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THE DILUTION EFFECT IN PLANT NUTRITION STUDIES

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I. INTRODUCTION

The "dilution effect" and its inverse, the "concentration effect," have been referred to in numerous studies of plant nutrition and soil fertility to explain

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results that arise when the concentration[1] of an element in plant tissue is decreased or increased due to a change in environmental conditions. "Environmental conditions," in this context, include changes in the soil environment due to the addition of inorganic and organic materials and water to soil, as well as temperature and light; the application of living organisms such as rhizobia and mycorrhizal fungi; and the inclusion of toxic materials such as heavy metals. An additional instance in which "dilution" is often alluded to is the change in nutrient concentration as a function of time. It has often been found, especially for N, P, and K, that young plants contain higher concentrations than do older plants (Loehwing, 1953).

A survey of the literature suggested that no extensive articles had been written on the dilution effect. Hughes *et al.* (1978) and Barea *et al.* (1980) both found that P additions decreased the concentration of N in plants, although because of increased yield greater total N accumulation was noted. Numerous articles have alluded to it, either to explain results or to describe basic plant behavior. For example, many articles in the Revised Edition of *Soil Testing and Plant Analysis* (Walsh and Beaton, 1973) refer to this phenomenon. Relevant statements from several of these articles follow:

> It is not unusual to find that the addition of certain nutrients reduces the amounts of other nutrients in the plant (Aldrich, 1973, p. 215).

> When nutrients such as N, P, or K are added, it is difficult to predict whether or not the concentration of a given element in the plant part will increase, remain unchanged, or be decreased. Much depends upon the influence of the other nutrients available in the soil and the direct and indirect effects the applied nutrient has on increased growth and yield (Munson and Nelson, 1973, p. 236).

> Analysis of plant tissue usually reveals only one deficiency at a time. A second nutrient, or even a third nutrient, may be in short supply but, due to reduced growth caused by the primary nutrient deficiency, all other nutrients will accumulate in the tissue (Ulrich and Hills, 1973, p. 286).

Conc. effect

> The problem area plants appeared to be N-deficient. Normal plants had few nodules on the roots and the plant tissue N levels were very low. Concentrations of certain elements in the normal plants were lower than corresponding nutrient levels in the problem plants. This was, in part, the result of dilution resulting from more rapid plant growth (Small and Ohlrogge, 1973, p. 324).

> When the total plant uptake (concentration times dry matter) was plotted versus time, the curves were fairly smooth. Therefore, most of the variations in element concentration were due to concentration or dilution associated primarily with change in dry matter production (Jones and Eck, 1973, p. 356).

> The basic principle of the use of plant analysis is that the chemical composition of the plant reflects the nutrient supply in relation to growth. We must, however, recognize that the chemical composition of any plant is a "result" of the interaction of nutrient supply and plant growth. Any factor that limits growth, be it light, moisture, temperature, or some nutrient, may cause other nutrients to accumulate in the plant (Martin and Matocha, 1973, p. 394).

> Samples taken at early stages of growth have high concentrations of N, P, K, and S. The concentration of such nutrients declines as the plant matures and approaches the bloom stage because of the dilution with carbohydrates and other structural solids (Martin and Matocha, 1973, p. 397).

> Generally, the "dilution effect" caused by the rapid growth of this grass will reduce the instances when "luxury consumption" of K will occur (Martin and Matocha, 1973, p. 412).

> Generally, if a particular element that is limiting growth is added in a fertilizer treatment and a subsequent growth response occurs, an increase in both that element concentration and content in tissues occurs. However, if a dramatic growth response follows treatment, it is possible that the addition of the limiting element may result in a lower concentration of that element in tissues. . . . On the other hand, the levels of other elements (nonlimiting growth) in the tissues may decrease in concentration due to dilution effects but increase in content due to increased biomass (Leaf, 1973, p. 445).

These citations represent to one degree or another the common conception of the "dilution" and "concentration" effects held by those who work with plant tissue sampling. In most instances, reference is made to changes induced by supplying a deficient nutrient to the plant; in one case, by removal of an environmental restriction such as nonoptimal light, temperature, or water. Responses due to microorganisms such as rhizobia or mycorrhizae have not generally been considered in the past.

As noted, the concentration of nutrient in a plant tissue is a single-point resultant of plant history, in particular the integration of two dynamic processes, nutrient uptake and transport and dry matter accumulation (Lundegardh, 1966; Martin and Matocha, 1973). Since the concentration is a resultant of the quantitative manner in which growth and accumulation vary, it would seem reasonable that a complete consideration of the crop's nutrient status could be separated into considerations of these fundamental processes. This article will analyze "dilution" and "concentration" effects in tissue on the basis of the relative rates of elemental uptake and dry matter accumulation.

II. SYSTEM FOR EXPRESSING RESULTS

Because the concentration in tissue is a ratio of two quantities, we believe it would clarify much of the following discussion if absolute responses were re-

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[1] Terminology relating to the quantities of nutrients in plants often relies on ambiguous terms (Leaf, 1973). In particular, "uptake" and "content" are not very precise terms. They may refer to either the total amount in the plant or to the concentration in the tissue. Throughout the course of our discussion, the term "concentration" will denote a mass or molar ratio such as milligrams or moles per kilogram. Further, "total accumulation" will be used when referring to the total quantity of nutrient in the plant, either the whole plant, the above-ground portion of the plant, or some tissue such as leaves or stems. Careful use of terms should minimize at least some points of confusion. "Uptake" will refer to the process of elemental movement into and within plants and not to quantity-based terms such as concentration or total accumulation.

(*a*) Direct dilution because of greater biomass.
(*b*) Extension or shortening of roots, e.g., root length density.
(*c*) Availability of energy to the root for uptake processes, e.g., rate of net photosynthesis, carbon assimilation, and translocation from top to roots.

Intermediate sorts of effects in which there was no clear separation between interactive and noninteractive types would include changes in the hormonal balance of the plant.

V. DILUTION EFFECTS

The situations considered in this section are those in which a deficiency of some type (nutrient, water, oxygen) is overcome by a change in the plant's chemical, physical, or biological environment. The concentrations of other elements in the leaf tissue are then measured as the plant dry matter increases due to treatment. Such changes help indicate how the plant is managing the supply of nutrients which are available to it, when a greater demand is placed upon this supply by a larger plant.

The discussions in this article will emphasize primarily research contributions in which total dry matter data are reported so that the dynamic effects of plant growth and nutrient uptake can be separated out in part at least and placed within the framework of the "types" presented in Table I. However, where it is felt to be important, the information from experiments dealing with changes in concentration only will also be discussed.

A. Dilution of Nutrient Applied

In this situation, a nutrient element is applied to a crop, the yield of the crop increases, but upon chemical analysis the average concentration of the element in some or all plant tissue is lower than in a deficient control plant (Piper, 1942; Steenbjerg, 1951; Steenberg and Jacobsen, 1963). This result is probably most surprising because of the implicit assumption, mentioned earlier in this article, that the adequacy of the plant's supply of a given nutrient is directly related to the tissue concentration of that nutrient (Lundegardh, 1966; Martin and Matocha, 1973). However, as Bates (1971) has summarized (see Table II), there are several explanations, consistent with physiological considerations, that may account for this. For example, plants may lose the potential for growth or response under acute nutrient stress and be unable to respond even though they have accumulated "adequate" concentrations of the limiting element in their tissue (Hiatt and Massey, 1958).

Table II
Examples of C-Shaped Yield–Nutrient Concentration Curves[a]

Nutrient	Culture	Tissue	Reference
Cu	Solution	Oats[b] (mature) (*Avena sativa*)	Piper (1942)
Cu	Soil	Oat (whole plant)	Steenbjerg (1945)
Mn	Soil	Oat (whole plant)	
P	Soil	Barley (straw) (*Hordeum vulgare*)	Poulson (1950)
Cu	Soil	Barley (straw)	Steenbjerg (1951)
Cu	Soil	Barley (grain)	
P	—	—	Prevot and Ollagnier (1956)
Mn	Sand	Tomato (lower stems) (*Lycopersicum esculentum*)	Hewitt (1956)
Zn	Field	Corn (whole plant) (*Zea mays*)	Hiatt and Massey (1958)
Zn	Field	Corn (whole plant)	
Mg	Soil	Oat (straw)	Jakobsen and Steenberg (1964)
Zn	Solution	Sugar beet (young blades)[c] (*Beta vulgaris*)	Rosell and Ulrich (1964)
Zn	Solution	Sugar beet (mature petioles)	
B	Solution	Birch (roots)[d] (*Betula* spp.)	Ingestad (1954)
SO_4-S	Soil	Grass (leaves) (*Lolium multiflorum*)	Saalbach and Judel (1966)
S[e]	Solution	Ryegrass (stems)	Ulrich (1968)
Zn	Solution	Alfalfa (stems)[d] (*Medicago sativa*)	Lo and Reisenauer (1968)

[a] From Bates (1971).
[b] Rye in the same experiment did not give a C-shaped curve.
[c] Mature blades did not give a C-shaped curve.
[d] Leaves did not give a C-shaped curve.
[e] A C-shaped curve was obtained with organic or total S but not with SO_4–S.

Whatever the actual physiological cause, when the growth-limiting element is supplied, the relative rate of dry matter accumulation increases more rapidly than the rate of nutrient accumulation, resulting in lower final concentrations in treated plants.

In all cases, even though the concentration of the element in the tissue has decreased, the total accumulation, as calculated by the product of concentration and dry matter yield, has increased significantly. Thus we represent the behavior as ↑↑↓, to indicate that plant growth has proceeded more rapidly than nutrient accumulation.

Piper (1942) and Steenbjerg (1951) both noted this effect on cereals with Cu. Steenbjerg found 16.6 mg Cu/kg tissue in untreated control plants, while on a whole plant basis (straw + grain) average concentrations of Cu-treated plants were 6.4–14.3 mg Cu/kg. Untreated grain had 3.2 mg Cu/kg, while treated grains ranged from 0.7 to 5.4, mean ± SD = 4.1 ± 1.7. Copper concentrations of straw, on the other hand, ranged from 8.3 to 14.4, with mean ± SD = 11.0 ± 2.2. In every case total uptake was greater than controls. So it is evident that higher concentrations could be achieved in grain with treatment, while in straw, or with all. ?

The harvest index (mass ratio of grain yield to straw yield) for control plants was 0.1/8.3 = 0.012, while for the best yield (1.02 g $CuSO_4 \cdot 5H_2O$/pot) it was 54.4/47.3 = 1.15, nearly 100 times as great (Fig. 1). Thus the grain provided a much greater sink for translocatable Cu in treated than in untreated plants.

By stunting the plant early during its growth period, there may be a synergistic negative effect due to the accumulation of elements to toxic concentrations in the tissue. This may even include the element in question that was initially deficient (see Section VI).

Gupta *et al.* (1976) found that a seed treatment with Mo may have significantly increased yield, of both onions and cauliflower, with a concomitant drop in Mo concentrations of leaf tissues. In both cases there were small but probably not significant decreases in total uptake as measured by [Mo] × yield, but cauliflower heads and onion bulbs were not considered in this case, and total uptake of treated plants was probably greater.

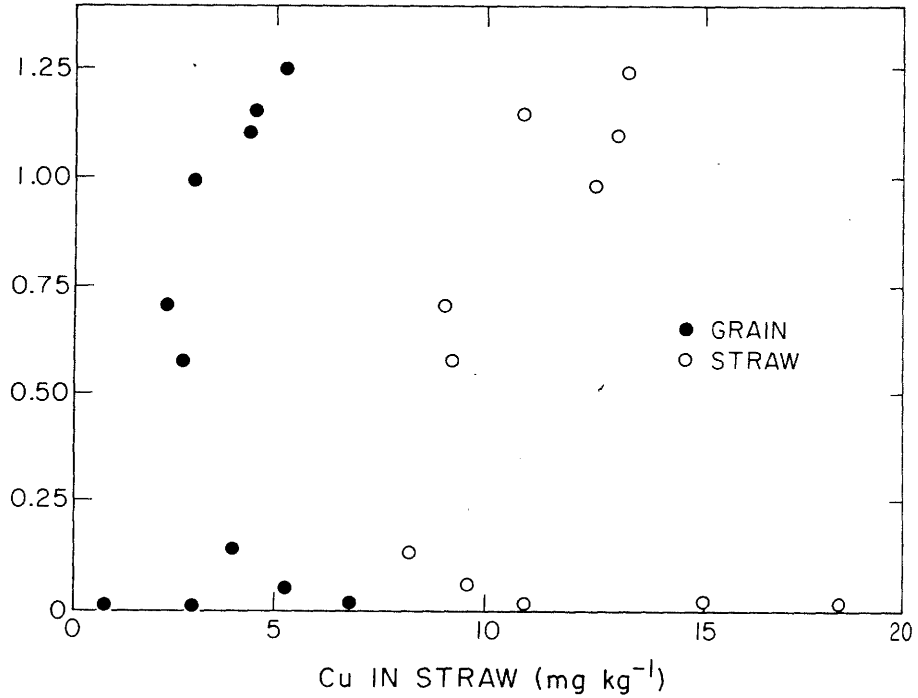

Fig. 1. Relationship between harvest index and [Cu] in straw (○) or grain (●). From Steenbjerg (1951).

Some of the data of Thomas and Mather (1979) on Fe application to sorghum suggest a dilution effect both in response to N, P, and K and to Fe applications. In the first crop, application of Fe with NPK increased yields substantially relative to −Fe treatments, but the tissue concentration of Fe dropped from 40 to 30 mg Fe/kg in leaves.

With Fe it is probably especially important to interpret total analysis data skeptically. Active Fe may be some variable fraction of the total Fe (Katyal and Sharma, 1980). Apparently the mineral or biochemical environment inside the cell may be more critical in determining the adequacy of Fe supply than is the total analytical concentration.

The dilution effect as shown in these examples is fundamentally interesting and may help in understanding biological problems. In a practical sense it is probably less significant in established agricultural settings. An exception to this case may be where virgin land is being converted to agriculture, or where new crops are introduced. But generally, few plants are as extremely deficient as the controls in these examples. In addition, since a yield response is obtained when these results are produced, there is little question about the limiting factor. However, where one is attempting to diagnose a given nutritional deficiency, or where a response is seen to the application of a complex (multielement) material, these problems become more real.

B. Dilution of Other Elements

This is by far the most common instance in which the "dilution effect" has been invoked to explain results. In most cases one analyzes a wide spectrum of elements in the plant after a change in the application rate of one or more other elements. In this situation, the crop is responding to the limiting element. Dry matter production increases. If uptake of some other element proceeds more slowly than dry matter accumulation, concentration will decrease.

The concentration of this other nutrient may decrease below levels of adequacy (however defined) and ultimately produce a deficient plant. It would be highly advantageous to be able to predict the second, third, etc. most limiting element simply by analyzing a single sample. However, much work needs to be done in order to predict response in a reasonable fashion.

Here, as in other sections, it is very important to distinguish between interactive and noninteractive effects. If the dilution in concentration is classic, that is, only because more dry matter is produced, the relationship may be termed noninteractive. However, if the elements interact directly at some uptake site o in the soil, such that uptake and/or translocation is partially inhibited by th added nutrient, then a direct interaction would be involved. It is often ver difficult, if not impossible, to separate these two mechanisms when a plant yiel response is obtained due to treatment. When yield is not changing, the effect may be separated a little more easily, although it is still not completely clear wha the mechanism would be.

A number of examples will be presented that demonstrate the range of observa tions of this type that have been made in the past.

Goh et al. (1979) measured levels of a wide variety of elements in ryegrass after treatment with N and S fertilizers, with the primary goal of looking at cation/anion balance. At one level of N, where yield increases were obtained, added S tended to decrease N concentrations in the plant.

Sulfur additions decreased both concentration and total accumulation of Se in alfalfa at two field sites, indicating that a direct interaction may have occurred (Westerman and Robbins, 1974). Where yields did not increase with S treatment, there was a decrease in Se total accumulation and Se concentration (↓0↓). Where yield responses were recorded, total uptake tended to increase while concentration decreased (↑↑↓), a more common observation of the dilution effect. The fact that total accumulation decreased where yields were unchanged or slightly increased suggests a direct interaction, but this would not be clearly indicated by results where the ↑↑↓ pattern occurred.

One of the most common dilution effects or interactions observations has been that involved with P × Zn interaction, frequently expressed as "P-induced Zn deficiency" (Thorne, 1957). Upon addition of P fertilizers to soils the concentration of Zn in tissue has often been observed to decrease. In terms of the symbolism used in this article, both ↑↑↓ and ↓↑↓ types of dilution effects have been observed.

Burleson *et al.* (1961), for instance, found decreases in total Zn accumulation by beans when P fertilizer was applied, even though total yield changed little (↓0↓). Where growth responses were observed with Zn and P additions, added P decreased both total Zn accumulation and Zn concentrations.

Safaya and Singh (1977) found that as P was increased slightly at low Zn plant yield increased and Zn concentration decreased slightly (↓↑↓). As P was increased further, yield and total Zn uptake decreased again, but concentration tended to increase (↓↓↑). At high Zn levels the first increment of P produced a ↑↑↓ response, typical dilution effect response, but additional P caused a change to ↓↓0. Such results suggest that at low Zn the added P was significantly decreasing the availability of P, but at higher concentrations this was not at all clear.

Schultz *et al.* (1979) found that application of K produced a Mg response of 0↑↓ in alfalfa and of ↑↑0 in white clover. This would suggest that perhaps the clover and alfalfa behave differently in their K–Mg relations, with the clover able to maintain its Mg uptake much better where K is applied.

Hughes *et al.* (1979) found that application of P to nonmycorrhizal red raspberries produced a dilution of N, K, Ca, Mg, Cu, B, and Zn in tissue, but total accumulation increased (↑↑↓). Manganese concentration remained constant (↑↑0). In mycorrhizal plants, dilution with added P only occurred for N, K, Ca, Mg, and B, with other nutrients showing no significant change.

fungi [illegible]

[illegible]

In summary, there are numerous situations where an increase in dry matter accumulation in response to the application of a nutrient element is accompanied by a decrease in the concentration of other elements within the plant. In some instances it is possible to separate out interactive and noninteractive types of effects. However, the background levels of nutrients present are very significant in determining how plants respond.

C. CHANGE IN pH

The favorable effects of optimizing pH on plant growth have been well-documented. In addition, there is some information available on the effects of pH on nutrient uptake by crops.

The pH of a soil can apparently affect both the concentration (solubility) of nutrients in the soil solution and the uptake of nutrients from solutions of constant ionic concentration. Since the effects of pH on plant growth are numerous, it is usually difficult to separate out those due to the increased availability of a single nutrient. However, in some instances improved growth may be due primarily to the increased availability of a single nutrient.

Experiments conducted in soil generally do not allow one to discriminate between increased solubility and an increased ability of the plant to absorb ions. Carefully conducted solution culture experiments allow one to examine plant behavior where pH but not nutrient ion activity is varied.

With tomatoes grown in the greenhouse (Jones and Fox, 1978), raising soil pH from 5.1 to 6.3 significantly increased yield and total Mn accumulation but decreased Mn concentration (↑↑↓). Above pH 6.3, both total Mn accumulation and Mn concentration decreased as pH increased (↓0↓). With Al a precipitous drop in total accumulation was observed as pH increased from 5.1 to 5.4 (↓0↓); total accumulation was roughly constant until pH 6.3 was exceeded, after which the ↓0↓ pattern occurred. Al

In flowing solution culture it was found that over the range of pH that increased plant growth rates, in nearly all cases increased pH increased both total accumulation and concentration of the macronutrients N, P, K, Ca, Mg, and S (Islam *et al.*, 1980). Only for S were concentrations diluted or unchanged. For