### **Dynamic Aspects of Plant Nutrition**

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## Life

- Life = f(G,E), an interaction of genetic and environmental factors.
- Life is composed of many different chemical and physical phenomena, the rates of which change with time.
- Understanding dynamic aspects of mineral nutrition in plants requires:
  - A conceptual framework.
  - Scientific tools.

### Understanding Genetic and Environmental Factors in Plant Nutrition

**Conceptual framework**: Plants can be thought of as systems composed of one or more compartments.

**Experimental design**: Test an hypothesis or hypotheses with genetic and/or environmental independent variable(s).

**Chemical tools**: Mineral nutrients and other dependent variables associated with conceptual compartments in plants can be measured at points in time.

**Mathematical tools**: Statistics, regression equations and differential calculus are useful to estimate net rates, or fluxes, of mineral nutrients in different parts of plants.

# Change in Living Organisms

- Changes occur atomic, molecular, metabolic, organelle, tissue, organ, and whole-plant dimensions.
- Rates vary through time.
- Measurements at one point in time are results of changing rates of processes prior to that point in time.
- Periodic measurements enable continuous estimates of rates of change.

### Conceptual Basis to Measure Changes of Rates in Plants

- Define a compartment or compartments of interest:
  - Organelle (e.g. nucleus, ribosome, vacuole)
  - Tissue (e.g. epidermis, xylem, phloem, parenchyma)
  - Organ (e.g. root, shoot, stem, leaf, flower, seed, fruit)

Conceptual Model of Fluxes, or Flows of a Substance into and out of a Compartment



 $I_i$  = influx to compartment *i* from outside the system containing compartment *i*.

 $F_{0i}$  = efflux from compartment *i* to outside the system containing compartment i.

 $y_i$  = the amount of substance y in compartment *i*.

 $F_{ii}$  = efflux of the substance from compartment *i* to compartment *j*.

 $F_{ij}$  = influx of substance to compartment *i* from compartment *j*.

# The Net Rate of Accumulation or Loss of a Substance in a Compartment:

$$\frac{\partial y_i}{\partial t} = \sum_{i \neq j} \left( F_{ij} - F_{ji} \right) + I_i - F_{0i}$$

The practical problem is, "How do we estimate the net rate, or change of the amount of the substance in a compartment as a function of time [and treatment]?"

## Three (+1) sequential steps to measure the flux, or net rate of accumulation or loss of a substance in a compartment:

- **1. Periodically measure** the amount of a substance (e.g. element or chemical compound) in the compartment.
- 2. Fit a curve to the measured data to provide a regression equation which is a continuous estimate of amount as a function of time.
- **3.** Calculate the first derivative of the regression equation to provide a continuous estimate of the net flux (net rate of accumulation or loss) of the substance as a function of time.
- 4. Calculate the second derivative to identify points in time when maximum and minimum rates occur.

#### Fluxes of Mineral Nutrients in Multi-Compartment Systems Affected by Genotype (G) and Environment (E)



 Effects of Mn on rates of growth and accumulation of minerals, dry matter and water in cucumber (Cucumis sativus L.) during vegetative growth. (E)





- Sources, fluxes and sinks of N in corn (Zea mays L.) during reproductive growth. (G, E)
- N translocation in genotypes of sorghum (Sorghum bicolor (L.) Moench differing in N use efficiency during vegetative and reproductive growth. (G)



 N source-sink relationships in cotton (Gossipium hirsutum) during vegetative and reproductive growth. (E)



Effects of Mn on rates of growth and accumulation of minerals, dry matter and water during vegetative growth of cucumber (Cucumis sativus L.) (E)

- **Hypothesis**: Rates of accumulation of dry matter, water, and seven essential elements differ in cucumber plants during vegetative growth as functions of time and Mn status of the rooting environment.
- **Treatments**: 0.013 mg Mn/L (deficient), 0.1 mg Mn/L (sufficient) and 10 mg Mn/L (toxic) in nutrient solution.
- Experimental design & sampling: RCB with 2 blocks, 38 pots, transplanted to one pot/plant after 28 d; 34 d to 58 d, every 3 days, 2 plants removed, washed with DI, separated into roots, stems and leaves, weighed, dried, weighed, and chemically analyzed.
- Measurements: DW, FW, N, P, K, Cu, Fe, Mn, Zn.
- **Mathematical model for regression**: a modification of the simple, exponential growth function:  $y = \beta_0 \exp(\beta_1 t) \quad 0.90 > R^2 > 0.99$

Effects of Temperature and Mn on Amount and Flux of FW, DW and Water in Whole Cucumber Plants during 27 d of Vegetative Growth



Figure 6. Low, mean, and high daily air temperatures (C) in the glasshouse in which cucumbers of the present study were grown. Vertical dashed lines indicate the three-day period in which temporarily and relatively high fluxes occurred for many variables with the Mn toxicity treatment.



Fig. 2. Changes in amounts per plant of fresh weight (a), dry weight (b), and water (c) and in accumulation rates per plant of fresh weight (d), dry weight (e), and water (f) for deficient, sufficient, and toxic treatments of Mn. Symbols represent means. The arrow on the X-axis indicates the beginning of the Mn deficiency and toxicity treatments.

#### Effects of Temperature and Mn on Flux of N, P, and K in Root and Shoot of Cucumber Plants during 27 d of Vegetative Growth



Figure 3. Fluxes of (a) N, (b) P, and (c) K in root of cucumber and fluxes of (d) N, (e) P, and (K) in shoot of cucumber. Arrows indicate the number of days after germination on which Mn deficiency and Mn toxicity were introduced, and dashed lines indicate zero flux.

#### Effects of Temperature and Mn on Flux of Mn, Cu, Fe, and Zn the Root and Shoot of Cucumber Plants during 27 d of Vegetative Growth



Figure 1. Fluxes of Mn in root of cucumber (a) with Mn sufficiency and Mn deficiency and (b) with Mn toxicity and in shoot of cucumber (c) with Mn sufficiency and Mn deficiency and (d) with Mn toxicity. Arrows indicate the number of days after germination on which Mn deficiency and Mn toxicity were introduced, and dashed lines indicate zero flux.



Figure 4. Fluxes of (a) Cu, (b) Fe, and (c) Zn in root of cucumber and fluxes of (d) Cu, (e) Fe, and (f) Zn in shoot of cucumber. Arrows indicate the number of days after germination on which Mn deficiency and Mn toxicity were introduced, and dashed lines indicate zero flux.



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#### Sources, fluxes and sinks of N in corn (Zea mays L.) (G,E)

- **Hypothesis**: rates of accumulation of N differ in parts of the shoot of corn as a result of genetic control by the opaque-2 gene and an N-status of the rooting medium during the reproductive phase of growth.
- **Treatments**: Pioneer 3369 hybrid corn with or without the *opaque-2* gene and during the 1<sup>st</sup> 36 d of reproductive growth, and N supplied (3.75 mM NO<sub>3</sub>-N enriched with <sup>15</sup>N) or not supplied to the plant.
- Experimental design & sampling: Replicated 2x2 factorial experiment in pots with sand and nutrient solutions; sampled 7 plant parts at pollination & at 12, 24 & 36 DAP: stalk, leaves below primary ear, leaves above primary ear, shank, husk, cob and grain. 3 days, 2 plants removed, washed with DI, separated into roots stems and leaves. Extracted amino acids and protein fractions from grain.
- **Measurements**: DW, Total N, exogenous N (15N-enriched, entered shoot after pollination), endogenous N (which entered the shoot before pollination), of the 7 parts of shoot and of amino acids and protein N fractions in grain.
  - **Mathematical model for regression**: polynomial functions such as:  $y = \beta_0 + \beta_1 x + \beta_2 x^2 + \beta_3 x^3$  where y = grams N and x = days after pollinationwith 1st derivative  $y = \beta_1 + 2\beta_2 x + 3\beta_3 x^2$  and  $2^{nd}$  derivative  $y = 2\beta_2 + 6\beta_3 x$  $0.18 > R^2 > 0.96$ , with most  $R^2 > 0.90$  for N in the grain and in grain amino acids.

#### Sources, fluxes and sinks of N in corn (Zea mays L.)



FIG. 1. Conceptual model of maize plant during reproductive growth phase, indicating N sources and sinks of the shoot. Arrows indicate N influx and efflux for all compartments, or regions, of the shoot except the grain for which only influx is assumed.



FIG. 2. Fluxes of total, endogenous, and exogenous N for (a) cob, (b) husk, (c) shank, (d) upper leaves, (e) lower leaves, and (f) stalk per maize plant during the first 36 d of reproductive growth when no N (— —) or 3.75 mm N (——) was supplied in the nutrient solution. Flux curves derived from regression equations with  $R^2 < 0.60$  are drawn as (- – ), and N treatments are indicated. Data of Pioneer 3369A and L3369 hybrids are pooled.

#### Sources, fluxes and sinks of N in corn (Zea mays L.)



TIME AFTER POLLINATION, DAYS

FIG. 3. Fluxes of total, endogenous, and exogenous N for grain per maize plant during the postpollination period with no N (- - -) or 3.75 mm N (--) in the nutrient solutions. Data of Pioneer 3369A and L3369 hybrids are pooled.

#### Sources, fluxes and sinks of N in corn (Zea mays L.) Amount and Flux of Amino Acid-N in the Grain

Dopoque -2

40

40



FIGURE 6. Amounts (A) and rates (B) of lysine nitrogen accumulation in normal (Pioneer 3369A) and opaque-2 (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 mM N treatments.



TABLE 2 - Number of days after pollination when maximum rate of nitrogen accumulation occurred in amino acid and acid-hydrolyzable ammonium fractions of maize grain during the first 36 days post-pollination.

Nitrogen Fraction	Genotype (1)	
	Normal	Opaque-2
Tryptophan	(2)	( <sup>2</sup> )
Methionine	20	24
Tyrosine	(3)	34
Isoleucine	27	28
Phenylalanine	32	32
Threonine	24	25
Serine	28	26
Glycine	23	23
Valine	25	25
Lysine	21	25
Histidine	29	30
Aspartate	20	25
Proline	28	27
Alanine	23	21
Arginine	24	27
Leucine	(2)	32
Glutamate	27	21
Ammonium	(2)	(2)

(1) Genotypes are normal (Pioneer 3369A) and opaque-2 (Pioneer L3369).

(2) Using second derivative of regression equation, when y'' = 0, x > 36.

(3) Using second derivative of regression equation, when y'' = 0, x = 36.





#### Sources, fluxes and sinks of N in corn (Zea mays L.) Amount and Flux of Amino Acid-N in the Grain



FIGURE 6. Amounts (A) and rates (B) of lysine nitrogen accumulation in normal (Pioneer 3369A) and opaque-2 (Pioneer L3369) maize grain per plant. Symbols represent means of replicates pooled from 0 and 3.75 mM N treatments.



FIGURE 8. Amounts (A) and rates (E of leucine nitrogen accumulation i normal (Pioneer 3369A) and opaque-(Pioneer L3369) maize grain per plant Symbols represent means of replicate pooled from 0 and 3.75 mM N treatments TABLE 2 - Number of days after pollination when maximum rate of nitrogen accumulation occurred in amino acid and acid-hydrolyzable ammonium fractions of maize grain during the first 36 days post-pollination.

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Serine	28	26
Glycine	23	23
Valine	25	25
Lysine	21	25
Histidine	29	30
Aspartate	20	25
Proline	28	27
Alanine	23	21
Arginine	24	27
Leucine	(2)	32
Glutamate	27	21
Ammonium	(2)	(2)

(1) Genotypes are normal (Pioneer 3369A) and opaque-2 (Pioneer L3369).

(2) Using second derivative of regression equation, when y'' = 0, x > 36.

(3) Using second derivative of regression equation, when y'' = 0, x = 36.







N translocation in genotypes of sorghum (Sorghum bicolor (L.) Moench differing in N use efficiency during vegetative and reproductive growth. (G)

- **Hypothesis**: Differences in NUE identified in two sorghum genotypes is be associated with differences in translocation of N in the shoot of those genotypes.
- **Treatments**: China 17 (N-efficient) and Tx623 (N-inefficient) genotypes of sorghum were used in a field experiment.
- **Experimental design & sampling**: RCB with 2 genotypes per block and 4 blocks; thinned to 70,000 plants/ha in 75 cm row spacing; Sharpsburg silty clay loam; 90 kg ammonium nitrate-N/ha broadcast and incorporated before planting; rainfed, no irrigation. Stem, leaves and grain were sampled (see paper for details); plant samples above soil surface were taken at 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, and 17 weeks after emergence, dried.
- Measurements: DW and total N (Kjeldahl method).
- **Mathematical model for fluxes**: a differential equation for growth and decay: dy/dx = ky where y = the amount of N in a compartment and k = a rate constant for the flux between compartments. Matrix models were tested to simultaneously estimate amounts of N in compartments and rate constants between compartments.

# N Fluxes in the Shoot of China 17 and TX623 Sorghum Plants

- Stalk 4: upper ½ of the upper ½ of the stalk.
- Leaf 4: the leaves attached to Stalk 4.
- Stalk 3: the lower 1/2 of the upper ½ of the stalk.
- Leaf 3: the leaves attached to Stalk 3.
- Lower ½ of the plant including the two lower parts of stem and two

lower sets of leaves.



#### Accumulation of N in Grain of China 17 and Tx623 Sorghum and N Fluxes





TX 623 has lesser NUE







N source-sink relationships in **cotton** (*Gossipium hirsutum*) during vegetative and reproductive growth. (E)

- **Hypothesis**: Different parts of the cotton plant act as sinks and sources of N at different times during the growth of the plant.
- **Treatments**: Expose plants to 15N-enriched nutrient solution for 5 plant growth periods of 30 days each: i) transplanting to appearance of 1<sup>st</sup> squares, ii) first squares to 1<sup>st</sup> flowers, iii) first flowers to peak flowering, iv) peak flowering to 1<sup>st</sup> open bolls, and v), first open bolls to 150 days.
- Experimental design & sampling: RCB with 5 treatments and 4 replicates. Plants (cv. Acala-SJ-2) were germinated in acid washed sand, and then transferred, 4 plants to a pot, to pots containing 20 L of nutrient solution. Plants were harvested at the end of each 30-d period and divided into 3 to 11 parts (bottom: 1<sup>st</sup> 5 nodes, middle: next 5 nodes, top next 5 to 11 nodes, depending upon plant age. Fallen leaves were collected, analyzed separately or combined with the other leaves if appropriate.
- Measurements: DW, Kjeldahl N, <sup>15</sup>N/<sup>14</sup>N ratio.
- Mathematical model for regression: not specified by the authors.

N source-sink relationships in **cotton** (*Gossipium hirsutum*) during vegetative and reproductive growth. (E)

- BOTTOM: 1<sup>st</sup> 5 nodes.
- MIDDLE: 2<sup>nd</sup> 5 nodes.
- TOP: next 5 to 11 nodes.
- N derived from solution in each period was added to the preceding period, so accumulated data are presented.

• Negative fluxes and NFS fluxes > than KN fluxes indicate exportation.

- 0-30 d: transplanting to 1<sup>st</sup> sq.
- 30-60 d: 1<sup>st</sup> sq to 1<sup>st</sup> bloom.
- 60-90 d: 1<sup>st</sup> bloom to peak bloom.
- 90-120 d: peak bloom to 1<sup>st</sup> open boll
- 120-150 d: 1<sup>st</sup> open boll to 150 days after transplanting



Figure 8: Nitrogen from the solution (NFS) and total nitrogen fluxes (KN) in some cotton plant regions and organs.

#### References

- Crawford, Jr. T.W, R.O. Kuehl, and J.L. Stroehlein. 1990. Net fluxes of mineral nutrients, water, and carbohydrate influenced by manganese in root and shoot of *Cucumis sativus* L. J. Plant Nutrition 13(7):759-786.
- Crawford, Jr. T.W., J.L. Stroehlein, and R.O. Kuehl. 1989. Manganese and rates of growth and mineral accumulation in cucumber. J. Amer. Soc. Hort. Sci. 114(2):300-306.
- Crawford, Jr., Thomas W., Victor V. Rendig, and Francis E. Broadbent. 1982. Sources, fluxes, and sinks of nitrogen during early reproductive growth of maize (*Zea mays* L.). Plant Physiol. 70:1654-1660.
- Crawford, Jr., T.W. and V.V. Rendig. 1982. Accumulation of amino acid nitrogen and acid-hydrolyzable ammonium nitrogen in *opaque-2* and normal maize grain. Maydica 27:11-26.
- Crawford, Jr., T.W., K.M. Eskridge, C.G. Wang, and J.W. Maranville. 2009. Multi-compartmental modeling of nitrogen translocation in sorghums differing in nitrogen use efficiency. J. Plant Nutrition 32(2):335-349.
- Crawford, Jr., T.W, K.M Eskridge, C.W. Wang, and J.W. Maranville. 2004. Nitrogen translocation in Nefficient and N-inefficient cultivars of sorghum. In Annual Meetings Abstracts. [CD-ROM]. ASA, CSSA, and SSSA, Madison, WI. (http://twc0etal.freeshell.org/tom/sorghum/sorghum.html)
- Rosolem, C.A. and D.S. Mikkelsen. 1989. Nitrogen source-sink relationship in cotton. J. Plant Nutrition 12(12):1417-1433.

### Thanks for attending. Questions?