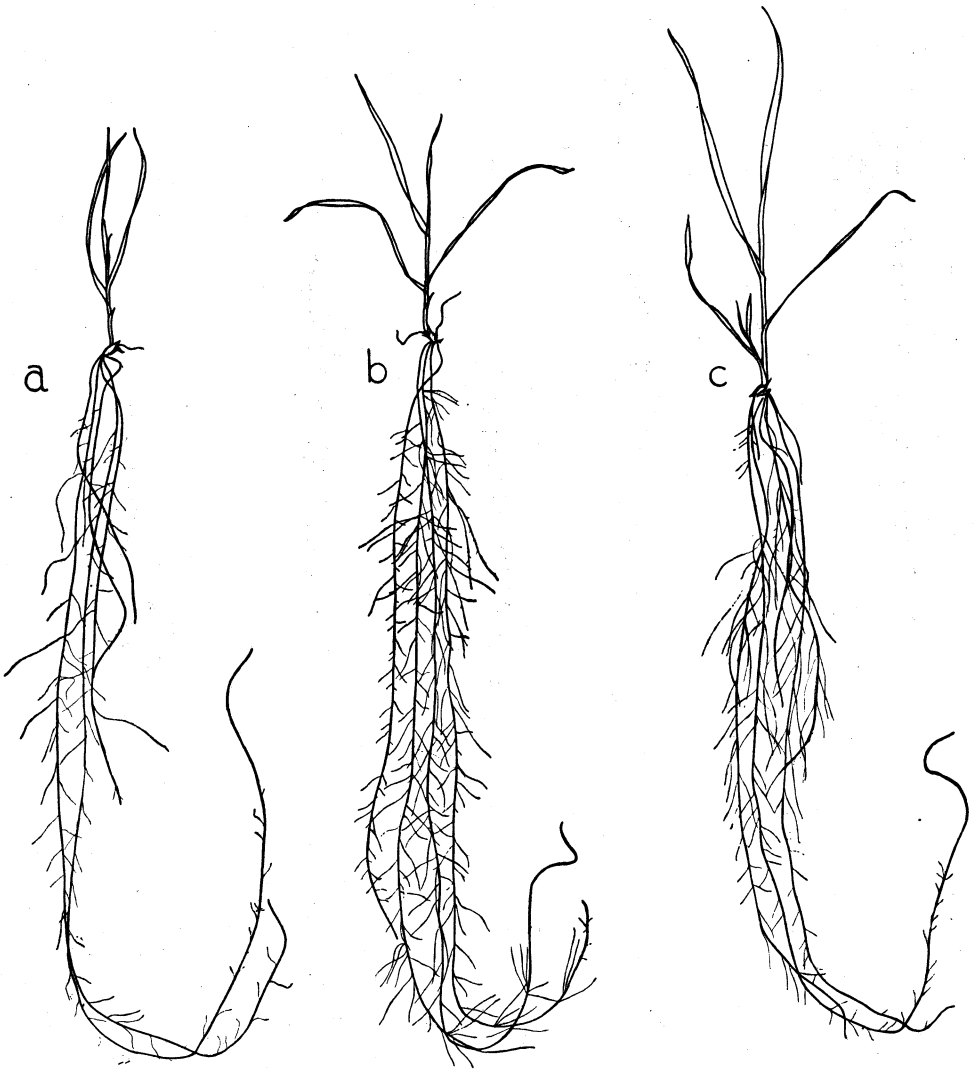

Fig. 4.—Appearance of typical plants grown in basal solution containing the following nitrogenous treatments: (a) no added nitrogen; (b) 3.5×10^{-4}M *trans*-oximinoglutarate; (c) 3.5×10^{-4}M potassium nitrate. (One-sixth natural size.)

The appearance of the plants after 54 days is illustrated in Figure 5 and results of analyses are set out in Table 5. The treatments included concentrations higher than those previously employed.

TABLE 4

DRY WEIGHTS (G./6 PLANTS), PROTEIN-N, SOLUBLE-N, AND TOTAL-N (MG./6 PLANTS) OF OAT PLANTS* GROWN IN NUTRIENT SOLUTIONS AT pH 7 WITH *TRANS*-OXIMINOGLUTARIC ACID AS SOLE SOURCE OF NITROGEN

Molarity	Shoots				Roots		Total Plant	
	D.W. (g.)	Prot.-N (mg.)	Sol.-N (mg.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)
Nil (Control)†	0.213	3.70	1.04	4.74	0.195	1.49	0.408	6.23
3.5×10^{-4}M Acid	0.287	6.77	2.52	9.29	0.207	4.06	0.494	13.35
3.5×10^{-4}M KNO_3	0.419	13.60	4.40	15.00	0.196	4.52	0.615	19.52

* Plants 34 days in nitrogenous solutions.
† All solutions changed every three days.
Experiment commenced 20.vii.46.

TABLE 5

DRY WEIGHTS (G./6 PLANTS), PROTEIN-N, SOLUBLE-N, AND TOTAL-N (MG./6 PLANTS) OF OAT PLANTS* GROWN IN NUTRIENT SOLUTIONS AT pH 6.8 WITH *CIS*-OXIMINOGLUTARIC ACID AS SOLE SOURCE OF NITROGEN

Molarity	Shoots				Roots		Total Plant	
	D.W. (g.)	Prot.-N (mg.)	Sol.-N (mg.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)	D.W. (g.)	Tot.-N (mg.)
Nil (Control)	0.094	0.74	0.76	1.50	0.132	1.35	0.226	2.85
5×10^{-4}M Acid	0.132	2.58	2.76	5.34	0.096	2.46	0.228	7.80
1×10^{-3}M Acid	0.198	4.32	3.54	7.86	0.106	3.06	0.304	10.92
5×10^{-3}M Acid	0.166	2.70	1.86	4.56	0.086	2.76	0.252	7.32
5×10^{-4}M KNO_3	0.214	4.74	2.22	6.96	0.094	2.00	0.308	8.96
1×10^{-3}M KNO_3	0.262	4.80	2.76	7.56	0.104	2.52	0.366	10.08
5×10^{-3}M KNO_3	0.560	14.52	6.72	21.24	0.224	4.86	0.794	26.10

* Plants 54 days in nitrogenous solutions. All solutions changed every ten days.
Experiment commenced 10.vii.47.

That *cis*-oximinoglutarate is more toxic in 5×10^{-3}M solution than in 1×10^{-3}M is reflected in the morphology of the plants and in their dry weight and protein contents, although N-synthesis occurred at the higher concentration. At the two lower concentrations of *cis*-oximinoglutarate root dry weight was approximately the same as that of plants grown in nitrate of the same molarity, although in the oxime solutions the root system was more compact. In shoots, dry weight was less in plants grown in oximes than those in nitrates, but protein synthesis occurred readily in plants grown in the oximinoglutarate solutions.

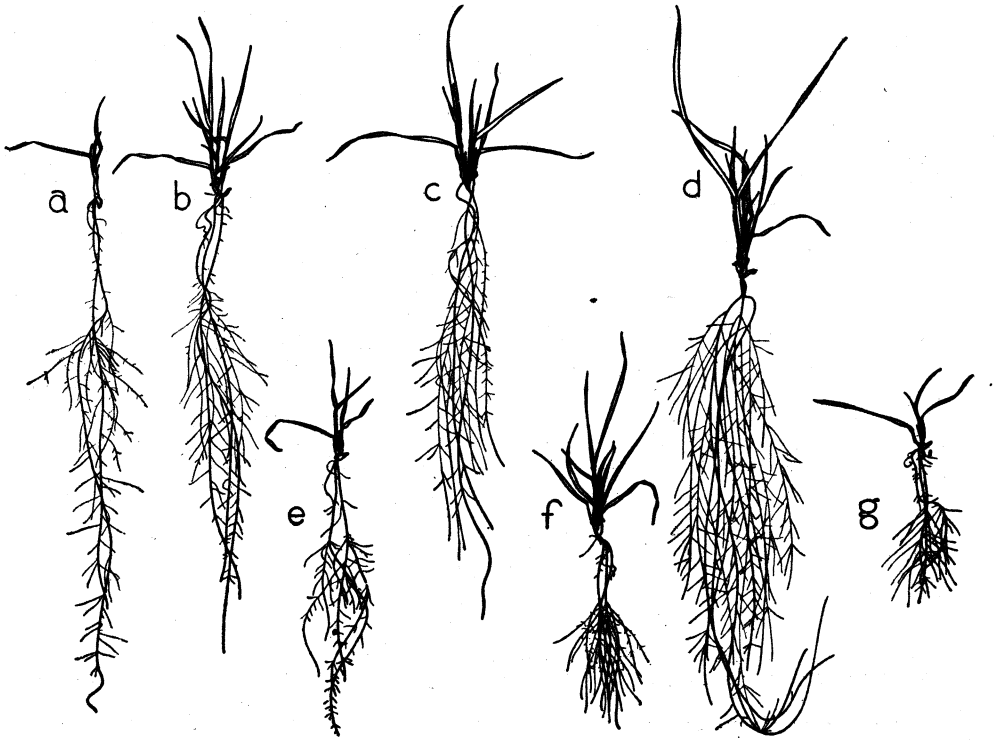

Fig. 5.—Appearance of typical plants grown in basal solution containing the following nitrogenous treatments: (a) no added nitrogen; (b) 5 x 10^{-4}M potassium nitrate; (c) 1.0 x 10^{-3}M potassium nitrate; (d) 5.0 x 10^{-3}M potassium nitrate; (e) 5 x 10^{-4}M *cis*-oximinoglutarate; (f) 1.0 x 10^{-3}M *cis*-oximinoglutarate; (g) 5.0 x 10^{-3}M *cis*-oximinoglutarate. (One-sixth natural size.)

IV. Discussion

The validity of the results described here depends upon showing that the oximino-acids are absorbed unchanged by plants. For reasons discussed earlier this is difficult to establish by analyses of plants but it has been shown that, under the conditions used, the oximes did not undergo reduction, oxidation, or hydrolysis in the culture solutions prior to adsorption by the plants.

The experiments described indicate that in non-toxic concentrations hydroxylamine, oximinopropionate, *trans*-oximinosuccinate, and both *cis*- and *trans*-oximinoglutarate can all be utilized by oat plants as sources of nitrogen to form proteins. With *trans*-oximinosuccinate and *trans*-oximinoglutarate the highest concentrations employed were respectively 7 x 10^{-4}M and 3.5 x 10^{-4}M, at both of which concentrations there was no evidence of toxic symptoms in plants grown in them. The optimum concentrations for protein synthesis appear to be about 1 x 10^{-5}M for hydroxylamine, about 1 x 10^{-4}M for oximinopropionic acid, and about 1 x 10^{-3}M for the three oximino-dicarboxylic acids which have been investigated.

Plants grown in both hydroxylamine and oximinopropionate solutions show marked depression of dry weight compared with control plants and this is probably connected with the known effect of hydroxylamine on photosynthesis; the depression in dry weight production is more marked in roots than in shoots.

With the oximino-dicarboxylic acids dry weight changes in plants, especially in shoots, are less obvious; the percentage protein content on a dry weight basis is approximately the same in plants grown in the oximino-dicarboxylic acids and nitrate solutions of equal molarity; this suggests interdependence of the processes of protein formation and dry weight production which is not apparent in plants grown in solutions of hydroxylamine and oximinopropionate.

The amount of protein formed in plants grown in solutions containing the oximino-dicarboxylic acids is relatively large and in some cases approaches that of plants grown on nitrate solutions of the same molarity.

The greater amount of growth and of protein synthesis in shoots compared with roots tend to support Burström's (1945) suggestion, discussed earlier, that oxime production occurs in leaves rather than in roots.

The interest in oximinosuccinate and oximinoglutarate and their ready utilization by oat plants for protein synthesis lies in their relationship with the corresponding keto-acids oxalacetic and α-ketoglutaric acids, and with the corresponding amino-acids aspartic and glutamic acids. Both these oximino-dicarboxylic acids are possible intermediates in the formation of amino-acids from keto-acids when nitrogen is supplied as nitrate. Figures published by Steward and Street (1947) for leaves of bean plants suggest connection between oxime and keto-acid contents, although for reasons described earlier such figures for oxime-nitrogen may have little meaning. At the present state of knowledge speculation seems idle and the results described in this paper do no more than show that the oximino-dicarboxylic acids readily, and oximinopropionic acid and hydroxylamine in non-toxic concentration, can be utilized by oat plants to synthesize proteins.

V. References

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