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**REGULATION OF NITROGEN UPTAKE AND ASSIMILATION: EFFECTS OF
NITROGEN SOURCE AND ROOT-ZONE AND AERIAL ENVIRONMENT ON
GROWTH AND PRODUCTIVITY OF SOYBEAN**

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CASE

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REGULATION OF NITROGEN UPTAKE AND ASSIMILATION

INTERDEPENDENCE OF ROOT AND SHOOT ACTIVITIES IN CARBON AND NITROGEN ACQUISITION

The interdependence of root and shoot growth produces a functional equilibrium that has been described in quantitative terms by a number of authors. BROUWER and DE WIT (1969) noted that bean seedlings grown in a constant environment tended to have a constant pattern of distribution of dry matter between roots and leaves that is characteristic of the set of environmental conditions. Disturbing the equilibrium by partial root excision or defoliation resulted in a change in relative growth of roots and leaves until the original ratio between roots and leaves was restored. They modeled this behavior by assuming that leaves and roots compete for carbohydrates and nitrogen and that the organ nearest to the source will be the most successful in obtaining its requirements. This simple assumption of carbon and nitrogen partitioning based on utilization priority, however, does not explain by itself frequent observations of declines in composition of nitrogen and the root-to-shoot ratio as plants age even when external supply of nitrogen is not growth limiting. THORNLEY (1976), on the other hand, developed a two-compartment, two-substrate model with root and shoot as the two compartments and nitrogen and carbon provided by roots and shoot as the two substrates. Accumulation of structural material (growth) by roots and shoot was assumed to depend on the presence of both substrates in the compartment. Rate of transport of each substrate between roots and shoot was assumed to be proportional to the difference in concentration of each substrate between compartments. While this partitioning scheme accounts for transport of carbon and nitrogen in response to differential growth and respiration of roots and shoot, it does not identify mechanisms for the mutual regulation of nitrogen acquisition by roots and carbon acquisition by the shoot.

To define a physiological basis for regulation of nitrogen uptake within the framework of a functional balance between root and shoot activities, we (LIM et al. 1990; RAPER et al. 1977, 1978; WANN and RAPER 1984; and WANN et al. 1978) combined the partitioning scheme of THORNLEY (1976) with the utilization priority assumption of BROUWER and DE WIT (1969). There are several critical assumptions and conditions for our combined scheme. (1) All carbon enters the plant through the photosynthetic process in leaves and all nitrogen enters the plant through active uptake by roots. (2) Nitrogen uptake by roots and secretion into the xylem for transport to the shoot are active processes (CLARKSON, 1986, 1993; CLARKSON and HANSON 1986; HANSON 1978) that require carbohydrates translocated from the shoot as an energy source. (3) Availability of exogenous nitrogen, by limiting proliferation of new roots (Drew 1975), determines concentration of soluble carbohydrates within the roots. When an exogenous source of nitrogen is available in nonlimiting amounts for uptake by roots, soluble carbohydrate arriving in roots is utilized rapidly for support of new root growth and proliferation rather than accumulated within a storage pool (GRANATO and RAPER 1989; GRANATO et al. 1989); thus, the concentration of soluble carbohydrates in roots remains relatively small in

the presence of nonlimiting exogenous nitrogen and increases when proliferative growth is restricted by limiting concentrations of exogenous nitrogen (CHAILLOU et al. 1994; HENRY and RAPER 1991). This condition defines a differential concentration gradient for soluble carbohydrates that favors translocation from shoot to root and indicates that for noninterrupted uptake of nitrogen roots depend on, and essentially compete with proliferative growth for, concurrent transport of soluble carbohydrates from the shoot. (4) Leaves are both a source and a sink for carbohydrate from the shoot pool and for a time during initiation and early expansion are net sinks for carbohydrate (TURGEON 1989). (5) The requirement for nitrogen by leaf growth is proportionally greater during initiation and early expansion (i.e. cell division) than during later expansion (i.e. cell elongation) and a decrease in nitrogen arrival to nitrogen-replete shoots restricts the rate of initiation and expansion of new leaves before it affects the photosynthetic rate of existing leaves (HENRY and RAPER 1991; TOLLEY-HENRY and RAPER 1986a, b).

Given these conditions and assumptions, a long-term pattern can be predicted of cyclic variations in carbohydrate availability for transport to roots of nitrogen replete plants with a nonlimiting supply of exogenous nitrogen. Beginning with a steady-state rate of photosynthesis per unit leaf area, fixed carbon would enter the nonstructural carbohydrate pool of the shoot at a rate proportional to leaf area. Carbohydrate in the shoot pool would be utilized as the source of carbon and energy for structural growth, growth respiration, and maintenance respiration. The nonstructural carbohydrate remaining in the shoot pool would be available for translocation to roots with the rate of translocation from shoot to root dependent on the concentration of carbohydrate in the shoot pool and the size of the carbohydrate pool and the metabolic activity of the roots. As a newly initiated leaf begins to emerge, for a time it would be a net sink rather than a net source of photoassimilates. As a consequence shoot utilization of carbohydrates from the shoot pool would increase, and the availability of carbohydrates for translocation to roots would decrease until the emerging leaf becomes a net contributor of photoassimilates to the shoot pool. A nonlimiting supply of exogenous nitrogen, by promoting proliferation of the roots, would assure continued meristematic activity and maximum respiratory utilization of carbohydrates within the roots (GRANATO and RAPER 1989; GRANATO et al. 1989). Flux of soluble carbohydrates from shoot to root thus would be proportional to the concentration of soluble carbohydrates in the shoot and should cycle in response to the successive periods of leaves as net sinks during emergence and net sources following expansion.

DOES CARBOHYDRATE FLUX FROM SHOOT TO ROOTS REGULATE NITROGEN UPTAKE?

There is a synchronization between oscillations in net rates of nitrogen uptake by roots (CHAILLOU et al. 1994; RAPER et al. 1991; SARAVITZ and RAPER 1994; TOLLEY and RAPER 1985; TOLLEY-HENRY et al. 1988) and intervals between emergence of successive leaves of nitrogen-replete soybean (HENRY and RAPER 1989a). Occurrences of minima in the oscillations of net rate of nitrogen correspond to the time of emergence

and early expansion of a leaf (or a group of leaves) when the projected flux of carbohydrate from shoot to roots would be low, and occurrence of maxima correspond to ca. the midpoint in expansion of the leaf immediately prior to emergence of the next leaf (or group of leaves) when the projected flux of carbohydrates to roots would be high. These observations suggest a possible regulatory function of the flux of carbohydrate from shoot to roots in net uptake of nitrogen.

It has been proposed that the shoot-mediated control of nitrogen uptake is exerted through the cycling of amino acids that occurs between shoot and roots (COOPER and CLARKSON 1989; IMSANDE and TOURAINÉ 1994). A majority of the nitrogen contained in the xylem of pea, barley, wheat, and rye plants originates from amino acids that have been translocated from shoot to roots in the phloem and are being returned to the shoot via the xylem (AGRELL et al. 1994; COOPER and CLARKSON 1989; DUARTE and LARSSON 1993; LARSSON et al. 1991). As nitrogen in shoot tissues declines to growth-limiting concentrations, cycling amino acids from shoot to roots and back declines (AGRELL et al. 1994; DUARTE and LARSSON 1993). Since a number of amino acids introduced into soybean roots either through the exogenous nutrient solution or through the phloem from cotyledons immersed in solutions containing amino acids can inhibit uptake of NO_3^- (MULLER and TOURAINÉ 1992), the decreasing concentration of amino acids in the phloem as shoot growth becomes nitrogen-limited could serve to activate the desuppression of nitrogen uptake that is observed when nitrogen is resupplied to roots of nitrogen-limited maize plants (IVANKO and INGVERSEN 1971; JACKSON and VOLK 1992; LEE et al. 1992). The certainty of such a mechanism, however, is lessened when nitrogen is supplied heterogeneously to a split-root system. When 80% of a growth-limiting NO_3^- supply was added to one half of a split-root system of barley and 20% was added to the other half, amino acid cycling from the shoot to each of the half-root systems was proportional to the external supply of nitrogen (AGRELL et al. 1994). If uptake is inversely related to amino acid translocation in phloem, one thus would expect the rate of nitrogen uptake to decline in portions of roots that are exposed to higher concentrations of exogenous nitrogen. The reverse, however, is the case. When a nonlimiting NO_3^- supply was discontinued in one-half of a maize split-root system, the net rate of NO_3^- uptake increased ca. 2-fold in the half that continued to receive a nonlimiting exogenous supply of NO_3^- (GRANATO and RAPER 1989). Based on growth and respiration rates of the two halves of the root system, however, flux of carbohydrate also increased ca. 2-fold to the half that continued to receive NO_3^- (GRANATO and RAPER 1989; GRANATO et al. 1989). These results would seem to support the concept that regulation by the shoot of nitrogen uptake is effected by carbohydrate flux, and not by amino acid recycling, from shoot to roots.

SITE OF REGULATION OF NITROGEN UPTAKE BY CARBOHYDRATE FLUX

The mechanism by which the flux of carbohydrate from the shoot may regulate nitrogen uptake is indistinct. Certainly, nonstructural carbohydrates in epidermal and cortical cells are necessary as an energy source for movement of nitrogen into the root symplasm from

the external solution. Changes in net uptake rates, however, are not correlated well with changes in concentrations of soluble carbohydrates in roots (RIDEOUT and RAPER 1994a), and it thus seems unlikely that as long as soluble carbohydrates in roots remain above some threshold concentration the long-term variations in uptake are a direct consequence of variations in carbohydrate availability in the epidermal and cortical cells. It seems more probable that changes in the availability of carbohydrate to the xylem parenchyma cells are related to the long-term pattern of variations in net rates of nitrogen uptake. Xylem parenchyma cells, which are connected to endodermal and cortical cells by plasmodesmata (LÄUCHLI et al. 1974), are involved in the active transfer of ions from the root symplasm into the xylem for transport to the shoot (CLARKSON 1993; LÄUCHLI 1976), and also are proximal to the phloem carrying carbohydrates from the shoot. The large cytoplasmic volume and high density of mitochondria in xylem parenchyma cells compared to epidermal and cortical cells (LÄUCHLI et al. 1974) are suggestive of a high metabolic activity, which would seem necessary for the relative increase in active flux of ions across the inner-wall plasmalemma as the ions absorbed into the much larger volume of epidermal and outer cortical cells are condensed into the xylem parenchyma cells for transfer into the xylem. Accordingly, short-term changes in concentration of carbohydrate in the phloem possibly would exert a more immediate effect as a source of metabolic energy in the transfer of nitrogen from the more proximate xylem parenchyma cells than they would on absorption of exogenous nitrogen into the more distal epidermal and cortical cells.

To account for the apparent association of carbohydrate flux to the roots and rate of acquisition of nitrogen by the plant, we propose that the long-term pattern of nitrogen uptake, i.e. the oscillations between maxima and minima in net rates of nitrogen uptake, results from separate regulation of active transport sites for entry of nitrogen from the external solution into the root symplasm and for transfer of nitrogen out of the symplasm into the xylem. It is reasonable to assume that transport of NO_3^- into the symplasm from the external solution involves multiple mechanisms that are dependent on external NO_3^- concentration (BRETELER and NISSEN 1982, HALLMARK and HUFFAKER 1978, RAO and RAINS 1976) and that influx of NO_3^- can approach a constant at a given external concentration of NO_3^- . Transport of nitrogen from the symplasm into the xylem likely is restricted by variations in translocation via the phloem of a substrate from the shoot (CLARKSON 1993). If, as we propose, this regulatory substrate is carbohydrate, then it also is reasonable to assume (1) that the capacity for rate of xylem loading of nitrogen is variable and contingent on shoot activity and (2) that net entry of nitrogen into the root symplasm restricts xylem loading only when symplastic nitrogen falls below an availability necessary to support the maximum shoot-mediated rate of xylem loading.

Consider the consequences of these assumptions. When solution NO_3^- is maintained at the concentration that facilitates net absorption into the root symplasm at a rate equal to the greatest rate of xylem loading, the symplastic pool of nitrogen can satiate this capacity during those intervals in which delivery of carbohydrate via the phloem facilitates the maximum rate of xylem loading. However, during intervals when delivery of carbohydrate facilitates less than the maximum rate for xylem loading, accumulation of

NO_3^- or its assimilation products within the root symplasm would decrease net uptake rate of NO_3^- by some combination of inhibition of influx (JACKSON and VOLK 1992, KING et al. 1993, LEE and RUDGE 1986) and enhanced efflux (BRETELER and NISSEN 1982, DEANE-DRUMMOND and GLASS 1983, JACKSON et al. 1976). The greater the restriction in xylem loading becomes, the greater will be the decrease in net uptake rate of NO_3^- . Thus, while net uptake rates will oscillate, cumulative export of nitrogen to the shoot will reflect cumulative delivery of carbohydrates to roots. In contrast, as NO_3^- in the nutrient solution is increased above the concentration that supports net absorption into the symplasm at rates equal to the shoot-mediated maximum capacity xylem loading, amplitudes of the oscillations in net uptake rate of NO_3^- should increase but without a change in accumulation of nitrogen in the shoot.

Experimental evidence supports this proposal. As concentrations of NO_3^- in the external nutrient solution were increased from 50 μM to 10 mM, the amplitude of oscillations in daily net rate of NO_3^- uptake of soybean progressively increased (SARAVITZ and RAPER 1994; TOLLEY-HENRY et al. 1988) without a detectable change in plant growth rate during a 3-week period of vegetative development. The increases in amplitude of oscillations with increases in solution concentration of NO_3^- from 50 to 100 μM and from 100 to 500 μM were accompanied by increases in total accumulation of nitrogen by the plants during the 3-week period; however, further increases in solution concentration from 500 to 1000 μM and from 1000 μM to 10 mM resulted in no further increase in total accumulation of nitrogen. The restricted acquisition of nitrogen by plants at the two lower solution concentrations can be attributed to an inability of plants at these exogenous concentrations to maintain nitrogen in the root symplasm at concentrations equal to or above the maximum capacity for xylem loading; in other words, the limiting mechanism in NO_3^- uptake was concentration-dependent transport into the root symplasm. The greatly increased amplitudes of oscillation in net rates of uptake, with no further increase in cumulative uptake, at external concentrations of 500 μM and above can be attributed to a shoot-mediated restriction in the capacity for xylem loading.

From this model, it would appear that some "futile-cycling" of NO_3^- into and out of roots is an inevitable consequence for the plant to accumulate nitrogen in non-growth-limiting amounts. If the external concentration of NO_3^- is sufficient for entry into the root symplasm at a rate equal to the greatest rate of transport into the xylem in response to delivery of phloem-transported substrate, then as a change in delivery of phloem-transported substrate restricts loading into the xylem enhanced efflux of NO_3^- would occur with its accretion in the symplasm. As external NO_3^- is decreased below the concentration that sustains uptake at a rate equal to the greatest shoot-mediated rate of transport into the xylem, a slower accumulation of NO_3^- in the symplasm during intervals of restricted xylem loading would constrain efflux but at the expense of an abatement in the cumulative uptake of nitrogen by the plant.

IS UPTAKE OF NO_3^- and NH_4^+ SUBJECT TO COMMON REGULATION?

The preceding discussion was based on responses of nitrogen-replete plants. Nitrogen-replete plants that have been pretreated with NO_3^- are able to utilize either NO_3^- or NH_4^+ as a nitrogen source with equal effectiveness when acidity of the nutrient solution is controlled near pH 6.0 (CHAILLOU et al. 1991; PEET et al. 1985; RAPER et al. 1991; RUFFY et al. 1983; TOLLEY-HENRY and RAPER 1986c; VESSEY et al. 1990). Moreover, in spite of the different pathways and locations of NO_3^- or NH_4^+ assimilation, similar oscillations in net rates of uptake occur for both ions (HENRY and RAPER 1989a; RAPER et al. 1991). It thus appears that at the whole-plant level regulation of nitrogen acquisition from both sources is subject to a common mechanism. On the other hand, entry of NO_3^- and NH_4^+ into the root symplasm is facilitated by separate transporters (GOYAL and HUFFAKER 1986), and the proportionality of net uptake of NO_3^- and NH_4^+ from mixed sources varies from day to day (RAPER et al. 1991) and is differentially influenced by ambient pH (VESSEY et al. 1990) and temperature (MACDUFF and WILD 1989) of the nutrient solution as well as by changes in nitrogen content or composition (MACDUFF and WILD 1989; MORGAN and JACKSON 1988) and carbohydrate or energy availability (CHAMPIGNY and TALOUZTE 1986) within roots. A common mechanism regulating uptake of NO_3^- and NH_4^+ thus would not seem to affect entry of the ions into the root symplasm; more likely, it would affect movement of nitrogen from the symplasm into the xylem. Responses in uptake of NO_3^- and NH_4^+ by nitrogen-depleted plants support this supposition.

Based on short-term uptake studies, the capacity of roots to absorb both NO_3^- and NH_4^+ increases as carbohydrate availability in roots increases (TALOUZTE et al. 1984) and concentrations of reduced and NO_3^- nitrogen decrease (CHAMPIGNY and TALOUZTE 1986; HENRY and RAPER 1991; LEE et al. 1992; LEE and RUDGE 1986; MORGAN and JACKSON 1988). For nitrogen-depleted soybean plants, which were supplied with 1.0 mM NO_3^- in hydroponic culture for the initial 2 weeks after germination and then supplied for the next 2 weeks with suboptimal applications of 200 $\mu\text{mol NO}_3^- \text{ d}^{-1}$ per plant, concentrations of soluble carbohydrates in roots increased more than 2-fold, concentrations of soluble amino acids decreased by 50%, concentrations of NO_3^- declined to barely detectable levels, and concentrations of free NH_4^+ decreased by 40% compared to nitrogen-replete plants that continued to receive 1.0 mM NO_3^- (RIDEOUT et al. 1994). Rather than net uptake of NO_3^- and NH_4^+ increasing when these nitrogen-depleted plants were resupplied with 1.0 mM nitrogen as NO_3^- alone, NO_3^- and NH_4^+ combined, or NH_4^+ alone, daily net rates of uptake per g root dry weight (specific net rates of uptake) were lower for nitrogen-depleted than for nitrogen-replete plants by 43% for NO_3^- resupply, 32% for NO_3^- and NH_4^+ resupply, and 83% for NH_4^+ resupply. Similarly, when NO_3^- was resupplied to one-half of a split-root system of nitrogen-depleted soybean and NH_4^+ was resupplied to the other half, daily specific net rates of uptake were considerably lower for the NH_4^+ -resupplied portion of the root system than for the NO_3^- -resupplied portion (SARAVITZ et al. 1994). In both of these experiments, however, monitoring of specific net rates of uptake at hourly intervals revealed intermittent hourly intervals of net influx (depletion of the exogenous solution) and net efflux (enrichment of the exogenous solution). Net rates

of uptake during the influx intervals were greater for the NH_4^+ -resupplied roots than for the NO_3^- -resupplied roots, but for the NH_4^+ -resupplied roots the intervals of efflux were more numerous than for the NO_3^- -resupplied roots. These results indicate that entry of nitrogen, and particularly of NH_4^+ , into the root symplasm was less restricted than translocation of nitrogen from the symplasm into the xylem.

DOES INHIBITION OF NH_4^+ UPTAKE INVOLVE RESTRICTED ENTRY OF NH_4^+ INTO ROOTS?

Net NH_4^+ uptake during short-term experiments involves both inward and outward movement across the plasmalemma of root cells (JACKSON et al. 1993; MORGAN and JACKSON 1988). If NH_4^+ enters the root symplasm but amino acids are not loaded into the xylem for translocation to the shoot, feed-back effects from accumulation of free NH_4^+ and/or amino acids could account for the limited net uptake of exogenous NH_4^+ by enhancing efflux of endogenous NH_4^+ from the root. During the initial 3 days of nitrogen resupply following a 2-week period of nitrogen-depletion, the frequency of hourly intervals of solution enrichment (i.e. net efflux) for NH_4^+ was about twice that for NO_3^- whether NH_4^+ was resupplied alone to either whole root systems or halves of split-root systems or was supplied in combination with NO_3^- to whole root systems (SARAVITZ et al. 1994). Together with the increased concentrations of free NH_4^+ during these first 3 days after resupply, this apparent enhancement in efflux of NH_4^+ , whether the effluxed NH_4^+ represented turnaround of nonassimilated NH_4^+ or NH_4^+ endogenously regenerated following assimilation, suggests the possibility that the restriction in net uptake of NH_4^+ by nitrogen-depleted plants involves a stimulation of efflux rather than a inhibition of influx (JACKSON et al. 1993). In turn, this suggests that inhibition of NH_4^+ uptake by nitrogen-depleted plants may involve restricted transfer of amino acids from the root symplasm into the xylem rather than restricted entry of NH_4^+ into the symplasm from the external solution.

The possible involvement of enhanced efflux in long-term regulation of NH_4^+ uptake by nitrogen-depleted plants, however, is contrary to the conclusion of LEE (1993) that uptake of NH_4^+ , as well as of NO_3^- , is controlled primarily by changes in influx. Uptake rates were determined by LEE (1993) only at the beginning of the seventh hour following resupply of nitrogen to deficient barley plants. Our observations (RIDEOUT et al. 1994; SARAVITZ et al. 1994), which extended for 3 days following resupply, indicate that the frequency of hourly periods of net efflux increased beyond the first 4 to 6 hours of resupply.

DOES NO_3^- REGULATE NH_4^+ UPTAKE IN NITROGEN-DEPLETED PLANTS?

Regardless of the mechanism of inhibition of NH_4^+ uptake, it appears that the capacity for roots of nitrogen-depleted plants to utilize NH_4^+ as a nitrogen source is enhanced by the availability of exogenous NO_3^- (RIDEOUT et al. 1994; SARAVITZ et al. 1994). A split-

root experiment was used to determine whether the effect of NO_3^- on facilitating uptake of NH_4^+ occurs within the root or whether it is integrated through NO_3^- translocated to and assimilated within the shoot (SARAVITZ et al. 1994). When NO_3^- and NH_4^+ are supplied to separate halves of a split-root system of nitrogen-replete soybean, the specific uptake rate of NH_4^+ is equal to or exceeds that of NO_3^- , although growth of the NH_4^+ -fed roots is less than that of the NO_3^- -fed roots (CHAILLOU et al. 1994). The uptake of NH_4^+ when resupplied to half of the split-root system of nitrogen-depleted soybean, however, was restricted relative to uptake of NO_3^- by the other half of the root system, especially during the last 4 days of the resupply period as both specific uptake rate and mass of the NH_4^+ -resupplied portion declined in comparison to the NO_3^- -resupplied portion. During these last 4 days, photosynthetic capacity of the shoot for the split-root plants had recovered to an extent comparable to that of plants resupplied with NO_3^- plus NH_4^+ and to an extent greater than that of NH_4^+ -resupplied plants. These results suggest that the effect of NO_3^- on enhancing NH_4^+ uptake, as occurred for whole root systems resupplied with NH_4^+ plus NO_3^- , was not expressed through enhanced availability of either photosynthates or assimilated nitrogenous compounds from the common shoot of the split-root system. Furthermore, since NO_3^- can be translocated through the phloem only in negligible quantities (HOCKING 1980) and NO_3^- did not appear in detectable quantities in the NH_4^+ -resupplied half of the split-root system (SARAVITZ et al. 1994), NO_3^- presumably was unavailable to the NH_4^+ -resupplied portion of the root. For nitrogen-depleted soybean, the role of NO_3^- in enhancing NH_4^+ uptake thus appears to be confined to availability of NO_3^- itself or to a product of NO_3^- reduction and assimilation within the absorbing root. The latter possibility seems unlikely. Only a small proportion of absorbed NO_3^- usually is reduced in roots of soybean (RUFTY et al. 1982), and the expected products of NO_3^- reduction and assimilation, i.e. amino acids and organic acids, in the NH_4^+ -resupplied portion of the roots were either higher than or comparable to concentrations in the NO_3^- -resupplied portion of split-root systems and in the whole root systems resupplied with NH_4^+ plus NO_3^- .

REGULATION OF NITROGEN UPTAKE DURING REPRODUCTIVE DEVELOPMENT

Seasonal profiles of nitrogen accumulation and NO_3^- uptake by soybean under field conditions indicate that rates of nitrogen acquisition decline during reproductive development (THIBODEAU and JAWORSKI 1975). Declines in net photosynthetic rates of leaves during seed fill frequently are associated with the declines in nitrogen acquisition (BOON-LONG et al. 1983; SESAY and SHIBLES 1980). THIBODEAU and JAWORSKI (1975) and SINCLAIR and DE WIT (1976) therefore have proposed that, as partitioning of carbohydrate is shifted from the support of root activity to fruit growth, nitrogen acquisition by roots also declines. In this so-called "self-destruction" hypothesis, the nitrogen requirement for fruit growth is presumed to be derived increasingly from remobilization of nitrogen out of leaves, further decreasing photosynthetic rates and accelerating the decline in rates of nitrogen acquisition. This relationship between limited

carbohydrate availability and nitrogen acquisition as a general characteristic during reproductive development of soybean also presumes that the photosynthetic capacity of leaves is exceeded by the requirement for carbohydrate during seed growth (SINCLAIR and DE WIT 1976) or is diminished by a senescence signal from the developing fruit (NOODÉN and GUIAMÉT 1989).

In field experiments soybean is subject to a number of environmental and biotic stresses throughout growth and development. Conversely, when grown under controlled-environment conditions and when exogenous nitrogen supply is not limiting to growth, external stresses can be avoided to enhance both rate of morphological development and the efficiency of carbon and nitrogen partitioning into reproductive growth. Under hydroponic culture in growth rooms a greater proportion of the total dry matter in the shoots (60%) is contained in the fruits than occurs under field culture (50%), maximum seed dry weight occurs within less than 8 weeks instead of more than 15 weeks, and productivity is ca. 20 g reproductive dry weight $m^{-2} d^{-1}$ compared to ca. 3.5 g $m^{-2} d^{-1}$ (cf. SARAVITZ et al. 1994 and VESSEY et al. 1990 with PATTERSON 1983). During reproductive development of soybean in the absence of external stresses, nitrogen accumulation from both N_2 -fixation (CURE et al. 1985) and NO_3^- sources (HENRY et al. 1992; SARAVITZ et al. 1994; VESSEY et al. 1990) does not decline, photosynthetic rates of leaves remain constant (HENRY et al. 1992; SARAVITZ et al. 1994), and concentrations of nonstructural carbohydrates in leaves and roots increase for both nodulated (CURE et al. 1985) and nonnodulated (HENRY et al. 1992; VESSEY et al. 1990) plants. In fact, when the supply of exogenous nitrogen was discontinued during seed fill of nonnodulated soybeans, the content of nonstructural carbohydrate in root, shoot and fruit tissue initially increased (HENRY et al. 1992); and when all or half of the pods were removed, photosynthetic rates of leaves declined in comparison to the constant rates for plants with a full pod load (SARAVITZ et al. 1994). These data indicate that carbohydrate is not limiting for growth of nitrogen-replete plants, and thus are inconsistent with the notions of the "self-destruction" hypothesis that (1) photosynthetic capacity of soybean is inherently inadequate for support of both seed growth and root functioning and that (2) remobilization of reduced nitrogen out of leaves to meet requirements of reproductive growth occurs to an extent that impairs photosynthetic activity of the leaves.

During reproductive growth of soybean, as occurs during vegetative growth, the net uptake rate of NO_3^- oscillates between maxima and minima (SARAVITZ et al. 1994; VESSEY et al. 1990). During vegetative growth, these oscillations appear to be associated with changes in flux of soluble carbohydrates from shoot to root in response to the successive periods of leaves as net sinks during emergence and net sources following expansion. If the oscillations in net uptake rate of NO_3^- during reproductive growth also are related to variations in flux of carbohydrate from shoot to root, the changes in carbohydrate flux obviously cannot be associated with emergence of new leaves since leaf number of soybean does not increase during reproductive growth (HENRY et al. 1992; SARAVITZ et al. 1994; VESSEY et al. 1990). An alternative possibility for changes in carbohydrate flux during reproductive development is that demands for carbon and nitrogen by seed growth, rather than being constant as is often presumed from destructive

sampling of whole plants (e.g. EGLI et al. 1981), are dependent on their mutual availabilities in the shoot pool for translocation to the fruits. In terms of the scheme developed for regulation of nitrogen uptake by carbohydrate flux during vegetative growth, we (SARAVITZ et al. 1994; VESSEY et al. 1990) propose the following scheme for reproductive growth. If, under conditions of relatively constant net CO₂ exchange rates when exogenous supply of NO₃⁻ is nonlimiting to growth, the pool size of available nitrogen in vegetative tissues of the shoot declines relative to that of soluble carbohydrates, then rates of transport of both nitrogen and carbohydrates to reproductive structures would decrease. The increased concentration of carbohydrate in the shoot pool would be available for translocation to roots and would enhance the uptake of nitrogen. Subsequently, as the enhanced uptake of nitrogen increases the pool of available nitrogen in the shoot, the availability of carbohydrate in the shoot pool for translocation to roots would be decreased by translocation of carbohydrate accompanying the increased flow of nitrogen to reproductive growth. This scheme would require that seed growth rate vary with the changes in relative availability of either nitrogen or soluble carbohydrate. While variations in seed growth rate are difficult to demonstrate *in vivo*, the *in vitro* growth rates of soybean embryos vary during their development with changes in concentration of either the carbon source (sucrose) or the nitrogen source (glutamine) in the culture medium (SARAVITZ and RAPER 1995).

REGULATION OF SEED COMPOSITION OF OIL AND PROTEIN

In addition to establishing the potential for growth rate of soybean seed to vary during their development with changes in ability of the mother plant to provide carbon and nitrogen to the seed, the *in vitro* culture of soybean embryos in the presence of different concentrations of sucrose and glutamine as the sole sources of carbon and nitrogen also demonstrate the potential for supplies of carbon and nitrogen from the mother plant to alter the composition of protein and oil in seeds (SARAVITZ and RAPER 1995). In these experiments, excised embryos of Ransom soybean were grown in culture media containing various concentrations of sucrose from 1.5 to 300 mM and of glutamine from 0.6 to 120 mM. The embryos were transferred to fresh media every 4 days to maintain the sucrose and glutamine concentrations of the culture media relatively constant. Embryos grown in the presence of 120 mM glutamine contained 69% protein and decreased availability of glutamine lowered protein concentration to 15% at 0.6 mM glutamine. These results, when compared to the ca. 42% protein characteristic of *in vivo* growth, suggest that soybean has the capacity to produce a greater proportion of protein than normally is observed *in vivo*. In combination with 150 mM sucrose, however, oil content increased from 13% to 24% as glutamine supply was increased from 0.6 to 6 mM and then decreased to 10% as glutamine supply was increased to 120 mM. Oil content also decreased as sucrose availability was decreased. Interestingly, when embryos were transferred midway through the 21-day culture period from 150 to 1.5 mM sucrose or from 0.6 to 6 mM glutamine, protein concentration was increased, but oil concentration was unaffected, relative to embryos that remained in 150 mM sucrose or 0.6 mM glutamine. These data thus suggest that the negative relationship commonly observed *in*

vivo between protein and oil content (Brim and Burton 1979) may be altered if availability of nitrogen to the seed can be increased during the later part of seed development.

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