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UNIVERSITY OF ALBERTA

A PHYSIOLOGICAL STUDY OF THE EFFECTS OF AN ABIOTIC STRESS ON COMPETITION

BY

KENNETH JOHN STADT

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

IN

ECOPHYSIOLOGY

DEPARTMENT OF BOTANY

EDMONTON, ALBERTA

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ABSTRACT

New techniques for interpreting the effect of an abiotic stress on competition and for conducting competition studies in solution culture are presented. The relative addition rate (RAR) technique, where nutrients are supplied at exponential rates to mimic the supply that becomes available to a plant in the soil, was adapted to a simple solution culture system. In this system, a background concentration of 36 μM nitrogen, plus other nutrients in proportion, was found necessary to stabilize the relative growth rate (RGR) of *Triticum aestivum* L. (wheat) at low RARs. Control of solution pH was improved by increasing the proportion of ammonium in the nitrogen supply to 25%. With these modifications, the response of RGR to RAR was 0.55 to 1. The RAR technique was used as an alternative to density to mimic nutrient competition. The effect of an abiotic non-resource stress, nickel toxicity, on nutrient competition was tested in two sets of experiments, one using density to induce nutrient competition, and the other using RAR. In both cases, ANOVA of raw growth data indicated that nickel stress reduced the absolute effect of competition on growth. However, it is argued that a relative effects model is a better basis for interpreting abiotic - competitive stress interactions. When the effects of nickel and competitive stress were interpreted using an ANOVA of proportional data and a non-linear regression technique, nickel had very little effect on competitive stress. Chemical interactions in the solution could have accounted for this apparent lack of an effect. However, the pattern of nutrient uptake could have also explained why nickel toxicity had little influence on the relative effect of competition. Plants exhibited increased nitrogen and phosphorus uptake in the presence of nickel. This increased nutrient demand at elevated levels of nickel stress may have maintained the relative intensity of competition. These results demonstrate the importance of selecting the appropriate model for interpreting stress interactions. The specific nature of these results also suggests that generalizations regarding the effects of non-resource stresses on competition may not be appropriate.

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CHAPTER 1
GENERAL INTRODUCTION

There has been a spirited debate among ecologists regarding the importance of competition in environments subject to abiotic stress. Insight into the relationship between competition and abiotic stress is essential for understanding how communities might respond to changes in their physical environment. In studies of plant competition to date, many authors have reported that the effect of competition on growth declines at high levels of physical stress. However, this view is not supported by all studies and has been recently challenged (Tilman 1987), chiefly on the basis of how the effects of competition and abiotic stress are interpreted. New distinctions in how competition is measured have also been proposed (Welden and Slauson 1986). The analysis of stress interactions has thus become a central issue in the competition *vs.* abiotic stress debate.

Definitions

The word 'competition' has broad use. To avoid ambiguity in discussing competition among plants, Harper (1961) proposed that 'competition' should be discarded in favor of a more precise term. More recently, Grace and Tilman (1990) have suggested that the word should be retained, and more careful attention should be paid to the way it is defined and measured in each case. Generally there is more agreement about what is meant conceptually by competition than how it should be measured and interpreted (Grace and Tilman 1990). A definition of competition offered by Welden and Slauson (1986) is "the induction of strain in one organism as a direct result of the use of resource items by another organism." This definition has the advantage that the stress, the factor inducing competition, is distinguished from the strain, or plant response in terms of size, fertility, or some other measure of fitness (Levitt 1980). Density and other methods of inducing competition are thus competitive stresses. Competition itself, however, does not occur until a strain is observed (Welden and Slauson 1986), so competitive stress levels below this threshold will not cause competition. Stress interactions are determined by the pattern of effects of factorial combinations of different levels of the component stresses. Thus, it is useful to call the factor that is varied to induce a response the stress, even though no actual response (strain) occurs at low stress levels. The question can then be clearly phrased: *is the* *strain caused by a given level of competitive stress equal at different levels of abiotic stress?*

Abiotic, or physicochemical, stress is distinct from competition or other biotic stresses such as predation or disease (Levitt 1980). Welden *et al.* (1988) defined abiotic stress as "an external condition, apart from the activities of other organisms, that induces physiological strain in an organism." Abiotic stresses include a broad array of factors, such as toxicants, chilling and heat stress, infertility, and drought (Levitt 1980). These can be broadly classified as non-resource or resource stresses (Grace 1990). As is the case with the definition of competition, the factor is the stress and the plant response the strain (Levitt 1980, Welden and Slauson 1986).

Studies of Competition Along Gradients of Abiotic Stress

The debate about the importance of competition along abiotic stress gradients was initiated by the assumptions of models of plant strategies (Grime 1977) and community development (Tilman 1988). Grime's (1977) theory of evolutionary strategies is based on the conditions imposed by two external factors: stress ("conditions that restrict production") and disturbance (the removal of existing biomass). He argued that plant species or ecotypes have developed adaptive characteristics for the three permutations of high stress with low disturbance (stress tolerant strategy), low stress with high disturbance (ruderal strategy), and low stress with low disturbance (competitive strategy), although most plants have strategies intermediate among these three. Grime surmised that the fourth permutation, high stress with high disturbance, would not support vegetation. The fundamental assumption of this theory is that the importance of competition decreases with increasing stress or disturbance. This causes the trade-off between stress tolerant or ruderal traits and competitive ability.

It should be noted that Grime (1977) used the term 'stress' in a more general sense than what is usually meant by abiotic stress. His definition of stress was simply "conditions that restrict production" and included low resource levels such as those caused by canopy closure or plant nutrient uptake (Grime 1977). However, he also distinguished competition from stress, although competition is clearly the cause of the above types of resource stress. Grace (1990) noted that this separation of the conditions caused by competition from the process itself limits competition to resource exploitation. It is also difficult to imagine how competition would cease among plants that had reduced resources to "stress" levels. Aside from cases of biologically-imposed resource stress, however, all other factors that "restrict production" are abiotic stresses in Grime's scheme.

There is considerable evidence that Grime's (1977) hypothesis that competition is less important in environments with high levels of abiotic stress is true. Early work by Suskatschev (1928, cited in Gause 1934) on forests in the U.S.S.R. indicated that trees growing in poor soils were smaller and had higher densities than similar-aged trees on fertile soil. Suskatschev also demonstrated in a pot experiment that a dense sowing of the annual, *Matricaria inodora*, had lower mortality on control soil compared to fertilized soil (Gause 1934, White and Harper 1970). Thus, plants that grew slowly due to nutrient stress showed little density-dependent mortality (self-thinning) over the course of the experiment. This reduced rate of self-thinning associated with low fertility has also been documented by Yoda *et al.* (1963). If the rate of self-thinning is a reasonable measure of the importance of competition, then these results are consistent with the view that competition is reduced by high abiotic stress.

Similar relationships have been reported in more recent studies. Mahmoud and Grime (1976) found that *Festuca ovina* was eliminated after 12 months' competition with *Agrostis tenuis* or *Arrhenatherum elatius* in well-fertilized soil. However, in a less fertile soil, *Festuca* persisted at a reduced size. Presumably competition was less important in the mineral-stressed soil. Wilson and Keddy (1986) measured competition by the absolute effect of removing lakeshore vegetation on the growth of transplants, and determined that competition declined in areas exposed to wave action (disturbance) and nutrient stress. Del Moral (1983) conducted selective removal experiments in subalpine meadows and estimated the importance of competition by the change in diversity and relative biomass once the dominant species had been removed. He concluded that competition was significant, but less important in less productive, drought-stressed sites.

It has also been noted that the competitive exclusion principle of one niche, one species, does not hold true in the case where fluctuating environmental stress prevents the competitively superior species from occupying all possible physical sites (Hutchinson 1957). Stressed sites have thus been seen as refuges from competition. Gurevitch (1986) studied populations of *Stipa neomexicana* in an Arizona grassland

and found that competition, measured by the absolute effect of removing other species on seedling establishment, seedling survival, flower production, and growth of mature plants, decreased in areas with high abiotic soil moisture stress. Although *Stipa*, with all other species removed, did best where moisture was abundant, it was excluded by other, more vigorous, species on all but the driest soils. In these areas of high moisture stress, the coexistence of the poor competitor, *Stipa*, with other species has been considered evidence that competition is less important (Goldberg 1990).

Although it might seem that the evidence for a decline in the importance of competition with increasing environmental stress is overwhelming, this view has been challenged. Tilman (1987) claimed that, in infertile areas, competition will be as intense as where resources are more abundant. He argued that, in all environments, competition is for a unique set of resource ratios, and the species tolerant of the lowest levels of these resources will be the superior competitor. Surprisingly, evidence of such interactions comes from some of the same sources cited by Grime (1977). Tilman (1987) suggested that rather than merely studying the survival of competing species, it is necessary to compare the relative abundances of the species at high and low levels of abiotic stress. He argued that a change in species proportion indicates a change in the resource competed for rather than a change in the importance of competition. Thus, in Mahmoud and Grime's (1976) experiment, the survival of *Festuca* and the reduced size of *Arrhenatherum* and *Agrostis* in the poorly fertilized soil indicated a shift in the outcome of competition, not a reduction of it (Tilman 1987).

A number of other reports are consistent with Tilman's view. Welden *et al.* (1988) found no consistent trend in either the intensity or the importance of competitive stress (this distinction is defined below) on canopy size along a drought stress gradient. Watkinson (1984) followed the response of *Vulpia fasiculata* to density under several nutrient levels over time. Although he did not attempt to do so, the relative effect of competition can be roughly estimated by the slope of his *log*-size *vs.* *log*-density plots. Early in these experiments competition was more severe at *high* levels of nutrient stress. However, as time progressed, competition became equally effective in reducing plant size in all nutrient treatments.

Still others have suggested that competition will become more important with increasing abiotic stress. Wiens (1977) argued that competition is most severe when an abiotic resource stress is high. Donald (1951) also suggested that competition for nutrients among plants will be most severe in infertile soils. Sherwood and Risser (1979) anticipated that in areas of low rainfall, the importance of competition in determining the dispersion of forest species would increase. However, there is little evidence available to support these claims.

Measuring Competition

Clearly there is a diversity of opinion regarding the effect of abiotic stress on competition. Recently, attempts have been made to reconcile these views. Several authors (Goldberg 1990, Grace 1990, Grace and Tilman 1990) have suggested that much of the ambiguity has been due to the way competition is defined, detected or measured. The discrepancy between how Mahmoud and Grime (1976) interpreted their experiment and how Tilman (1987) addressed it is a good example. The authors measured competition by its absolute effects, the persistence of a species and size reduction due to the presence of competitors. Tilman and colleagues (Tilman 1987, Grace and Tilman 1990), on the other hand, interpreted the effects of competition relative to the productivity of the environment, the quotient of size reduction to total biomass present. The absolute effect of competition is necessarily greater at low levels of resource stress due to increased growth, even though the ability of competitive stress to cause growth reductions relative to the growth possible in the absence of competition remains the same.

Welden and Slauson (1986) also hypothesized that the interaction between abiotic stress and competitive stress can be interpreted differently if relative, rather than absolute, scales of stress measurement are used. They suggested that the absolute effect of competitive stress, such as the reduction in size brought about by an increase in crowding, is a measure of its intensity. The reduction in size from competition relative to the reduction caused by all stress, biotic and abiotic, affecting the plant is a measure of its importance. Welden and Slauson (1986) claimed that while the intensity of competition is related to the physiology of the plant and the resource competed for, the importance of competition is related to the role of competition to in the community. Thus, the importance of competition would be the appropriate measure for questions regarding the community. However, the relative measure of competitive stress that Welden and Slauson (1986) called importance is different from the relative measures used in stress physiology. Importance is calculated by ratios of growth *reductions* rather than growth itself, which leads to a more complex interaction model. Intensity can be calculated on the basis of growth, as well as growth reduction. Therefore, the analysis of abiotic and competitive stress interactions presented here will focus on applying the absolute and relative effects models on the intensity of competition.

There are compelling reasons why the relative rather than the absolute effects of competition should be measured when another stress is present. Several analyses of stress interactions (Lockhart 1965, Mohr 1972) have indicated that relative effects[a] models are far common more in biological systems than absolute effects[b] models. Several authors have argued that when high levels of multiple non-resource stresses are present, the absolute effects model breaks down because it begins to predict a negative growth response (Christensen *et al.* 1979, Taylor 1989). McCarthy and Schroeder (1973) pointed out that biological growth models which describe the effect of resource supply rates should be dimensioned according to a relative effects model, since absolute dimensions predict that growth will still occur in the absence of an essential substance. At a mechanistic level, two stresses will show relative effects if they act sequentially on growth processes, or absolute effects if they act on the same site (Berry and Wallace 1981, Mohr 1972). If the stresses affect multiple processes, an analysis of sequential *vs.* similar effects is still possible, but complex (Lockhart 1965). Christensen and Chen (1985), however, suggested that one effect of a stress is frequently dominant, and its other effects can be ignored in predicting the outcome of an interaction with other stresses.

Resource vs. Non-Resource Abiotic Stress

Grace (1990) noted that Grime's (1977) and Tilman's (1988) theories ignore the difference between abiotic stress caused by low resource levels and abiotic stress caused by non-resource conditions. The debate between Grime and Tilman (Thompson 1987, Thompson and Grime 1988, Tilman 1987) has focused on the importance of competition at different levels of resource stress, which can be partially reconciled

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[a] The relative effects model has also been described as the proportional (Lockhart 1965), sequentially additive (Berry and Wallace 1981), or response multiplication model (Christensen 1984, Christensen and Chen 1985). It is most commonly known as the multiplicative model (Christensen *et al.* 1979, Lockhart 1965, Sokal and Rohlf 1981, Taylor 1989).

[b] The absolute effects model is usually called the additive model (Sokal and Rohlf 1981, Taylor 1989), but is also known as the response addition model (Christensen 1984, Christensen and Chen 1985).

through comparable measures of the effects of competition and attention to the perceived role of competition in community development (Grace 1990, Grace and Tilman 1990). However, non-resource stresses may have effects on competition which, regardless of the measurement method, lead to a decrease in the effect of competition. Grace (1990), for example, reported a trade-off between competitive ability and deep water (a non-resource stress) tolerance in two *Typha* species. Each species grew well in shallow water when the other was removed (Grace 1987), but *Typha domingensis* was better able to maintain root aeration and could tolerate deeper water than *Typha latifolia* (Grace 1988). Greenhouse and field experiments indicated that *T. latifolia* was always the superior competitor where it could grow, thus limiting *T. domingensis* to deep water sites (Grace 1987). Observations of long-established populations in ponds indicated that there was minimal overlap between the species (Grace 1987). However, where they did overlap, interspecific competition was presumably unimportant, owing to the anoxic stress caused by the water depth.

There is good reason to expect competition to be less important at high levels of a non-resource stress. Since competition is for resource items, the effect of competition should be reduced if the rate of resource depletion is reduced. The impact of a non-resource stress is a reduction in plant growth rate, which should subsequently reduce the rate of resource consumption. Competition should thus be less intense under increasing non-resource stress conditions. I addressed this hypothesis using nickel toxicity to impose a non-resource stress on a competitive stress response. The importance and known mechanisms of nickel toxicity are outlined below. Although a detailed analysis of the mechanisms by which nickel causes strain could lead to a precise prediction of the effect of nickel on competition, the lesions caused by nickel are not fully understood. At this point, only a general prediction is possible.

Nickel Stress

Nickel toxicity is a localized problem in areas having outcrops of nickel-bearing rock and soils affected by mine and smelter waste (Adriano 1986). Nickel has been shown to be toxic in the serpentine soils of Scotland (Hunter and Vergnano 1952). Numerous other regions have serpentine soils high in nickel: Scandinavia (Brooks and Crooks 1980), Southern Europe (Brooks *et al.* 1979), Zimbabwe (Brooks and Yang 1984), Southeast Asia (Wither and Brooks 1977), Australia (Anderson *et al.* 1973), New

Zealand (Lee *et al.* 1983), Western North America (Reeves *et al.* 1983), and Quebec (Hutchinson 1981). These areas are often vegetated by endemic species, some able to accumulate nickel to concentrations that would be lethal to other species (Hutchinson 1981).

Industrial nickel pollution has been well documented in the province of Ontario, Canada (Hutchinson 1981, Mudroch and Capobianco 1979, Temple and Bissessar 1981, Whitby *et al.* 1976). Whitby *et al.* (1976), for instance, found soils within 7.4 km of a nickel, copper, and iron smelter near Sudbury, Ontario, had levels of nickel, copper and aluminum that were lethal to radish seedlings. Sublethal concentrations of nickel extended some 15 km from the largest Sudbury smelter (Hutchinson 1981). Industrial nickel contamination has also been reported in Wales, Sweden, Australia, Germany, Poland, Holland, the United States, and Japan (Kabata-Pendias and Pendias 1984 p.254). Nickel in municipal sewage sludge is also of concern, since the sludge is often applied to fields as a soil amendment (Alexander *et al.* 1978).

Nickel is known to cause numerous toxic lesions in plants. Roots of plants grown in nickel-contaminated solutions are typically stunted, brittle, and curled. Robertson (1985) reported that nickel arrested mitosis in root tips of *Zea mays* and suggested that this might be the cause of the curled roots. He also found that nickel reduced cell elongation, making roots stiff and brittle. Some accounts (reviewed in Mishra and Kar 1974) have shown that nickel increases total nitrogen and phosphorus concentrations in plants. Foy *et al.* (1978) reported that foliar iron is reduced by toxic concentrations of nickel. Other accounts (reviewed in Mishra and Kar 1974) suggest that nickel causes local, rather than whole-plant iron deficiency. Nickel uptake appears to be an active process in *Avena sativa* (Aschmann and Zasoski 1987). Nickel toxicity is thus likely to affect internal as well as root surface processes. After uptake, nickel is rapidly transported to the shoot. *Solanum tuberosum* and *Artemesia spp.* accumulate more nickel in their above-ground parts than in their roots or tubers (Mishra and Kar 1974). A number of species accumulate nickel in their above-ground parts in excess of soil nickel levels (Hutchinson 1981). Cataldo *et al.* (1978) found that nickel was readily mobile within *Glycine max*, and would be re-translocated to seeds at senescence. Nickel toxicity has also been shown to reduce photoassimilate translocation in *Phaseolus vulgaris* (Peterson and Rauser 1979), and may alter the normal sequence of organ development and senescence. In the leaves of monocots, nickel toxicity causes a distinctive pattern of alternating healthy and chlorotic bands, while dicot leaves show a generalized chlorotic mottling (Hutchinson 1981). When nickel is present in small amounts it appears to accumulate in the vacuole and other plastids. When supplied in higher concentrations, nickel accumulates in the cell wall and causes damage to the membranes of the chloroplasts, leading to chloroplast agglutination (Mishra and Kar 1974). Chlorophyll (both *a* and *b*) content is typically decreased by toxic levels of nickel. This effect has been chiefly attributed to reduced chlorophyll synthesis, either by direct interference with the incorporation of magnesium into protoporphyrin, or by inhibiting other iron porphyrin-containing enzymes involved in this process (Mishra and Kar 1974). Carlson *et al.* (1975) reported reduced rates of net photosynthesis in *Helianthus annuus* and *Zea mays* in the presence of a toxic dose of nickel. However, these authors traced this effect to the inhibition of stomatal opening by nickel, rather than chloroplast damage. The activity of a number of enzymes has been shown to be inhibited by high levels of nickel, possibly due to interference with micronutrient cofactors, but generalized mechanisms of inhibition are not clear, since as many other enzymes are stimulated by the presence of nickel (Mishra and Kar 1974).

Clearly nickel toxicity is a geographically localized, though significant, abiotic stress. It is also a relatively simple stress to administer and control. Nickel salts are readily soluble in water and do not hydrolyze, so the toxicity of nickel in solution is relatively pH stable. There is also ample toxic response data available for *Triticum aestivum* grown in nickel solutions (Taylor *et al.* 1991). Nickel was used in this study to induce an easily controlled non-resource stress.

Growth Techniques

Descriptive surveys (Sherwood and Risser 1979, Welden *et al.* 1988), field experiments (Mahmoud and Grime 1976, Grace 1987, 1988, Gurevitch 1986, Welden *et al.* 1988, Wilson and Keddy 1986) and greenhouse trials (Grace 1987, Watkinson 1984) have been used to characterize the effects of abiotic stress gradients on plant competition. However, for a precise study of the effect of a particular abiotic stress on competition, solution culture offers considerable advantages. Solution culture has been used extensively for testing interactions between toxic metals. Johnson and Proctor (1981) were able to separate the relative importance of magnesium and nickel toxicity in a serpentine area using solution culture to simulate the conditions of the soil. Recently, Berendse and Elberse (1990) used the nitrogen productivity values determined in solution culture experiments (Ingestad 1979, Ingestad and Kahr 1985, Jia and Ingestad 1984) to correlate species' habitats with the species' maximum growth rate. In this study I used solution culture to impose nickel and competitive stress under controlled conditions.

The use of solution culture for competition studies raises questions about how the solution medium should be controlled to mimic plant growth in the soil. The use of different densities and concentrated nutrient solutions may not be representative of different levels of competition in the soil. The nutrient supply available to a soil-grown plant is a function of the plant's growth rate (which the nutrient supply also controls), the soil nutrient content and the rates of mineralization, diffusion and mass flow of the essential nutrients (Chaney 1975). Ingestad (1982) developed an method of supplying nutrients to solution-grown plants that mimics the nutrient supply that becomes available to a plant growing in the soil. This technique is particularly suited for growth under conditions of nutrient scarcity, such as occurs when competition is primarily for nutrients. Nutrients are added frequently, in exponentially increasing amounts to maintain plants at a level of nutrient stress which is constant relative to the plants' size. This relative nutrient addition rate (RAR) technique was used in this study as well as density as two separate mimics of nutrient competition in the soil.

Objectives

The overall objective of this study was to determine the effect of an abiotic non-resource stress on the effects of competitive stress. I used monocultures of *Triticum aestivum* L. (wheat) grown in solution culture to measure the effects of nutrient competition and nickel stress as well as their combined effect. Although the interplay between shoot and root competition is an important issue, the scope of this study was limited to competition for nutrients alone. I used density in one experiment and the relative addition rate (RAR) technique in three others to mimic nutrient competition. Regardless of the method used to mimic competition, it was expected that the toxic effect of nickel would diminish the relative effect of competition through reduced resource depletion. The theoretical basis for the RAR method and experiments conducted to tailor this technique to a relatively simple solution culture apparatus are described in Chapter 2 and in Stadt *et al.* (1992). The nickel stress *vs.* nutrient competition experiments are described in Chapter 3. Ion interactions in solution as well as nickel and nutrient uptake were studied to attempt to account for the observed effects of nickel on competition. This work is also described in Chapter 3.

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CHAPTER 2

ADAPTATION OF THE RELATIVE ADDITION RATE TECHNIQUE TO A TRADITIONAL SOLUTION CULTURE SYSTEM[a]

INTRODUCTION

Solution culture offers a number of advantages for studies of competition. Conditions in the rhizosphere can be monitored and controlled, root competition can be easily separated from shoot competition, and roots of individual plants can interact in a common solution without becoming physically entwined. The disadvantage of solution culture, however, has been a peculiar set of nutrient dynamics caused by the way nutrients are supplied.

In classical solution culture work, plants are either provided with a large single dose of nutrients and permitted to draw these down, or the growth solutions are replaced frequently to avoid nutrient deficiencies. In either case, the initial nutrient concentrations are typically higher than those found in the soil solution. If growth solutions are replaced, high concentrations are maintained, resulting in luxury consumption of nutrients. Furthermore, the exponential character of plant growth requires that the solutions be changed more and more frequently to continue meeting plant requirements. If the plants are permitted to grow on a single dose of nutrients, severe competition for nutrients develops rapidly. There is no reserve supply of nutrients adsorbed to colloids, present as sparingly soluble solids, or released by mineralization processes to maintain the low concentrations typical of the soil solution.

Recent developments in solution culture research have addressed these problems. Several workers have shown that adding nutrients frequently in an exponentially increasing supply results in constant plant nutrient concentrations over time (Ericsson 1981, Ingestad and Lund 1979, Jia and Ingestad 1984). Provision of exponentially increasing amounts of nutrients meets the demand of plants in the exponential growth phase. Furthermore, plant relative growth rates (RGR = dW/[dt·W], where W is plant weight and t is time) have been shown to be directly related to

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internal plant nutrient concentrations (Ingestad 1982, Ingestad and Agren 1988, McDonald *et al.* 1986). Supplying nutrients (Nutr) at exponential rates (relative addition rates, RAR = dNutr/[dt·Nutr]) that are less than that required to sustain the maximum relative growth rate can produce reduced RGRs that are approximately equal to the RARs. Thus, restricting the RAR permits control of growth rate by maintaining plants under constant levels of nutrient stress. Plants remain in a steady nutritional state over time and, like field plants, show minimal deficiency symptoms (Ingestad 1981, 1982; Ingestad and Lund 1979, 1986).

Ingestad (1982) suggested that such nutrient stresses are representative of natural ecosystems. Plant communities seldom show nutrient deficiency symptoms, yet respond to fertilization with increased growth rates (Ingestad 1981, 1982; Ingestad and Lund 1979). Classical solution culture methods (e.g. Epstein 1972), where nutrients are supplied at concentrations higher than soil solution levels and changed regularly, are clearly poor mimics of field conditions. Permitting plants to deplete these nutrient solutions results in the development of pronounced deficiency symptoms and a reduction or cessation of growth. Although such nutrient regimes might be found in the field, they are not typical. Flowing solution culture systems with large volumes which provide a constant rate of nutrient supply have also been used to investigate nutrient stress (*e.g.* Asher 1981, Asher and Loneragan 1967, Asher and Ozanne 1967, Clement *et al.* 1978). This technique can also result in changing levels of nutrient stress and progressive deficiency symptoms when the plant nutrient demand exceeds the supply (Ingestad 1982). With the relative addition rate technique however, nutrient supply increases with the normal exponential growth pattern of the plant. Deficiency symptoms do not appear, or appear only as small and constant differences in leaf color, similar to symptoms that have been observed among field plants in sites of varying fertility (Ingestad 1981, 1982; Ingestad and Lund 1979, 1986).

A further argument in support of the RAR technique is that the exponential nutrient addition mimics the supply of nutrients that becomes available to an exponentially expanding root system in the soil (Ingestad and Lund 1979). Although the nutrient supply per unit soil volume changes through the growth season as plants deplete soil reserves and as seasonal conditions alter natural mineralization rates, effects on nutrient supply are dampened as root/shoot partitioning and root morphology respond rapidly to nutrient stress (Ingestad and Agren 1988, Ingestad and

Lund 1979). In situations where root competition and seasonal change do lead to increasing nutrient limitation (Freijsen *et al.* 1990, Ingestad 1982), exponential root growth is still a reasonable assumption for the initial vegetative phase of growth.

Thus, the state of the art in solution culture is the low volume, well-stirred system with frequent replenishment of nutrients using a constant RAR (Freijsen and Otten 1987, Ingestad and Lund 1986). Notwithstanding the clear advantages of this technique, it is technologically intensive and expensive to set up, particularly when nutrients are dispensed under automatic control. I believe, however, that a technology-intensive approach is not a prerequisite for use of the RAR technique. The criteria for the maintenance of a constant RGR under a RAR treatment are (1) provision of nutrients in exponentially increasing quantities, but at a constant RAR, (2) the presence of nutrients at the minimum background concentration required for adequate uptake, (3) use of an appropriate NH_4/NO_3 ratio which provides access to nitrogen without toxicity due to NH_4, and (4) supply of nutrients in the optimum proportions for growth at the level of stress selected (Ingestad 1971, Ingestad and Lund 1986). All these criteria can be met using a conventional solution culture system.

The focus of this work was to apply the RAR technique to a simple solution culture system in order to mimic the nutrient stress caused by competition. To supplement experiments where density was used to induce competition for a single dose of nutrients, I wished to use a series of RARs to simulate different levels of competition. The density method results in a very rapid increase in the intensity of competition as nutrients are rapidly depleted. The RAR technique allows plants to be grown under constant nutrient stress, mimicking stable intensities of competition in the field.

To adapt the RAR technique to this purpose, I sought to determine the minimum background concentration of nutrients which would permit uptake at a rate that could sustain a constant RGR, and to minimize pH changes by determining the highest NH_4/NO_3 ratio that was not toxic. In keeping with my desire to develop a simple adaptation of the RAR technique, standard nutrient proportions for traditional solution culture work were used (Taylor and Stadt 1990). To check the overall effectiveness of this modified system, I tested the effect of a series of RARs on plant RGR to determine how closely the growth rate approximated the treatment variable.

Theory

A plant growing exponentially increases in weight according to equation 2.1:

[2.1] $$W_t = W_o \cdot e^{RAR \cdot (t - t_o)}$$

where W_t is plant weight at time t and W_o is the initial weight at t_o, the start of the measurement period. A nutrient that remains at a stable concentration in the plant will increase as:

[2.2] $$Nutr_t = C \cdot W_o \cdot e^{RGR \cdot (t - t_o)}$$

where $Nutr_t$ is the total amount of nutrient present in the plant (in grams) at time t and C is the plant nutrient content (g nutrient [g plant]$^{-1}$). Under these conditions, the required relative rate of increase of the nutrient (RAR) is equal to the RGR.

A number of authors have shown that plants adjust their RGR to match suboptimum relative rates of nutrient supply (RAR), if nutrients are added at frequent intervals during the growth period (Ericsson 1981, Ericsson and Ingestad 1988, Freijsen and Otten 1987, Ingestad 1981, Ingestad and Lund 1979, Jia and Ingestad 1984). Thus, equation 2.2 can be rewritten with RAR as the control variable:

[2.3] $$Nutr_t = C \cdot W_o \cdot e^{RAR \cdot (t - t_o)}$$

To determine how much nutrient to add in a given time interval (A_t) the difference in the total amount of nutrient present in the plant at the start and finish of the interval is calculated:

[2.4] $$A_t = Nutr_{t+1} - Nutr_t = C \cdot W_o \cdot e^{RAR \cdot (t+1-t_o)} - C \cdot W_o \cdot e^{RAR \cdot (t-t_o)}$$

Simplifying this equation yields equations 2.5 or 2.6:

[2.5] $$A_t\,(g) \quad = C \cdot W_o \cdot e^{RAR \cdot (t-t_o)} \cdot [e^{RAR} - 1] \quad \text{or}$$

[2.6]
$$A_t\,(mol) = [C\cdot W_o/M]\cdot e^{RAR\cdot(t-t_o)}\cdot[e^{RAR} - 1]$$

where M is the molecular weight of the nutrient (adapted from Ingestad 1982). Rather than calculate a separate addition schedule for each nutrient, values for nitrogen alone can be computed and the rest of the nutrients can be supplied in proportion to nitrogen (Ericsson 1981, Ingestad 1981).

MATERIALS AND METHODS

Growth Technique

For all experiments, seeds of *Triticum aestivum* cv. Neepawa were surface sterilized in 1.2% sodium hypochlorite for 20 minutes, then imbibed overnight in an aerated solution of Vitavax fungicide (0.005 g L^{-1}). Seedlings were grown on aerated solutions containing background concentrations of nutrients to which additional nutrients were added on a daily basis according to equation 2.6. The background concentrations used were low doses of nutrients intended to provide an adequate rate of uptake so that constant RGRs would be maintained.

In order to minimize variation between experimental plants within treatments, all plants were subjected to a pretreatment period of 9 days beginning with germination. During this period, nutrient additions were calculated from an initial dry weight of 0.018 g $plant^{-1}$ (W_o, value obtained from previous experiments), an estimate of the plant nitrogen content of 5% by weight (Ingestad and Stoy 1982), and a series of RAR treatments. For the first 4 days of pretreatment, approximately 150 plants were supported on mesh-covered plastic frames in each of a number of 10 L Plexiglas aquaria, one aquarium per treatment (and one per replicate where possible). These plants were thinned to about 50 per aquarium and supported by perforated Plexiglas sheets on the same solutions for the remaining 5 days. Daily nutrient additions were adjusted according to the number of plants. Solution pH was set initially to 5.0 with HCl in the pretreatments and was not readjusted.

On day 10, the start of the treatment period, plants of uniform size were selected and mounted on the opaque Plexiglas covers of 10 L polyethylene buckets, 4 plants per cover (8 in experiment 2.1). Fresh aerated solutions in these buckets contained background levels of nutrients adjusted to pH 5.0. Plant RGRs during the pretreatment period were larger than the RARs at lower RAR treatments (probably due to the mobilization of seed resources). Nutrient addition schedules were therefore recalculated for the treatment period based on the dry weights of the plants at the end of day 9. Four plants per replicate (8 for experiment 2.1) were harvested before planting on day 9, then at intervals during the 3 week treatment period. After harvest, vestigial seeds were discarded and roots and shoots were separated, dried at 60°C for 3 days, then weighed.

Table 2.1 indicates the nutrient proportions by weight and molar ratio (relative to nitrogen=100) used in the background and nutrient addition stocks. Three stocks were prepared, the first containing N (1.8 M), Ca, and Mg, the second P, K and S, and the third Fe and micronutrients. Nitrogen was supplied as 8.3% NH_4 / 91.7% NO_3 for experiments 2.1 and 2.2 and 25% NH_4 / 75% NO_3 for experiment 2.4. The Fe source in all experiments was Fe(III)EDTA.

Growth Conditions

Plants were grown in a controlled environment room with a 16 hour photoperiod. Air temperatures at leaf height ranged from 19 to 25°C during the light period and 17 to 20°C during darkness. Solution temperatures were maintained between 17 to 20°C in light and darkness by standing all the buckets in a common water bath. Relative humidity varied from 35 to 60% in the light period and 50 to 70% in darkness, except for experiment 2.2 where RH was 30 to 40% in the light and 35 to 50% in darkness. Light was provided by 12 high intensity discharge (HID) mercury halide lamps and 4 HID high pressure sodium lamps located 1.3 m above the Plexiglas covers. Photosynthetic flux density at the plant bases was 268 ± 5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (mean ± s.e.) for experiment 2.1 and 305 ± 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for experiments 2.2 to 2.4. Conductivity and pH of the growth solutions were monitored throughout the treatment period and were measured just before the daily nutrient addition.

Table 2.1. Background and addition stock nutrient proportions by weight and molar ratio (relative to nitrogen=100).

Nutrient	Proportion		Nutrient	Proportion	
	Weight	Molar		Weight	Molar
N	100	100	Na	3.7	0.56
Ca	64	22	Fe	1.1	0.28
K	62	22	Mn	0.22	0.056
Cl	31	12	Cu	0.19	0.0042
S	19	8.4	B	0.13	0.17
Mg	15	8.3	Zn	0.065	0.014
P	6.1	2.8	Mo	0.019	0.0028

Statistical Analysis

Relative growth rate was determined by orthogonal polynomial regression of *ln*-transformed plant dry weight on time for experiments having more than two harvests. The *ln*-transformation converts true exponential growth over time to a straight line (log-linear growth) with slope equal to the RGR. The orthogonal regression allows the first order, linear, component of the RGR to be compared among treatments independently of the second order, curvilinear, component. This way a linear estimate of the RGR can be determined independently of the degree of change in RGR over time (curvilinearity). In experiment 2.3, RGR was calculated using equation 2.7 since only initial (W_o) and final (W_t) harvests were taken:

$$[2.7] \qquad RGR = (\ln W_t - \ln W_o) / (t - t_o)$$

All statistical analyses were performed using the analysis of variance, general linear models, and regression procedures available on SAS release 5 (SAS Institute 1985). Significance was defined at the 5% level.

Experiment 2.1. Background Concentration

To determine whether plant growth is sensitive to the minimum background concentration at varied RARs, a series of 3 background levels (0, 180, and 360 μ*M* nitrogen) was supplied to plants growing under two RARs, 0.05 and 0.15 d^{-1}, in a factorial design with 3 replicate blocks. All other nutrients were added in proportion to nitrogen both in the background and in the addition stock. Plants were destructively harvested 9, 13, 17, 21, 25, and 30 days after germination.

Experiment 2.2. Fine-tuning Background Concentration

To determine the background concentration which allowed reasonably log-linear growth over time at low RARs, 6 background levels (0, 7.2, 18.0, 36.0, 90.0, and 180.0 μ*M* nitrogen, plus other nutrients in proportion) were supplied to plants growing under a single relative addition rate treatment of 0.05 d^{-1}. Harvests were taken after 9, 13, 17, 21, 25, and 29 days. Space limitations allowed only two replicates per treatment at each harvest. However, RGR estimates were obtained from this data by regression, so replication, though desirable, was not required.

Experiment 2.3. Ammonium to Nitrate Ratio

Using an intermediate RAR of 0.12 d^{-1} and a background concentration of 36 μM nitrogen (plus other nutrients in proportion), the nitrogen source ratio in both background and nutrient addition stock was supplied at 10 levels (NH_4/NO_3): 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20, 90/10, 100/0. All other nutrients were kept in the proportions of Table 2.1 except SO_4, the counter ion for NH_4, which increased from 8.4% of nitrogen (by molar ratio) to 98.4%. Two additional treatments were tested, one with 8.3% NH_4 / 91.7% NO_3, the ratio used in experiments 2.1 and 2.2, and one with 10% NH_4 / 90% NO_3 with additional $CaSO_4$ to bring SO_4 to 98.4% of N as a check on SO_4 effects. Three replicate blocks were prepared. Harvests were taken after 9 and 30 days.

Experiment 2.4. Response of RGR to RAR

Plants were grown under 6 RAR treatments (0.03, 0.06, 0.09, 0.12, 0.15, and 0.18 d^{-1}) on 36 μM background nutrients in three replicate blocks. Harvests were taken after 9, 16, 23, and 30 days. The linear component of the RGR was regressed on RAR to determine the degree of control that RAR imposed on RGR.

RESULTS AND DISCUSSION

Experiment 2.1. Background Concentration

Background concentration had no effect on growth at an RAR of 0.15 d^{-1} (Figure 2.1, filled symbols). All background treatments within this RAR had a linear RGR estimate of approximately 0.20 d^{-1} (Table 2.2). Significant 2nd order coefficients (curvilinearity) in the 0 and 180 μM background treatments were not large, and were not different from the nonsignificant 360 μM coefficient by Tukey's hsd (Sokal and Rohlf 1981).

Within the 0.05 d^{-1} RAR treatment, addition of background nutrients clearly

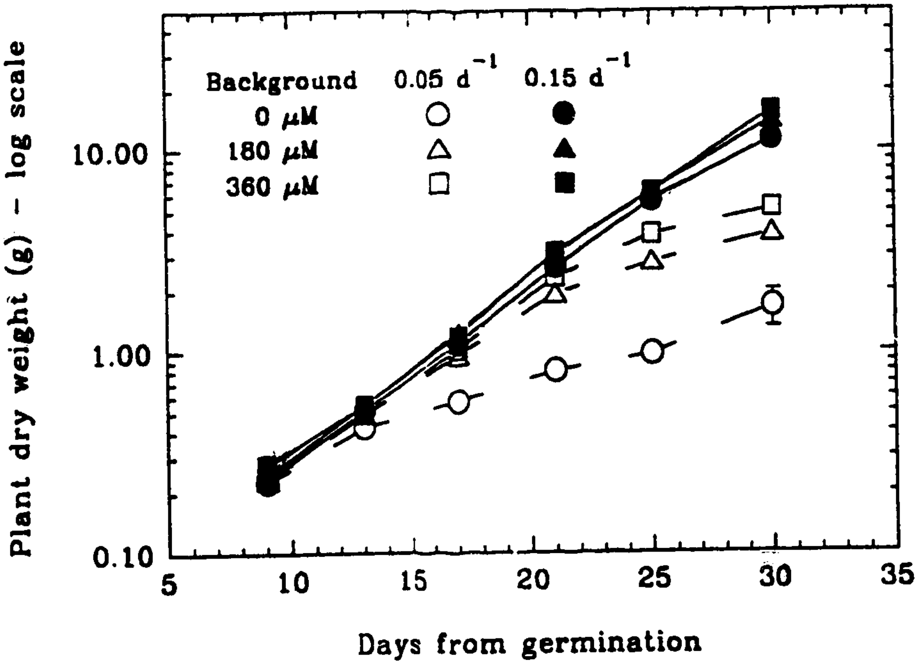

Figure 2.1. Experiment 2.1. Growth of plants under 0.05 and 0.15 d^{-1} relative addition rate treatments and background concentrations of 0, 180, 360 μ*M* nitrogen plus other nutrients in proportion.

Table 2.2. Experiment 2.1. Linear (β_1) and curvilinear (β_2) components and standard errors (SE) of relative growth rate under two relative addition rate and three background concentration treatments.

Relative Addition	Background Level	Relative Growth Rate					
Rate (d^{-1})	(μM Nitrogen[a])	β_1 (d^{-1})	SE	T[b]	β_2 (d^{-2})	SE	T[b]
0.05	0	0.090^{***}	0.007	a	-0.0018^{ns}	0.0013	ab
0.05	180	0.142^{***}	0.004	b	-0.0055^{***}	0.0008	a
0.05	360	0.158^{***}	0.004	b	-0.0055^{***}	0.0008	a
0.15	0	0.195^{***}	0.003	c	-0.0028^{***}	0.0006	ab
0.15	180	0.200^{***}	0.005	c	-0.0024^{*}	0.0010	ab
0.15	360	0.203^{***}	0.003	c	-0.0012^{ns}	0.0006	b

[a] Plus other nutrients in proportion to nitrogen

[b] Tukey's honestly significant difference test. Regression coefficients followed by the same letter are not significantly different ($P<0.05$).

^{ns} $P>0.05$, * $0.01<P<0.05$, ** $0.001<P<0.01$, *** $P<0.001$

elevated growth rates (Figure 2.1, open symbols). In the 0 μM background treatment, plants grew at a linear RGR of 0.090 d^{-1} (Table 2.2). Relative growth rate increased significantly to 0.142 d^{-1} at 180 μM background concentration, but increasing the background further to 360 μM had no additional effect on RGR. Significant curvilinearity in two background treatments (180, 360 μM) was not great, being within Tukey's hsd of the nonsignificant 0 μM coefficient.

Plants growing under the 0.05 d^{-1} treatment with 0 μM background demonstrated chlorosis and tip necrosis of the older leaves which progressed during the 21 days following germination. Newly emerging leaves were green but needle-like. After day 21, new growth emerged with a normal, unrolled, appearance. The progression of these symptoms was delayed by the addition of 180 or 360 μM background nutrients for about one or two weeks respectively. These first 21 days of growth delineate the "lag phase" of adjustment to the RAR (Ingestad and Lund 1979). Healthy new growth after this period indicated that internal nutrient concentrations had stabilized and that growth thereafter should match the RAR. In contrast, plants growing under the 0.15 d^{-1} RAR treatment showed no signs of nutrient stress for the duration of the experiment and no obvious differences among background treatments. This addition rate apparently supported growth close enough to the maximum RGR of the plants that no adjustment period was necessary. Thus, since high RARs supply nutrients in sufficient quantity, the effects of background concentration are not a concern.

The potential for sustained exponential growth over the 3-week length of these experiments was shown by the lack of curvilinearity in the regression of *ln*-dry weight on time for the treatment with the largest nutrient supply (0.15 d^{-1}, 360 μM). Clearly, self-shading was not a problem in plants of this size. The significant negative curvilinearity in the 0.15 d^{-1}, 0 μM treatment may have indicated the onset of nutrient limitation due to unbalanced nutrient proportions (Ingestad 1971, 1982). The magnitude of this decline in RGR was small over the experimental period however, so the nutrient proportions were adequate.

In experiment 2.1, the linear RGR estimates were larger than the RAR treatments, even with zero background nutrient concentration. This is contrary to the one-to one relationship established by previous work (Ericsson 1981, Ericsson and

Ingestad 1988, Ingestad 1981, Ingestad and Lund 1979, Jia and Ingestad 1984). This could reflect a lower plant nitrogen content (C) than was used in the daily addition calculation (equation 2.6). Overestimating the nutrient content would result in an oversupply of nutrients for the early part of the experiment and a correspondingly higher overall estimate of the linear RGR. This could also account for the negative curvilinearity in the RGR, since RGR would decline once the plants achieved a size where the nutrient content was stable at the treatment RAR (note that overestimating the nutrient content is equivalent to overestimating the initial plant size in equation 2.6). Elevated RGRs may also be due to continued mobilization from nutrient reserves in the seeds.

Despite the lack of a simple one-to-one relationship between RAR and RGR, this experiment indicated that RAR exercised considerable control on RGR, and that background nutrients only affect RGR when RAR is low (under high nutrient stress). Within the 0.05 d^{-1} RAR treatment, the large difference in the linear RGR between 0 and 180 μM background concentrations suggests that the minimum background concentration for log-linear (exponential) growth under RAR control is less than 180 μM.

Experiment 2.2. Fine-tuning Background Concentration

To pinpoint the appropriate background nutrient level I sought an initial nutrient concentration that would produce as near to log-linear growth as possible under low RAR (0.05 d^{-1}) conditions. In this experiment, high background concentrations (90, 180 μM) produced initially rapid growth which declined 21 days after germination (Figure 2.2). Components of these RGRs (Table 2.3) indicate that the linear estimate of RGR (0.129, 0.155 d^{-1}) was considerably higher than the RAR of 0.05 d^{-1} and the curvature (-0.0042, -0.0048 d^{-2}) comparatively large. Clearly the background concentration was too high, initially accelerating the RGR, and causing it to decline once the solution concentration became depleted.

At low background levels (0, 7.2 μM), the linear RGR (0.065, 0.066 d^{-1}) was much closer to the 0.05 d^{-1} RAR (Table 2.3) but growth virtually ceased before the last harvest (Figure 2.2; a plot with a slope of 0.05 d^{-1} is provided for comparison). Seed resources likely sustained growth early in these treatments and growth declined

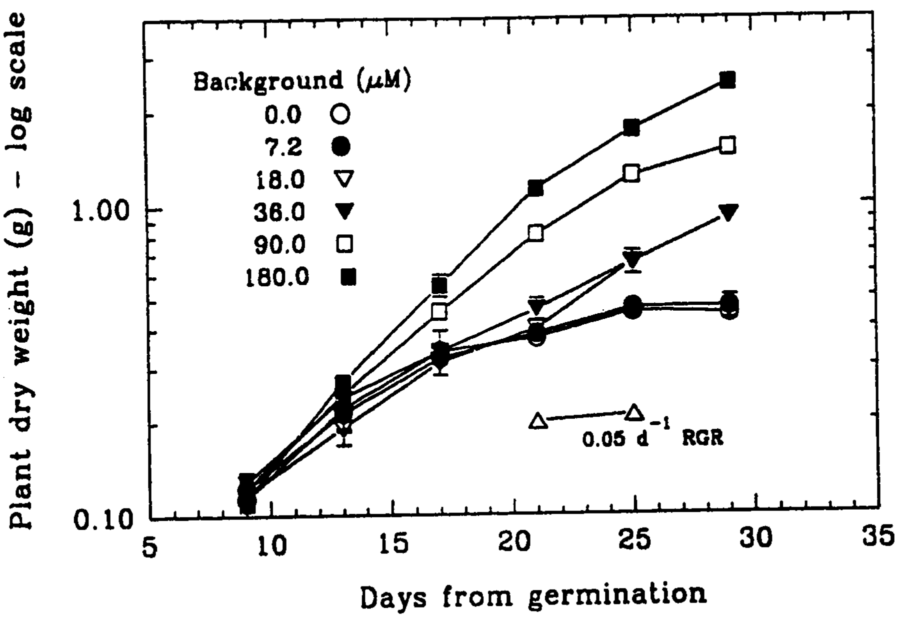

Figure 2.2. Experiment 2.2. Growth of plants under 0.05 d^{-1} relative addition rate treatment and six background concentrations. Inverted triangles indicate the slope corresponding to a 0.05 d^{-1} RGR.

Table 2.3. Experiment 2.2. Linear (β_1) and curvilinear (β_2) components and standard errors (SE) of relative growth rate under 0.05 d^{-1} relative addition rate and six background concentration treatments.

Relative Addition Rate (d^{-1})	Background Level (μM Nitrogen[a])	Relative Growth Rate β_1 (d^{-1})	SE	T[b]	β_2 (d^{-2})	SE	T[b]
0.05	0	0.065^{***}	0.005	a	-0.0048^{***}	0.0009	ab
0.05	7.2	0.066^{***}	0.004	a	-0.0042^{***}	0.0008	ab
0.05	18.0	0.082^{***}	0.004	ab	-0.0027^{**}	0.0007	ab
0.05	36.0	0.093^{***}	0.004	b	-0.0016^{*}	0.0006	a
0.05	90.0	0.129^{***}	0.003	c	-0.0036^{***}	0.0006	ab
0.05	180.0	0.155^{***}	0.003	d	-0.0042^{***}	0.0005	b

[a] Plus other nutrients in proportion to nitrogen

[b] Tukey's honestly significant difference test. Regression coefficients followed by the same letter are not significantly different ($P<0.05$).

^{ns} $P>0.05$, * $0.01<P<0.05$, ** $0.001<P<0.01$, *** $P<0.001$

toward the end of the experiment as this resource was exhausted. If the treatment period were extended, I would expect that RGRs in these low background treatments would increase again to match the RARs once sufficient nutrients had been added to permit an adequate rate of uptake. I could not test this latter hypothesis further, since the increase in solution pH became prohibitively rapid after 30 days, and was difficult to compensate for without automated equipment.

Based upon the remaining data of experiment 2.2, the target background concentration appears to be in the range of 18-36 *μM*. Growth under these treatments is most closely exponential, as shown by the small curvilinear RGR components (Table 2.3). The log-linear character of growth under these background concentrations is further improved if the first harvest is ignored (Figure 2.2). Since the 36 *μM* background treatment produced the truest exponential growth curve (least curvilinearity on the log plot), this concentration was selected as the optimum background for all RARs in subsequent experiments.

The direction of the interaction of RAR on background level permits the use of the 36 *μM* concentration for higher RARs. Experiment 2.1 indicated that much higher background levels (180, 360 *μM*) were unimportant at an RAR of 0.15 d^{-1}. This low background concentration suitable for an RAR of 0.05 d^{-1} should then have a minimal effect at intermediate RARs.

Ericsson (1981) and Ingestad (1981) found no clear relationship between background concentration and uptake rate or RGR down to concentrations as low as 6 or 7 *μM* N when the RAR technique was used. Ingestad (1981) concluded that the minimum concentration required for adequate uptake at a given RAR depends more on physical factors, such as the rate of nutrient renewal, the extent of stirring, and resistance of the boundary layer to nutrient diffusion, than on a physiological requirement. In a recent study, where NO_3 was added continuously to well-stirred solutions at a 0.10 d^{-1} RAR, NO_3 concentrations in solution of 0.39 *μM* or less were maintained by plant uptake and growth at a constant RGR (Freijsen *et al.* 1989). It is likely the response I have observed to low background concentrations is attributable to limited stirring in my solutions compared to that provided in these other experiments.

Experiment 2.3. Ammonium to Nitrate Ratio

Solution pH values in experiments 2.1 and 2.2 typically decreased from 5.0 to 4.0 in the first week of the experimental period (9 to 16 days after germination) then rose during the remainder of the experiment to as high as 7 (see for example Figure 2.3, 8.3% NH_4). Furthermore, higher RARs produced higher final pH values (data not shown) due to increased growth. This rise in pH has been attributed to a greater anion than cation uptake, coupled with a greater internal oxyanion (NO_3, SO_4) reduction than ammonium oxidation (Troelstra 1983). Increasing the proportion of ammonium in the nitrogen supply to some value above 8.3% should therefore stabilize the solution pH (Ericsson 1981, Ingestad and Lund 1986). Ammonium, however, is toxic to many plants, particularly cereals (Ingestad and Stoy 1982), so selection of an ammonium/nitrate ratio must be restricted to the nontoxic range.

The results of experiment 2.3 clearly indicate that increasing the percentage of NH_4 in the nitrogen supply reduced the rate of pH rise (Figure 2.3). A 3-way repeated measures analysis of variance, where replicates were the random factor (Sokal and Rohlf 1981), indicated substantial main effects of NH_4 on pH (P=0.0000). Growth, measured as root and leaf dry weight, was increased slightly when NH_4 was increased from 8.3 to 10% of the N supply, but further increases up to 30% NH_4 had no significant effect (Figure 2.4). When NH_4 contributed 40% or more of the nitrogen supply, NH_4 became toxic, particularly to roots (Figure 2.4). Increasing SO_4 from 8.4 to 98.4% of nitrogen at 10% NH_4 had no significant effect on pH (Figure 2.3) or growth of roots or leaves (Figure 2.4). This indicates that treatment effects were due to the NH_4/NO_3 ratio alone, assuming that SO_4 had the same effect at high as well as low NH_4/NO_3 ratios. In addition to biomass effects, symptoms of NH_4 toxicity were apparent in higher NH_4 treatments. Leaves developed chlorotic stripes, and roots were notably thicker, stunted and had fewer branches in treatments with more than 30% NH_4. For further experiments I chose a NH_4/NO_3 ratio of 25%/75% to improve pH control without risk of ammonium toxicity.

These results differ from those of Ingestad and Stoy (1982) who found that as little as 10% NH_4 in the nitrogen supply decreased the growth of *Triticum aestivum* as well as that of *Secale cereale*, *Hordeum vulgare* and *Avena sativa*. In contrast to my experiments, those seedlings were grown only at optimal RARs where toxic levels of

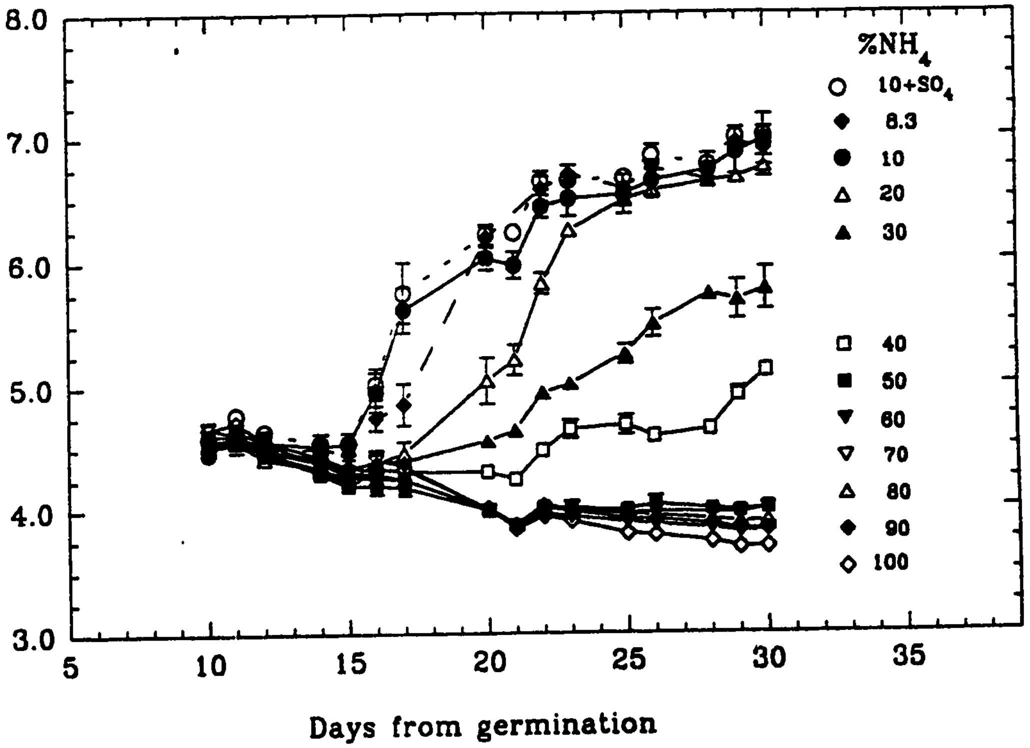

Figure 2.3. Experiment 2.3. Changes in solution pH over time under different percentages of NH_4 in the nitrogen supply. The "10+SO_4" treatment is a check on SO_4 effects (see text).

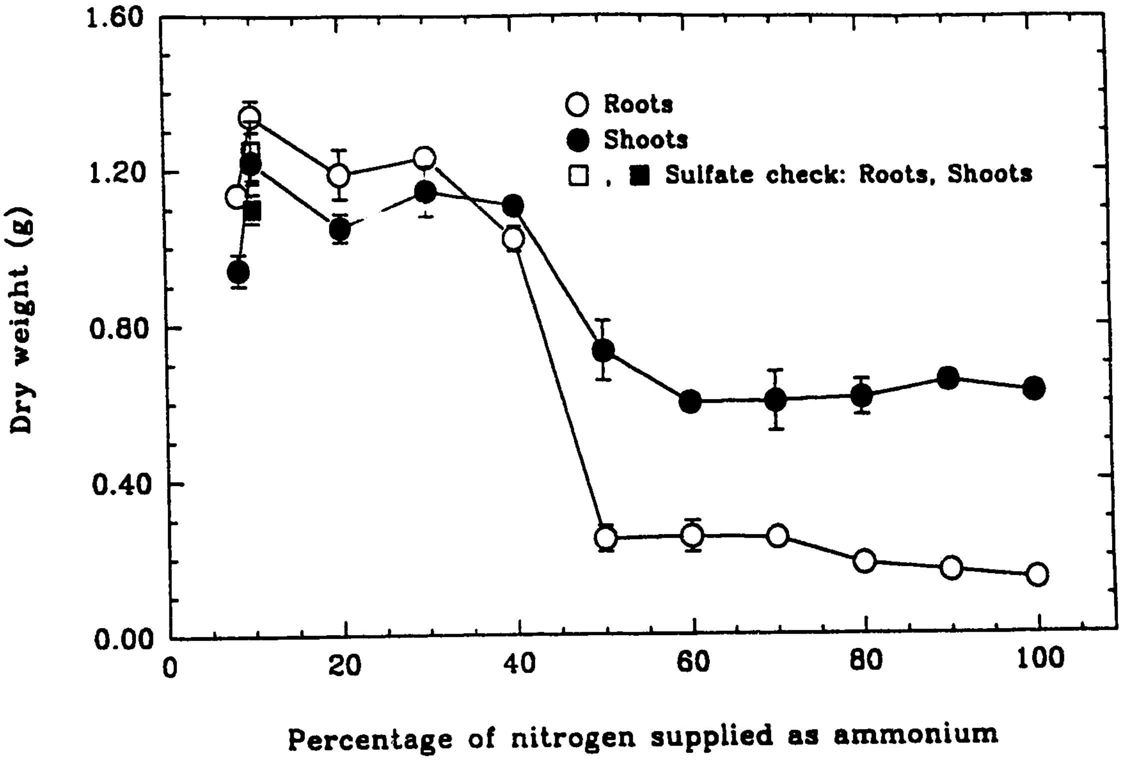

Figure 2.4. Experiment 2.3. Growth of plants under different percentages of NH_4 in the nitrogen supply.

NH_4 could be accumulated through luxury consumption.

Acid/base additions are an alternative method for controlling pH. I have found, however, that when the NH_4/NO_3 ratio of the nutrient stock is low, the pH climbs so rapidly that even twice-daily acid additions will not keep it at reasonable values. If pH control is improved by increasing the percentage of NH_4 in the nitrogen supply, acid additions can better control the remaining variation.

Experiment 2.4. Response of RGR to RAR

Growth of wheat plants under a range of RAR treatments from 0.03 to 0.18 d^{-1} was closely log-linear (Figure 2.5). Curvilinearity was significant in most RAR treatments, but was not great (Table 2.4). It is clear, however, that RAR did not exercise full control over RGR. Low RAR treatments (0.03-0.12 d^{-1}) produced linear RGRs that were greater than the RARs while the highest RAR (0.18 d^{-1}) resulted in a RGR that was smaller than the addition rate. This diminishing response is evident in the regression of RGR on RAR (Figure 2.6) which had a slope of 0.55 ± 0.04 d^{-1} and an intercept of 0.07 ± 0.01 d^{-1} (R^2=0.99, *P*=0.0009).

The theory for the RAR technique (Ingestad 1982, Ingestad and Lund 1986), as well as previous experiments with *Betula verrucosa* (Ingestad and Lund 1979), *Salix spp.* (Ericsson 1981), *Alnus incana* (Ingestad 1981), and *Populus simonii* and *Paulownia tomentosa* (Jia and Ingestad 1984), indicate that RGR should respond directly to RAR with a linear slope of one and zero intercept. The reason for the reduced dependence in the experiments reported here has not been investigated. Elevated RGRs at low RARs are likely due to the overestimation of the plant nitrogen content. I used an estimate of 5% nitrogen content, obtained from experiments on wheat grown at near-maximum RGR (Ingestad and Stoy 1982), for all RAR treatments. However, nutrient content is lower at lower RARs (Ericsson and Ingestad 1988, Ingestad 1981), accounting for the increasing elevation in RGR and curvilinearity at lower RARs. At high RARs (>0.15 d^{-1}) the plants may have been close to the maximum growth rate possible under these light and temperature conditions, and thus responded poorly to further increases in RAR. Nonetheless, the highly significant regression of RGR on RAR indicates that this modified RAR technique can be used to control the RGR of plants using a conventional solution culture apparatus.

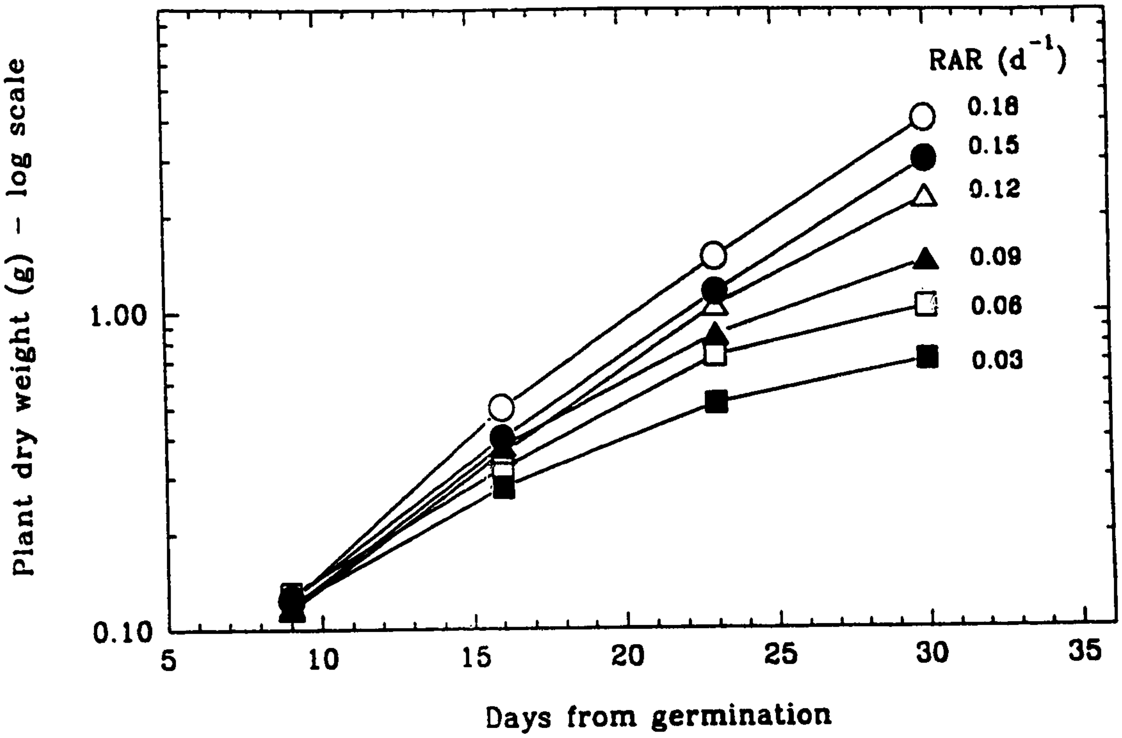

Figure 2.5. Experiment 2.4. Growth of plants under a series of relative addition rate (RAR) treatments.

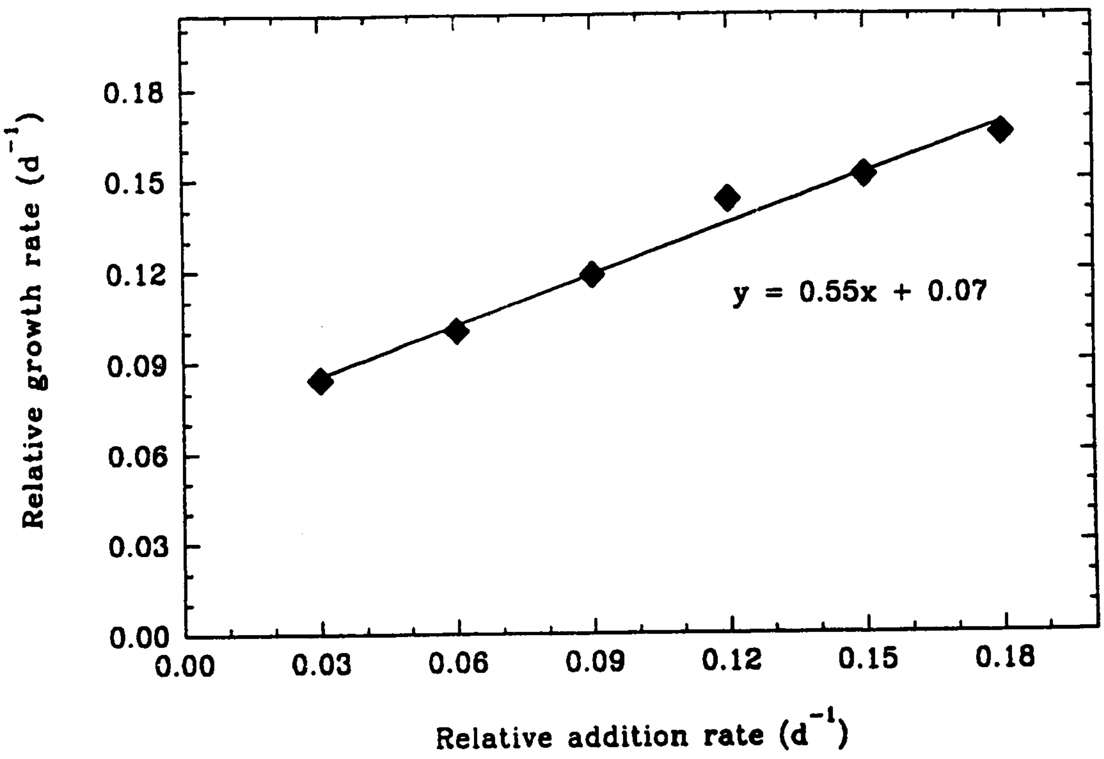

Figure 2.6. Experiment 2.4. The effect of relative addition rate on the linear component of relative growth rate.

Table 2.4. Experiment 2.4. The effect of varied relative addition rate on the linear (β_1) and curvilinear (β_2) components of relative growth rate.

Relative Addition	Relative Growth Rate					
Rate (d^{-1})	β_1 (d^{-1})	SE	T^a	β_2 (d^{-2})	SE	T^a
0.03	0.085^{***}	0.003	a	-0.0026^{***}	0.0004	a
0.06	0.101^{***}	0.004	b	-0.0028^{***}	0.0006	a
0.09	0.119^{***}	0.003	c	-0.0032^{***}	0.0005	a
0.12	0.143^{***}	0.003	d	-0.0018^{**}	0.0004	a
0.15	0.151^{***}	0.003	d	-0.0010^{ns}	0.0005	a
0.18	0.165^{***}	0.003	e	-0.0020^{**}	0.0004	a

[a] Tukey's honestly significant difference test. Regression coefficients followed by the same letter are not significantly different ($P<0.05$).

^{ns} $P>0.05$, * $0.05<P<0.01$, ** $0.01<P<0.001$, *** $P<0.001$

CONCLUSIONS

These experiments have demonstrated that the RAR technique applied to a simple solution culture system permits nutritional control of whole plant relative growth rate. Although this technique did not confer full control of RGR, distinct growth curves for RARs of 0.03 to 0.15 d^{-1} were obtained. The steps for tailoring the RAR technique to a simple solution culture system are straightforward, requiring only an estimate of the plant's nitrogen content, a complete nutrient solution with reasonable nutrient proportions, and a preliminary experiment to determine the appropriate initial background nutrient concentration. Solution pH control can be improved by increasing the ammonium to nitrate ratio within the nontoxic range. Using this technique, the experimenter can maintain plants under steady levels of nutrient stress, representative of conditions in the field. This permits precise testing of the interactions between nutrient stress, such as that caused by nutrient competition, and other stresses in a solution culture environment.

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CHAPTER 3

MEASURING THE EFFECT OF AN ABIOTIC STRESS ON COMPETITION[a]

INTRODUCTION

The effects of abiotic stress on competition have been the subject of speculation since Sukatschev's observation that self-thinning proceeds more rapidly in fertile compared to less fertile soils (Sukatschev 1928, cited in Gause 1934). In reviewing evidence of competition in different environments, Gause (1934) noted that "in better conditions of existence competition proceeds with greater intensity." Abiotic-stressed environments have thus been seen as refuges from competition. Grime (1977) extended this proposition to develop a theory of adaptive strategies for plants, where competitive ability and stress tolerance are opposites on a continuum of adaptive characteristics. However, others have argued that competition is more important in environments that are resource stressed (Donald 1951, Wiens 1977, Sherwood and Risser 1979). Tilman (1988), on the other hand, has contended that competition is always important in determining community structure, regardless of the abiotic environment.

According to Grace and Tilman (1990), much of the confusion regarding the effects of competition along gradients of abiotic stress is due to the operational definition of competition used. They pointed out that if competition is measured by the improvement in growth of one individual when neighboring individuals are removed, competition will have a lower absolute effect when abiotic stress has already severely limited growth. If the measure of competition is adjusted for the total amount of biomass present, then, in this relative sense, competition may well be as intense in highly stressed as in less stressed environments.

Stress Interaction Models

There are compelling reasons for interpreting the relationship between abiotic stress and competition by their relative rather than their absolute effects. A relative effects model implies that the proportional effect of two or more stresses in

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[a] A version of this chapter is being prepared for submission to *Ecology*.

combination will be equal to the product of each stress's proportional effect when applied alone. If two stresses are each able to reduce plant growth to 40% of an unstressed control, for example, then their effect when applied together will be a reduction to 16% of control. The relative effects model is more frequently called multiplicative for this reason (Sokal and Rohlf 1981, Taylor 1989). An absolute effects model, on the other hand, implies that the effect of two stresses in combination will be equal to the growth possible in the absence of these stresses less the sum of the growth reductions caused by each stress when applied alone. In the above example, the absolute effects model predicts that growth will be reduced 120%, the sum of the two 60% reductions. This model is more commonly called additive. One of the reasons the additive model is questionable in interaction studies is because its predictions are unreasonable at high levels of the component stresses (Christensen *et al.* 1979, Taylor 1989). McCarthy and Schroeder (1973) have also argued that the effects of resource supply rates in biological growth models should be dimensioned in a multiplicative fashion. Additive dimensions lead to the prediction that some growth is possible in the absence of an essential substance.

There are a few cases where additive effects might be anticipated, but they require that the stresses act on identical sites. A perfectly multiplicative model, on the other hand, implies sequential effects on a multi-step process (Berry and Wallace 1981, Mohr 1972). *A priori* determinations of appropriate stress interaction models from the mechanisms of the stresses' effects are used in relatively simple biochemical systems (*e.g.* Mohr 1972). Complex relationships that are partially additive and partially multiplicative in character are possible when individual stresses act on multiple sites (Lockhart 1965). However, Christensen and Chen (1985) note that one site frequently forms the "weak link" where most of the effect of that particular stress takes place. Thus, a simple analysis may suffice. Although the additive model has been used in multiple stress experiments (*e.g.*, Allison and Dzialo 1981, Burton *et al.* 1986, Coughtrey and Martin 1978, Davis and Carlton-Smith 1984, de Villaret and Himmelblau 1973, McGrath *et al.* 1980, Wu and Antonovics 1975) it has usually been uncritically applied (Taylor *et al.* 1992). Lockhart (1965) reviewed the available literature on chemical and physical factors and concluded that, in almost all cases, a multiplicative model was the appropriate basis for describing the relationship between these factors on plant growth. More recently, the multiplicative model has been shown to be appropriate for the effects of multiple toxicants (Christensen *et al.* 1979, Taylor

1989, Taylor and Stadt 1990) and multiple resource stresses (McCarthy and Schroeder 1973).

Statistical techniques

Space constraints often make it difficult to prepare a replicated, fully-factorial experiment with enough levels of each factor to characterize the full response to each at different levels of the others. An alternative for two factor experiments is to limit the number of levels of one factor and determine the effect of these few on the response to a full range of levels of the other stress. For clarity, I designate the primary stress in each experiment to be the one whose response is fully characterized and the secondary stress to be the one with the fewer levels.

A multi-way analysis of variance (ANOVA) is the conventional method of analyzing factorial experiments. Analysis of variance is based on an additive model, however, so that if the relationship between the factors is multiplicative, the interaction term will be significant when the relative effect of one stress on the other has not changed (Sokal and Rohlf 1981, Taylor 1989). A number of techniques are available for interpreting data using the multiplicative model. A *log*-transformation or dividing data by the control values for the appropriate secondary stress treatment group will convert multiplicative into additive effects. An ANOVA can then be legitimately applied (Sokal and Rohlf 1981). However, several authors (Kinraide and Parker 1989, Taylor 1989, Taylor and Stadt 1990) have indicated that growth is seldom reduced to zero, even at extreme levels of stress, either because of biomass present when the stress treatments are imposed, or because of growth that occurs before the stresses exert their full effect. This non-zero minimum can obscure the relationship between the stresses, since growth at any level of a secondary stress will decline to the same value at extreme values of the primary stress. An ANOVA will always yield a significant interaction term in these cases, suggesting that one stress reduced the effect of the other, regardless of the true relationship (Taylor 1989).

To overcome these problems, Taylor (1989) developed a weight index (WI) transformation that models multiplicative effects above a non zero minimum value (Equation 3.2, Materials and Methods). However, in the analysis of a series of multiple metal stress experiments, the transformation was found to propagate error to the extent that the interpretation of the WI-ANOVA became unclear (Taylor and Stadt

1990). Subsequently, Taylor *et al.* (1992) developed a non-linear regression technique as an alternative to the WI-transformation and found it effective for interpreting multiple toxic metal interactions. Briefly, this technique fits a modified Weibull function (Equation 3.3, Figure 3.1) to raw growth data at a full range of levels of the primary stress. A separate function is fit to the primary stress response data for each level of the secondary stress (each graph in Figure 3.1 shows 5 functions for 5 secondary stress levels). The function's parameters (Figure 3.1) estimate the minimum biomass achieved at extremely high levels of the primary stress (parameter *a*), the amount of growth above this minimum at the lowest level of the primary stress (parameter *b*), the scale of response to the primary stress (parameter *c*), and the shape of the response (parameter *d*). The value x_o is fixed at the lowest value of the primary stress. To model a multiplicative relationship between two stresses above a non-zero value, changes are necessary in the growth response (*b*) parameter only. This can be clearly seen in Figure 3.1. If only parameter *b* changes, growth at a higher level of the secondary stress remains a constant proportion of growth at a lower level of the secondary stress, above the minimum biomass and across the range of primary stress levels tested. The *relative* effect of the primary stress thus remains constant with changes in the secondary stress. The significance of changes in each parameter can be determined by a *t*-test, using the estimates and standard errors of the parameters.

Definitions

Welden and Slauson (1986) defined competition as "the induction of strain in one organism as a direct result of the use of resource items by another organism." This definition is useful because it separates the stress, the factor that induces competition (traditionally density), from the strain, the plant response to this factor (such as growth, survivorship, or fertility). Competition occurs only if there is strain, that is, if some level of the stress used to induce competition causes a decrease in growth or a change in some other physiological parameter compared to the optimum condition (Levitt 1980). However, stress interactions are interpreted by the effect of one stress on the response of a plant to another stress. So it is not really competition (the strain) that is in question at different levels of abiotic stress: competitive stress levels are usually chosen so that strain will occur. It is the effect of abiotic stress on the ability of competitive stress to cause strain that is relevant. Thus, the question can be clearly phrased: *is the strain caused by competitive stress equal at different levels of abiotic*

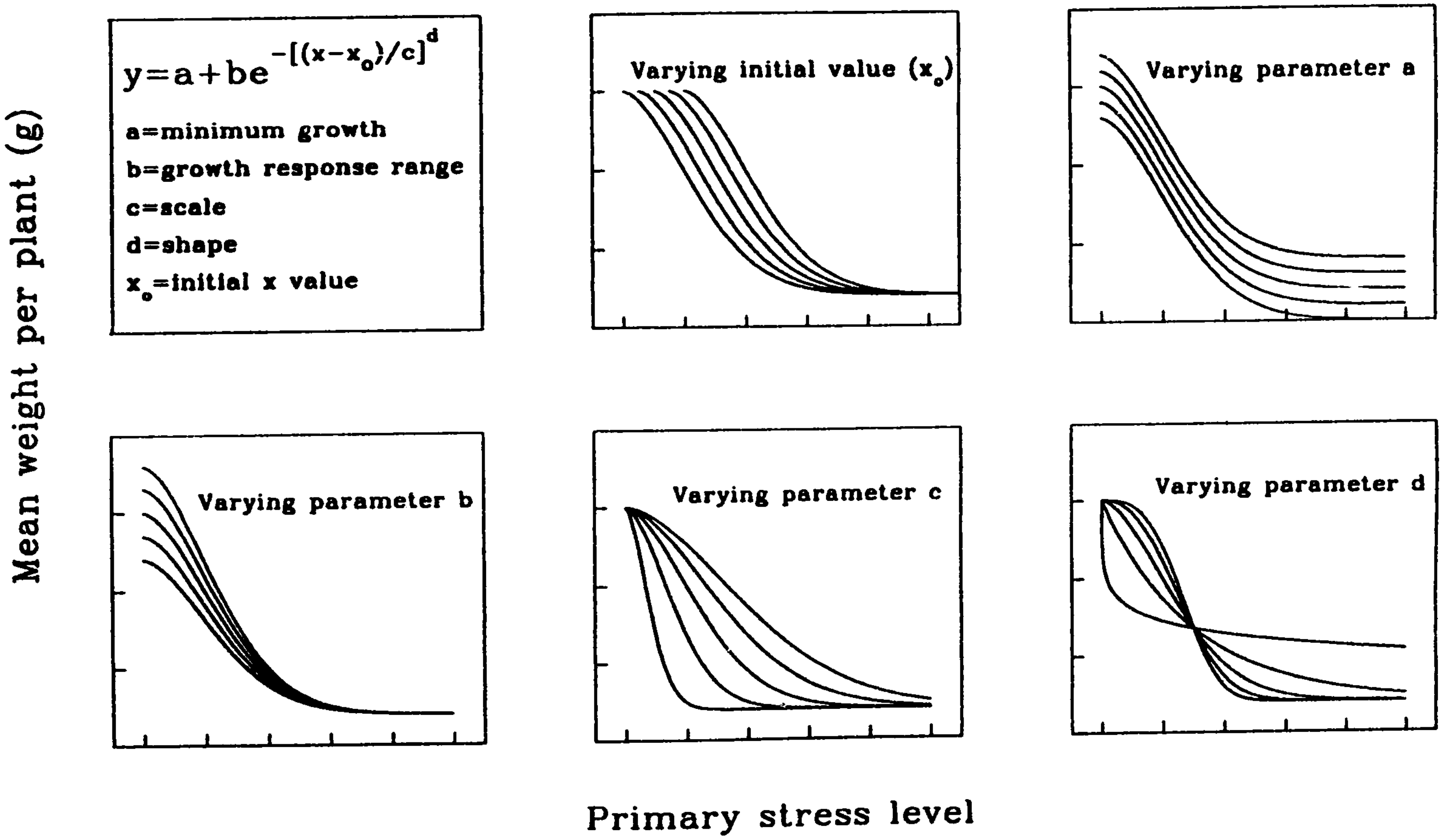

Figure 3.1. Flexibility and biological meaning of the parameters of the modified Weibull function.

stress? Changes in strain can then be interpreted by an absolute or relative effects model.

Abiotic stresses include low resource levels imposed by the abiotic environment, such as drought or infertility, as well as non-resource stresses such as chilling, heat, or toxic stresses. Welden *et al.* (1988) defined abiotic stress as "an external condition, apart from the activities of other organisms, that induces physiological strain in an organism. As is the case with the definition of competition, the condition is the stress and the plant response the strain.

Welden and Slauson (1986) distinguished between the intensity of competition and its importance. They suggested that the intensity of competition is a physiological parameter that relates strain to stress, such as the slope of a size *vs.* density regression. The importance of competition, on the other hand, is a measure of the ecological and evolutionary significance of competition. The distinction made in the present study between absolute and relative effects applies to measures of the intensity of competition, *i.e.* absolute and relative intensity.

Resource vs. Non-Resource Abiotic Stress

Grace (1990) suggested that non-resource stresses may have different effects on competition than abiotic resource stresses. An abiotic resource stress will decrease the resource competed for. This will decrease the maximum amount of growth possible, so the absolute effect of competitive stress will be reduced, but since the stresses are sequential in their action, the relative effect of competitive stress should remain unchanged. Non-resource stresses, on the other hand, do not alter the total amount of resources available, but do change the rate of resource use by plants. Non-resource stresses have specific effects, but their common action (and that of resource stresses) is to slow growth processes down. If they do so without altering the pattern of resource use, the result will be reduced rates of resource use and depletion. The reduced depletion of resources should reduce the strain due to competition at any given time. Thus, the negative feedback of non-resource stresses on competition through their action on growth and resource use might reduce the relative (as well as the absolute) intensity of competition.

Nickel Toxicity

Nickel toxicity is a significant problem in the serpentine soils of Scotland (Hunter and Vergnano 1952, Johnson and Proctor 1981), and is potentially toxic in serpentine regions in Scandinavia, Southern Europe, Zimbabwe, Southeast Asia, Australia, New Zealand, Western North America, and Quebec (Hutchinson 1981). Nickel from industrial sources has severely contaminated some regions of Ontario (Hutchinson 1981, Mudroch and Capobianco 1979, Temple and Bisessar 1981, Whitby *et al.* 1976). Industrial nickel pollution is also a problem in Wales, Sweden, Australia, Germany, Poland, Holland, the United States, and Japan (Kabata-Pendias and Pendias 1984). Municipal sewage sludge, a common soil amendment, has also been implicated as a source of nickel contamination in crops and pastures (Alexander *et al.* 1978, Davis and Carlton-Smith 1984). Nickel toxicity affects a number of plant processes, including root development, nutrient uptake, chlorophyll production, chloroplast integrity, stomatal function, photoassimilate translocation, and a suite of enzyme systems (Mishra and Kar 1974). Nickel salts are readily soluble, and pH has little effect on their behavior in solution. Nickel toxicity was used here as an easily-controlled non-resource stress.

Experimental Technique

Solution culture is commonly used in physiological ecology to answer questions about species' adaptations to soils of varied mineral composition (*e.g.* Johnson and Proctor 1981, Ingestad 1974). Recently, data obtained from solution culture experiments has been used to analyze adaptations for competition in particular habitats (Berendse and Elberse 1990). Solution culture was used here as a convenient method of growing plants for competition studies, permitting accurate control of nutrient resources and determination of root growth.

The use of different densities to induce different degrees of competition within solution culture may not be satisfactory however. In soil, mineral nutrients reach the roots by bulk flow, diffusion, and root exploration (Chaney 1975). Moreover, nutrients are adsorbed and precipitated as sparingly soluble solids that maintain large storage reserves, but low soil solution concentrations (Bohn *et al.* 1985). Models of nutrient supply that take into account these factors indicate that the amount of each nutrient reaching the plant increases exponentially with time, at least for the early phase of vegetative growth (Hoffland *et al.* 1990, Ingestad 1982). Ingestad and colleagues (Ingestad and Lund 1979, Jia and Ingestad 1984) have grown plants with exponentially increasing nutrient supplies and determined that this technique maintained stable nutrient concentrations in the plant over time. Furthermore, plants treated with low relative addition rates (RARs) of nutrients grew slowly, at relative growth rates (RGRs) similar to the RARs, without showing nutrient deficiency symptoms. Thus the RAR technique provides a convincing mimic of plant growth in the field, where nutrient restriction is the norm, but deficiency symptoms are seldom seen.

This technique of exponential, or relative addition rates (RAR) of nutrient supply can be carried one step further. An exponentially increasing nutrient supply at a low RAR places plants under a constant nutrient stress that closely mimics nutrient stress conditions found in the field (Ingestad 1982). Under different levels of nutrient competition, the nutrient stress would be expected to differ. Rather than impose competition by dividing a given nutrient supply between different numbers of plants (*i.e.* by using different densities), the number of plants was kept constant and different exponential nutrient addition rates imposed. High RAR treatments received an ample supply of nutrients to correspond to a situation where competitive stress was low. Low RAR treatments received a paltry supply of nutrients to correspond to severe competitive stress.

Objectives

In this chapter, I report on an investigation of the effect of a non-resource stress, nickel toxicity, on nutrient competition in monocultures in *Triticum aestivum* (wheat) grown in solution culture. Density was used in one experiment and the relative nutrient addition rate in three others to impose a range of competitive stress levels. In all experiments it was expected that nickel toxicity would reduce the relative effect of competitive stress through reduced rates of nutrient depletion. Analysis of variance of weight-index transformed data and the Weibull regression technique were used to compare the observed responses to a multiplicative model. An ANOVA of raw data was also performed to compare the interpretation based on the multiplicative model to one based on an additive model.

MATERIALS AND METHODS

DENSITY EXPERIMENT

Experiment 3.1. Density *vs.* Nickel Stress

This experiment tested the effect of two levels of nickel stress on nutrient competition induced by density. Wheat seeds were surface sterilized in 1.2% (v/v) sodium hypochlorite for 20 minutes then imbibed overnight in a 0.05% (w/v) solution of Vitavax fungicide. Emerging seedlings were grown for 3 days on an aerated 10 L solution with initial concentrations (µM) of NO_3-N (2900), NH_4-N (300), Ca (1000), Mg (300), then for 5 days on 10 L of a complete nutrient solution containing (µM) NO_3-N (3300), NH_4-N (300), Ca (1000), Mg (300), K (800), SO_4 (101), PO_4 (100), Cl (34), Na (20), Fe-EDTA (10), B (6), Mn (2), Zn (0.5), Cu (0.15), and Mo (0.10). The pH of these solutions was initially set to 5.0 with HCl and was then allowed to vary.

Nine-day old seedlings were transplanted to density and nickel stress treatments. The treatment vessels were polyethylene pails of 10 L capacity, placed in a water bath to maintain even solution temperatures. The growth solutions were aerated and initially contained complete nutrients in the amounts listed above. Plants were permitted to deplete these nutrients for the length of the experiment. A mildly phytotoxic dose of nickel, designed to reduce growth to 70% of control (15 µmol $NiSO_4$ L^{-1}), was added to half the pails. Solution pH was again set to 5.0 initially, then allowed to vary. The level of the solution in the pails was periodically restored to 10 L with distilled water to compensate for transpiration losses.

Seedlings were mounted with open-cell foam in slots on opaque Plexiglas lids. There were 4 slots in each lid, arranged in a "+" shape to minimize plant-to-plant shading and competition for light. Densities of 1, 3, 5, 7, 10, 15, 20, 30, 40 and 50 plants per pail were prepared, with the seedlings evenly distributed among the 4 slots. The design was fully factorial, with 10 densities, 2 metal treatments, and 3 replicate blocks, for a total of 60 pails.

The experiment was carried out in a controlled environment [illegible]. Light was supplied by 12 high intensity discharge (HID) mercury halide (400 W) lamps and 4 HID sodium lamps (400 W) located 1.3 m above the Plexiglas covers. Photosynthetic flux density was 287 ± 4 µmol m^{-2} s^{-1} (mean ± se) at the level of the covers. Air temperatures ranged from 18 to 25°C during a 16-hour photoperiod, and 17 and 23°C during darkness. Solution temperatures remained between 18 and 20°C during light and darkness.

Plants were harvested after 21 days of treatment (30 days after germination). For each pail, roots were separated from shoots, dried at 60°C for three days, and weighed.

RELATIVE ADDITION RATE EXPERIMENTS

The relative addition rate (RAR) technique was developed with technologically sophisticated apparatus (Ingestad and Lund 1986). In Chapter 2, I report on adaptations introduced to tailor the RAR technique to a relatively simple solution culture system (see also Stadt *et al.* 1992). I have followed this adapted technique for these experiments. Relative nutrient addition rates were used in these experiments instead of density to provide a better simulation of nutrient competition in the soil.

Wheat seeds were surface sterilized and imbibed in fungicide as for the density *vs.* nickel experiment. Seedlings were grown on 10 L solutions containing initial concentrations (µM) NO_3-N (27), NH_4-N (9), Ca (10), Mg (3), K (8), SO_4 (1.01), PO_4 (1), Cl (0.34), Na (0.2), Fe-EDTA (0.1), B (0.06), Mn (0.02), Zn (0.005), Cu (0.0015), and Mo (0.001). These low levels of nutrients provided a background concentration to drive uptake at a rate sufficient to give near constant relative growth rates (Chapter 2, Stadt *et al.* 1992). Additional amounts of nutrients were added daily to provide an exponentially increasing supply to the plants, keeping pace with their exponential growth pattern. The amount of a nutrient to be added on a given day, A_t (measured in µmol), is determined by the plants' nutrient content, C (g nutrient [g plant]$^{-1}$), the nutrient's molecular weight, M (g mol^{-1}), the initial weight of the plants, W_o (g), at the start of the experiment, t_o (in days, d), the relative rate of nutrient increase or relative addition rate, RAR (d^{-1}), and the time, t (d):

$$A_t = [C \cdot 10^6 \cdot W_o / M] \cdot e^{RAR \cdot (t - t_o)} \cdot [e^{RAR} - 1] \qquad [3.1]$$

(Ingestad 1982). Rather than calculate an addition schedule for all nutrients in this way, the daily amounts were calculated for nitrogen, based on a plant nitrogen content of 0.05 g N [g plant]$^{-1}$ (Ingestad and Stoy 1982), then the other nutrients were supplied in proportion to nitrogen. The nutrient proportions were the same as those used in the background concentrations. I used three stock solutions which contained (m*M*): Stock 1: NO_3 (1350), NH_4 (450), Ca (400), Mg (150), Cl (200); Stock 2: K (400), SO_4 (150), PO_4 (50); Stock 3: Cl (17), Na (10), Fe-EDTA (5), B (3), Mn (1), SO_4 (0.33), Zn (0.25), Cu (0.075), and Mo (0.05). The pH was set to 5.0 with HCl and was allowed to vary.

After 9 days, the seedlings were transferred to polyethylene pails containing 10 L of fresh background solutions at the same concentrations as the background solutions in the aquaria. A sample of plants was harvested from each RAR treatment and daily nutrient additions were continued using the new W_0 values obtained from this harvest. The initial pH was again set at 5.0 and was maintained close to this value with daily HCl additions. At harvest, the plants were placed in a distilled water rinse for 3 minutes, then in 1.0 m*M* $CaSO_4$ for 30 minutes to desorb minerals from the root surface, then in another distilled water rinse for 3 minutes. Vestigial seeds were discarded and roots were separated from shoots. The material was dried for 3 days at 60°C and weighed.

Experiment 3.2. Relative Addition Rate *vs.* Nickel Stress

This experiment was designed to test the effects of abiotic stress on the RAR mimic of nutrient competition. Multiple RAR levels were imposed in the presence and absence of a mild nickel stress. Ten RAR treatments were prepared: 0 (no nutrients other than the background concentrations provided), 0.025, 0.05, 0.075, 0.10, 0.125, 0.15, 0.175, 0.20, and 0.225 d^{-1}. Two nickel levels, 0 and 1.9 µ*M* $NiSO_4$, were imposed on the treatments, the latter intended to reduce growth to 70% of control (0 µ*M* Ni) growth. Three replicate blocks of each treatment combination were prepared, a total of 60 pails. Plants were harvested 30 days after germination.

Mineral Analysis

As a preliminary investigation into the mechanism of the growth response, mineral analysis was also carried out on plants from this experiment. Following the method of Novozamsky *et al.* (1974), 0.03 - 0.3 g of dried, finely chopped (<3 mm) roots or shoots were placed in 100-mL volumetric Pyrex tubes with 3.3 mL of a mixture of 0.39 *M* salicylic acid in slightly diluted H_2SO_4 (100 mL concentrated H_2SO_4 added to

18 mL water = 15.25 *M*) and left to sit for 1 hour. This 1-hour pretreatment in the presence of salicylic acid converted free NO_3 and NO_2 to aminosalicyclic acid, so that total nitrogen, rather than NH_4 + organic N, could be determined (Novozamsky *et al.* 1974). The tubes were placed in an aluminum heating block and the temperature was raised to 100°C for 1 hour, then to 280°C for 3 hours. During the 3 hour period, 0.5 mL aliquots of 30% H_2O_2 were added periodically until the digests cleared (approximately 5 mL was required). Following digestion, the samples were made up to 100 mL with distilled water. Standards were prepared from $(NH_4)_2SO_4$, Na_2HPO_4, K_2SO_4, and $NiSO_4$ in 0.37 *M* H_2SO_4.

Reagents for the nitrogen assay were prepared as follows.

NITROGEN MIXED REAGENT 1:
(1) 100 mL of an 8.8% (w/v) solution of phenol in 1.0 *M* NaOH
(2) 200 mL of a freshly prepared 0.05% (w/v) sodium nitroprusside solution
(3) 10 mL of a 4% (w/v) Na_2EDTA solution.

NITROGEN MIXED REAGENT 2:
(1) 400 mL of a 1.335% (w/v) solution of $Na_2HPO_4{\cdot}2H_2O$, adjusted to a pH of 12 with NaOH
(2) 100 mL of a 0.7% (w/v) sodium hypochlorite solution.

Samples from the digests and standards were diluted 1 in 5. A 3.0 mL aliquot of mixed reagent 1 and 5.0 mL of mixed reagent 2 were added to 0.20 mL of these diluted solutions, mixing after each reagent. After 90 minutes, absorbance of the indophenol blue complex was read at 630 nm.

Phosphorus content was determined after Prepas and Rigler (1982). Samples and standards from the previous digestion were diluted 1 in 50. 0.5 mL of the following mixed reagent (freshly prepared) was added to 5 mL of the diluted solutions. After 15 minutes, absorbance was read at 885 nm.

PHOSPHORUS MIXED REAGENT
(1) 0.737 g ammonium molybdate
(2) 0.0165 g potassium antimony tartarate
(3) 1.03 g ascorbic acid
(4) 100 mL 1.34%(v/v) H_2SO_4 (0.24 *M*)

Potassium and nickel content of the digested samples were determined by atomic absorption spectrophotometry with a 1 in 25 and no dilution respectively.

Experiment 3.3. Nickel Stress *vs.* Relative Addition Rate

This experiment was designed to test the effect of a full range of nickel stress levels on RAR-induced nutrient competition. There were 2 RAR treatments, 0.10 and 0.14 d^{-1}, 10 nickel treatments, 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 10 μM $NiSO_4$, and 3 replicate blocks for a total of 60 pails. Plants were harvested 30 days after germination.

Experiment 3.4. Relative Addition Rate *vs.* Nickel Stress *vs.* Time

The intent of this experiment was to test the effect of abiotic stress on nutrient competition as Experiment 3.2, and to follow this relationship over time. Five RAR treatments were tested: 0, 0.04, 0.08, 012, and 0.16 d^{-1}. There were two abiotic stress treatments, 0 and 1.5 μM $NiSO_4$, two harvests, 20 and 31 days after germination, and 3 replicate blocks for a total of 60 pails.

Nickel Speciation

To investigate the possibility that chemical interactions in the growth solutions could explain the effects of nickel on competition, the activity of free ionic nickel was calculated at each RAR. Higher RARs resulted in higher conductivity readings (higher concentrations of nutrients) over the course of the experiment. This analysis was conducted to determine if this effect of RAR on the solution nutrient concentrations might alter the toxicity of nickel to the plants. The nutrient concentrations in the growth solutions were estimated from conductivity data and the proportions of nutrients added to the solutions. Two assumptions were necessary. The first was that nutrients were taken up in the same proportion as they were added. Experiments designed to adapt the RAR technique for use in these experiments indicated that addition proportions were reasonably close to uptake proportions (Chapter 2, Stadt *et al.* 1992). The second assumption was that exudation of soluble organic substances by the roots made no significant contribution to the measured conductivities. Conductivity changed very little in treatments where no nutrients were added (0 d^{-1} RAR), supporting this assumption. At higher RARs, it was impossible to separate the contribution of exudation from the contribution of nutrient additions to conductivity. The relationship between conductivity and nutrient concentration was determined by preparing a series of nutrient solutions at known concentrations and measuring the conductivity. Both the mean conductivity over the entire experiment and the conductivity on the last day of the experiment were used to estimate the typical and "most different" solution nutrient concentrations respectively. The effect of solution concentration on nickel activity was then calculated using the chemical speciation software, GEOCHEM.

STATISTICAL ANALYSIS

The absolute effects of nickel and competition (either density or RAR) on mean weight per plant of roots, shoots, and whole plants were analyzed by 2-way analyses of variance (ANOVAs). Although the variance of the data increased with its mean, the *log*-transformation was not applied to achieve homoscedasticity (Sokal and Rohlf 1981), because this would alter the additive interpretation of the relationship between these stresses. According to Sokal and Rohlf (1981), moderate heteroscedasticity does not seriously effect the accuracy of overall tests of significance, such as the test for interaction. The relative effects of nickel and competition were analyzed using Taylor's (1989) weight index (WI) transformation and the Weibull technique of Taylor *et al.* (1992). Although this transformation generates proportional data that is likely to be heteroscedastic (Sokal and Rohlf 1981), no adjustment was made, since further transformation would again change the interpretation of the interaction.

The root, shoot, or plant weight index (RWI, SWI, PWI) transformation is calculated as shown in Equation 3.2:

$$[3.2] \qquad WI = (W - W_{min})/(W_{max} - W_{min})$$

where W is the mean root, shoot, or total weight per plant for that particular pail, W_{min} is the lowest weight within that particular secondary stress treatment group and replicate block, and W_{max} is the highest weight within the secondary stress treatment group and block (Taylor 1989). For instance, the SWI for Experiment 3.3, pail 15, which had a primary stress of 1 μM Ni, a secondary stress of 0.10 d^{-1} RAR, and was in block 3, was calculated from the difference between the mean shoot weight (SW) of the 4 plants in pail 15 and the SW of the 4 plants in pail 30 (the lowest SW in block 3 subject to the 0.10 d^{-1} RAR treatment), divided by the difference between the SW of pail

A modified Weibull cumulative frequency distribution was fit to the untransformed mean weight per plant data for roots, shoots, and plants using the "doesn't use derivatives" (DUD) algorithm of the nonlinear regression procedure (PROC NLIN) available on SAS release 6.06 (SAS Institute 1985). Use of the DUD option eliminated the need to supply the partial derivatives of the function. The modified Weibull function has the form:

$$y = a + b \cdot e^{-[(x-x_o)/c]^d} \tag{3.3}$$

where y is the strain variable, x is the stress level, x_o is the lowest stress level, and a, b, c, and d are parameters (Taylor *et al.* 1991, 1992). The regression algorithm requires initial estimates of the parameters, and solves for the set of parameters which minimizes the residual sum of squares. Previous experience indicated that the choice of starting values for the parameters (required to initialize the algorithm) did not affect the final solution obtained.

Mean root, shoot, and total weight per plant (RW, SW, PW) were separately regressed on density or 1/RAR in the absence (function 1) and presence (function 2) of a mildly toxic dose of nickel in Experiments 3.1, 3.2, and 3.4. In Experiment 3.3, RW, SW, and PW were regressed on nickel concentration at an RAR of 0.14 d^{-1} (function 1) and 0.10 d^{-1} (function 2). Since RAR treatments place plants under increasing nutrient stress as RAR decreases, the direction of this variable was reversed in experiments 3.3 and 3.4 by taking the reciprocal of the RAR. This competitive stress index (CSI = 1/RAR) is an appropriate transformation because a linear decrease in RAR results in an exponential decrease in the total amount of nutrients supplied, and the reciprocal transformation approximates this relationship. For treatments with an RAR of 0, I assigned an arbitrary reciprocal value of 4000 d, indicating severe nutrient stress. Reversing the direction of the RAR axis also allows the same Weibull-type function as for the previous experiments to be fit to the data.

Manipulations of the stress variables, unlike the strain variable, should have no effect on the interpretation of the relationship between the stresses. The scale of these transformations is unimportant, much as the coding of the class levels in an ANOVA is unimportant. It is the changes in the strain variable at several different combinations of the stress variables that indicate the stress's relationship. The key factor in choosing a transformation for an independent variable is that the spacing of the values allows the function to accurately characterize the shape of the stress response. Dassel and Rawlings (1988) recommended that independent values for the Weibull function should be selected so that one point is at or close to zero strain (x_o), at least one point is in or near the "bottomed-out" region of extreme strain, and the majority of points are in the actively changing region. The reciprocal transformation of RAR met these criterion (see Figures 3.4, 3.6, 3.7) and permitted the Weibull function to model the stress response accurately.

Once these criterion are met, lack-of-fit testing (Cornell and Berger 1987) can be used to confirm that the function is appropriate for the data. The lack-of-fit test requires replicate observations at each level of x, and has the form:

$$\text{lack-of-fit } F = \frac{(SS_{res} - SS_{err})/(k-m)}{SS_{err}/(N-k)} \tag{3.4}$$

Here SS_{res} is the residual sum-of-squares obtained from the regression, SS_{err} is the error sum-of-squares obtained from a one-way ANOVA of the data used only in that particular regression, k is the number of independent (x) levels, m is the number of parameters in the function, and N is the total number of observations ($N = \Sigma r_i$, where r is the number of replicates at level i of the independent variable). The lack-of-fit statistic is compared to the F-distribution with (k - m) and (N - k) degrees of freedom. The lack-of-fit statistic measures whether the function maps the data within the limits of the replicate variation. A significant F statistic indicates poor mapping. This test is a better indicator of a function's suitability than R^2, which indicates the proportion of the total variation in the data that is explained by the function (Cornell and Berger 1987).

Parameters of the two functions within each experiment were compared using a t-test:

$$z = (\beta_1 - \beta_2)/(s_1^2 + s_2^2)^{1/2} \tag{3.5}$$

Here β_1 and β_2 are corresponding parameters of functions 1 and 2 respectively, and s_1 and s_2 are their standard errors. The statistic z is compared to the Student's t distribution with $[\Sigma(N_i-m)]$ degrees of freedom, where i is the number of functions (2 in this case), N is the number of observations for each function, and m is the number of parameters in the function (Zar 1984, Taylor *et al.* 1992).

If the functions differed in the growth response (*b*) parameter only, a multiplicative interaction would be indicated between competitive stress and nickel stress. Differences in the functions' scale (*c*) or shape (*d*) parameters would indicate that nickel stress modified the effect of competitive stress or vise-versa (growth in the presence of nickel was not a constant proportion of growth in the absence of nickel above the minimum biomass). By plotting the function expected if the relationship were perfectly multiplicative, it can then be determined whether nickel toxicity increased the importance of competition (growth in the presence of both stresses was less than expected), or decreased it (growth in the presence of both stresses was greater than expected).

Statistical analyses were carried out using the general linear models and nonlinear regression procedures available on SAS Release 6.06 (SAS Institute 1985). Significance was defined at the 5% level.

RESULTS

Experiment 3.1. Density *vs.* Nickel Stress

There was no mortality so densities were as planted. Total plant yield per pail was compared to density at both nickel levels (Figure 3.2) and indicated that constant yield was approached, though not obtained over this range of densities. Mean weight per plant (PW = roots + shoots) and mean shoot weight per plant (SW) declined with increasing density in a hyperbolic manner to a minimum value of approximately 0.6 g PW and 0.4 g SW, both in the absence and presence of the 15.0 μM nickel dose (Figure 3.3a,b). Mean root weight per plant (RW) was initially unchanged by density then declined to approximately 0.2 g (Figure 3.3c). The nickel dose reduced PW and RW substantially at low densities but, owing to the apparent common minimum, had a negligible absolute effect at high densities. Similarly, the absolute effect of competition on growth was less when nickel was present than when it was absent. Mean shoot weight per plant (SW) was also affected by nickel, but at low densities this effect was not significantly greater than at high.

The ANOVA of the untransformed data indicated significant main effects of density and nickel for all strain variables (PW, SW, and RW; Table 3.1). When nickel was present, the absolute reduction in PW and RW in response to density was much less than the absolute magnitude of the same growth response when nickel was absent, accounting for the significant density x nickel interaction term for PW and RW. The SW data show a similar pattern, but the change in the competition response due to nickel was not great enough to yield a significant interaction term. When the data were weight-index transformed for comparison to a multiplicative model, only density had a significant effect on plant-, shoot-, or root-weight-index (PWI, SWI, and RWI; Table 3.1). This indicates that the mild nickel stress did not change the relative effect of density on root, shoot or whole-plant growth. Weight-index values were calculated separately for each secondary stress (nickel) treatment group (see Equation 3.2), so that, if the data conformed to a multiplicative model, the effect of nickel as well as the interaction term would disappear. If a non-multiplicative relationship was present, a significant interaction and nickel effect should have been evident (Taylor 1989).

The Weibull function fit the strain variables, PW, SW, and RW, accurately at both levels of nickel (Figure 3.3). Over 95% of the total variation was accounted for by the regressions (R^2, Table 3.2) and the function fit the response pattern within the limits of the error variation (lack-of-fit tests were all non-significant: $P > 0.05$, Table 3.2). Significant differences were detected only in the growth response parameter (parameter *b*) between functions fit to the density response at both levels of nickel for PW, SW, and RW data (Table 3.2). Thus, growth above the minimum biomass in the presence of a 15.0 μ*M* nickel stress remained a constant proportion of growth above the minimum biomass in the absence of nickel over the range of densities tested. Like the WI-ANOVA, the results of the Weibull analysis suggest that a multiplicative model adequately explained the relationship between density and nickel stress, *i.e.* relatively speaking, nickel stress had no effect on competition.

Experiment 3.2. Relative Addition Rate *vs.* Nickel Stress

The response of mean weight per plant, mean shoot weight per plant and mean root weight per plant (PW, SW, and RW) to the relative addition rate (RAR) mimic of competitive stress (Figure 3.4) was similar to the strain observed under density treatment (Experiment 3.1, Figure 3.3). All strain variables showed a hyperbolic

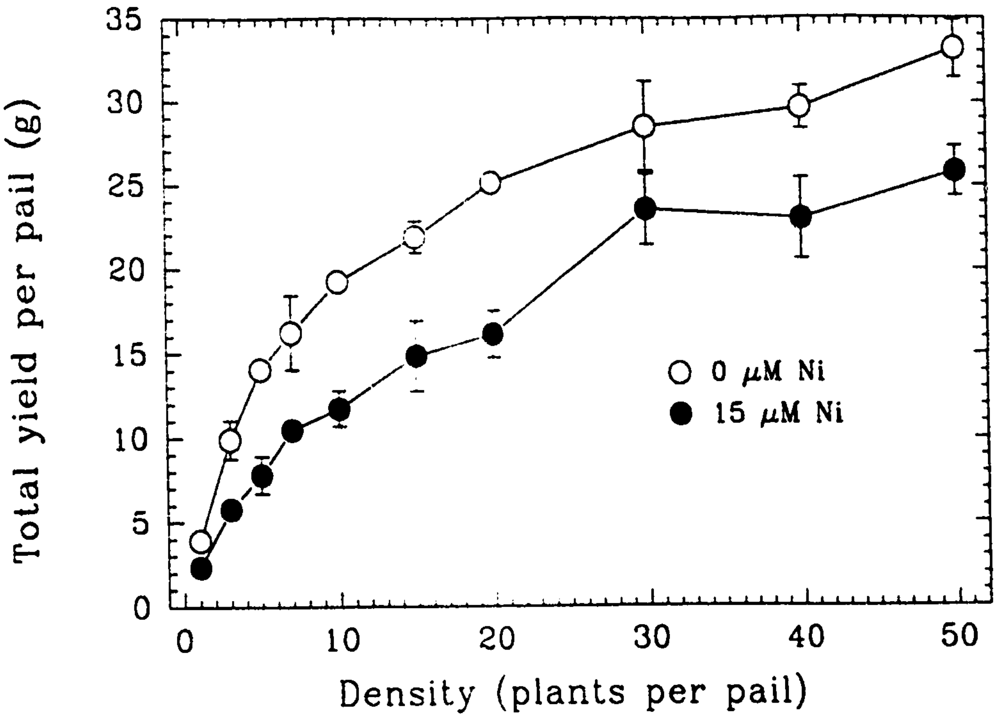

Figure 3.2. Experiment 3.1. Total weight per pail as a function of density and nickel concentration.

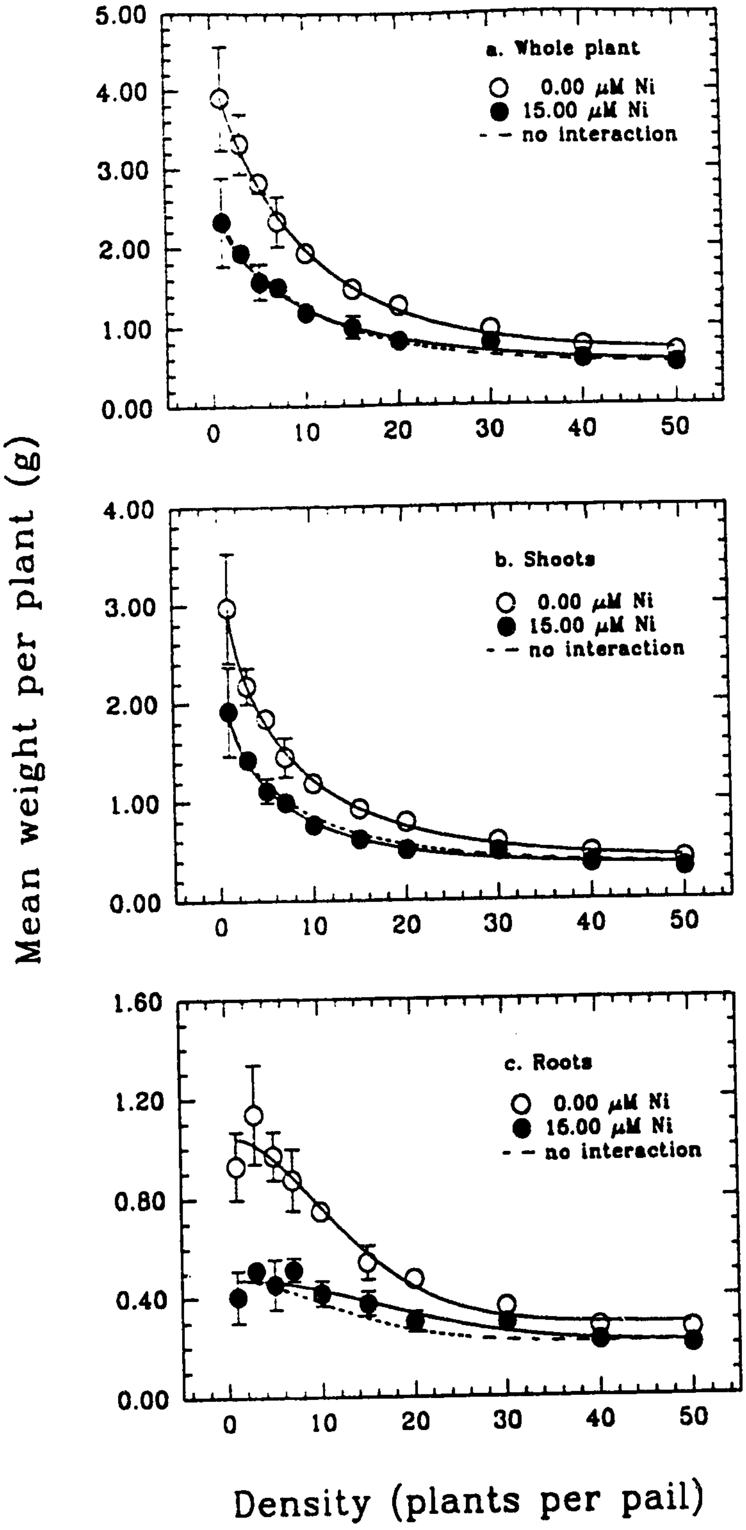

Figure 3.3. Experiment 3.1. The response of mean weight per plant (PW), shoot (SW), and root (RW) to density in the absence and presence of a mildly toxic nickel stress.

Table 3.1. Experiment 3.1. Density *vs.* nickel. Analysis of variance of raw mean weight per plant (W) and weight index transformed[a] (WI) whole-plant (P), shoot (S), and root (R) data.

Effect	*P* level[b]					
	PW	PWI	SW	SWI	RW	RWI
Ni	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Density	0.0001*	0.2161	0.0001*	0.3116	0.0001*	0.2792
Ni x Density	0.0227*	0.9803	0.1334	0.9982	0.0017*	0.8829

[a] see Equation 3.2, Materials and Methods, for transformation.

[b] significant statistics ($P<0.05$) are highlighted by asterisks (*).

Table 3.2. Experiment 3.1. Weibull parameters and statistics for density *vs.* nickel interaction on mean weight per plant (PW), mean shoot weight per plant (SW), and mean root weight per plant (RW).

Strain Variable	Secondary Stress Level	x_o	Parameter				R^2	Lack-of-fit *P*
			a	*b*	*c*	*d*		
PW	0.00 μM Ni	1	0.66 ± 0.25	3.27 ± 0.36	9.92 ± 2.20	0.91 ± 0.21	0.971	0.9992
	15.00 μM Ni	1	0.50 ± 0.25	1.83 ± 0.33	9.92 ± 4.05	0.79 ± 0.27	0.956	1.0000
			(0.3234)[a]	(0.0025*)	(0.4996)	(0.3635)		
SW	0.00 μM Ni	1	0.37 ± 0.21	2.60 ± 0.29	7.72 ± 2.10	0.74 ± 0.18	0.965	0.9992
	15.00 μM Ni	1	0.33 ± 0.14	1.60 ± 0.21	6.79 ± 2.12	0.74 ± 0.22	0.952	0.9979
			(0.4267)	(0.0033*)	(0.3776)	(0.4982)		
RW	0.00 μM Ni	1	0.29 ± 0.06	0.75 ± 0.10	14.40 ± 2.52	1.68 ± 0.62	0.960	0.7324
	15.00 μM Ni	1	0.21 ± 0.06	0.26 ± 0.07	21.73 ± 7.32	1.94 ± 1.35	0.950	0.6727
			(0.1900)	(0.0001*)	(0.1745)	(0.4317)		

[a] Probability of obtaining the *z*-statistic (Zar 1986, see text) based on the null hypothesis that the parameters are equal; an asterisk (*) highlights a significant difference between the parameter pair ($P<0.05$).

decline with increasing competitive stress index level (CSI=1/RAR) to a non-zero minimum value of about 0.1 g PW, 0.07 g SW, and 0.03 g RW (Figure 3.4). The 1.90 μM nickel dose had a larger absolute effect at low levels of competitive stress than at high, so that in the presence of nickel, there was a smaller absolute decline in PW, SW, or RW in response to competitive stress than when nickel was absent. At high levels of competitive stress (25 and 4000 d CSI), however, nickel still had a small toxic effect on growth, such that the minimum biomass in the presence of nickel was approximately half that of the minimum reached in the absence of nickel.

The ANOVA of untransformed data indicated significant effects of RAR and nickel, as well as a significant RAR x nickel interaction term on PW, SW, and RW (Table 3.3). The interaction signifies that, in absolute terms, nickel stress reduced the effect of competition. The ANOVA of SWI data indicated a non-multiplicative relationship between competition and nickel since the nickel and RAR x nickel effects were significant. Comparing the SW data to a perfectly multiplicative model (Figure 3.4b, dotted line) indicates that nickel reduced the relative effect of competition: higher levels of competitive stress were required to reduce SW to a given proportion at a mild level of nickel stress than in its absence. In the ANOVA of RWI, a significant effect of the primary stress (RAR), only, indicated that the RW response was explained by a multiplicative relationship between nickel and RAR. The ANOVA of PWI data indicated significant RAR and nickel effects, but no interaction effect. Because of the design of the WI-transformation, it is difficult to explain a significant secondary main effect such as nickel without corresponding interactions with the primary stress (RAR in this case). It is likely this "orphan" effect of nickel is implies an influence of nickel on RAR that was too weak to generate a significant interaction. Likewise, significant interactions without corresponding main effects are problematic. Taylor and colleagues (Taylor and Stadt 1990, Taylor *et al.* 1992) suggested that the increase in variance introduced by the weight-index transformation tends to obscure the interpretation of an ANOVA.

The Weibull function provided accurate fits to the data ($R^2 > 0.98$, lack-of-fit $P > 0.05$, Table 3.4). The minimum biomass estimate (parameter *a*) was significantly less when nickel was present than when it was absent for all strain variables. Non-multiplicative effects of nickel on competition were indicated for PW, SW, and RW. The scale parameter (*c*) and, for SW and RW, the shape parameter (*d*) changed significantly. The direction of this non-multiplicative effect was a decrease in the relative effect of

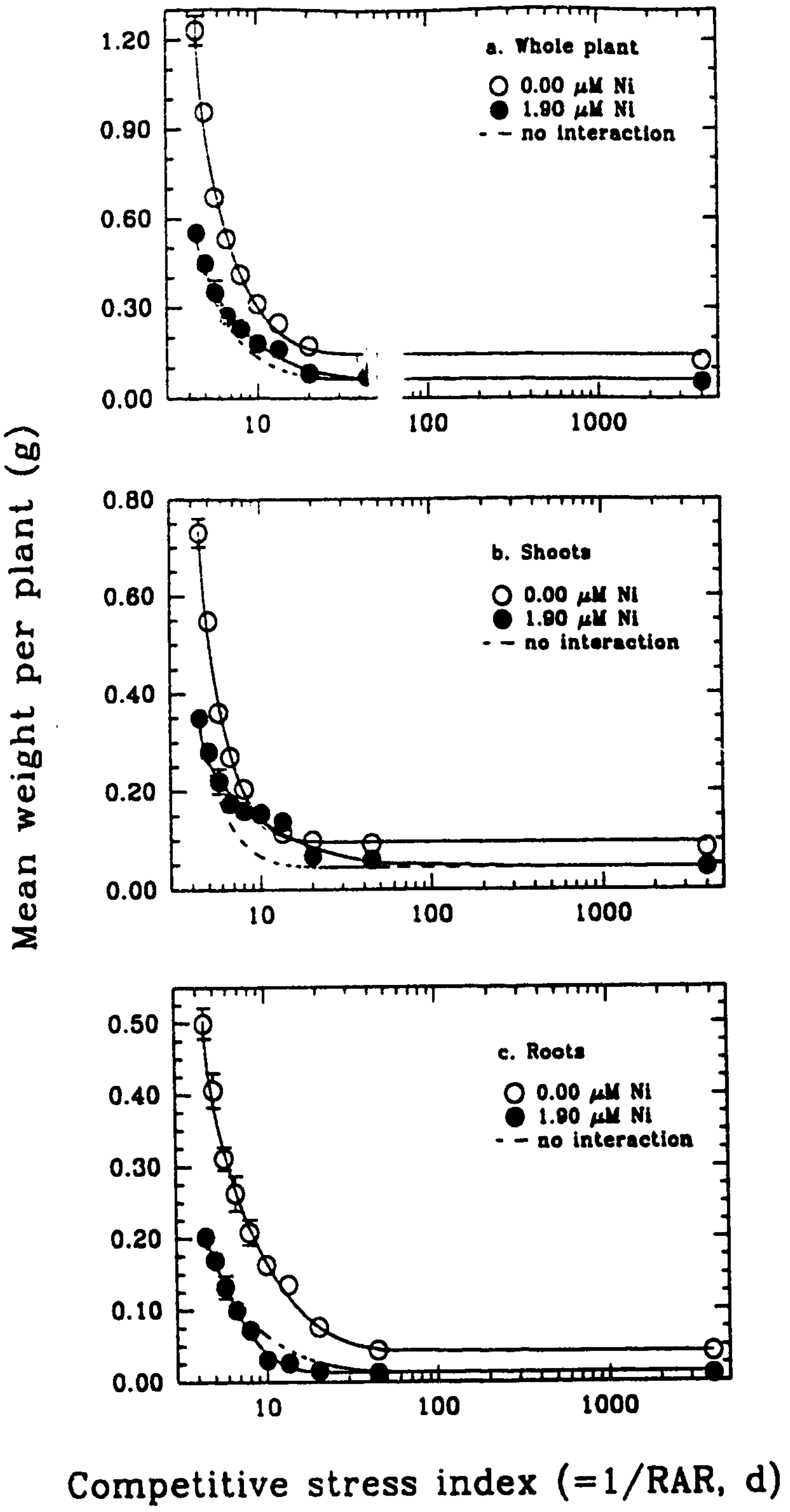

Figure 3.4. Experiment 3.2. The response of mean weight per plant (PW), shoot (SW), and root (RW) to the relative addition rate (RAR) mimic of competitive stress in the absence and presence of a mildly toxic nickel stress.

Table 3.3. Experiment 3.2. Relative addition rate (RAR) *vs.* nickel. Analysis of variance of raw mean weight per plant (W) and weight index-transformed[a] (WI) whole-plant (P), shoot (S), and root (R) data.

Effect	*P* level[b]					
	PW	PWI	SW	SWI	RW	RWI
Ni	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
RAR	0.0001*	0.0004*	0.0001*	0.0001*	0.0001*	0.0706
Ni x RAR	0.0001*	0.4331	0.0001*	0.0002*	0.0001*	0.1652

[a] see Equation 3.2, Materials and Methods, for transformation.

[b] significant statistics ($P<0.05$) are highlighted by asterisks (*).

Table 3.4. Experiment 3.2: Weibull parameters and statistics for nickel *vs.* relative addition rate (RAR) interaction on mean weight per plant (PW), mean shoot weight perplant (SW), and mean root weight per plant (RW).

Strain Variable	Secondary Stress level	x_o	Parameter				R^2	Lack-of-fit *P*
			a	*b*	*c*	*d*		
PW	0.00 μM Ni	4.44	0.143 ± 0.015	1.112 ± 0.033	2.22 ± 0.15	0.71 ± 0.05	0.996	0.1147
	1.90 μM Ni	4.44	0.062 ± 0.014	0.503 ± 0.026	3.30 ± 0.42	0.65 ± 0.07	0.990	0.4984
			(0.0001*)[a]	(0.0001*)	(0.0091*)	(0.2709)		
SW	0.00 μM Ni	4.44	0.096 ± 0.008	0.640 ± 0.017	1.69 ± 0.10	0.82 ± 0.06	0.995	0.2210
	1.90 μM Ni	4.44	0.045 ± 0.012	0.308 ± 0.018	4.14 ± 0.78	0.53 ± 0.07	0.988	0.0690
			(0.0003*)	(0.0001*)	(0.0015*)	(0.0008*)		
RW	0.00 μM Ni	4.44	0.042 ± 0.011	0.462 ± 0.019	3.66 ± 0.41	0.66 ± 0.06	0.992	0.7486
	1.90 μM Ni	4.44	0.013 ± 0.004	0.190 ± 0.008	2.84 ± 0.23	1.01 ± 0.11	0.988	0.8353
			(0.0083*)	(0.0001*)	(0.0422*)	(0.0030*)		

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[a] Probability of obtaining the *z*-statistic (Zar 1986, see text) based on the null hypothesis that the parameters are equal; an asterisk (*) highlights a significant difference between the parameter pair ($P<0.05$).

competition on PW and SW at the higher level of nickel. Higher levels of competitive stress were required to reduce growth to a given proportion of control in the presence of nickel than in its absence (compare the 1.90 μM nickel curves with the dotted lines in Figure 3.4a,b). For roots the effect was reversed. Lower levels of competitive stress were required to reduce RW to a given proportion of control in the presence of nickel than in its absence (Figure 3.4c). Thus, nickel increased the relative effect of competition on RW.

The difference in minimum growth (parameter *a*) does not affect the nature of the interaction. Both the Weibull analysis and the weight-index transformation were developed under the assumption that growth would decline to the same minimum value at extreme levels of a primary stress, regardless of the level of the secondary stress. However, if the primary stress takes time to exercise its full effect (the "lag time" cited by Taylor 1989), the secondary stress may have a small effect on this residual growth. In this case, seed reserves and background levels of nutrients in the solution provided a nutrient reserve that reduced the effect of the most extreme competitive stress [illegible] 4000 d). The nickel dose likely had an impact on the rate of uptake and utilization of this reserve, causing the difference in the response minima. Since this small nutrient reserve was present in all treatments, the growth difference it caused (under the assumption that plant nutrient content was roughly the same in the presence and absence of nickel) could be subtracted at all levels of competitive stress, shifting the response curve in the absence of nickel downward till the minima are equal. Doing so would not change the shape of the stress response curve. Thus, the interpretation of the stresses' relationship (multiplicative *vs.* non-multiplicative) can be made on the basis of changes in parameters *b*, *c*, and *d* only.

Mineral Analysis

Both in the absence and presence of nickel, whole-plant nitrogen, phosphorus, and potassium concentrations increased significantly with increases in RAR, particularly above 0.10 d^{-1} (Figure 3.5a,b,c; Table 3.5). Nickel caused a small increase in nitrogen content at low RARs (Figure 3.5a), but this effect disappeared as RAR increased. Phosphorus content increased some 40% in the presence of nickel, except at an RAR of 0.075 d^{-1} where nickel had no effect (Figure 3.5b). Potassium was decreased by nickel at low RARs and increased by nickel at high (Figure 3.5c). These

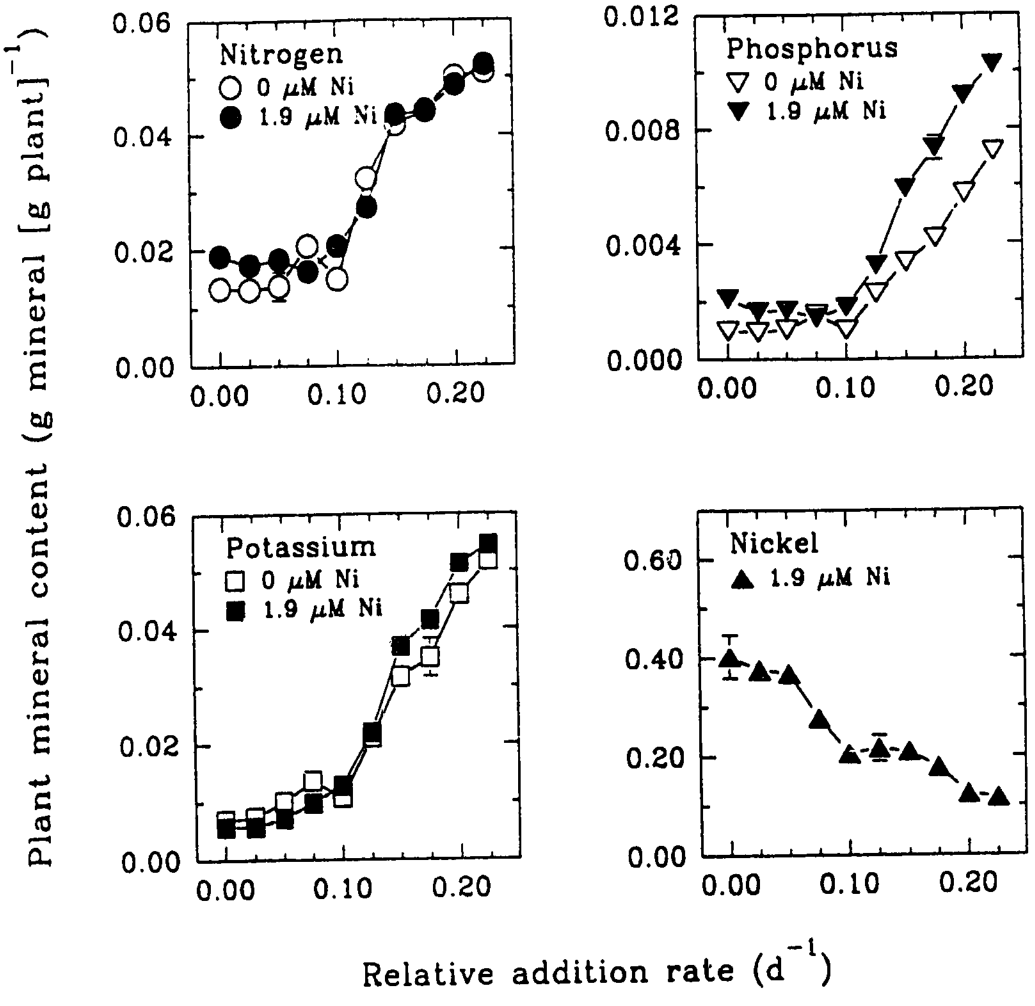

Figure 3.5. Experiment 3.2. The effect of relative addition rate (RAR) and nickel on plant nitrogen, phosphorus, potassium, and nickel content.

Table 3.5. Experiment 3.2. Analysis of variance of the effect of relative addition rate (RAR) and nickel on plant nitrogen, phosphorus, potassium, and nickel content.

Effect	*P* level[a]			
	Nitrogen	Phosphorus	Potassium	Nickel
RAR	0.0001*	0.0001*	0.0001*	0.0001*
Ni	0.0229*	0.0001*	0.0303*	
RAR x Ni	0.0001*	0.0001*	0.0031*	

[a]significant statistics ($P<0.05$) are highlighted by asterisks (*).

effects were small, however. Nickel content declined with increasing RAR in the nickel-treated plants and was undetectable in untreated material (Figure 3.5d; Table 3.5).

Experiment 3.3: Nickel Stress *vs.* Relative Addition Rate

Nickel was the primary stress and RAR the secondary stress in this experiment. Mean weight per plant, mean shoot weight per plant, and mean root weight per plant (PW, SW, and RW) declined in a sigmoid fashion with increasing nickel concentrations from 0 to 5 μM at RARs of 0.10 d^{-1} and 0.14 d^{-1} (Figure 3.6). Above 5 μM Ni the stress response remained at a minimum value of approximately 0.05 g PW, 0.04 g SW, and 0.01 g RW at both RARs. Relative addition rate treatment had a large absolute effect at 0 μM Ni but none at 10.0 μM.

The ANOVA of untransformed data indicated significant effects of nickel, RAR, and nickel x RAR on PW, SW, and RW (all $P = 0.0001$, Table 3.6). Clearly, as nickel stress increased, the effect of competitive stress (RAR) on growth was reduced in absolute terms. When the data were transformed to test for a multiplicative relationship, ANOVA again indicated significant effects of nickel, RAR, and nickel x RAR for PWI and RWI. Comparing the data to a multiplicative response (Figure 3.6a,c dotted lines) indicates that the relative effect of competitive stress was increased at low levels of nickel stress (growth was less than predicted by the multiplicative model), and, for PW only, decreased at intermediate levels of nickel stress (growth was greater than predicted). For SWI data, only the effect of the primary stress, nickel, was significant, indicating multiplicative effects (Figure 3.6b).

Weibull fits for PW and RW in this experiment were less accurate. At a RAR of 0.14 d^{-1} there was a significant additive component in the residual variance compared to the error mean square (PW lack-of-fit (LOF) $P = 0.0064$, RW LOF $P = 0.0001$; Table 3.7). However, there was a anomalous value at a nickel concentration of 0.25 μM (Figure 3.6) which, when removed, yielded non-significant LOF statistics. The function fit the PW and RW 0.10 d^{-1} RAR data as well as all the SW data adequately (LOF $P > 0.05$) and in all cases accounted for 98% of the total variation (R^2, Table 3.7). Comparison of the parameters of the 0.10 d^{-1} and 0.14 d^{-1} functions fit to PW and SW indicated significant differences between the growth response (*b*) parameters only (Table 3.7). Growth at a high level of nutrient competition (at an RAR of 0.10 d^{-1}) remained a constant proportion of growth at a lower level of nutrient competition (at an

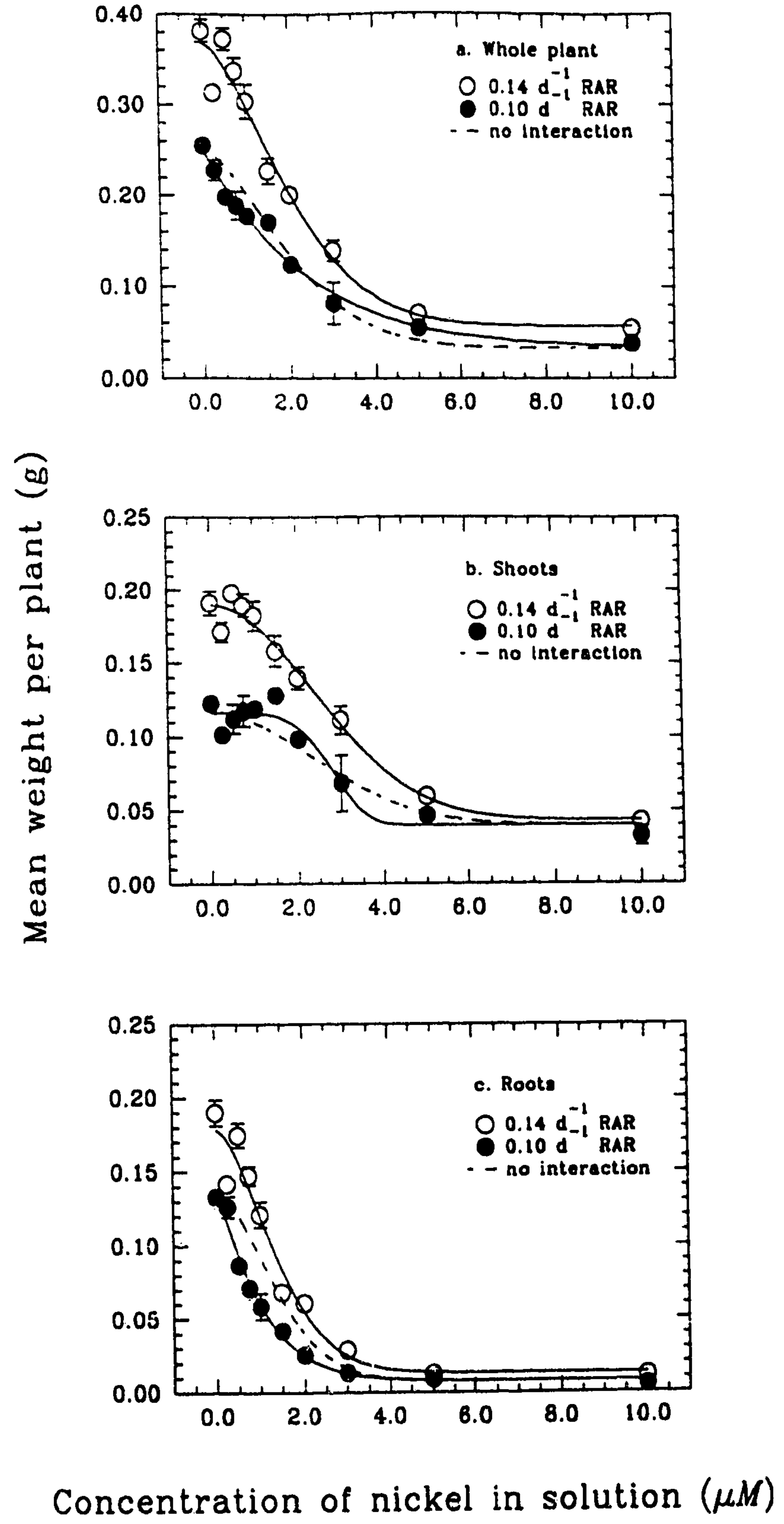

Figure 3.6. Experiment 3.3. The response of mean weight per plant (PW), shoot (SW), and root weight (RW) to nickel stress at two relative addition rates (RARs).

Table 3.6. Experiment 3.3. Nickel *vs.* relative addition rate (RAR). Analysis of variance of raw mean weight per plant (W) and weight index-transformed[a] (WI) whole-plant (P), shoot (S), and root (R) data.

Effect	*P* level[b]					
	PW	PWI	SW	SWI	RW	RWI
Ni	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
RAR	0.0001*	0.0262*	0.0001*	0.3569	0.0001*	0.0001*
Ni x RAR	0.0001*	0.0083*	0.0001*	0.3420	0.0001*	0.0001*

[a] see Equation 3.2, Materials and Methods, for transformation.

[b] significant statistics ($P<0.05$) are highlighted by asterisks (*).

Table 3.7. Experiment 3.3. Weibull parameters and statistics for nickel *vs.* relative addition rate (RAR) interaction on mean weight per plant (PW), mean shoot weight per plant (SW), and mean root weight per plant (RW).

Strain Variable	Secondary Stress Level	x_o	Parameter				R^2	Lack-of-fit *P*
			a	*b*	*c*	*d*		
PW	0.14 d^{-1} RAR	0	0.057 ± 0.014	0.311 ± 0.020	2.37 ± 0.20	1.58 ± 0.25	0.991	0.0064
	0.10 d^{-1} RAR	0	0.033 ± 0.013	0.217 ± 0.018	2.34 ± 0.34	1.04 ± 0.16	0.989	0.3828
			(0.1029)[a]	(0.0004*)	(0.4604)	(0.3760)		
SW	0.14 d^{-1} RAR	0	0.043 ± 0.008	0.147 ± 0.010	3.23 ± 0.28	1.98 ± 0.34	0.993	0.1746
	0.10 d^{-1} RAR	0	0.040 ± 0.006	0.076 ± 0.008	2.99 ± 0.22	4.78 ± 2.40	0.979	0.2291
			(0.3704)	(0.0001*)	(0.1760)	(0.1273)		
RW	0.14 d^{-1} RAR	0	0.013 ± 0.007	0.165 ± 0.010	1.65 ± 0.13	1.65 ± 0.13	0.983	0.0001
	0.10 d^{-1} RAR	0	0.008 ± 0.004	0.129 ± 0.006	1.08 ± 0.09	1.15 ± 0.13	0.987	0.1945
			(0.2274)	(0.0019*)	(0.0003*)	(0.0434*)		

[a] Probability of obtaining the z-statistic (Zar 1986, see text) based on the null hypothesis that the parameters are equal; an asterisk (*) highlights a significant difference between the parameter pair ($P<0.05$).

RAR of 0.14 d^{-1}) over the range of nickel concentrations tested. For PW, this interpretation was different than the WI-ANOVA; however, the level of significance of the WI-ANOVA RAR and interaction terms were close to the critical value of 0.05 (Table 3.6). For RW there were significant differences between the growth response (*b*), scale (*c*), and shape (*d*) parameters (Table 3.7). The direction of change in scale and shape was such that, above the minimum biomass, growth in the presence of nickel was a decreasing proportion of growth in the absence of nickel as nickel increased from 0 to 1.5 μM. Since growth was less than predicted by a multiplicative model (see Figure 3.6c, dotted line), increasing nickel stress increased the relative effect of competition (RAR) on root growth.

Experiment 3.4. Relative Addition Rate *vs.* Nickel Stress *vs.* Time

The response of PW, SW, and RW to competitive stress was again similar to the strain observed under density treatment (Figures 3.7 and 3.3). All strain variables showed a hyperbolic decline to a non-zero minimum value (Figure 3.7). However, at the early harvest, the effect of nickel was complex. On day 20, nickel had a negligible effect on PW at low levels of competitive stress (CSI = 6.25 d, Figure 3.7a). This effect *increased* in absolute terms as competition increased, so that, at extreme levels of competitive stress (CSI=4000 d), the minimum biomass was approximately 0.03 g less in the presence of nickel than in its absence (0.05 compared to 0.08 g). Nickel treatment *increased* SW at low levels of competitive stress on day 20 (Figure 3.7b), but, as with PW, competition reduced SW to a lower minimum value when nickel was present (0.06 compared to 0.04 g). In other words, nickel *increased* the absolute effect of competition on PW and SW. Mean root weight per plant (RW) was reduced more by nickel, in absolute terms, at low levels of competitive stress than at high (Figure 3.7c). However, RW still declined to a lower minimum at high CSI levels in the presence of nickel (0.01 g) than in its absence (0.02 g). At the later harvest (day 31), PW, SW, and RW at low levels of competitive stress (6.25 d) were higher than at day 20 (Figure 3.7). At high levels of competitive stress, biomass also increased with time, but more so in the presence of nickel. At day 31, the nickel dose decreased the strain variables by 30% at low levels of competition (Figure 3.7d-f). As competitive stress increased, PW, SW, and RW declined to a common minimum of approximately 0.1 g PW, 0.07 g SW, and 0.03 g RW at both levels of nickel. Thus, nickel *reduced* the absolute effects of competition at this time.

A full three-way ANOVA of untransformed data indicated that all main and interaction effects were significant (*P* < 0.05, Table 3.8). Since the absolute growth rate of the plants was affected by both competitive and nickel stress, there were significant RAR x time and nickel x time interactions for all the strain variables. The significant RAR x nickel interaction implies that, in absolute terms, the overall effect of nutrient competition over both harvests was changed by an increase in nickel stress. Since the unexpected increase in the absolute effect of competitive stress on PW and SW due to nickel at day 20 changed to a decrease in the effect of competitive stress due to nickel at day 31, there was a three-way RAR x nickel x time interaction on PW and SW. The RAR x nickel x time interaction was significant for RW as well, owing to the change in the effect of nickel on the minimum biomass over time.

When the data were transformed before the ANOVA, evidence was ambiguous for a multiplicative relationship. The WI-transformation was extended so that the nickel, time, and interactive effects should disappear if a multiplicative relationship existed among RAR-induced competitive stress, nickel toxicity, and time. The effect of the primary stress, RAR, was significant for all variables as expected (Table 3.8). For PWI data, the interaction effects did disappear, but main effects of nickel and time were still present. Likewise for RWI, the RAR x nickel and nickel x time interaction terms were not significant, while the main effects of nickel and time were. The significant RAR x time interaction, coupled with the main effect of time, indicated non-multiplicative effects of RAR and time on RWI, since RAR had a greater proportional effect on root growth at day 31 than day 20. For SWI, the effects of nickel and RAR x nickel were not significant, indicating a multiplicative relationship between these factors on shoot growth. However, there was also a significant time and nickel x time effect on SWI. Again, it is difficult to explain significant secondary main effects such as nickel and time without corresponding interactions with the primary stress (RAR in this case). Weak effects of nickel and time on RAR may be indicated here. The most important feature of this analysis was that, for all strain variables, the RAR x nickel x time interaction was not significant, indicating that, in relative terms, the relationship between RAR and nickel was not changed significantly with time.

Again the Weibull function adequately characterized the data ($R^2 > 0.97$, lack-of-fit $P > 0.05$, Table 3.9). At the earlier harvest (day 20) there were significant differences between the functions fit to the 0 and 1.50 μM nickel treatments in the minimum growth (*a*) and growth response (*b*) parameters. If the differences in

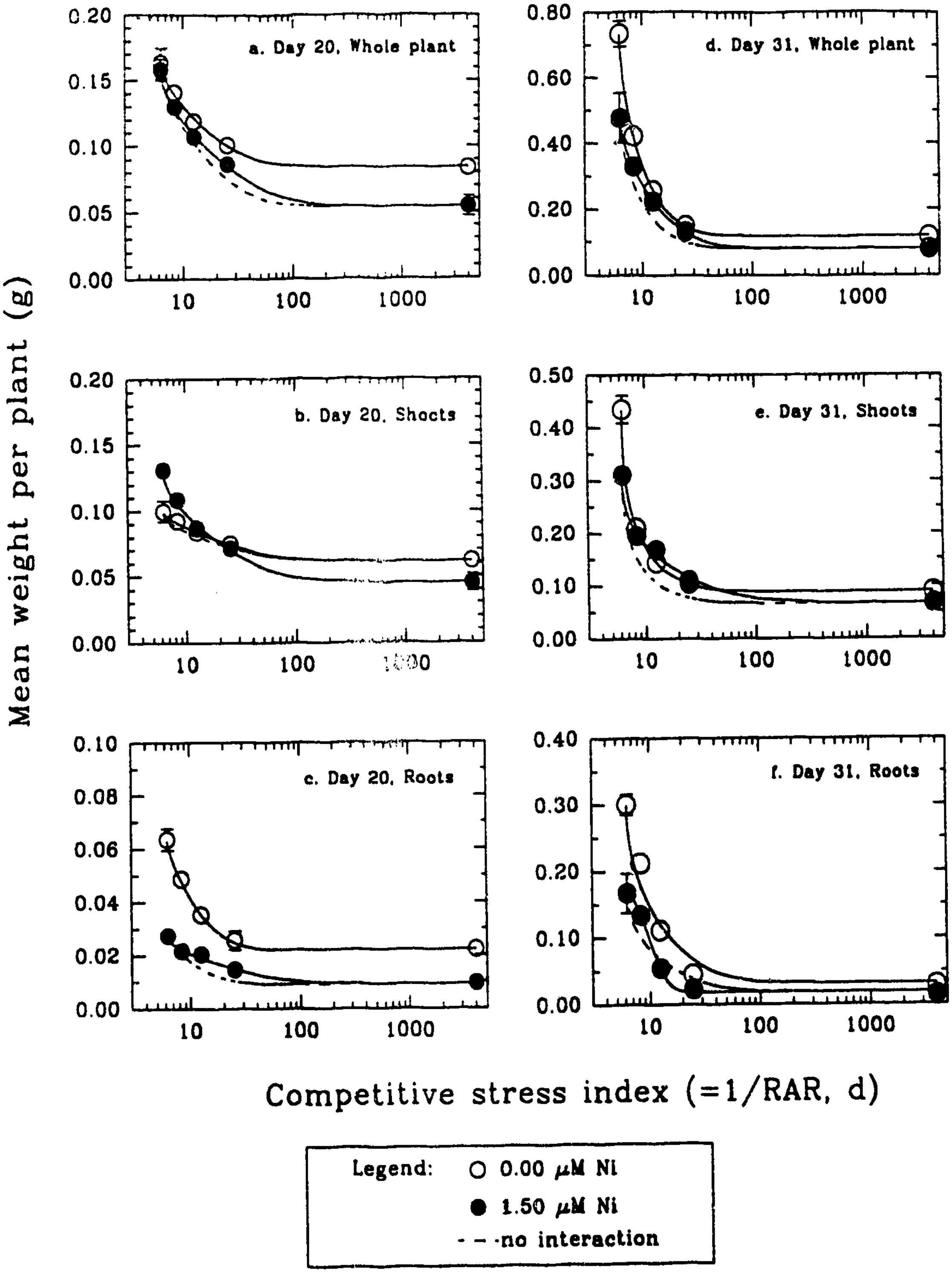

Figure 3.7. Experiment 3.4. The response of mean weight per plant (PW), shoot (SW), and root weight (RW) to the relative addition rate (RAR) mimic of competitive stress in the presence and absence of nickel stress over time.

Table 3.8. Experiment 3.4. Relative addition rate (RAR) *vs.* nickel *vs.* time. Analysis of variance of raw mean weight per plant (W) and weight index-transformed[a] (WI) whole-plant (P), shoot (S), and root (R) data.

Effect	*P* level[b]					
	PW	PWI	SW	SWI	RW	RWI
RAR	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Ni	0.0001*	0.0079*	0.0211*	0.0877	0.0001*	0.0290*
Time	0.0001*	0.0017*	0.0001*	0.0009*	0.0001*	0.0403*
RAR x Ni	0.0001*	0.2822	0.0007*	0.7454	0.0001*	0.1966
RAR x Time	0.0001*	0.1027	0.0001*	0.0645	0.0001*	0.0125*
Ni x Time	0.0001*	0.1058	0.0004*	0.0032*	0.0001*	0.1030
RAR x Ni x Time	0.0001*	0.4282	0.0001*	0.0960	0.0002*	0.0572

[a] see Equation 3.2, Materials and Methods, for transformation.
[b] significant statistics ($P<0.05$) are highlighted by asterisks (*).

Table 3.9. Experiment 3.4. Weibull parameters and statistics for nickel *vs.* relative addition rate (RAR) interaction on mean weight per plant (PW), mean shoot weight per plant (SW), and mean root weight per plant (RW).

Harvest/ Strain Variable	Secondary Stress Level	x_o	Parameter				R^2	Lack-of-fit P
			a	*b*	*c*	*d*		
Day 20	0.00 µM Ni	6.25	0.084 ± 0.003	0.080 ± 0.006	9.31 ± 3.16	0.68 ± 0.38	0.995	0.9750
PW	1.50 µM Ni	6.25	0.054 ± 0.005	0.104 ± 0.008	13.26 ± 3.49	0.58 ± 0.12	0.995	0.9737
			(0.0001*)[a]	(0.0104*)	(0.2052)	(0.4078)		
Day 20	0.00 µM Ni	6.25	0.061 ± 0.004	0.038 ± 0.006	17.10 ± 6.16	0.67 ± 0.60	0.994	0.8174
SW	1.50 µM Ni	6.25	0.045 ± 0.008	0.086 ± 0.007	12.87 ± 3.57	0.58 ± 0.13	0.995	0.3916
			(0.0071*)	(0.0001*)	(0.2792)	(0.4462)		
Day 20	0.00 µM Ni	6.25	0.022 ± 0.002	0.041 ± 0.003	5.41 ± 1.23	0.81 ± 0.19	0.993	0.7215
RW	1.50 µM Ni	6.25	0.009 ± 0.001	0.018 ± 0.002	15.23 ± 6.26	0.59 ± 0.20	0.989	0.2918
			(0.0001*)	(0.0001*)	(0.0691)	(0.2205)		
Day 31	0.00 µM Ni	6.25	0.118 ± 0.017	0.616 ± 0.025	3.46 ± 0.45	0.67 ± 0.10	0.996	0.9974
PW	1.50 µM Ni	6.25	0.081 ± 0.017	0.396 ± 0.027	6.15 ± 1.23	0.67 ± 0.12	0.991	0.9945
			(0.0694)	(0.0001*)	(0.0264*)	(0.4949)		
Day 31	0.00 µM Ni	6.25	0.088 ± 0.012	0.346 ± 0.017	1.89 ± 0.45	0.50 ± 0.12	0.994	0.9519
SW	1.50 µM Ni	6.25	0.066 ± 0.011	0.243 ± 0.018	6.59 ± 1.95	0.45 ± 0.10	0.991	0.2569
			(0.0967)	(0.0003*)	(0.0143*)	(0.3723)		
Day 31	0.00 µM Ni	6.25	0.031 ± 0.007	0.269 ± 0.011	5.24 ± 0.55	0.97 ± 0.13	0.995	0.4867
RW	1.50 µM Ni	6.25	0.018 ± 0.007	0.149 ± 0.014	5.00 ± 0.72	1.57 ± 0.44	0.975	0.5731
			(0.1051)	(0.0001*)	(0.3941)	(0.0989)		

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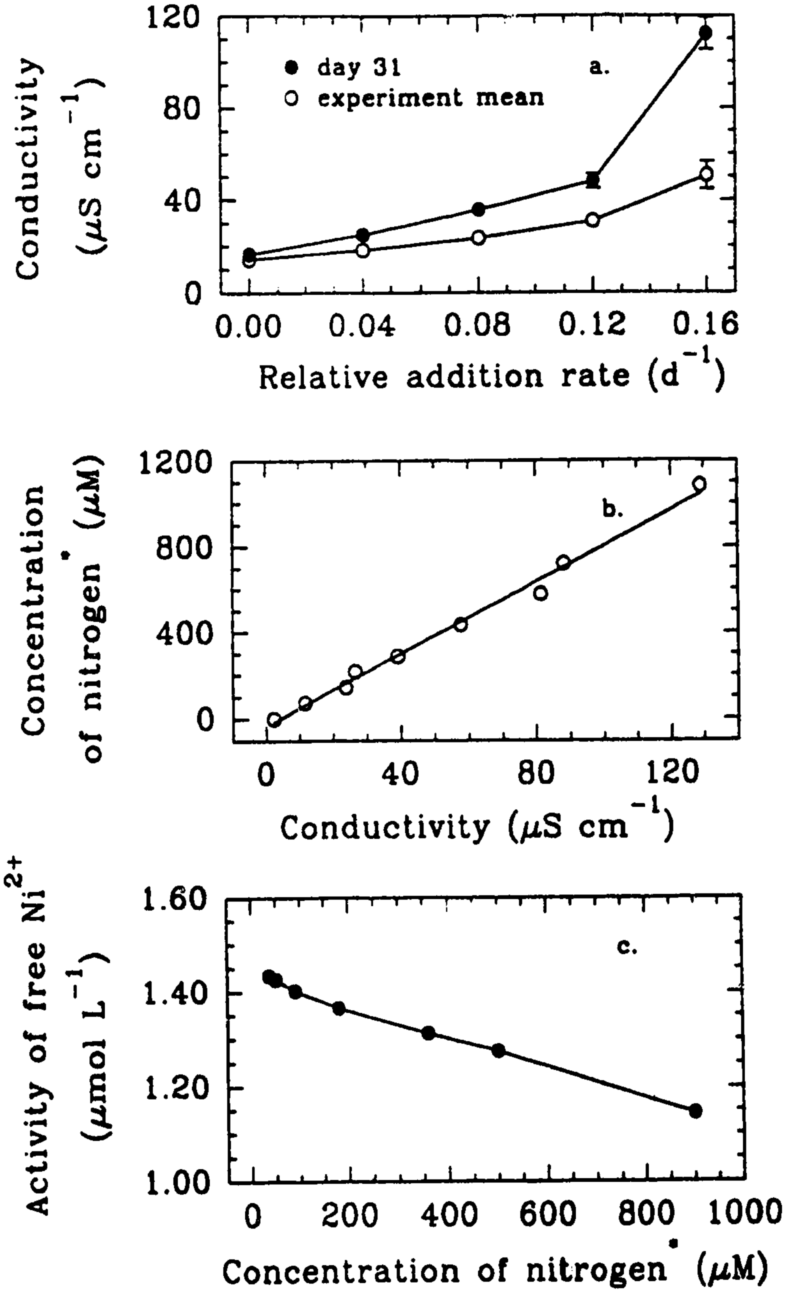
[a] Probability of obtaining the *z*-statistic (Zar 1986, see text) based on the null hypothesis that the parameters are equal; an asterisk (*) highlights a significant difference between the parameter pair ($P<0.05$).

minimum biomass are disregarded, the effects of nickel and competition at day 20 are adequately explained by a multiplicative model. Significant changes in the growth response parameter (*b*), but not the scale (*c*) or shape parameter (*d*), signify that growth, above the minimum biomass, at one level of nickel remained a constant proportion of growth, above the minimum biomass, at the other level of nickel. For RW, 1.50 μM nickel reduced the amount of growth response (parameter *b*) as might be expected. However, for PW and SW, the growth response was greater in the presence of nickel than in its absence.

Data for the harvest taken 31 days after germination indicate a different relationship. There were no significant differences between the minimum growth parameters at the two nickel levels for any of the strain variables (Table 3.9). For RW, the growth response parameter was decreased significantly in the presence of nickel; the scale (*c*) and shape (*d*) parameters were not significantly changed. A multiplicative relationship adequately explained the relationship between nickel and competition for RW. Nickel decreased the growth response (*b*) parameter and increased the scale parameter (*c*) for PW and SW. This change in scale indicated that nickel toxicity partially alleviated the relative effect of competition on shoot and whole-plant growth (see Figure 3.7a,c).

Nickel Speciation

Solution electrical conductivity increased with time in all but the lowest (0, 0.04 d^{-1}) RAR treatments. Conductivity was higher in the higher RAR treatments and in the presence of nickel. The most extreme differences in conductivity among the nickel treated solutions were on the last day of the experiment (day 31). However, the mean conductivity over the duration of the experiment is a better estimate of the solution conditions (Figure 3.8a). Nutrient composition and concentrations were obtained from these values using the empirical relationship shown in Figure 3.8b. Nitrogen concentration is shown, but this relationship was determined with all other nutrients present in the same proportion to nitrogen as in the addition solution (see Materials and Methods, Relative Addition Rate Experiments). The predicted activity of free nickel (Ni^{2+}), as calculated by GEOCHEM on the basis of these nutrient concentrations, is shown in Figure 3.8c. As RAR increased from 0 to 0.16 d^{-1}, increases in conductivity indicated that the average nutrient concentration in the solution over the experiment increased from 100 to 400 μM. In consequence, the



*Other nutrients present in proportion

Figure 3.8. Experiment 3.4. The effect of relative addition rate on the activity of free nickel in solution.

speciation calculations predicted that the activity of free nickel would be decreased from 1.4 to 1.3 μmol L^{-1}. This change was primarily due to the tendency of nickel to pair with EDTA at high nutrient concentrations. If the most extreme conductivity data (day 31) is considered, the change in free nickel activity would be from 1.4 to 1.1 μmol L^{-1}.

DISCUSSION

INTERPRETING THE RELATIONSHIP BETWEEN NUTRIENT COMPETITION AND NICKEL STRESS

Whole-Plant Effects

If stress caused by competition and stress caused by nickel toxicity are interpreted by their absolute effect on the growth of individual plants, nickel toxicity reduced the effect of nutrient competition. In all the experiments performed here, analysis of variance (ANOVA) of untransformed whole-plant (PW) data indicated a significant competition x nickel interaction. This interaction was such that the slope of the competition response curve (Experiments 3.1, 3.2, and 3.4) or the nickel response curve (Experiment 3.3) was less when nickel or competitive stress was more severe (Figures 3.3a, 3.4a, 3.6a, and 3.7a). The corollary is that competition also reduced the absolute effect of nickel (although this is not how the abiotic stress *vs.* competition question is usually viewed).

While it is clear that the effects of competition were reduced by nickel stress in an additive sense, the consensus among stress physiologists (Christensen *et al.* 1979, Lockhart 1965, McCarthy and Schroeder 1973, Taylor 1989, Taylor and Stadt 1990, Taylor *et al.* 1992) is that a multiplicative relationship best describes the effect of multiple stresses on growth. Two techniques for analysis using a multiplicative model, the weight-index transformation and the Weibull regression analysis, indicated little or no effect of nickel on competition, when the effects of these stresses were measured on *whole-plant* growth. When density was manipulated to induce different degrees of nutrient competition (Experiment 3.1), nickel had no effect on competition. In an experiment, where the daily relative addition rate (RAR) of nutrients was manipulated instead to mimic competition (Experiment 3.2), nickel stress caused a small reduction in the relative effect of competitive stress. When multiple levels of nickel stress were imposed on two RAR levels (Experiment 3.3), a small reduction in the relative effect of competitive stress with increasing nickel stress was indicated by the weight-index ANOVA, but not by the Weibull analysis.

Over time, the effect of nickel on competition appeared to change from a multiplicative relationship early on, to one where nickel stress reduced the relative effect of competition (Experiment 3.4). This was a complex change, however, since, early in the experiment, competitive stress had a *greater* absolute effect on plant growth (PW) when nickel was present than when it was absent. This peculiar result may have been due to the action of nickel toxicity on the utilization of seed and background solution nutrient reserves at extreme levels of competitive stress (see Results, Experiment 3.2). Toxic effects of this dose of nickel at all levels of competitive stress were manifest later in the experiment (day 31), presumably once tissues had accumulated more nickel. At this time nickel also caused a slight reduction in the relative effect of competitive stress.

These results generally support Grace and Tilman's (1990) suggestion that if the effects of abiotic stress on competition are measured relative to the productivity of the environment (*i.e.* amount of growth possible in the presence of the abiotic stress), the intensity of competition will be little affected by abiotic stress. Arguments about the intensity of competition in different environments can thus be largely reconciled by similar operational definitions of competition. It would appear that Grime (1977) was correct in claiming that the effect of competition decreases with increasing abiotic stress, but only because he interpreted the effects of stress on competition using an additive model. Welden and Slauson (1986) and Welden *et al.* (1988) argued that physiological approaches to studying the effects of abiotic stress on competition provide a poor reflection of the contribution of these stresses to plant fitness. They suggested that the importance of competition in community regulation should be measured by its impact relative to the total amount of biotic and abiotic stress in the environment (rather than the amount of growth possible, as in a multiplicative model). In environments subject to severe abiotic stress, this would make competition an unimportant stress, much as Grime (1977) predicted. While it is true that in such environments, tolerance of the abiotic conditions is of prime importance, competition may still be as important a factor controlling the distribution of the tolerant species as it is in less stressed environments. This is the main point of the multiplicative model: the absolute size of an effect, even relative to other effects, is not the sole factor indicating its importance. Rather, stress effects must be measured relative to the amount of growth possible under the conditions imposed by all other stresses.

Root/Shoot Partitioning

The effects of nickel were primarily on roots. In all experiments, mean root weight per plant (RW) was decreased relatively more by a given concentration of nickel than mean shoot weight per plant (SW). This is consistent with the view that the primary site of metal toxicity is in the roots (Taylor 1989), despite evidence that nickel is rapidly translocated and accumulates in above-ground tissues (Mishra and Kar 1974). In contrast, the effects of nutrient competition were manifest more in shoots than roots. When density provided the competitive stress, RW showed no significant response at densities below 7 plants per pail (Figure 3.3c). Increasing competitive stress was compensated for by a shift in root/shoot carbon partitioning, so that root size was preserved at the expense of the shoots. This shift in partitioning is an indication of nutrient stress, and suggests that competition was primarily for nutrient resources. Under RAR treatment, RW also declined relatively more than SW in response to increases in competitive stress (Experiments 2, 3, and 4; Figures 3.4, 3.6, and 3.7), but the differences were less marked. Since nickel had its primary effect on roots and nutrient competition on shoots, the interaction of these stresses may be best interpreted by whole-plant measurements.

THE EFFECT OF A NON-RESOURCE STRESS ON COMPETITION

Grace (1990) suggested that non-resource stresses may act differently from abiotic resource stresses and reduce the effect of competition on plant growth. If nickel toxicity simply reduced plant growth rate, the consequent reduction in resource use, and thus the strain induced by competition, could account for this effect. However, in these experiments, reductions in the relative effect of competition on whole-plant growth as a result of increased nickel stress were small or absent. The response patterns were closely described by a multiplicative model. Thus, nickel must have had an effect on the processes involved in nutrient competition. Three explanations are possible. The effect of competition on the nutrient concentration of the growth solution may have altered the phytotoxicity of the nickel provided, so that the apparent effect of competition was partially due to increasing nickel toxicity. Nickel toxicity may have increased the amount of nutrient uptake required for a given amount of growth, so that competition remained intense among the smaller nickel-treated plants. Nickel may also have altered the plants' ability to take up nutrients from the solution, requiring higher solution concentrations for a given amount of growth. The first two hypotheses were investigated.

The Effect of Competitive Stress on Solution Nutrient Concentrations and Nickel Toxicity

The toxicity of nickel is clearly dependent on the ionic conditions of the growth solution. A dose of 15 μM Ni was required in the density experiment to reduce plant size about 30%. When RAR was used to mimic competition, a similar growth reduction was obtained with about 1.5 μM Ni. In the RAR experiments, nutrient concentrations were kept low by frequent additions of the amount required for the day's growth. Since nutrients were added in a single large dose in the density experiments, nutrient concentrations would have been considerably higher, at least for the early part of the experiment. High ionic concentrations have been shown to alleviate metal toxicity. Johnson and Proctor (1981), for example, demonstrated that an increase in the concentration of calcium or micronutrients in the growth solution decreased the toxicity of nickel to *Festuca rubra*. Thus it is possible that, within an experiment, decreases in the solution nutrient concentration caused by increasing competitive stress could have increased the toxicity of nickel. This could have masked a greater effect of nickel on competition. A speciation analysis was carried out for this reason.

Speciation modeling of the growth solutions indicated that chemical interactions preceding uptake may have changed the toxicity of nickel over the range of competitive stress levels tested. If the mean conductivity values for the experiment are representative of speciation effects, the activity of free ionic nickel decreased 7% in Experiment 4 as RAR increased from 0 to 0.16 d^{-1} (Figure 3.8c). If the most extreme values were selected instead, the activity of free nickel ion declined 20% over the same RAR range. Most of this change was due to the tendency of nickel to pair with EDTA. DeKock and Mitchell (1957) showed that Ni-EDTA was much less toxic than Ni^{2+} in solution culture, so it is possible that this pairing decreased the toxicity of nickel at low levels of competitive stress (high RARs). It is also possible that a similar effect occurred in the density experiment. While conductivity was not measured in this experiment, the nutrient concentrations would have been depleted less quickly at low densities where there were fewer plants sharing the same nutrient supply. These higher solution nutrient concentrations may have resulted in decreased nickel toxicity.

Thus, a larger decrease in the relative effect of competitive stress due to increased nickel toxicity may have been masked by this decline in the activity of free nickel at lower levels of competitive stress. Furthermore, EDTA may not be taken up in proportion with the rest of the nutrients, as assumed in the speciation analysis. In dicots, iron is reduced and removed from the EDTA chelate before uptake (Marschner 1986). Thus, at high RARs, when ample nutrients are supplied, a lot more EDTA may be left in the solution to complex with nickel than would be expected if EDTA was taken up in proportion with the rest of the elements supplied in the nutrient addition solution. However, monocots appear to be able to take up iron as intact iron-phytochelate complexes (Marschner 1986). It is not clear yet whether synthetic chelates such as EDTA are taken up as well. Therefore, changes in the toxicity of a single *dose* (concentration) of nickel with changes in the competitive stress level may mean that 7% of the effect of a given *toxic level* (activity) of nickel on competition is masked, and that this masking may be greater yet, if EDTA is not taken up in proportion with the nutrients supplied. An increase in the activity of nickel, independent of the levels of competitive stress applied, may then decrease the relative effect of competition on plant growth substantially.

The Effect of Nickel on Plant Mineral Content

It is clear that nickel did affect the uptake of the macronutrients nitrogen, phosphorus, and potassium. Nickel-stressed plants accumulated higher nitrogen and phosphorus concentrations than non-stressed plants. Phosphorus content was higher at all but one intermediate competitive stress level, while nitrogen was only increased at high stress levels (low RARs). This increase in concentration was not simply due to luxury consumption of nutrients, since increasing the nutrient supply (increasing RAR) caused an increase in growth. Furthermore, phosphorus was supplied in low proportion compared to other nutrient solutions used for wheat (Ingestad and Stoy 1982) so is likely the most limiting nutrient. It appears that nickel increased plant phosphorus and possibly nitrogen requirements, thus maintaining the relative intensity of competition for phosphorus in the presence of this abiotic stress.

Plant nickel content was reduced by increases in RAR. This concurs with the speciation calculations which suggested that nickel toxicity was more severe at high levels of competitive stress (low RARs or high densities). However, although nickel content is inversely correlated with growth, the relationship is not linear (Taylor, unpublished data). The form of nickel in the plant (Cataldo *et al.* 1978), its compartmentation, and the physiological status of the plant also contribute to the toxic response. Taylor and Foy (1985) have also suggested that the primary site of metal injury may be at the plasma membrane, particularly the root membranes, so the correlation between the internal concentration of a metal with its toxicity may be spurious.

Changes in the chemical behavior of nickel and increases in whole-plant nitrogen and phosphorus content could both account for the maintenance of the relative intensity of competition when nickel toxicity was increased. These results are contrary to Grace's (1990) prediction that there should be a trade-off between the importance of competition and the importance of non-resource stress. However, the effects of nickel toxicity may be specific to nickel, and not to non-resource stresses in general. Grace's experiments (Grace 1987, 1988) suggest that this may be so. Deep water stress (caused mainly by low oxygen) was found to diminish the intensity of competition between *Typha domingensis* and *Typha latifolia*. Clearly more attention is required on the effects of non-resource stresses on competition to establish whether their effects are indeed stress-specific.

CONCLUSIONS

Theoretical criteria indicate that the effect of an abiotic stress on competition should be interpreted using a multiplicative model. When this model was applied, the effect of nickel toxicity on nutrient competition was quite different than that indicated by an interpretation based on additive effects. The additive model indicated that nickel always decreased the effects of competitive stress on growth. However, nickel did not change the relative effect of density-induced competitive stress. When the relative nutrient addition rate was used to mimic competition, nickel caused a small relative decrease in the effect of competitive stress on whole-plant growth in two of three experiments. The chemical behavior of nickel in the growth solutions as well as an increased plant phosphorus requirement could have accounted for the lack of change in the relative effects of competitive stress when nickel contaminated. If these specific effects of nickel are responsible for the observed relationship between nickel and competitive stress, generalizations about the effects of non-resource stresses on competition may not be possible. The effects of non-resource abiotic stresses on competition may need to be interpreted on a stress-by-stress basis.

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CHAPTER 4
GENERAL DISCUSSION

As studies of competition have progressed, the definition of competition has been refined (Grime 1977, Harper 1961, Welden and Slauson 1986, Grace 1990). It has been recently suggested that the ambiguity about the role of competition along gradients of abiotic stress has been chiefly caused by differences in the operational definition of competition used, and that careful attention to the methods used to calculate and interpret the effects of competitive stress might go a long way to clarifying the issue (Grace 1990). In this vein, the work presented here applied the conventions of stress physiology to interpret a series of abiotic stress *vs.* competition experiments. In addition, a new method, relative addition rate, was tested for conducting competition experiments in solution culture.

Relative Addition Rate vs. *Density*

The relative addition rate (RAR) technique provided an effective method for mimicking nutrient competition in this study (Chapter 2). Although the control of plant relative growth rate (RGR) by RAR was not the 1:1 relationship obtained by others (Ericsson 1981, Ingestad and Lund 1979, Jia and Ingestad 1984), a strong response (0.55:1) was obtained. Plants could be grown at pre-determined and nearly constant levels of nutrient stress for the duration of the experiment. Solution nutrient concentrations were kept at very low levels, representative of those found in soil pore water (Reisenauer 1966). Furthermore, lower concentrations of nickel were required to produce a toxic response when the RAR technique was used (Chapter 3). These low concentrations were close to the concentrations found in the pore water of a serpentine soil known to be toxic to oats and native species (Johnson and Proctor 1981). Different levels of competitive nutrient stress and nickel stress could thus be more realistically imposed on plants by changing the RAR rather than the density. Despite these differences, the whole-plant response to RAR-induced competitive stress was similar to the response to density-induced stress. The effect of nickel on competitive stress was also similar when RAR or density provided the latter stress. Apparently the method of inducing competition had little effect on the results.

Since RAR- or density-induced competition experiments did not yield substantially different results, it would not be enlightening to test a hybrid of the two methods to study the effect of nickel stress on competition. However, it might be desirable to develop a hybrid method for testing other competitive-abiotic stress interactions to more closely mimic competition in field conditions. Nutrient supply models such as that of Hoffland *et al.* (1990) could be substituted for the exponential model used in the RAR experiments to cause nutrient stress to increase with time (see Freijsen *et al.* 1990). Empirical studies of the rate of nutrient supply to plants over the growing season in conditions where competition is important would also be helpful. Improvements to the RAR technique itself could be achieved by removing the seeds from the plants early in an experiment, to reduce uncontrolled sources of nutrients. This would improve the control of RGR at low RARs substantially. If fluctuations in pH could be reduced, experiments could also be continued for a longer period, so that seed effects would become trivial.

A Stress Interaction Model for Competition vs. *Abiotic Stress Studies*

The importance of choosing an appropriate stress interaction model was clearly illustrated by this study. A different view of how an abiotic stress affects competition emerged when a multiplicative model was applied and competitive stress measured by its relative effect on growth compared to when an additive model was assumed and competitive stress measured by its absolute effect. The experiments performed in Chapter 3 indicated that the relative intensity of competition at different levels of nickel stress was not greatly changed. In two experiments, where a restricted relative addition rate (RAR) of nutrients was used to impose a range of nutrient competition levels, there was a small decrease in the relative effect of competition on whole-plant growth when the level of nickel stress was increased. Nickel had no effect on competition in another experiment using the RAR technique to mimic two levels of competition on a full range of nickel stress levels, and in one experiment where nutrient competition was induced by different densities. However, when competition was measured by its absolute effect, nickel toxicity decreased its intensity in all experiments.

This concurs with Grace and Tilman's (1990) suggestion that, if competition is measured relative to the productivity of the environment, its effects will not be greatly changed by changes in the abiotic environment. Grime's (1977) hypothesis that competition is unimportant in stressed environments is consistent with an interpretation based on the additive model. Tilman's (1987) assertion that competition is always important is supported by the same data, interpreted by the multiplicative model. However, *a priori* considerations indicate that the multiplicative model is the appropriate standard for this comparison. Lockhart's (1965) review of stress interactions indicated that the multiplicative model accounts for the majority of multiple physical and chemical stress effects. High levels of multiple stresses lead to improbable predictions if the additive model is applied. Furthermore, the mechanisms by which abiotic resource stresses and competitive stress take effect are likely to be sequential, and thus have multiplicative effects on growth (Berry and Wallace 1981). Clearly, these properties show that an interpretation of abiotic stress *vs.* competition interaction should be based on the multiplicative model.

In a plant community, establishment, growth, survival, and fecundity are controlled by competition, other biotic factors (predation, disease), and the abiotic environment. Grime (1977) suggested that competition is unimportant when abiotic stress is high, because competitive strain is less in absolute terms in these environments. Welden and Slauson (1986) recommended that the importance of competition be determined by measuring the growth reduction due to competition relative to the total growth reduction from optimum in a particular environment. However, this measure leads to the same prediction as Grime (1977). In a severe abiotic environment, the contribution of competition to the total growth *reduction* from the optimum condition for the plant would be small. In a better environment, competition has the potential to contribute more to the total reduction, and therefore have more "importance." The argument advanced here is that competition can be equally important even if it has a smaller absolute effect. Studies of competition in severe environments (Inouye *et al.* 1980, del Moral 1983) indicate that competition does have an effect on community structure. In other studies (*e.g.* Gurevitch 1986), transformation of growth response variables to a relative scale may indicate similar effects of competition at high abiotic stress levels. The role of competition in self-thinning and survivorship experiments (*e.g.* Gause 1934, Yoda *et al.* 1963, Mahmoud and Grime 1976) is more difficult to determine in relative terms. A detailed study of self-thinning at different levels of abiotic stress would clarify whether self-thinning proceeds faster in fertile soils because the plants are larger, or because the plants grown in the fertile conditions reach a larger biomass at a given density.

The Effect of Non-Resource Stresses on Competition

Although Grace (1990) suggested that non-resource abiotic stresses may alleviate the effects of competition, little reduction in the relative effect of competitive nutrient stress due to increased nickel stress was found here. Speciation modelling suggested that one reason for the near constant effect of nutrient competition at different levels of nickel may be due to an increase in the toxicity of the nickel dose at higher densities or lower RARs. Rapid uptake of nutrients at high levels of competitive stress may have reduced the formation of the relatively nontoxic nickel-EDTA complex, increasing the activity or effective concentration of free nickel. Mineral analysis indicated that whole plant nitrogen and phosphorus concentrations were increased in the presence of nickel stress. Since phosphorus was proportionately low in the nutrient solutions, it is possible that an increased requirement for phosphorus maintained the importance of competition among plants whose growth was reduced by high nickel levels. Since these speciation and uptake effects are unique to nickel stress, the observed effects of nickel on competition cannot be generalized to all non-resource stresses. Further studies of the effects of other toxic stresses, chilling, and heat stress on competition are required to establish trends among non-resource abiotic stresses.

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