

This gives $n_2 = \sqrt{\frac{2.5 \cdot 33}{1.5 \cdot 89}} = 1.43$ coleoptiles per vial

The corresponding number of samples is $n_1 = 4.74$ with resulting variance of the mean, $V\bar{x} = 23.7$. Practically, either one or two coleoptiles per vial may be used. The corresponding numbers of samples n_1 and variance $V\bar{x}$ are:

one coleoptile per vial, $n_1 = 5$, $V\bar{x} = 24.4$

two coleoptiles per vial, $n_1 = 4$, $V\bar{x} = 26.3$

Despite the slightly greater efficiency with one coleoptile per vial (using five samples) many investigators may feel safer with two (using four samples).

The number of samples may be varied at will depending on the precision required and on the available time and material.

SUMMARY

Methanol extracts of dormant peach buds were analyzed for possible growth promoting and inhibiting substances. Paper chromatography techniques and the wheat coleoptile straight growth test were used for separation and measuring respectively of the growth substances present. A growth inhibitor (R_f 0.53) occurred in all of the peach bud samples and is being investigated further. Results of the statistical study are summarized below:

1. Replications of coleoptiles treated similarly is an underestimate of the experimental error; true replication is by use of separate samples including all sources of variation.

2. The largest component of experimental error was the failure of known and unknown growth substances chromatographed always to appear in the same section.

3. Growth of coleoptiles follows approximately a normal distribution curve indicating additivity. This is confirmed by a statistical test of additivity. Therefore, ordinary statistical techniques can be used directly without transforming the data. Changing the growths to percentages of control introduces unnecessary complications.

4. It was shown that the variance of the mean is decreased more by increasing the number of replica-

tions (samples) than by increasing the number of coleoptiles per vial.

5. For experiments of the type described, one or two coleoptiles per section are indicated as furnishing the greatest information per hour spent.

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EFFECT OF METAL CHELATES ON GROWTH OF CORN IN SOLUTION CULTURES^{1,2}

S. K. MAJUMDER AND STUART DUNN

BOTANY DEPARTMENT, UNIVERSITY OF NEW HAMPSHIRE AGRICULTURAL EXPERIMENTAL STATION,
DURHAM, NEW HAMPSHIRE

Most of the basic studies on metal chelates in plant nutrition relate to soil applications. Not as much is known about their use in solution cultures where effects of variables such as hydrogen ion con-

centration, essential micro- and macro-elements and their interactions can be better controlled and studied than in soil. This paper presents results of two series of experiments with corn grown under greenhouse conditions in which chelating agents were employed in solution cultures.

Corn seeds (Kingscrot, Ke) were germinated in sand. The seedlings were transplanted to culture

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jars of 3.8 liters capacity (1 gallon) 6 days after planting, when the shoots were about 7 cm tall. The most uniform plants were selected from a large number of seedlings. Roots were thoroughly washed in distilled water before transplantation.

For each treatment, there were five jars with two plants in each (unless otherwise stated). The solutions were changed weekly and were adequately aerated once in the middle of each week. The greenhouse temperature was maintained at approximately 25° C. Plants were harvested after 21 days from the date of transplantation and dry weights of tops and roots were taken as a measure of the effects of the different treatments.

The basic composition of the solution was as follows (per liter):

Macro-salt	Concentration in millimoles	Micro-salt	Concentration in micromoles
Ca-acetate	0.26	H ₂ BO ₃	8.3
NaNO ₃	0.40	MnCl ₂	4.0
(NH ₄) ₂ SO ₄	0.27	ZnSO ₄	0.3
NH ₄ NO ₃	0.14	CuSO ₄	0.1
MgSO ₄	0.33	FeCl ₃	62.0
KCl	0.21		
Ethyl Ammonium Phosphate	0.48		

The composition of the solution is obviously different from any conventional type. It was developed to suit the purpose of this experiment. Calcium acetate was used because it acted as a buffer and helped to keep the hydrogen ion concentration of the solution fairly constant during the experiment. The use of ethyl ammonium phosphate instead of inorganic phosphates, commonly used, calls for special explanation. This compound is a water-white to yellow tinged liquid of ammoniacal odor (specific gravity 1.23 at 25° C). Chemically it is a mixture of 35 % monoethyl diammonium phosphate, 50 % diethyl ammonium phosphate and 15 % monoethyl ammonium hydrogen phosphate. Thirty eight percent of this

compound is phosphorus as P₂O₅. Unlike inorganic compounds of phosphorus, it does not form precipitates of iron phosphate with ferric chloride. Moreover, this organic compound was found to be not only physiologically non-toxic, but also a good source of phosphorus in nutrient culture.

The 1st experiment was concerned with the effects of the disodium salt of EDTA³ on corn seedlings, grown in solutions as given above, except for the varying amounts of EDTA. The 2nd experiment was concerned with the substitution of chelated magnesium ("Versenol" magnesium chelate) for the usual source, magnesium sulfate.

The results of the 1st experiment are shown in table I. It appears from our results that there is a definite correlation between the amounts of EDTA in solution and plant growth, which was progressively better with decreasing concentrations. Amounts of 80 micromoles or higher were sooner or later lethal to the plants. At 200 micromoles concentration plants died immediately after transplantation. There was no increase in dry weight at this concentration. For this reason the weights of these plants were taken as a base and were subtracted from the mean dry weights in all other treatments to show the mean dry weight increase (columns 5 and 6, table I). Plants receiving 160 micromoles of EDTA survived for about a week, whereas those receiving 120 micromoles and 80 micromoles died in about 8 and 12 days respectively from the date of transplantation. At 40 micromole and 20 micromole levels plants were very chlorotic and stunted. Concentrations of 10 micromoles, 5 micromoles and 2.5 micromoles not only were non-toxic, but appeared to be growth-promoting as compared to the control which contained no EDTA. It seems from this that the upper non-toxic level of EDTA concentration approximated 10 micromoles per liter. It also may be noted that plants receiving non-toxic concentrations of EDTA grew more luxuriant root systems than those without this compound.

³ EDTA: Ethylene diamine tetraacetic acid.

TABLE I
EFFECT OF DIFFERENT CONCENTRATIONS OF EDTA ON THE GROWTH OF CORN IN SOLUTION CULTURE

Na ₂ EDTA MICROMOLES PER LITER	MEAN DRY WT G/PLANT		STANDARD ERROR		MEAN DRY WT INCREMENT AFTER TRANSPLANTATION		MEAN DRY WT INCREMENT AS % OF CONTROL	
	Top	Root	Top	Root	Top	Root	Top	Root
0 (control)	1.53	0.61	0.109	0.045	1.40	0.49	100	100
2.5	1.90	1.02	0.096	0.062 **	1.77	0.89	126.7	182.4
5.0	1.75	0.91	0.088	0.094 **	1.62	0.79	115.7	160.5
10.0	1.67	0.91	0.109	0.045 *	1.54	0.78	110.2	160.3
20.0	0.67	0.43			0.54	0.31	38.9	63.0
40.0	0.40	0.28			0.27	0.15	19.4	31.3
80.0	0.29	0.23			0.16	0.11	11.7	21.7
120.0	0.18	0.16			0.05	0.04	3.8	7.4
160.0	0.14	0.13			0.01	0.01	0.9	1.4
200.0	0.13	0.12			0.0	0.0	0.0	0.0

* Difference compared with control significant at 0.05 level.

** Difference compared with control significant at 0.01 level.

The chelating agent (Na_2EDTA) when mixed with a solution of various inorganic salts, forms complexes with metals in an order depending on the stability of the corresponding complexes. It forms more stable compounds with iron and copper than with any other metals, and chelated iron has been successfully used as a source of iron in solution culture (5, 6, 7). It may be noted, however, that at all levels of Na_2EDTA below 20 micromoles the molar ratio of EDTA to Fe^{+++} was less than 1 : 1. This means that at these levels all the Fe^{+++} ions were not chelated. Therefore, Fe from FeCl_3 rather than from Fe-EDTA was probably the more dominant source of iron for the plants. Besides, the supply of iron was adequately insured even without the addition of EDTA to the solution since ethyl ammonium phosphate did not form precipitates of iron phosphate with ferric chloride. Therefore, the beneficial effect of low concentrations of EDTA on plants growing in solution culture must be attributed to a growth-promoting property of this compound.

Heath and Clark (3, 4) have suggested that EDTA at very low concentrations acts as a growth substance and that 3-indole acetic acid affects growth in the same way as a chelating agent. Gross (2) reported an increase in activity of adenosine triphosphatase at lower concentrations of EDTA, but a significant inhibition in its activity at higher concentrations. Weinstein et al (8) found that 10 ppm of EDTA is optimum for soybeans grown in solution cultures. Results by Jacobson (5) on corn were in substantial agreement with this.

There is some evidence from the work of Gross (2) that chelates in low concentrations may provide some protection to enzyme systems from traces of heavy metals. Whether this would be a factor in the beneficial effect of low concentrations of EDTA on plants grown in culture solutions is a question needing further investigation.

In the 2nd experiment, chelated magnesium ("Versenol" magnesium chelate) replaced magnesium sulfate in the culture solutions (table II). Dunn and Roberts (1) found no beneficial effect of magnesium Versene⁴ on corn and apple seedlings grown in solution cultures. Previous work in the Horticultural Department of the University of New Hampshire by R. Eggert (unpublished data) showed that Mg-EDTA in high concentrations was very toxic to plants either as a foliar spray or in soil applications.

In this experiment there were 10 culture jars per treatment with two plants in each jar. Except as noted in table II the solutions used were identical in composition with those used in the control of the previous experiment. Plants were harvested 21 days from the date of transplantation.

Treatment A was considered to be the control, and the plants received magnesium sulfate and "Versenol" iron chelate as sources of Mg and Fe. Plants in both treatment A and B looked very vigorous as compared

⁴ Magnesium Versene: Sequestrene Na_2Mg of Geigy Chemical Corporation.

TABLE II
ROLE OF VERSENOLO MAGNESIUM CHELATE AS A SOURCE OF
MAGNESIUM FOR CORN IN SOLUTION CULTURE

TREATMENTS, MICROMOLES PER LITER	MEAN DRY WT G/PLANT		STANDARD ERROR	
	Top	Root	Top	Root
A. Versenol Fe-chelate, 9 micromoles (instead of FeCl_3)	0.94	0.33	0.015 **	0.006 **
B. Versenol Mg-chelate, 6 micromoles (instead of MgSO_4), Versenol Fe-chelate, 4.5 micromoles (instead of FeCl_3)	1.05	0.35	0.152 *	0.016 **
C. No magnesium and Versenol iron chelate, 9 micromoles (instead of FeCl_3)	0.55	0.25	0.034	0.008

* Significant at 0.05 level over treatment C.

** Significant at 0.01 level over treatment C.

Versenol iron and magnesium chelate are metals chelated with "Versenol" (Dow Chemical Company) which is a trisodium salt of *N*-hydroxy ethyl ethylene diamine triacetic acid.

to those of treatment C which were distinctly stunted and had all the symptoms of magnesium deficiency (chlorosis, necrotic leaf spots, poor root growth, etc.). The dry weight yields of tops and roots in treatment B were found to be significantly higher than those of treatment C.

The following points may be considered in explanation of the results. The total concentration of "Versenol" metal chelate in any of the treatments did not exceed 10.5 micromoles which was found to be the optimum concentration for "Versenol" under these conditions. The "Versenol" magnesium chelate in treatment B should dissociate in the solution to a very negligible extent throughout the experiment. This is because both iron and magnesium were in chelate form and there would be no possibility for the iron to replace the magnesium from its complex. The percentage of magnesium present as metal in the "Versenol" magnesium chelate, however, is considerably lower than that in magnesium sulfate (treatment A). In spite of this low concentration of magnesium in treatment B, the plants did not show any signs of magnesium deficiency (with treatment C as an index). Instead they were just as vigorous as those in treatment A. This indicates that the magnesium of the "Versenol" chelate was as readily utilized by plants as that of magnesium sulfate. There appears to be no other published account of the successful use of chelated magnesium as a plant nutrient in solution culture.

SUMMARY

Ethylene diamine tetraacetic acid in low concentrations (10 micromoles and below) was distinctly

beneficial to plant growth, especially to root development, in nutrient cultures under greenhouse conditions.

The magnesium salt of "Versenol", if supplied in non-toxic concentrations and with proper adjustment of the solution composition, can substitute successfully for magnesium sulfate as a source of magnesium for corn grown in solution cultures.

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THE WATER CONTENT OF MAPLE STEMS. I. APPLICATION OF THE KARL FISCHER METHOD FOR THE ANALYSES OF WATER IN MAPLE BARK AND WOOD^{1,2}

MARY T. GREENE AND JAMES W. MARVIN

DEPARTMENT OF BOTANY, UNIVERSITY OF VERMONT, BURLINGTON, VERMONT

In physiological studies in which water determinations are a problem the oven-dry method may have definite limitations. In such experiments alcohol extraction of the water followed by titration with Karl Fischer reagent is a useful alternative method. Karl Fischer first reported in 1935 on the reagent which now bears his name (3). Since then the very general usefulness of this reagent in analyses for water has been recognized. Mitchell and Smith (14) have critically reviewed the literature describing the various applications of the Karl Fischer titration. In the determination of moisture in native wood samples, Mitchell (13) obtained good agreement between the titration and oven-dry methods. In general he found higher values with the Fischer titration. His data are for samples of air-dried Douglas fir, kiln-dried cypress and partly-dried oak. No data on the water content of fresh wood as determined by the Karl Fischer method were available.

Although references to analyses for water in wood using the Fischer method are few, there is a considerable amount of published data on the moisture content of wood, determined by oven-drying or by other methods. These data relate to two distinct problems concerning water content; either its relation to the physical and mechanical properties of wood as lum-

ber, or its relation to the physiology of the living tree. As concerns lumber, McMillen (12) states that even though wood must have proper moisture content for maximum utility, none of the standards associations has adopted a standard testing procedure. When water content must be determined the industrial wood users depend on oven-drying.

The method of sampling fresh wood for water determinations becomes more of a problem in studies of tree physiology. McDermott (11) using the oven-dry method, showed that the percent moisture of tree branches varied significantly with the method of cutting the samples. His samples were taken when the water in the xylem elements was presumably under tension; i.e., under conditions of rapid transpiration. Cutting breaks this tension, causing the water to pull away from the cut end. Determinations on such samples gave significantly lower values than those on samples where a simultaneous cutting method prevented the removal of any of the water. Gibbs (4) had previously considered the possibility of changes during sampling and avoided it by using a method of punching out cores of wood. In a later report (6) Gibbs considered McDermott's findings in relation to a natural gradient in the water content along the branch. He confirmed McDermott's results and found that natural gradients were too small to affect them.

Another sampling problem originates from a difference in water content of successive annual rings or between sapwood and heartwood. Huber (7) presented data from Langer on the wood, water, and

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