“Turfgrasses do not eat cookies – your turf soil solutions”

STMA
WEST Palm Beach
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OBJECTIVES
- Plant Roots and Nutrient Uptake
- All the “3-3-3... Golden Rules” of plant nutrients and nutrient physiology
- Turfgrass Nitrogen
- Fe and Ni Nutrition and Studies at Clemson University

Plant Roots and Nutrient Uptake

Soil Solution

Objectives Diagram
Part II of Chapter 2
Review of Plant Roots and Nutrient Uptake
Tap roots of plants

Fibrous roots of bamboo

Primary and developing secondary fibrous roots of a perennial ryegrass plant

Longitudinal diagram of a grass root showing zones of growth and absorption

Grass root cross sections

Old Kentucky bluegrass root  Young creeping bentgrass root

J. Beard, 1973
EPIDERMIS
CORTEX
ENDODERMIS (PRIMARY)
STELE
XYLEM (VESSEL ELEMENT)
PHLOEM (SIEVE ELEMENT)

LATERAL TRANSPORT ROUTES IN A GRASS ROOT

TRANSPORT ROUTES
— APOPLASTIC
— SYMPLASTIC
— TRANSCELLULAR

Cross Section of a Root with a Branch

Root architecture and root hairs

Same species with different root growth

Shallow Intermediate Deep

Rhizosphere

Rhizobia Mycorrhizae

The Golden Rule
First Plant Nutrient Rule

- Unreplaceable
- Unique Metabolic and biological functions
- Deficiency symptoms appear under stresses

C, O, H, N, P, K, Ca, Mg, S, Fe, Cl, Mn, B, Cu, Zn, Mo, Ni

Essential elements

Plants require 17 essential elements:
The 3 elements from air and water

- Carbon C
- Hydrogen H
- Oxygen O

C + H + O = 90-95% of the dry weight

The rest 14 elements are from soils called Plant Nutrients

Mineral nutrients (14)

Primary nutrients: 0.2 to 6%
N, P, and K
Secondary nutrients: 0.1 to 0.6%
Mg, Ca, and S
Micronutrients: 0.0001 to 0.05%
Fe, Mn, Cu, Zn, B, Mo, Ni, and Cl
## Micronutrients

<table>
<thead>
<tr>
<th>Element</th>
<th>Formula</th>
<th>Available form</th>
<th>Dry wt. ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>Fe^{2+}, Fe^{3+}</td>
<td>100-200</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>Mn^{2+}</td>
<td>30-70</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>Zn^{2+}</td>
<td>20-50</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>Cu^{2+}</td>
<td>5-20</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
<td>Ni^{2+}</td>
<td>0.01-10</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Cl</td>
<td>Cl^{-}</td>
<td>100-200</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>MoO_{4}^{2-}</td>
<td>0.1-2</td>
</tr>
<tr>
<td>Boron</td>
<td>B</td>
<td>B(OH)_{3} (Boric acid)</td>
<td>20-40</td>
</tr>
</tbody>
</table>

The above 8 elements are micro-nutrients for plants. Crops often do not require application of micro-nutrients.

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## Micronutrient Concentrations In Plants and Soil

<table>
<thead>
<tr>
<th>Element</th>
<th>Formula</th>
<th>Dry wt. in plants ppm</th>
<th>In Soils ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron</td>
<td>Fe</td>
<td>100-200</td>
<td>20,000-50,000</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn</td>
<td>30-70</td>
<td>500-5,000</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Cl</td>
<td>100-200</td>
<td>50-500</td>
</tr>
<tr>
<td>Boron</td>
<td>B</td>
<td>20-40</td>
<td>20-200</td>
</tr>
<tr>
<td>Zinc</td>
<td>Zn</td>
<td>20-50</td>
<td>17-160</td>
</tr>
<tr>
<td>Nickel</td>
<td>Ni</td>
<td>0.01-10</td>
<td>5-100</td>
</tr>
<tr>
<td>Copper</td>
<td>Cu</td>
<td>5-20</td>
<td>5-50</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Mo</td>
<td>0.1-2</td>
<td>0.1-5.0</td>
</tr>
</tbody>
</table>

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# 5

The Golden Rule

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# 6

The Golden Rule

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# 7

The Golden Rule
Movements of Ions from Soils to Roots

- Mass flow
  - Diffusion
    \[ \text{NO}_3^- > \text{K}^+ > \text{H}_2\text{PO}_4^- \]
  - Interceptions
    \(<1\%\)

Plant nutrient uptake
- Active transport cost energy
- Passive transport no energy expenses
- Combination of both
**Nutrient Uptake Kinetics**

- Less Genetically controlled
- Genetically controlled
- Most genetically controlled

**Michaelis Menten Equation**

\[ V = \frac{V_{max} \times [S]}{K_m + [S]} \]

External nutrient concentration \( S = m\text{M} \) (or \( u\text{M} \))

Please notice the differences of two \( K_m \).

**The Most Important Law of Plant Nutrition**

Law of the Minimum

Justus von Liebig (1803 – 1873), German chemist

Justus von Liebig formulated the **Law of the Minimum**, stating that a plant's development is limited by the one essential mineral that is in the relatively shortest supply, visualized as "Liebig's barrel". This concept is a qualitative version of the principles used to determine the application of fertilizer in modern agriculture.
**Nitrogen:**
3 - 6% in dry tissue
$\text{NO}_3^-$, $\text{NH}_4^+$, and urea
Mobile in turfgrasses and soils
Most frequently deficient
Older leaves turn yellow and reduced shoot growth

**Functions of Nitrogen in Plants**
- Chlorophylls
- Proteins
- DNA, RNA
- Hormones
- Secondary metabolites

**Chloroplasts**

**A TURFGRASS LEAF**
- Lower epidermis
- Guard cells
- Upper epidermis
- Mesophyll cells
- Vascular bundle
- Air boundary layers

**Chloroplast**
Symptoms of N Deficiency
Soil Solution

Uptake by plants

OM and Microorganisms

Exchange and surface adsorption

Solid minerals
Iron
Symbol: Fe
Atomic Number: 26
Atomic Mass: 55.845 amu
Melting Point: 1535.0 °C (2862.0 °F)
Boiling Point: 2750.0 °C (4982.0 °F)
Number of Protons/Electrons: 26
Number of Neutrons: 30
Classification: Transition Metal
Crystal Structure: Cubic
Density @ 293 K: 7.86 g/cm³
Color: Silvery

Iron Functions in Plants
- Chlorophyll development and function.
- It plays a role in energy transfer within the plant.
- It is a constituent of certain enzymes and proteins.
- Iron functions in plant respiration, and plant metabolism.
- It is involved in nitrogen fixation
Two major mechanisms of iron acquisition studied in plants are based either on reduction of organic ferric iron chelates, via a membrane-associated ferrireductase called strategy I plants, mainly broadleaf plants. Gramineae (grass) family produces phytosiderophores that bind ferric ions and introduce them into the cell via a special receptor, called strategy II plants.

What are phytosiderophores?
Phytosiderophores (PS) are organic substances (such as nicotinamine, mugineic acids (MAs) and avenic acid etc) produced by plants under Fe-deficient conditions, which can form organic complexes or chelates with Fe$^{3+}$, and increase the movement of iron in soil. It is non-proteinous, low molecular weight acids released by the graminaceous species under the iron and Zn deficiency stress. The PS mobilize micronutrients Fe, Zn, Mn, and Cu from the soils to plant in deficient conditions.

Evolutionary Lineages of Life

Iron absorption strategy in roots of grasses and transport under light and dark (Schmidt, 2008).
Factors Affecting Fe Availability
- Soil pH and (HCO₃⁻) bicarbonate;
- Water-logging conditions;
- Low soil OM and calcareous soils;
- In most cases, Fe³⁺ is reduced to Fe²⁺ for plant absorption and plants differ in Fe²⁺ absorption;
- Interactions with other nutrients: high Cu and Mn in soils can worsen Fe deficiency;
- NO₃⁻ can worsen Fe deficiency than NH₄⁺.

Highly Responsive Crops to Fe
Alfalfa, asparagus, barley, beans (white), beets, broccoli, brussel sprouts, cabbage, cauliflower, celery, citrus, grass, oats, peanuts, rye, rice, soybean, sorghum, spinach, strawberry, sudangrass, tomato, and turfgrasses.

Iron Management for Turfgrasses
- Iron is an essential micronutrient for turfgrasses (.01 to 0.1%).
- Because iron in the soil is often insoluble, it is not always readily available for plant uptake.
- The best responses to iron fertilization occur when the turfgrass is under stress.
- Chelated iron is more readily absorbed by grasses.
- Frequently mowed turf requires more frequent iron applications.

Iron Deficiency
- Alkaline soils pH > 7.0
- Poor rooting
- Excessive thatch
- Cold, wet soils in spring
- Low organic matter soils
- Heavy metals such as Cu, Zn, Mn
- Poor irrigation water with high concentrations of HCO₃⁻, Ca, P, and other metals

Fruit Tree Iron Deficiencies
Apple  Pear  Peach

Citrus Iron Deficiency
NUTRIENT DEFICIENCY SYMPTOMS OF TWO COOL-SEASON TURFGRASSES

- Minus Phosphorus
- Minus Iron
- Minus Magnesium
- Minus Potassium
- Minus Sulfur

Iron Deficiency of Three Cool Season Turfgrasses

- Kentucky bluegrass
- Creeping bentgrass
- Red fescue

Fe deficiency of St. Augustinegrass
Iron deficiencies can be corrected by application of numerous iron containing fertilizers. Here Ironite is one of examples.
Most products only last three to four weeks.

Iron Toxicity
- Blacken the canopy can cause tissue injury or heat stress
- High Fe can induce Mn deficiency
- Acid and poor drained soils can produce toxic level of Fe.
- Black layer for turfgrasses

Rice Fe disorders
Deficiency
Toxicity

Common Fe Fertilizers

<table>
<thead>
<tr>
<th>Source</th>
<th>Formula</th>
<th>%Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulfate</td>
<td>FeSO₄·7H₂O</td>
<td>19</td>
</tr>
<tr>
<td>Ferric sulfate</td>
<td>Fe₃(SO₄)₂·4H₂O</td>
<td>23</td>
</tr>
<tr>
<td>Ferrous oxide</td>
<td>FeO</td>
<td>77</td>
</tr>
<tr>
<td>Ferric oxide</td>
<td>FeO</td>
<td>69</td>
</tr>
<tr>
<td>Ferrous ammonium phosphate</td>
<td>Fe(NH₄)PO₄·H₂O</td>
<td>29</td>
</tr>
<tr>
<td>Ferrous ammonium sulfate</td>
<td>(NH₄)SO₄·FeSO₄·6H₂O</td>
<td>14</td>
</tr>
<tr>
<td>Iron ammonium polyphosphate</td>
<td>Fe(NH₄)HP₂O</td>
<td>22</td>
</tr>
<tr>
<td>Iron chelates</td>
<td>NaFeEDTA</td>
<td>5-14</td>
</tr>
<tr>
<td></td>
<td>NaFeEDDHA</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>NaFeDTPA</td>
<td>10</td>
</tr>
<tr>
<td>Natural organic materials</td>
<td></td>
<td>5-10</td>
</tr>
</tbody>
</table>
**Complex**
When a metal ion combines with an electron donor, the resulting substance is said to be a complex or a coordination compound.

(CH3)-N-COO-metal

**Chelate**
The main difference between a metal complex and a chelate is that in the chelate, the donor atoms are attached not only to the metal but also to each other.

![Metal Oxalic Acid Donor]

**Common Synthetic and Natural Chelate Compounds**

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Abbrev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>EthyleneDiamineTetraAcetic acid</td>
<td>C_{10}H_{16}O_{8}N_{2}</td>
<td>EDTA</td>
</tr>
<tr>
<td>DiethyleneTriaminePentaAcetic acid</td>
<td>C_{16}H_{22}O_{14}N_{3}</td>
<td>DTPA</td>
</tr>
<tr>
<td>CyclohexaneDiamineTetraAcetic acid</td>
<td>C_{10}H_{20}O_{14}N_{2}</td>
<td>CDTA</td>
</tr>
<tr>
<td>EthyleneDiamineDi-o-HydroxyphenylAcetic acid</td>
<td>C_{18}H_{20}O_{6}N_{2}</td>
<td>EDDHA</td>
</tr>
<tr>
<td>Citric acid</td>
<td>C_{6}H_{8}O_{4}</td>
<td>CIT</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>C_{2}H_{2}O_{4}</td>
<td>OX</td>
</tr>
<tr>
<td>Pyrophosphoric acid</td>
<td>H_{4}P_{2}O_{7}</td>
<td>PPA</td>
</tr>
</tbody>
</table>

**Why Use Strong Chelated Products?**
- Compatibility and stability mixing with other nutrients and products
- Protects nutrient from falling out (precipitating) and assists in plant uptake and translocation

**Synthetic Chelating Agents**
- Synthetic chelates used extensively in turf products:
  - EDTA, DTPA, CDTA, etc.
  - Very popular but are effective only on the metal micronutrients.
  - Were developed for soils and are not as effective when used as a foliar.
  - Are not biodegradable and may remain as a residue in the plant tissue or soil.
  - Can be toxic to the plant tissue under some conditions and concentrations.

**Organic Chelating and Complexing Agents**
- Organic products used as complexing and chelating agents:
  - Humic, citric, fulvic, yucca extracts & amino acids
  - Lignosulfonates
  - Wood pulp derivatives & hydrolyzed protein mixes
  - Some generally have low stability constants.
  - Mixed with phosphate may result in precipitate
  - When added to the soil or plant, high organic concentrations are biodegradable.
  - Some organics may also provide an energy source to plants and soil microorganisms thus aiding in nutrient availability.
Products with Organic Chelating Agents

- The difference in the various products on the market is in the type and amount of Organic facilitating agents as well as the type and formulation of nutrients.
- Many products are not 100% chelated and although classified by law as a chelate may not be effective.
- Even among organics some chelating agents are far superior to others and all are classified as chelating agents by law.
- Many products have an improper pH.

Objective

- To determine whether the application of iron (Fe) will affect Diamond zoysia grass and 'Tifgrand' bermudagrass under a shaded and full sun environment.
- By improving:
  - Turf color
  - Turf quality
  - Water dormancy color
  - Spring green-up

Material and Methods

Field Study

- Testing done on Diamond zoysia grass and 'Tifgrand' bermudagrass at Cornell's University's turf research plots.
- In full sun and under shaded conditions (50% shade).
- Iron rates of 1, 3, and 5 kg/ha applied every 3 weeks from May to October.
- Ferrous sulfate is the iron fertilizer used.
- Data will be collected by:
  - Turf quality (visual rating)
  - Non-leaf ingrowth index (NAI)
  - Chlorophyll Content
  - Turf Cippings Clipping Nutrients (Fe)
Materials and Methods

Warm-season Turfgrasses
• ‘Diamond’ Zoysiagrass
  • (Zoysia matrella (L.) Merr)
• ‘Tifgrand’ Bermudagrass
  • (Cynodon transvaalensis Burt-Davy x C. dactylon)

Light Treatments
• Control (full-sun)
• 40% continuous shade
Utilizing Iron In Turfgrass Management

Nickel
Symbol: Ni
Atomic Number: 28
Atomic Mass: 58.6934 amu
Melting Point: 1453.0 °C (1726.15 K, 2647.4 °F)
Boiling Point: 2732.0 °C (3005.15 K, 4949.6 °F)
Number of Protons/Electrons: 28
Number of Neutrons: 31
Classification: Transition Metal
Crystal Structure: Cubic
Density @ 293 K: 8.902 g/cm³
Color: white

Kentucky bluegrass treated with excessive rates of ferrous sulfate (foreground) and iron chelate (background) shows impact 24 hours after application (foliar).

Ni Functions in Plants
- Nickel is a functional constituent of seven enzymes.
- Among the seven, urease (i.e., urea amidohydrolase) is extremely important to N metabolism in plants.
- Urease assists in the hydrolysis of urea.
- Nickel works as a cofactor to enable urease to catalyze the conversion of urea into the ammonium ions, which plants can use as a source of N.
Cowpea seedlings

+Ni

-Ni

cowpea seedlings

Pecan Ni deficiency

Some Ni Fertilizers

<table>
<thead>
<tr>
<th>Product</th>
<th>Chemical Formula</th>
<th>B %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel Sulfate</td>
<td>NiSO₄·6H₂O</td>
<td>32.1</td>
</tr>
<tr>
<td>Anhydrous nickel sulfate</td>
<td>NiSO₄</td>
<td>37.5</td>
</tr>
<tr>
<td>Nickel nitrate</td>
<td>Ni(NO₃)₂·6H₂O</td>
<td>20.2</td>
</tr>
<tr>
<td>Nickel Chloride</td>
<td>NiCl₂·6H₂O</td>
<td>37.2</td>
</tr>
<tr>
<td>Sewage sludge composite</td>
<td>composite</td>
<td>2.4-5.3</td>
</tr>
</tbody>
</table>

TifEagle bermudagrass growth reduction under four Ni²⁺ levels

Control, 400, 800, 1600 μM

Diamond zoysiagrass growth reduction under four Ni²⁺ levels

Control, 400, 800, 1600 μM
Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under control treatments.

Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 400 µM Ni treatments.

Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 800 µM Ni treatments.

Nickel toxicity symptoms on leaf tissue of Diamond (L) and TifEagle (R) under 1600 µM Ni treatments.
Thank you and any questions!

Foliar Fertilization
Plant Leaf Morphological Characteristics Related to Hydrophobicity and Foliar Absorption

Team Work Efforts
- Investigators
  - Dr. Haibo Liu
  - Dr. Frank Bethea
- Team Members
  - Dr. Terri Bruce
  - Dr. Hong Luo
  - Dr. William Bridges
- Turf Team Members
  - Dr. Bert McCarty
  - Dr. Dara Park
  - Dr. Bruce Martin
  - Dr. JC Chong
  - Mr. Don Garrett
  - Mr. Mike Echols
- Clemson Light Microscopy Facility
  - Rhonda Powell
  - Sarah Howell
  - Caroline Ashworth
- Clemson EM Facility
- Clemson Multilaser Analytical Lab
- Other Colleagues and Students
  - Dr. Bob Cross
  - Dr. Jeff Atkinsion
  - Mr. Nate Gambrell
  - Dr. Brad Shaver
  - Dr. Nick Menchyk
  - Mr. Nickles Mirmow
  - Dr. Zhigang Li
  - Ms. Emily Wakefield

Overall Goal
- Investigate the factors affecting foliar absorption
  - Solution chemistry
  - Solution concentration
  - Plant leaf surface characteristics
    - Leaf morphology
    - Cuticle analysis
  - Stomata size and density
  - Leaf hydrophobicity
  - Solution uptake

When it is too hot, potential of chemical burn is high

When plant, environmental, and soil conditions are restricted or less favorable for root nutrient absorption

Severe lose of roots during the summer Poor drainage or high soil moisture content
When it is colder, root activity slows. 

**Turfgrass Industry**

- **Economic Impact**
  - $40 Billion estimated value annually in US
  - ~ 50 million acres of land in US
- **South Carolina**
  - $2.7 billion from golf courses alone (Jackson, 2012)
  - Golf Course Expenses ~ $540 m
  - $27 million in fertilizers
  - 5% Course operating expenses

**Turfgrass Management**

- **Foliar Fertilizer Applications**
  - **Benefits:**
    - Quick Response
    - Reduce growth surge
    - Accurate application
    - Environmental friendly
    - Labor Efficient
    - Tank Mixing
  - **Major Problem**
    - Inconsistent uptake (10-60%)

**Solution Chemistry**

- **Foliar Fertilizer and Pesticide Applications**
  - **Products**
    - Fertilizers
    - Adjuvants
    - Pesticides
  - **Rates/Carrier Volumes**
    - Product variation
    - Area applied

**Plant Leaf Surface**

- **Leaf Size**
- **Leaf Morphology**
  - Cell Structure and Orientation
  - Surface Roughness
- **Stomata**
  - Size (µm)
  - Stomata mm⁻²

**Plant Leaf Cuticle**

- **Cuticle Composition**
  - Protective waxy coating
  - Long chain carbon compounds
- **Cuticle Morphology**
  - Crystalloids
Plant Leaf Hydrophobicity

- Contact Angle
  - solid, liquid and air interface
- Hydrophobic
  - $> 90^\circ$
- Hydrophilic
  - $< 90^\circ$
- Quantifies the wettability of a surface
- Surface chemistry and surface roughness

Question: Does solution hydrophobicity differ among plant species?

Part I: Solution Hydrophobicity Interaction with Three Plant Leaf Surfaces

Part I: Objective

- To determine the hydrophobicity of various popular foliar application products (fertilizers, adjuvants, and pesticides) on three plant species (St. Augustinegrass, tall fescue and white clover)

Part I: Materials and Methods

<table>
<thead>
<tr>
<th>Purpose</th>
<th>Product</th>
<th>Concentration</th>
<th>Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td>CO(NH$_2$)$_2$</td>
<td>0.60 M</td>
<td>500 L ha$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>NBNO</td>
<td>1.26 M</td>
<td>200 L ha$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>KNO$_3$</td>
<td>1.38 M</td>
<td>200 L ha$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>K$_2$HPO$_4$</td>
<td>0.44 M</td>
<td>200 L ha$^{-1}$</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>Nonionic Surfactant</td>
<td>2.5 ml</td>
<td>0.250% v/v</td>
</tr>
<tr>
<td></td>
<td>Nonionic Silicone</td>
<td>2.5 ml</td>
<td>0.250% v/v</td>
</tr>
<tr>
<td>Fungicide</td>
<td>Chlorothalonil</td>
<td>17.5 g L$^{-1}$</td>
<td>500 L ha$^{-1}$</td>
</tr>
<tr>
<td>Growth Regulator</td>
<td>Trinexap-acid</td>
<td>0.78 ml L$^{-1}$</td>
<td>200 L ha$^{-1}$</td>
</tr>
</tbody>
</table>

Objective I: Materials and Methods

- Hydrophobicity Test
  - 1μl droplet
  - Adaxial leaf side
  - Beattie and Marcell (2002)
Part I: Results

Fertilizer Group

1) Water
2) Urea
3) Ammonium nitrate
4) Potassium nitrate
5) Potassium phosphate

Adjuvant Group

1) Water
2) Drift Reducer
3) Nonionic Surfactant
4) Nonionic Siloxane Surfactant

Pesticide Group

1) Water
2) Trinexapac-ethyl
3) Chlorothalonil

Conclusions

- Products significantly reduce hydrophobicity
  - Urea reduced hydrophobicity the most by the fertilizer group
  - Chlorothalonil significantly reduced hydrophobicity, compared to trinexapac-ethyl
  - Nonionic siloxane surfactant had the lowest average contact angle

- Plant species differed in hydrophobicity
  - White clover consistently had the highest contact angle, with St. Augustinegrass and tall fescue second and third, respectively

Question: What plant leaf surface characteristics influence leaf hydrophobicity?
Part II: Plant Leaf Surface Characteristics related to Leaf Hydrophobicity

Part II: Objective

- The objectives were to investigate the plant leaf surface characteristics that influence the hydrophobicity of the leaf surface

Part II: Materials and Methods

Plant Species

- 15 different species
- 5 species with multiple cultivars
- 32 different plants
- C₃ & C₄ plants
- Various applications
  - Golf Courses
  - Sports Fields
  - Home Lawns
  - Utility Areas
  - Weed Species

Plant Leaf Morphology

- LEXT OLS4000 Optical Profiler
  - 20X Objective
- Imaging:
  - Color
  - Laser
  - Topographical Map
- Surface Roughness ($S_u$)
  - Average of the deviation distances from the Mean Line of all the Peaks to all the Valleys within a sample

Plant Stomata

- Imprints of plant leaves
- Size
  - $\mu$m
- Stomata density
  - stomata mm$^{-2}$ leaf area
  
  (Radoglou and Jarvis, 1990)
Plant Cuticle Analysis

- Cuticle Composition and Quantification
  - Jenks et al. (1995)
  - Cuticle extracted in hexane
  - Gas Chromatography/Mass Spectroscopy

- Cuticle Morphology
  - Hitachi S4800 (SEM)
  - Air-dried (Pathen et al., 2008)
  - Sputter-coated with platinum

Plant Leaf Hydrophobicity

- Contact Angle measurement
- 1 μl water droplet
- Horizontal Sterescope
- Beattie and Marcell (2002)

Part II: Results

Warm-Season Leaf Surface Roughness

Examples of 3-D light micrographs of four warm-season grass species leaf surface roughness. 1-4) Ultradwarf bermudagrass, zoysiagrass, seashore paspalum, centipedegrass, respectively. a-b) Adaxial and abaxial (Scale Bars = 150μm)

Averages:
- Adaxial: 2.42
- Abaxial: 1.44

Cool-Season Leaf Surface Roughness

Examples of 3-D light micrographs of four cool-season grass species leaf surface roughness. 1-4) Tall fescue, Kentucky bluegrass, creeping bentgrass, perennial ryegrass, respectively. a-b) Adaxial and abaxial (Scale Bars = 150μm)

Averages:
- Adaxial: 3.14
- Abaxial: 1.71

Stomata Analysis

<table>
<thead>
<tr>
<th>Warm-season</th>
<th>Cool-Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adaxial</td>
<td>Adaxial</td>
</tr>
<tr>
<td>Mean Stomata mm² : 28.3</td>
<td>Mean Stomata mm² : 11.9</td>
</tr>
<tr>
<td>Range: 9.0-65.1</td>
<td>Range: 0-18.3</td>
</tr>
<tr>
<td>Mean Stomata size : 18.1 μm</td>
<td>Mean Stomata size : 25.8 μm</td>
</tr>
<tr>
<td>Range: 14.7-36.9</td>
<td>Range: 0-36.9</td>
</tr>
<tr>
<td>Abaxial</td>
<td>Abaxial</td>
</tr>
<tr>
<td>Mean Stomata mm² : 23.0</td>
<td>Mean Stomata mm² : 6.0</td>
</tr>
<tr>
<td>Range: 5.15-40.4</td>
<td>Range: 0-17.5</td>
</tr>
<tr>
<td>Mean Stomata size : 23.0 μm</td>
<td>Mean Stomata Size : 28.9 μm</td>
</tr>
<tr>
<td>Range: 15.2-40.7</td>
<td>Range: 0-36.9</td>
</tr>
</tbody>
</table>
Stomata Analysis

- Log transformed relationship among plant leaf stomata size and plant leaf stomata density. Each point represents the adaxial or abaxial means or stomata size and density. (R2=0.7009, p = <0.0001)

Cuticle Analysis: Warm-Season

- Celebration had highest amount of total wax and primary alcohols
- Primary alcohol content was variable for species
- Composition revealed multiple compounds comprise the primary alcohol group, contrary to cool-season

Cuticle Analysis: Cool-Season

- Abbey, Kentucky bluegrass had the most total wax
- Considerably more total wax for cool-season than warm-season
- Primary alcohol content mostly composed of one compound, C26 hexacosanol

Cuticle Morphology

Examples of scanning electron micrographs of grass species cuticle morphology. A-I) Bermudagrass, zoysiagrass, seashore paspalum, St. Augustinegrass, Kentucky bluegrass, perennial ryegrass, tall fescue and creeping bentgrass respectively. Images were acquired at 5,000X, Scale Bar = 5 μm.

Leaf Hydrophobicity: Warm-Season

Light micrographs of water droplet on six warm-season grass species used to test hydrophobicity. A-B) Common bermudagrass, zoysiagrass, seashore paspalum, St. Augustinegrass, Kentucky bluegrass, and perennial ryegrass respectively. a-b) adaxial and abaxial (Scale Bar = 500 μm)

Leaf Hydrophobicity: Cool-Season

Light micrographs of water droplet on six cool-season grass species used to test hydrophobicity. A-B) Kentucky bluegrass, perennial ryegrass, tall fescue, white clover, and creeping bentgrass respectively. a-b) adaxial and abaxial (Scale Bar = 500 μm)
Correlation Analysis
Cool-Season Adaxial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contact Angle</th>
<th>Stomata Size</th>
<th>Stomata Density</th>
<th>Leaf Surface Area</th>
<th>Leaf Surface Roughness</th>
<th>Total Cuticle Wax</th>
<th>Primary Alcohol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle</td>
<td>-0.0005</td>
<td>-1.1052</td>
<td>-0.5623</td>
<td>-0.7585</td>
<td>-0.0493</td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td>Stomata Size</td>
<td>0.7322</td>
<td>0.0818</td>
<td>0.6126</td>
<td>0.6127</td>
<td>-0.1990</td>
<td>0.0780</td>
<td></td>
</tr>
<tr>
<td>Stomata Density</td>
<td>0.3315*</td>
<td>0.4008</td>
<td>0.4895</td>
<td>-0.1318</td>
<td>0.3395</td>
<td>0.3500*</td>
<td></td>
</tr>
<tr>
<td>Leaf Surface Area</td>
<td>0.5805*</td>
<td>0.5289*</td>
<td>0.5496</td>
<td>0.3069</td>
<td>0.3400</td>
<td>0.0640*</td>
<td></td>
</tr>
<tr>
<td>Leaf Surface Roughness</td>
<td>0.8235*</td>
<td>0.6753*</td>
<td>0.6995</td>
<td>0.4354</td>
<td>0.0106*</td>
<td>0.0405</td>
<td></td>
</tr>
<tr>
<td>Total Cuticle Wax</td>
<td>0.3138*</td>
<td>0.6878</td>
<td>0.9826</td>
<td>0.1398</td>
<td>0.0106*</td>
<td>-0.0000*</td>
<td></td>
</tr>
<tr>
<td>Primary Alcohol Content</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Correlation coefficients for plant leaf characteristics for adaxial leaf side of cool-season plants

Question: Do the plant leaf surface characteristics influence actual foliar N absorption?

Part III: Microscopic Investigation of Solution Uptake and $^{15}$N Absorption

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Contact Angle</th>
<th>Stomata Size</th>
<th>Stomata Density</th>
<th>Leaf Surface Area</th>
<th>Leaf Surface Roughness</th>
<th>Total Cuticle Wax</th>
<th>Primary Alcohol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contact Angle</td>
<td>-0.1850</td>
<td>-0.8484</td>
<td>-0.7266</td>
<td>-0.7550</td>
<td>-0.1615</td>
<td>0.1056</td>
<td></td>
</tr>
<tr>
<td>Stomata Size</td>
<td>0.5602</td>
<td>0.3466</td>
<td>0.5304</td>
<td>0.3779</td>
<td>-0.5562</td>
<td>-0.3417</td>
<td></td>
</tr>
<tr>
<td>Stomata Density</td>
<td>0.8923</td>
<td>0.3466</td>
<td>0.5304</td>
<td>0.3779</td>
<td>-0.5562</td>
<td>-0.3417</td>
<td></td>
</tr>
<tr>
<td>Leaf Surface Area</td>
<td>0.3355</td>
<td>0.1144</td>
<td>0.0952</td>
<td>-0.6258</td>
<td>-0.4515</td>
<td>-0.1442</td>
<td></td>
</tr>
<tr>
<td>Leaf Surface Roughness</td>
<td>0.0242*</td>
<td>0.6678</td>
<td>0.2518</td>
<td>0.6407*</td>
<td>0.4605</td>
<td>0.4288</td>
<td></td>
</tr>
<tr>
<td>Total Cuticle Wax</td>
<td>0.0160*</td>
<td>0.6186</td>
<td>0.0769</td>
<td>0.1652</td>
<td>0.6143</td>
<td>0.9257</td>
<td></td>
</tr>
<tr>
<td>Primary Alcohol Content</td>
<td>0.0176*</td>
<td>0.6522</td>
<td>0.0882</td>
<td>0.5874</td>
<td>0.1905</td>
<td>-0.0000*</td>
<td></td>
</tr>
</tbody>
</table>

* Correlation coefficients for plant leaf characteristics for adaxial leaf side of warm-season plants

Part III: Objective

The objectives were to investigate urea fertilizer solution uptake using confocal microscopy and to determine $^{15}$N absorption on various warm- and cool-season plant species.

Conclusions

- Leaf surface roughness
  - Variable for species and leaf side
  - Correlated with contact angle
- Cuticle Analysis
  - Variable for species
  - Cool-season vs. warm-season plants
  - Primary alcohol related to cuticle morphology
  - TCW and PA correlate with hydrophobicity
- Stomata analysis
  - Stomata density: Adaxial > Abaxial
  - Warm-season > Cool-Season
  - Stomata size negative relationship with stomata density

Question: Do the plant leaf surface characteristics influence actual foliar N absorption?
Part III: Materials and Methods

Plant Leaf Solution Uptake

- Solution Uptake
  - FITC (Fluorescein isothiocyanate) or HCCA (7-hydroxycoumarin-3-carboxylic acid) was added to the solution (2mg/ml)
- Harvested 24h after application
- Leaves fixed with 50/50 (v/v) 1X PBS/glycerol
- Leica SP8X-MP (63X oil immersion objective, 2X zoom)

15N Solution Absorption and Stomata Uptake

- 15N-labeled urea solution
  - 0.98 g N m⁻²
  - 2.67 % atom ¹⁵N in fertilizer
- Applied with spray chamber
- 280 L ha⁻¹ carrier volume
- Stomata Involvement
  - ¹⁵N-labeled urea solution
  - applied with brush
  - adaxial vs. abaxial leaf sides

Part III: Results

Imaging Solution Uptake

Examples confocal microscopy images demonstrating foliar applied solution deposition and penetration on four grass species 1-4) Celebration, common bermudagrass, Abbey, Kentucky bluegrass, Palisades, zoysiagrass, Palmetto, St. Augustinegrass, respectively a-b) Overlay of red and blue channels and depth coding imaging, respectively.

15N Absorption

- Percent ¹⁵N-labeled urea absorption 24 h after foliar application by 12 turfgrass cultivars
- Mean ¹⁵N Absorption: 20.7 %
- Warm-Season: 23.3%
- Cool-Season: 15.6%
**15N Absorption Correlation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>%15N Absorption</th>
<th>Leaf Surface Roughness</th>
<th>Total Cuticle Wax</th>
<th>Primary Alcohol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf Surface Roughness</td>
<td>0.0019 ±*</td>
<td>0.8086</td>
<td>0.0116</td>
<td></td>
</tr>
<tr>
<td>Total Cuticle Wax</td>
<td>0.0033 ±*</td>
<td></td>
<td>0.9064</td>
<td></td>
</tr>
<tr>
<td>Primary Alcohol Content</td>
<td>0.0654 ±*</td>
<td>0.0013 ±*</td>
<td>&lt;0.0001 ±*</td>
<td></td>
</tr>
</tbody>
</table>

* Correlation Coefficients for %15N Absorption and Adaxial Plant Leaf Characteristics

* Correlation Probability for %15N Absorption and Adaxial Plant Leaf Characteristics

**Regression Model Accuracy**

Regression analysis for actual %15N absorption by predicted %15N absorption.

- \( R^2 = 0.9769, \ p = <0.0001 \)

**Conclusions**

- Solution Uptake
  - Collected at and around stomata complexes and valleys
  - Penetration maybe be occurring at stomata, not conclusive

- 15N Absorption
  - Variation between species
  - Warm-season greater uptake than cool-season grasses
  - Stomata density increased foliar uptake for St. Augustinegrass
  - Correlated with surface roughness, total cuticle wax and primary alcohol

- Multiple Regression Analysis
  - Stomata size, leaf surface roughness and total cuticle
  - As predictors increase, 15N absorption decreases
  - Complex process

**Summary and Perspectives**

**Part I:**
- Solution chemistry and concentration affect hydrophobicity
- Determine solution and plant species interaction

**Part II:**
- Plant leaf surface characteristics influence hydrophobicity (Sx, TCW, PA)
- Stomata density greater for adaxial leaf side and warm-season plants

**Part III:**
- Plant leaf surface characteristics = good predictors of foliar 15N absorption
- Stomata, leaf surface roughness and total cuticle wax

**Future Research:**
- Expand species (broad leaf) and solution uptake
- Application timing
- Identify second chance uptake
References


Funding Sources

Thank You

Questions?