SHOOT RESPONSES TO ROOT STRESS - A RESOURCE GATHERING POINT OF VIEW

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Abstract. Understanding the control and integration of resource gathering is one of the primary problems in plant physiology today. Information on the interactions between root stresses and whole plant integration is scant. The theme I have examined is that plants are resource gatherers and that carbon gathering is coupled to the integration between roots (sinks) and shoots (sources). Plants must balance resource acquisition between roots and shoots. Environmental stress can alter the gathering ability of organs. It is possible that major mechanisms to maintain the balance of resource acquisition between roots and shoots include monitoring of carbon flow and changes in the flux of root sourced metabolites which may function as biochemical messengers. In studies of suboptimal root environments, it appears that decreased sink strength of the roots influences the export of assimilates out of leaves, resulting in the accumulation of leaf metabolites. This may work in conjunction with changes in the flow of metabolites from roots to leaves. It is possible that these mechanisms form part of the basic framework of plant responses to environmental stress.

Studying coordinated functions of shoots and roots is necessary to understanding whole-plant physiology. To be successful, plants must acquire resources from above and below ground. For proper functioning, resource acquisition must be balanced between roots and shoots relative to the availability of these resources. The above ground portion (shoot) of the plant must capture light energy to fix carbon dioxide into sugars. The sugars produced by the above ground portion of the plant provide the basic raw materials to build and maintain the entire plant, including the roots. The below ground portion (roots) of the plant must gather water and mineral nutrients for the shoot as well as anchor the plant in the ground.

Without adequate supplies of certain above and below ground resources, the plant cannot acquire other resources. For example, if a plant cannot take up and transport adequate water to the leaves, stomata will close, resulting in reduced photosynthesis and growth. Reduced photosynthesis will impact the roots because a large root system requires a high input of carbon from leaves. If photosynthesis is limited, root development could be restricted by inadequate building materials normally supplied by leaves. Thus, the growth of either root or shoot is dependent upon the complementary functions of both the roots and shoots. This functional equilibrium of plants has been extensively studied (2, 12, 20).

When plants are grown under environmental stress, the resource gathering ability of roots or shoots is limited. This can result in changes of growth patterns of the plant. An example of the balance between roots and shoots can be demonstrated when plants are grown in containers with limited space for root growth. When the plant has filled the available rooting volume, the rate at which leaves grow is reduced. This results in smaller leaves and less total leaf area on the plant relative to plants in unrestricted soil volumes (Figure 1).

In nurseries and landscape sites, woody species are often grown under conditions that would not be encountered in the natural environment. This is especially true of below ground environments where soils can be flooded, water-deprived or roots restricted by container plantings. Exposure to root zone stresses can limit the long-term fitness of woody species in the landscape. With this in mind we have taken on a number of projects to understand how leaves of woody species respond to various perturbations in the root environment. Here, results are presented from current and previous studies of poplar responses to oxygen deficiency around the roots (root hypoxia) and root restriction, acknowledging that they may or may not act through the same mechanisms. Since little is known of the mechanisms of leaf responses to specific root stresses, studying related stresses can provide an avenue for understanding how shoots integrate carbon, water and hormonal systems into an early warning signal of unfavorable root-zone conditions. The goal of such studies is to provide examples of the integra-

tion between carbon, water and hormonal systems of plants. Ultimately an understanding of basic plant responses to root stresses could provide a way to inform tree care professionals about the vigor of a tree and to study the soil conditions that influence tree health.

Methods and Materials

Plant Material. Young ramets of a *Populus trichocarpa x P. deltoides* hybrid obtained from a clone bank (6) were propagated from greenwood cuttings and grown in a greenhouse as previously described (22). Plants were used 5 to 8 weeks after propagation, when they were approximately 30 cm in height.

Root Treatments. For the root hypoxia experiments, the plants were grown in aerated or hypoxic solution cultures as previously described (23). For the root restriction experiments, ramets were transferred to small (0.5 L) and large (3.5 L) pots. Plants were carefully watered at all times to prevent water deficits, particularly in the smaller pots. All measurements were taken after two weeks.

Physiological Measurements. Growth was measured with a ruler (tip to petiole) on leaves in the linear phase of the sigmoidal growth curve at the same time each day. Leaf water potential measurements were made with a Scholander pressure chamber (Soil Moisture Corp., Model 3005, Santa Barbara, California, USA). Osmotic potential was estimated using sap expressed from frozen-thawed tissue with a thermocouple psychrometer (Decagon, Model SC-10, Pullman, WA), and turgor pressure was calculated from the difference.

Wall extensibility of growing leaves was measured using a CAWL (capacity for acid-induced wall loosening) assay. Estimates of CAWL of leaf strips were made using a constant stress creep apparatus using a 10 g load (18). Three to four strips (2.2 mm x 5 mm) from growing leaves (2-3 plants) were frozen, thawed and extended while bathed in neutral pH solution (50 mM Na acetate buffer, pH 6.0). After 25 min, the pH was changed to 4.5 and the rate of extension was recorded for the next 6 h.

For hormone measurements, abscisic acid (ABA) and zeatin riboside (ZR) concentrations in extracts of xylem sap were determined by immunoassay after partial purification as previously described (23). Measured hormone values were corrected for losses during handling by counting an internal standard of [3H]-($\pm$)-ABA or [3H]-dihydrozeatin at the beginning and end of the purification procedure.

For carbohydrate extraction and analysis, mature source leaves were dried and ground in a mortar. Starch concentration was determined as described by Robbins and Pharr (19).

In all whole plant experiments, a random design with three to six replicates were used in each treatment. All experiments were repeated at least three times with similar results.

Leaf Disk Growth Assay. The residual effects of root stress on leaf growth were measured using an *in vitro* growth assay of leaf disks. Five or six leaf disks (7.2 mm diameter) were excised from leaves in the linear stage of growth on plants with non-restricted and restricted roots. Disks were incubated on control media (KCl plus sucrose, 10mM each), with or without added hormones, at a range of concentrations (1mM to 100 pM) and on two-fold concentrated expressed xylem sap collected from roots of non-restricted or restricted plants as previously described (14). The disks were grown *in vitro* under lights (250 μM/m^{2}s PAR) for 24 h. Growth rates were determined by measuring the increase in disk diameter after 24 h.

Results and Discussion

Effects of Root Hypoxia and Root Restriction on Leaf Growth and Water Relations. Experiments were aimed at describing and understanding the effects of root stresses on leaf expansion. Both root restriction and root hypoxia decreased the rate of leaf growth (Table 1) resulting in smaller leaves on stressed plants than leaves on control plants (Figure 1). To determine whether changes in turgor were associated with the measured changes in leaf growth, bulk leaf water potential was measured by pressure chamber on the same samples that osmotic potential was determined using sap expressed from frozen-thawed leaves. Values obtained on leaves of control and root-stressed plants showed no decrease in bulk leaf water potential or turgor in either root restricted or
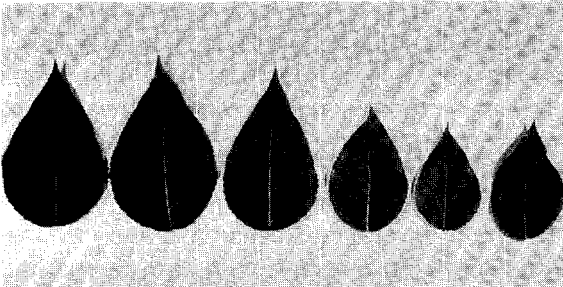
Figure 1. Effect of root restriction on final leaf size of hybrid poplars. Plants were grown in large (3.5 L) and small (0.5 L) pots for two weeks. Leaves of root restricted plants are on the right.

in plants with hypoxic roots (Table 1).

Effects of Root Stress on Cell Walls. Since cell growth is controlled by turgor and wall characteristics, the reduction in leaf growth could be related to changes in wall rheology. Root restriction and root hypoxia caused a reduction in the capacity for acid-induced wall loosening (Table 1).

In vitro Growth Measurements. To study the residual effects of root restriction on leaves, leaf disks were excised from the growing leaves of plants exposed to root restriction for two weeks. Leaf disks from control plants grew less on twofold concentrated xylem sap from plants with restricted roots than on sap collected from plants with non-restricted roots (Table 2). Disks treated with xylem sap from restricted roots grew less than disks incubated on sap collected from non-restricted roots. abscisic acid caused a reduction in the *in vitro* growth of leaf disks from both plants with restricted and non-restricted roots. Zeatin riboside increased the *in vitro* growth of all leaf disks.

Endogenous Plant Hormone Measurements. The concentration of abscisic acid and zeatin riboside-type cytokinins in xylem sap was reduced in roots of restricted plants (Table 1).

Starch Partitioning. The concentration of starch was increased in source leaves by both root restriction and root hypoxia (Table 1).

Summary and Discussion

Why is Leaf Growth Inhibited in Plants with Stressed Roots? The development of leaf area is related to the rate of cell expansion, which is

Table 1. Summary of work describing physiological responses of hybrid poplars to root hypoxia and root restriction

	Control	Hypoxic	Restricted
(A) Avg growth rate (mm/ h)	0.83±.09	0.52±.07	0.41±.05
(B) Leaf water relations			
Water potential (MPa)	-0.68±.01	-0.71±.01	-0.72±.02
Solute potential (MPa)	-1.41±.02	-1.39±.02	-1.38±.01
Pressure potential (MPa)	0.74±.25	0.69±.04	0.66±.02
(C) CAWL (μm/ min)	1.12±.17	0.75±.05	0.42±.07
(D) Xylem sap hormones			
ABA (pmol/ml)	7.7±1.0	6.1±.9	2.2±.9
Zeatin riboside (pmol/ml)	0.79±.11	0.40±.18	0.48±.04
(E) Starch (mg/cm²)	3.9±.3	15.9±1.1	12.3±1.0

All measurements were made after 12-14 days of root hypoxia or root restriction. Growth and CAWL (capacity for acid-induced wall loosening) measurements were made on leaves in the linear phase of growt. (A) Average growth rate. Growth was measured for 24 h during the linear phase of growth. B) Leaf water relations (midday). (C) CAWL. Data are expressed as the linear rate of extension (μm/min). (D) Xylem sap hormones. Data are expressed as the concentration in xylem sap expressed from roots under 0.3 MPa pressure. (E) Starch concentration in mature photosynthetic (source) leaves. Starch concentration was measured spectrophotometrically following hydrolysis. Data are expressed on a leaf area basis. All data are the means for 5-6 plants with standard error.

determined by cell turgor and characteristics of the cell walls. On one hand, it is possible that leaf growth is inhibited due to altered supply of water. In some cases root stresses are correlated with substantial reduction in water uptake (22). It is easy to imagine some type of hydraulic signaling in which the water supply to leaves is reduced and acts as a source of information on the status of the roots. In the leaves of poplars with hypoxic or restricted roots, however, leaf growth is not limited by reduced turgor (Table 1). In poplar plants with hypoxic roots, the reduction in leaf growth may be partially explained by reduced cell wall extensibility. Wall extensibility can be expressed as the capacity for acid induced wall loosening (CAWL). CAWL is a biologically mediated response, correlated with growth rate (26,15). Reduced cell wall extensibility has also been demonstrated in leaves of plants with dehydrated roots (26). How the root

Table 2. In vitro growth of leaf disks from hybrid poplars with non-restricted (control), and restricted roots.

Growth media	Source of leaf disks	
	Non-restricted	Restricted
a) Xylem sap, non-restricted	34.8±0.9	26.5±0.9
Xylem sap, restricted	20.6±1.3	16.2±1.5
b) KCl/sucrose	37.1±1.3	28.4±1.4
c) Zeatin riboside, 10μM	41.2±1.3	30.4±1.4
d) Abscisic acid, 1mM	11.4±1.4	4.9±1.0

Leaf disks were grown on: a) 2-fold concentrated xylem sap expressed from non-restricted or restricted roots, b) 10 mM KCl and 10 mM sucrose (standard media), c) 10 μM zeatin riboside and d) 1 mM ABA. Data are expressed as percent increase in leaf diameter.

environment alters leaf cell wall extensibility is currently unknown.

How Does the Plant "Know" That Its Roots are Stressed and "Tell" the Shoot to Slow Growth? Evidence shows that the development of both roots and shoots depends upon their complementary functions such that carbon from leaves is necessary for root growth and chemical signals from roots may regulate leaf functions (3,4).

In addition to water, roots supply both nutritional and hormonal factors necessary for leaf function. Studies have shown that changes in the root environment will affect active ion uptake and ion concentration in the xylem (21). However, instead of being a direct signaling mechanism in the growth response of root-stressed plants, it is possible that the nutritional status of plants modifies plant responses to other factors (17,27). Nutrient delivery itself may be unreliable as an indicator of root zone conditons because delivery occurs through the transpiration stream, which is subject to many aerial factors resulting in fluctuations in the rate of water movement through the plant (7).

Blackman and Davies (1) suggested that roots and shoots are integrated through changes in the flux of hormones in response to soil perturbations. Results from several laboratories suggest that ABA is exported at high levels from stressed roots and has been shown to be important in leaf responses of flooded and drought-stressed plants (10,13,28,29). Others have suggested that suboptimal soil conditions which reduce growth

and root meristematic activity also reduce the production of growth enhancing compounds, such as cytokinins, by roots (8). A number of experments using hybrid poplars have been performed to describe the nature of the root-sourced factors generated by changes in the root environment (14,23,24). These studies found: 1) that the growth of poplar leaf disks was decreased when disks were grown on sap expressed from hypoxic roots, which suggests that the xylem sap may contain or be lacking a compound which influences leaf growth, 2) a correlation between reduced leaf growth and decreased cytokinin transport in root stressed plants, and 3) that cytokinins increased the growth rate of leaf disks *in vitro*. It was also shown that although ABA decreased the rate of growth of leaf disks *in vitro*, there was no increase of ABA in xylem sap of poplars with hypoxic roots. Experiments of this sort suggest that poplar roots may normally supply factors to leaves which stimulate growth, and when root growth is restricted, the leaves no longer receive as much of the growth stimulating compound.

Is the Production of Leaf Area Related to the Sink Strength of the Roots? Leaves store starch during the day, which they break down and send to the roots as sugar during the night for growth. Roots are sinks for photoassimilates, they influence carbon partitioning in woody plants, and are particularly responsive to changes in carbon allocation within shoots (4). In herbaceous plants, the removal of reproductive sinks alters photosynthetic rates and assimilate partitioning (5,16). There may be similar effects on woody plants when the sink strength of the roots is limited by the rooting environment. This effect can be shown with results from the studies presented here with plants grown in small (0.5 L) pots and in large (3.5 L) pots. Starch concentrations were highest in the leaves of plants with stressed roots suggesting that growth does not appear to be limited by photosynthates (Table 1). Instead, it appears that it is the utilization of photoassimilates rather than the production of photoassimilates which is most sensitive to root stress. It is possible that the decline in leaf growth reduces the demand for carbon resulting in accumulation of carbohydrates in source leaves. Root stresses may, therefore, simply result in

fewer growing roots resulting in a lower demand for assimilates (reduced sink strength) which is sensed in leaves as the build-up of assimilates and subsequently slows leaf growth. It is possible that a change in the balance of carbohydrates would have the effect of acting as a "warning system" that results in reduced growth before there is a severe imbalance in the resource gathering capacity of the plant. It is also possible that leaves respond to subtle changes in the flux of carbon instead of accumulated starch or through the ratio of carbon to some other resource (e.g. nitrogen) which is somehow "sensed" by leaves resulting in reduced leaf growth (4).

Conclusion

There appears, to be at least two ways by which plants are integrated and diminished root growth can be sensed in leaves. On one hand, roots and leaves are linked by the flow of plant hormones. Prime candidates are cytokinins and abscisic acid. It is unlikely, however, that the message system consists of a single compound. Until more is known of the compartmentation and active sites of all the plant hormones, it will be difficult to determine the importance of mechanisms involving hormonal action (8).

Additionally, plants appear to respond to source-sink imbalances. This would suggest that leaves and roots communicate via source-sink relationships which would then enable a plant to coordinate responses at the whole plant level. This discussion has considered some of the mechanisms by which plants may coordinate growth under limited resources. It appears that part of the basic framework of plant responses to environmental stress includes monitoring of carbon flow and root-sourced metabolites. Finally, it is clear that there is a lack of information about the integrative functioning of plants and rigorous research is needed in this area.

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Résumé. Les plantes sont des accumulateurs de ressources
et l'accumulation des carbones est intégrée entre les racines
(entreposage et dissipation; «sink») el les pousses (sources).
La dynamique entre les surplus et les demandes n'est pas
statique. Les stress environnementaux peuvent altérer la
capacité d'accumulation des organes. Les mécanismes
majeurs pour maintenir l'équilibre de l'acquisition des
ressources entre les racines et les pousses impliquent un
contrôle sur la circulation des carbones et des modifications
dans la fluctuation des métabolites de source racinaire. Dans
les études sur des environnements de racines en sous
optimisation, il apparaît qu'une diminution de la capacité
d'accumulation et de dissipation («sink») des racines influ-
ence l'exportation des substances assimilées hors des feuilles,
ce qui résulte à une accumulation de métabolites foliaires.
Ceci peut se faire en conjonction avec des modifications dans
la circulation des métabolites des racines vers les feuilles. Il est
possible que ces mécanismes constituent une partie de la
structure de base des réponses des végétaux aux stress
environnementaux.

Zusammenfassung. Pflanzen sind Sammler natürlicher
Reichtümer und die Aufnahme von Kohlenstott ist integriert
zwischen den Wurzeln (sinks) und den oberirdischen Treiben
(sources). Die Dynamik von Angebot und Nachfrage is nicht
statisch. Umweltbedingter Streß kann die Aufnahmefähigkeit
der Organe verändern. Die Hauptmechanismen zur Erhaltung
des Gleichgewichts der Nährstoffaufnahme zwischen Wurzeln
und Treiben beinhalten die Überwachung des
Kohlenstofftransports und des Wechsels im fluß von
wurzeleigenen Metaboliten (Umsetzungsprodukten). In Studien
über die suboptimalen wurzelräumen kommt es vor, daß eine
herabgesetzte Filterkraft der Wurzeln den Export von
Assimilaten aus den Blättern beeinflußt und zu einer
Akkumulation der Blattmetaboliten führt. Das könnte sich
auswirken im Zusammenhang mit den Veränderungen im
Transport der Metabolite von den Wurzeln zu den Blättern. Es
ist möglich, daß diese Mechanismen einen Teil des
grundsätzlichen Rahmens der Pflanzenreaktion auf
umweltbedingten Streß bilden.