Black Kernel Rot Malady of Pecan

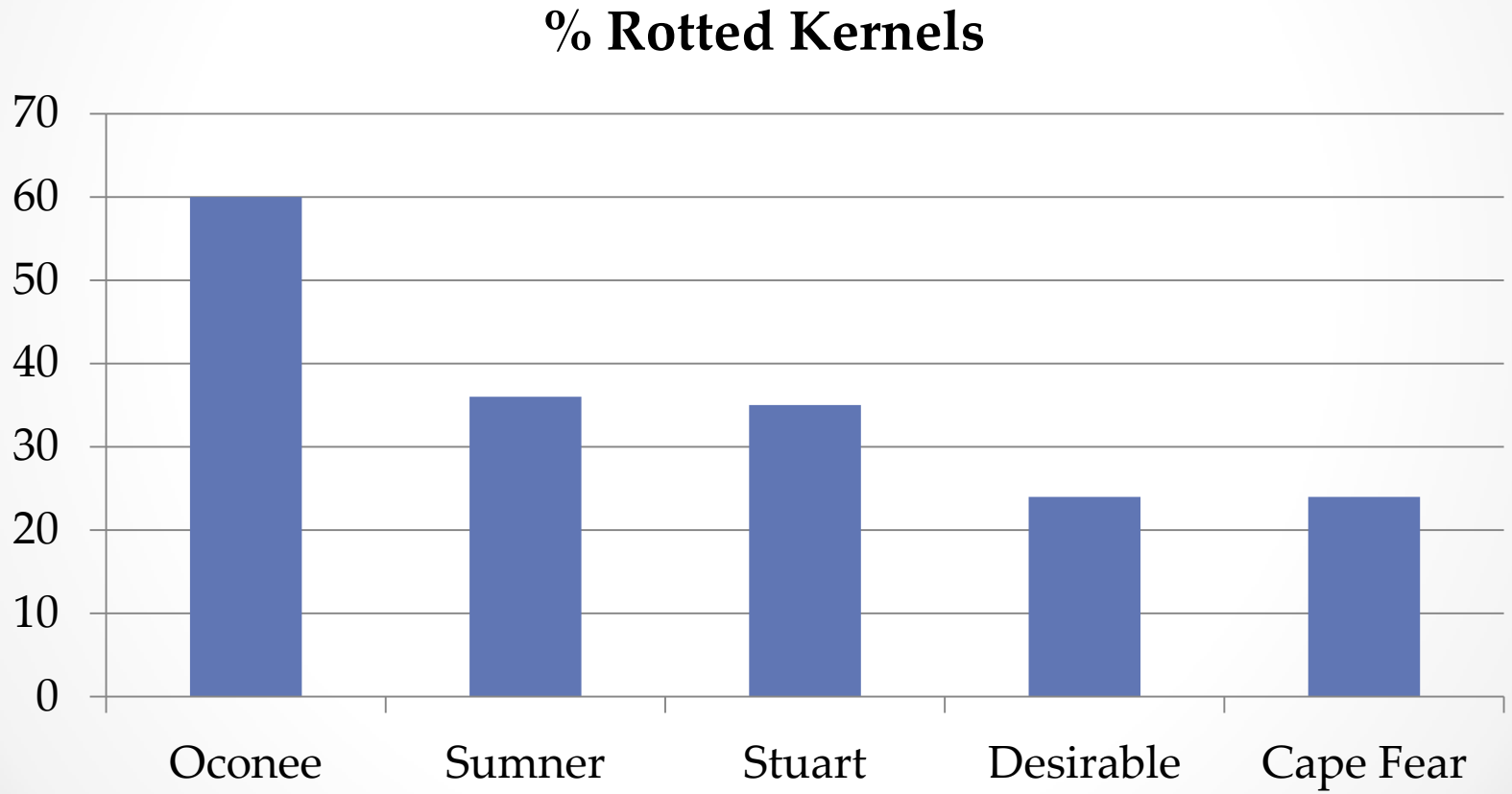
B Wood, C Bock, I Wells, T Cottrell, M Hotchkiss

Black Kernel Rot: What is it?

Black Kernel Rot: 'Black Phase'

Black Kernel Rot: 'Brown Phase'

Sampling of Fruit for Black Kernel Rot Malady (Lenny Wells)*



Wells, L. 2015. Quality Problems Affecting 2015 Pecan Crop. The Pecan Grower Magazine 26(6):4, 6-8.

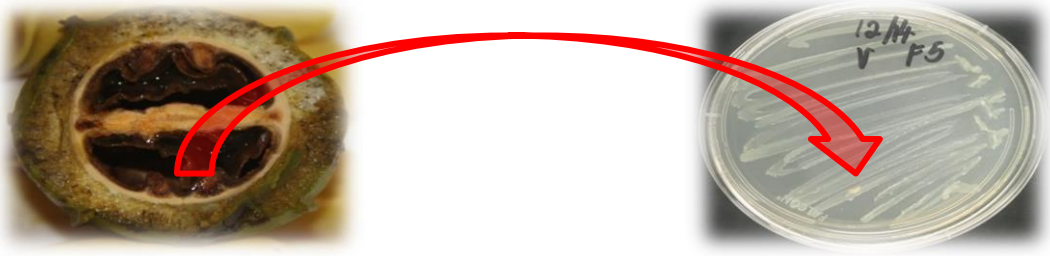
Possible Causes?

- Insect Feeding Damage
- Fungal or Bacterial Pathogen
- Phosphite Toxicity
- Nutrient Element Toxicity
- Herbicide Damage
- Pollination Associated Latent Pathogen
- Self Pollination Defect (inbreeding)
- Immobile Nutrient Element Deficiency to Seed Coat
- Water-stage Fruit Split & Bacterial Interaction

Cause?

- ~~Insect Feeding Damage~~
- ~~Fungal or Bacterial Pathogen~~
- ~~Phosphite Toxicity~~
- ~~Nutrient Element Toxicity~~
- ~~Herbicide Damage~~
- ~~Pollination Associated Latent Pathogen~~
- ~~Self Pollination Defect (Inbreeding)~~
- Immobile Nutrient Element Deficiency to Seed Coat?
- Water-stage Fruit Split & Bacterial Interaction?

‘Black Phase’
What is the Black Stuff ???



- **Drs. Bock and Hotchkiss:**
  - **DNA extraction and then 16S rRNA gene sequencing through Lucigen Laboratories by Dr. John McInroy from Auburn University) of 8 culture samples from kernels exhibiting symptoms**
  - **Endophytic bacteria (a parasitic plant organism living within its host’s body)**
  - ***Rhizobium larrymoorei*** **(formally** ***Agrobacterium larrymoorei*****; originally isolated from aerial gall on cut branches of weeping fig)**
  - **So, the bacteria appears to be non pathogenic and therefore not the causal factor of the malady.**

Rhizobium larrymoorei: a Close Relative to Crown Gall on Pecan Roots

- http://www.bing.com/images/search?q=image+of+crown+gall+on+pecan&view=detailv2&&id=FD5B28A0BB9FBE79F0D1CBC1F2B8212851FF059F&selectedIndex=8&ccid=1z2N%2bNdm&simid=608053772389712015&thid=OIP.Md73d8df8d766e49ac2ef0c598d0996a2o0&ajaxhist=0

‘Brown Phase’ Due to Lack of Bacterial Colonization ?

- ***The malady without colonization by <u>Rhizobium larrymoorei</u> endophytic bacterium***

Anecdotal Evidence

- **So, if a bacteria is unlikely the fundamental causal factor, then what is?**
  - **Clues:**
    - **1)** All carefully dissected symptomatic kernel seed coats exhibited splits or tears (i.e., brittle inelastic seed coats), usually in the dorsal (upper side) basal (stem end) half of the seed coat.
    - **2)** Basal half's of kernel seed coats were darker brown in color than normal (i.e., likely accumulation of polyphenols due to blocked metabolism)
    - **3)** Seed coat tissue is fed by the phloem, so there is a missing factor that is only conditionally phloem mobile (i.e., a non-mobile nutrient)
    - **4)** There was water-stage fruit split in the trees (i.e., slow movement of K+ across seed coat plasma membrane)

Anecdotal Evidence

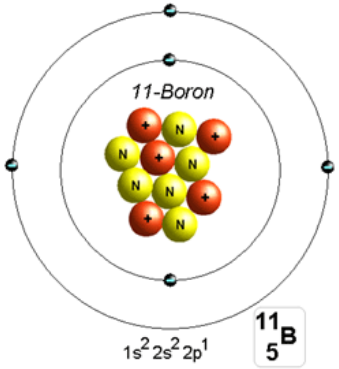
- **So, if bacteria is unlikely to be the fundamental causal factor, then was is?**
  - **Clues**
    - **5)** Delayed lignification of shell (i.e., interference of lignin metabolism)
    - **6)** The malady, and water split, were simultaneously random within a tree (i.e., transitory deficiency of a non-mobile micronutrient element)
    - **7)** Incidence of the malady appeared to increase with crop load (i.e., a limited supply, of dilution, of a sparingly-mobile factor)
    - **8)** Poor kernel filling (i.e., inhibited phloem downloading or impaired membrane permabilty)
    - **9)** Exhibited by trees growing on southeastern soils but not by trees in southwestern soils (i.e., a factor limiting in SE soils but not in SW soils)

So, what is the simplest common denominator explaining all of the above symptoms?

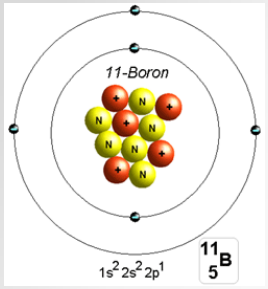
Simplest Common Denominator?

Characteristic	Zn	Cu	B	Mo	Ni	Fe	Mn	N	P	K	S	Ca
Major Limiting Factor in SE Soils	X	X	X	X	X	X	X	X	X	X	X	X
Seed Coat Polyphenol Accum.	X	X	X		X	X	X					
Non Mobile Nutrient Element			X				X					X
Inelastic Seed Coat			X									
K+ Movement in Fruit			X							X		
Blockage of Lignin Metabolism	X	X	X		X	X	X					
Transitory Deficiency in Rapid Growing Fruit Tissue			X		X		X					X
Impaired Kernel Filling	X	X	X	X	X	X	X					
Risk of Timely Availability to Seed			X				X					X

The Common Denominator: Boron (B) Deficiency???



- ***Wait !!!*** --- How can B be deficient if July-August leaf analysis shows B sufficiency (i.e., 50-60 ppm), and B is often applied annually with fertilizer in Feb-Mar. ???



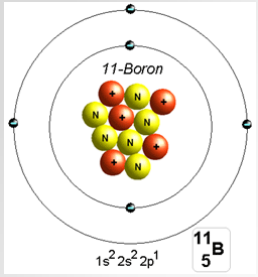
Boron Deficiency???

- How can there be 50-60 ppm B in July-August leaf analysis but nuts still be B deficient ?

Only a phloem connection to seed coat

No xylem connection to seed coat

- Because B is an <u>immobile</u> element in tree species, like pecan, that move sugar as sucrose. B does not mobilize once deposited in tissue.
  - B rarely moves (i.e., conditionally mobile) in the phloem portion of the vascular system
  - This means that no matter how much B is in foliage, there can be transitory deficiency in rapidly growing fruit or tissues; especially if roots are unable to access B in soils while fruit are rapidly growing.



Most Likely Cause!

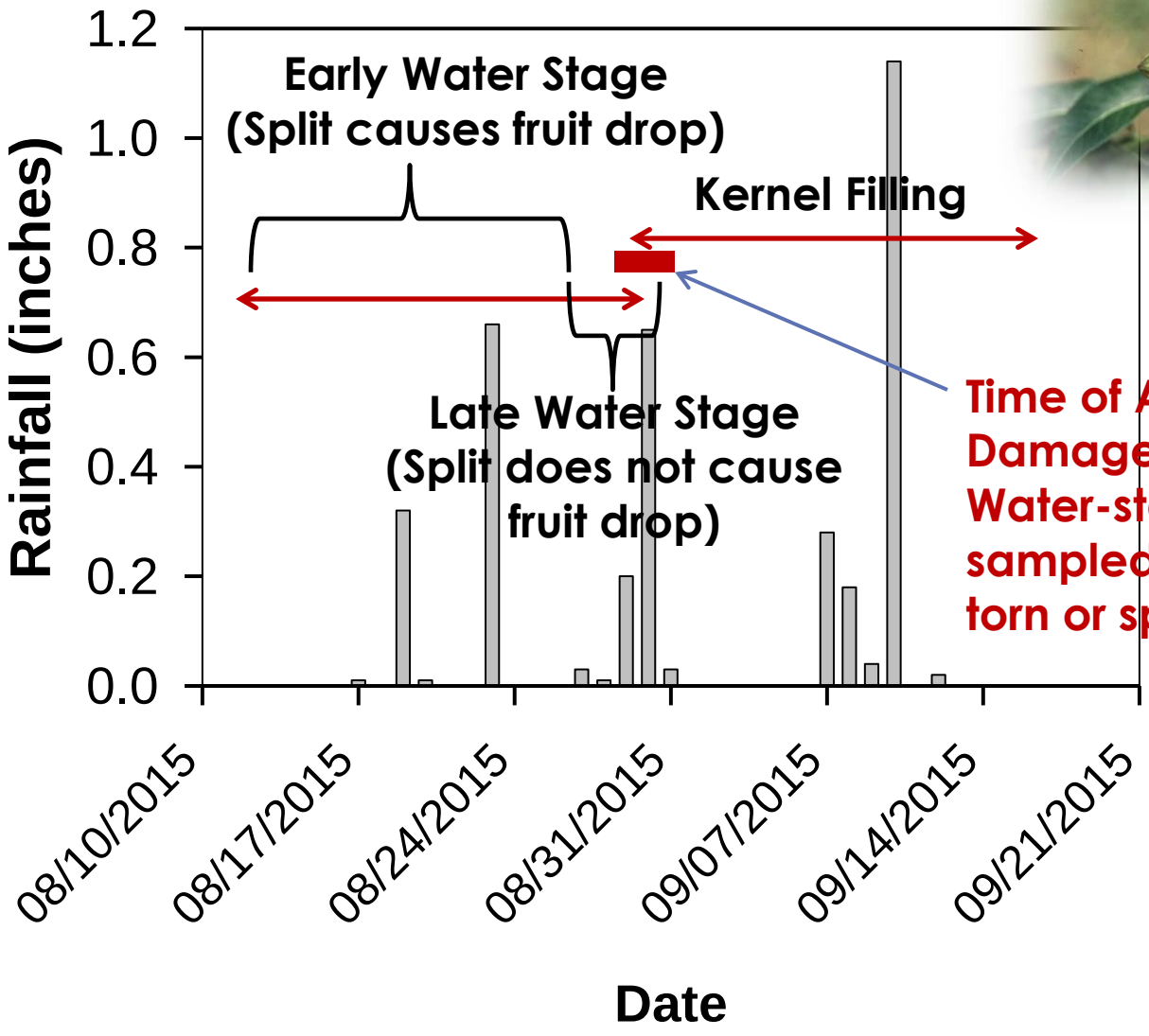
Boron Deficiency Interacting with the Turgor Physiology of Water-stage Fruit Split and Delayed Shell Lignification

[Possibly Accentuated by a Transition Metal Deficiency (Mn) With Incidental Bacterial Colonization]?

Typical Water-stage Fruit Split

Fruit drop 7 days after seed coat splits

Rainfall During Late Kernel Development



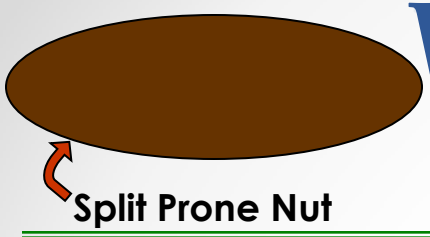
Time of Apparent Latent Damage Due to Late Water-stage Split? All sampled kernels had torn or split seed coats.

Atypical Water Split

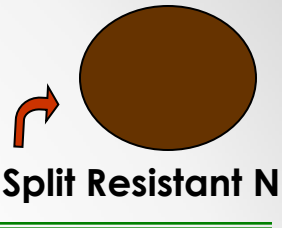
Or

Instead of typical water-stage split that causes fruit to drop, we get very late stage seed coat split which in turn causes the 'Brown/Black Kernel Rots', but not drop?

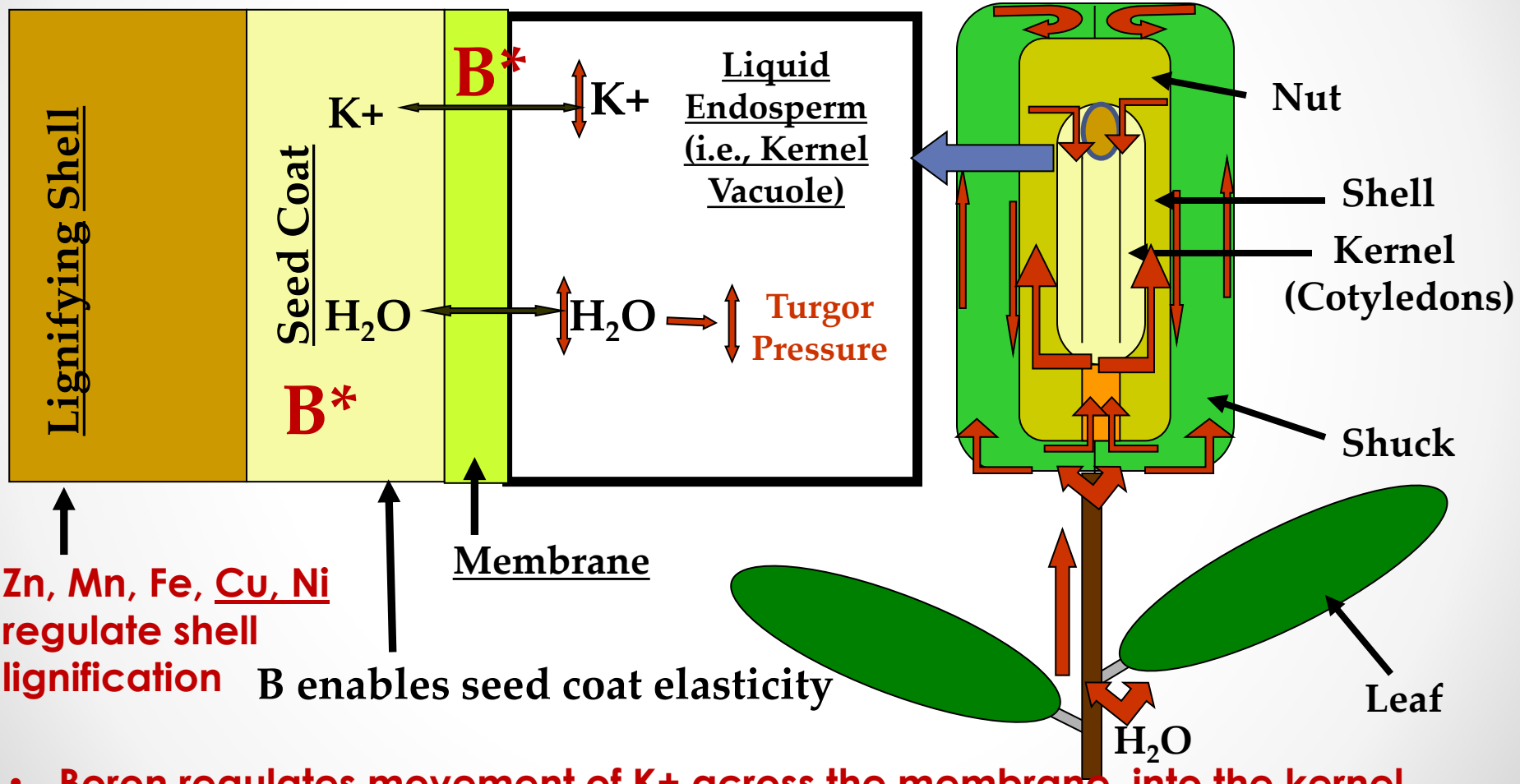
Water Stage Fruit Split



Split Prone Nut

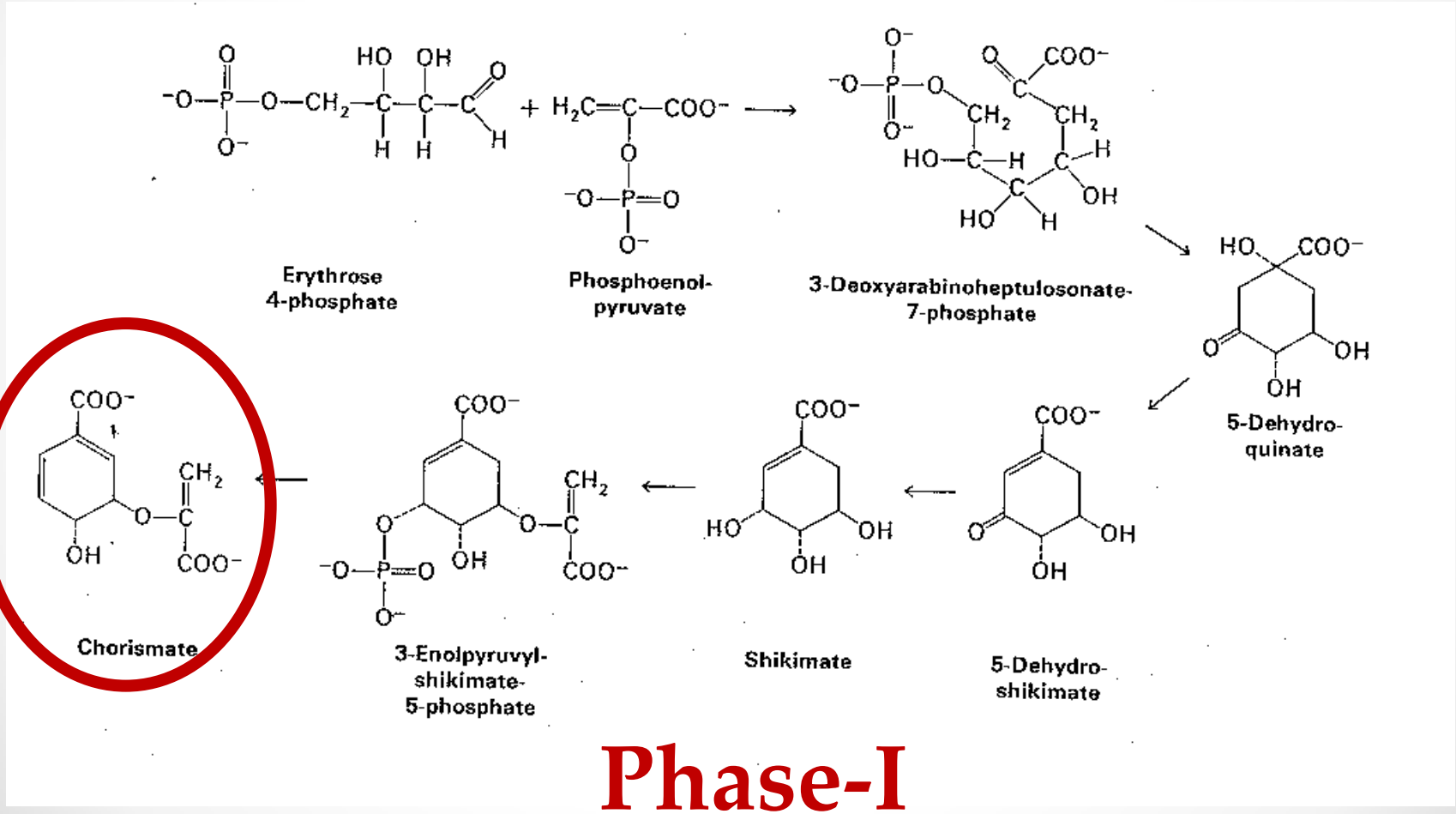


Split Resistant Nut

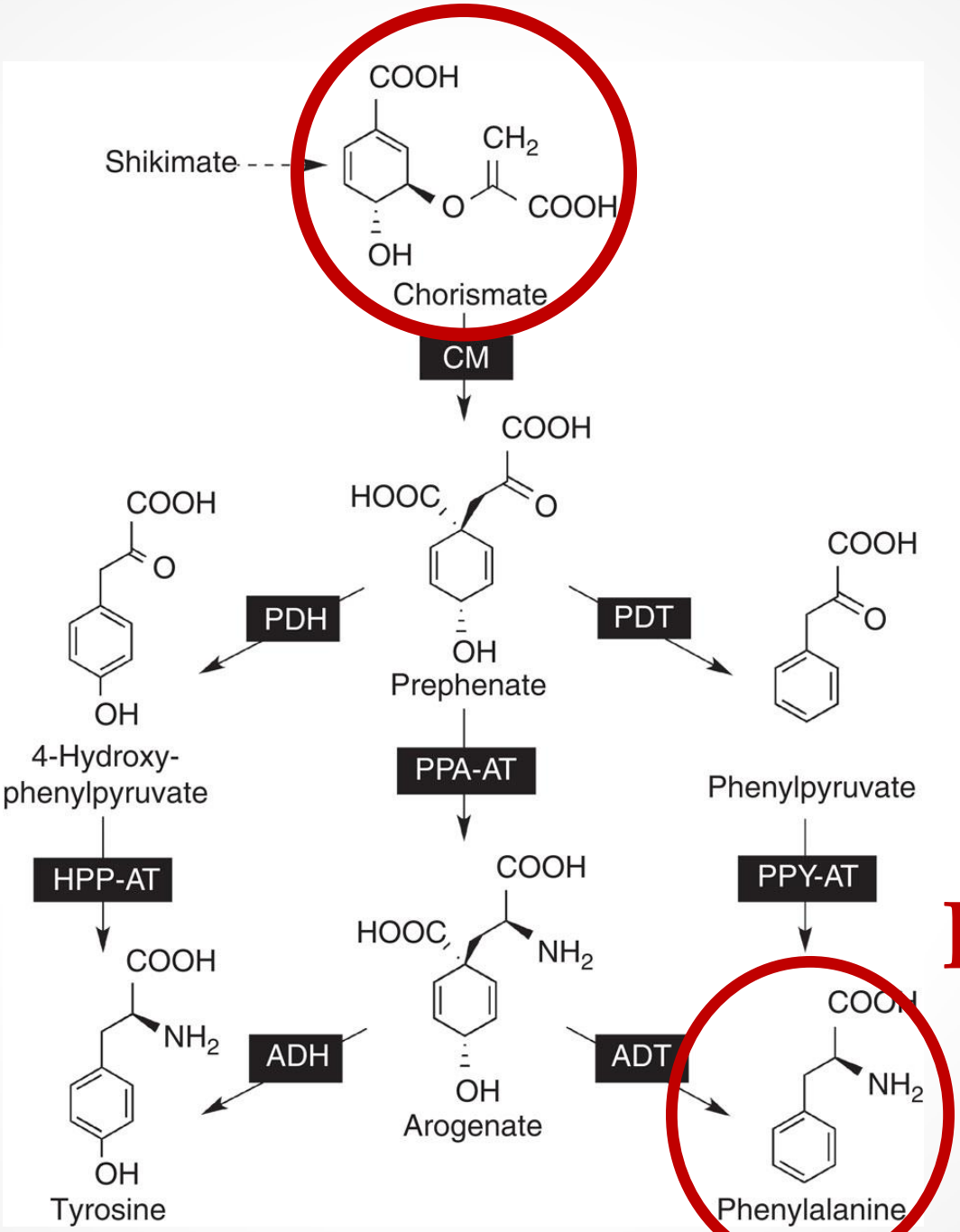


- Boron regulates movement of K+ across the membrane into the kernel vacuole, and K+ controls the pressure on the seed coat and shell.

Lignin as a Secondary Factor: Pathway for Lignin Biosynthesis in Pecan Shells

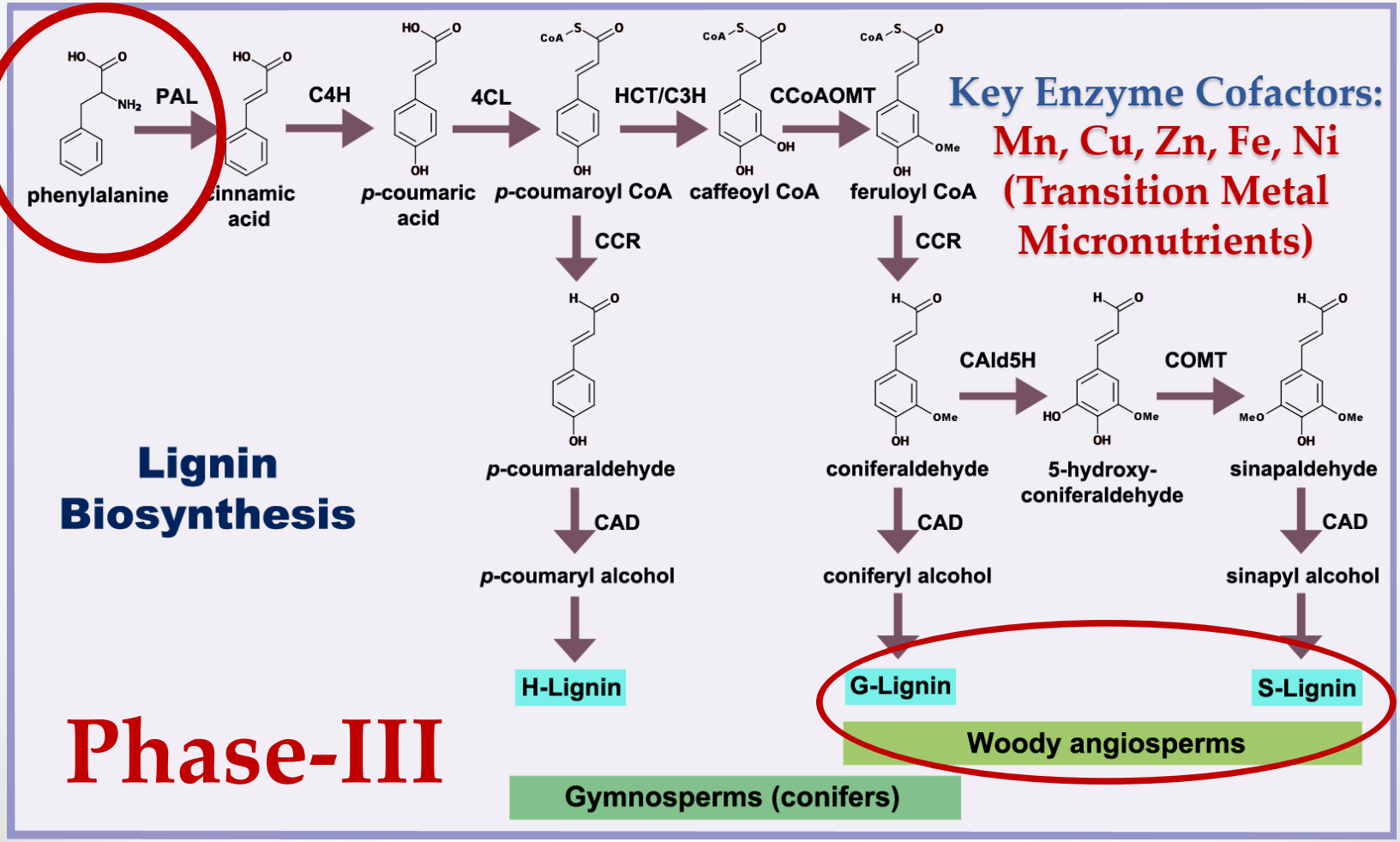


Phase-I

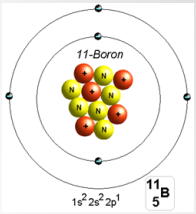

Shikimate
COOH
CH2
O
COOH
OH
Chorismate
CM
COOH
HOOC
O
OH
Prephenate
PDH
PDT
COOH
O
OH
4-Hydroxy-
phenylpyruvate
COOH
O
Phenylpyruvate
PPA-AT
HPP-AT
PPY-AT
COOH
HOOC
NH2
OH
Arogenate
COOH
NH2
OH
Tyrosine
ADH
ADT
COOH
NH2
Phenylalanine

Phase-II

Insufficient B, Mn, Cu, Zn, Fe, or Ni reduces lignin biosynthesis, so shells are slow to stiffen enough to withstand the turgor pressure exerted from the liquid endosperm---so nuts split.

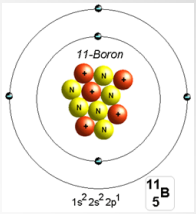


Boron



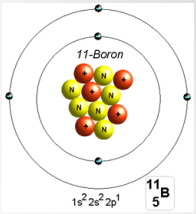
- Plant requirement for B is higher than for any other micronutrient !
- World wide, B deficiency is more extensive than deficiency of any other plant micronutrient !
- Most Southeastern soils are low in B, with the B problem accentuated during rapid growth phases, especially if soil is dry when leaves or fruit are rapidly growing
- B enables elasticity of seed coat, membranes, and cell walls

Boron



- B deficiency (e.g., transitory deficiency) causes poor seed yield and quality (i.e., poor filling) and increases fruit drop.
- B has great impact on reproductive organs. B is also required for lignin deposition.
- B is required for pollen tube growth or the tubes burst due to inelasticity; pollen tube follows B gradient to locate the egg.
- B indirectly regulates seed turgor pressure via determining rate of K^+ influx and efflux of the associated liquid endosperm.
- In most plants, B is immobile once deposited in tissue. B is not remobilized in pecan; so, can have plenty of B in leaves; yet, there is a deficiency in the nut due to dry soil during rapid fruit sizing.

Boron



- Low B causes accumulation of polyphenols in seed coats—i.e., seed coats that are darker brown, or amber grade
- B is xylem mobile, but only 'conditionally' mobile in pecan phloem; thus, there is only limited mobility to the developing seed and seed coat.
- High soil Calcium and/or Aluminum reduces B uptake, so high soil (in feeder root zone) or plant Ca means that the tree's B requirement is higher (Ca:B ratio of about 3,000:1)
- The higher leaf B, the more likely B in seed coat will be sufficient, unless soil is dry or high in Ca or Al
- Probably should manage for leaf B at 75-100 ppm DW and
- with season-long moist soil?

Areas of Boron Deficient Soils

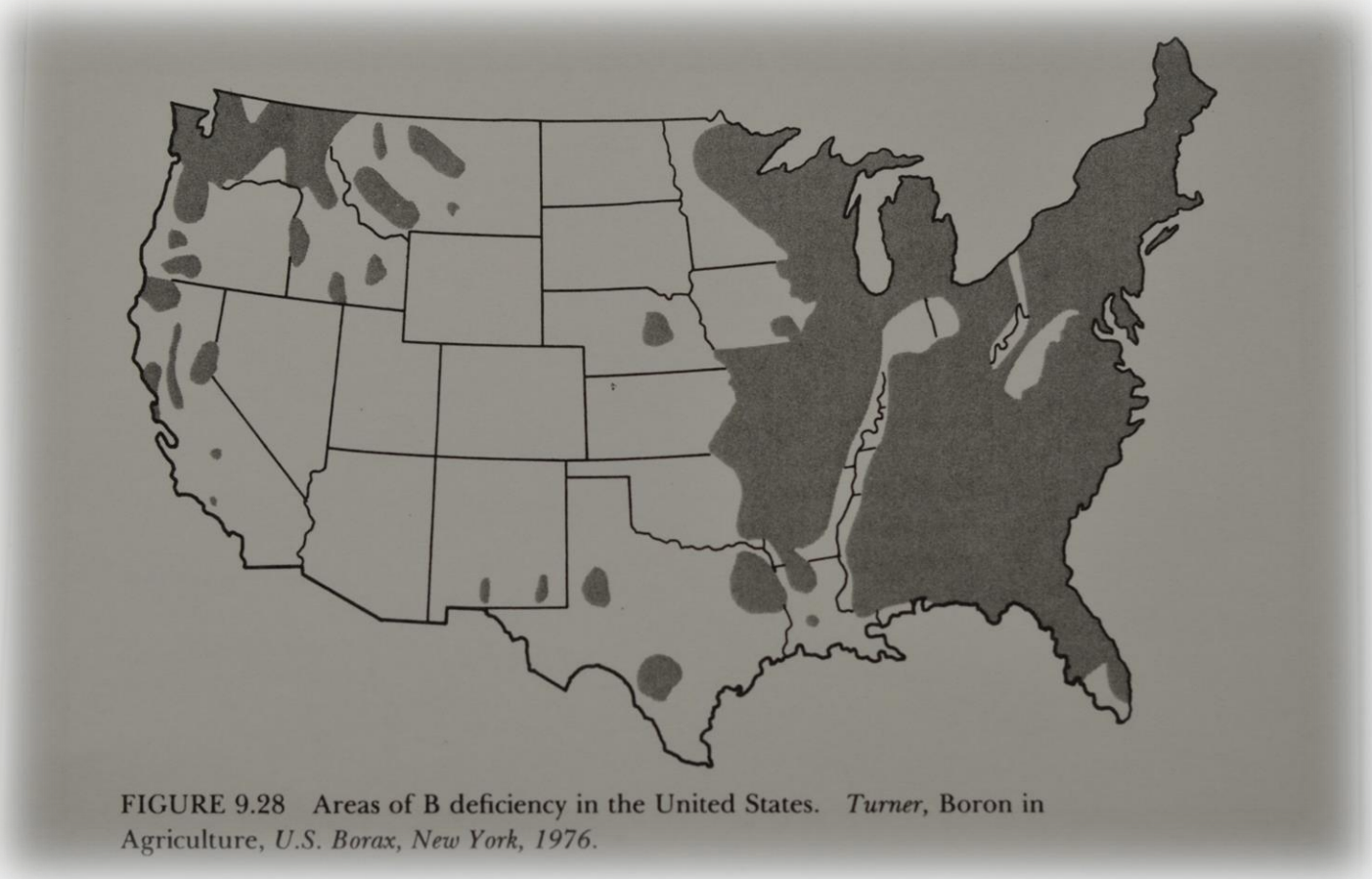
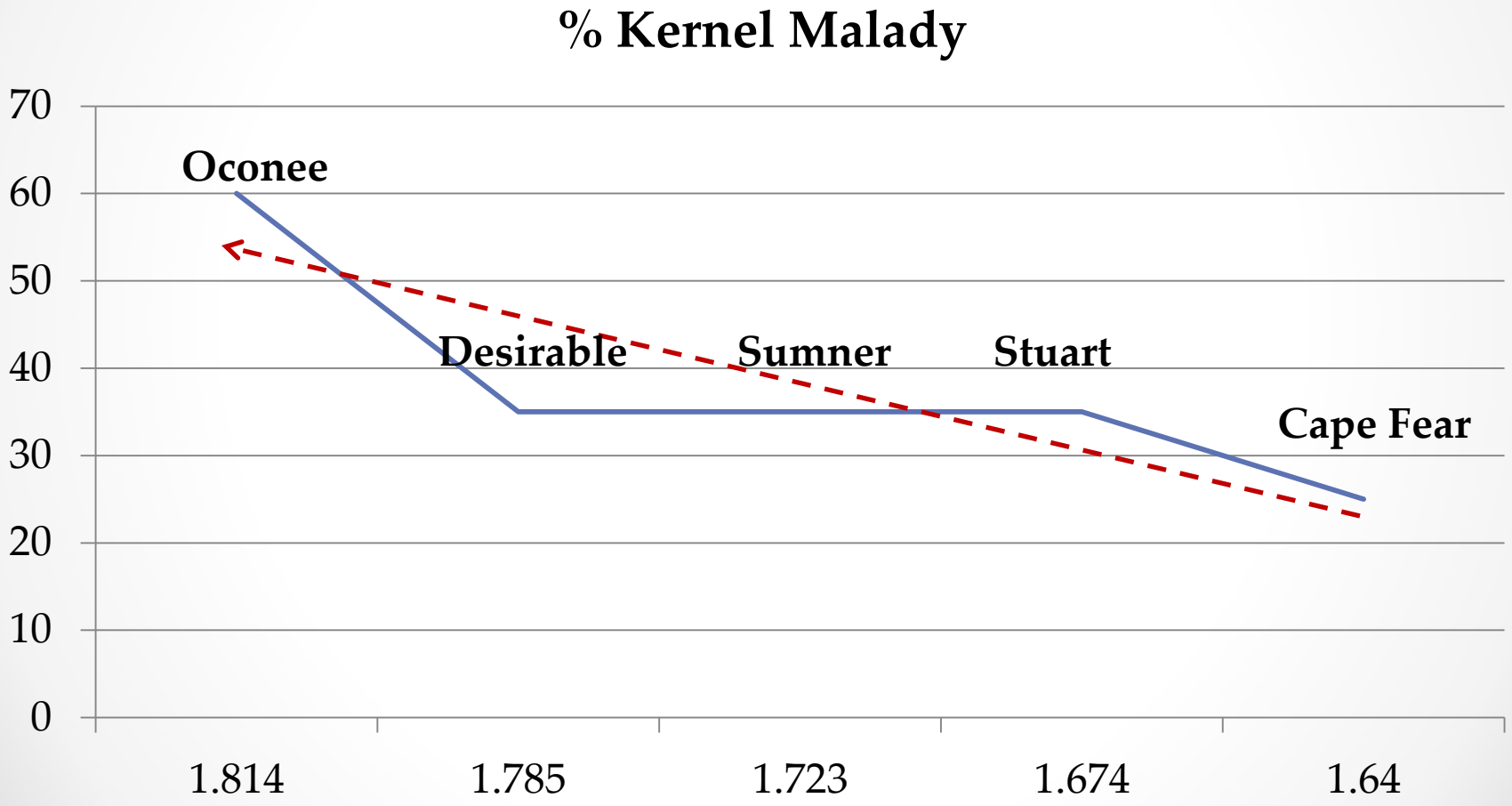


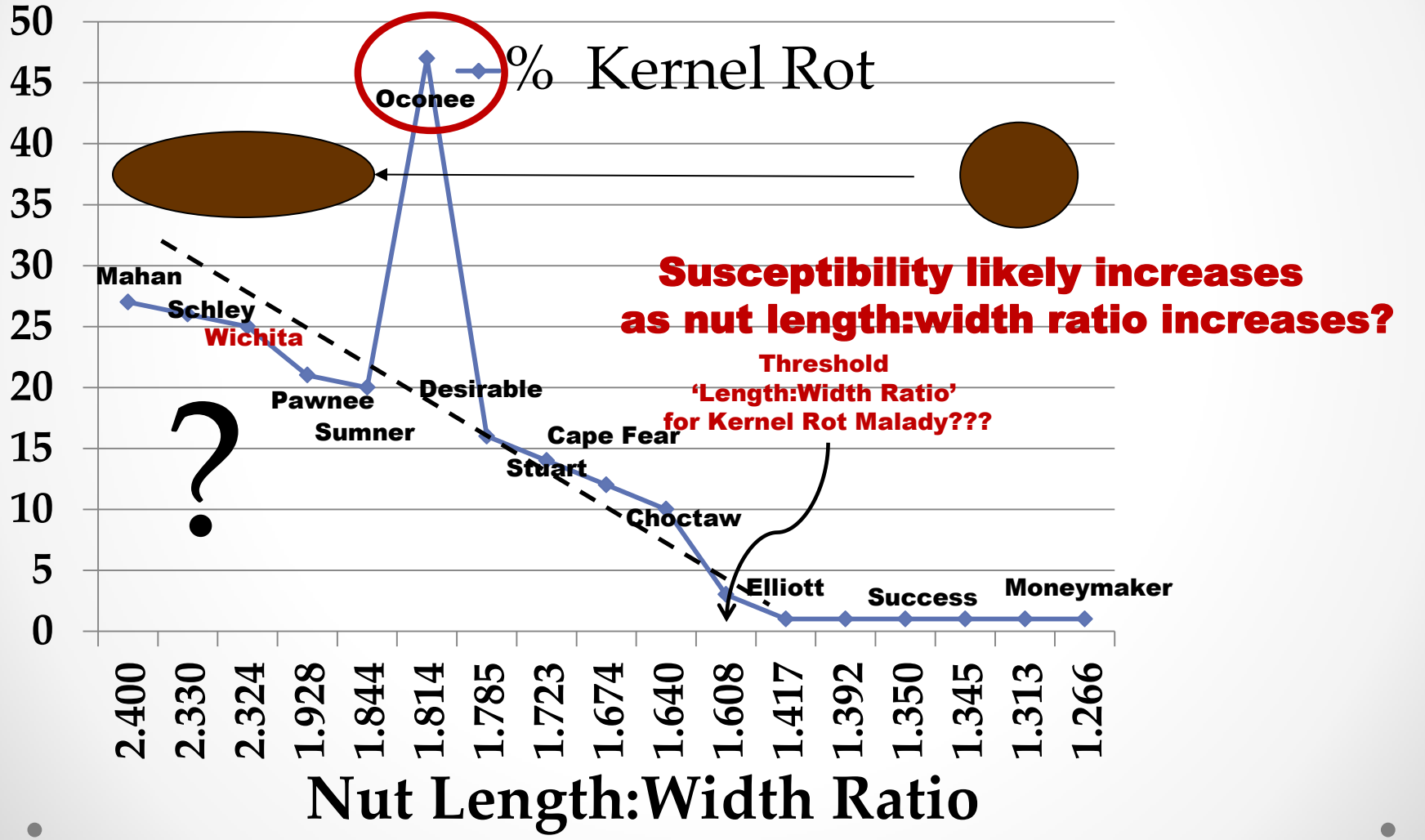
FIGURE 9.28 Areas of B deficiency in the United States. *Turner*, Boron in Agriculture, *U.S. Borax, New York, 1976.*

Relationship Between Black Kernel Malady and 'Nut Length:Width Ratio'*



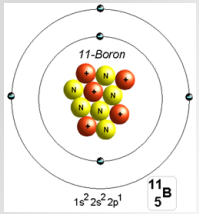
* L. Wells. 2015, Pecan Grower

Nut Shape vs. 'Black/Brown' Kernel Rot



Oconee (I)

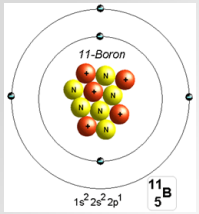
- **Oconee ('Schley' X 'Barton';56-7-72)**
  - **Released in 1989 for use in the southern U.S. pecan belt**
  - **48 nuts/lb, 56% kernel; elliptic, with obtuse apex and base; round in cross section**
  - **Type-I (Protandrous), with early to mid-season pollen shed and mid- to late-season pistil receptivity, similar to Desirable**
  - **Good precocity and yield potential**
  - **Nuts ripen with Cheyenne, about 21 days after Pawnee**
  - **Moderate scab resistance, fair resistance to downy spot and vein spot**



Managing Orchards to Minimize Incidence of Black Kernel Rot

1) Ensure excellent Boron availability during fruit sizing

- 2-3 foliar B sprays during mid to late August (Metalosate-B @12-18 fluid oz./100 gal/acre, or Solubor @ 1/16 - 1/8 lb B/100 gal/acre; and others)?
- Imparts seed coat elasticity, so it stretches instead of tears; enables kernel filling and lignin deposition, and lighter colored kernel meats
- Enables rapid regulation of turgor pressure in liquid endosperm (K+ transfer)
- B @75-100 ppm DW?



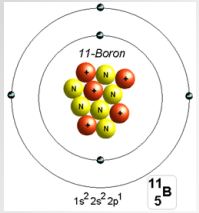
Managing Orchards to Minimize Incidence of Black Kernel Rot

2) Minimize tree water stress during late water stage of fruit development (August), especially just prior to beginning of the gel stage

- Greatly reduces incidence of excessive turgor pressure, rapid expansion and splitting of the seed coat with rainfall or cloud cover

3) Ensure tree Mn, Zn, Fe, Cu and Ni nutrition is in the mid sufficiency range

- Ensures timely lignin biosynthesis and deposition to shell, so shell will be strong enough to withstand the pressure generated by the liquid endosperm upon sudden rainfall, irrigation or cloud cover.



Ramifications?

- **If 'Black Kernel Malady' is fundamentally a 'transitory tissue specific Boron deficiency', then low tissue B during rapid growth is likely causing other profit limiting problems:**
- **1) Reduced fertilization of flowers by pollen (i.e., bursting of pollen tubes)**
- **2) Premature fruit abortion/drop (i.e., inhibited movement of assimilates into new fruit)**
- **3) Water-stage fruit split (i.e., K+ pressure regulation)**
- ***4) Poorly filled kernel meats (i.e., low % kernel; unable to load assimilates into cotyledons)***
- **5) Darker seed coats of kernels (i.e., polyphenol accumulation)**

Areas of Boron Deficient Soils

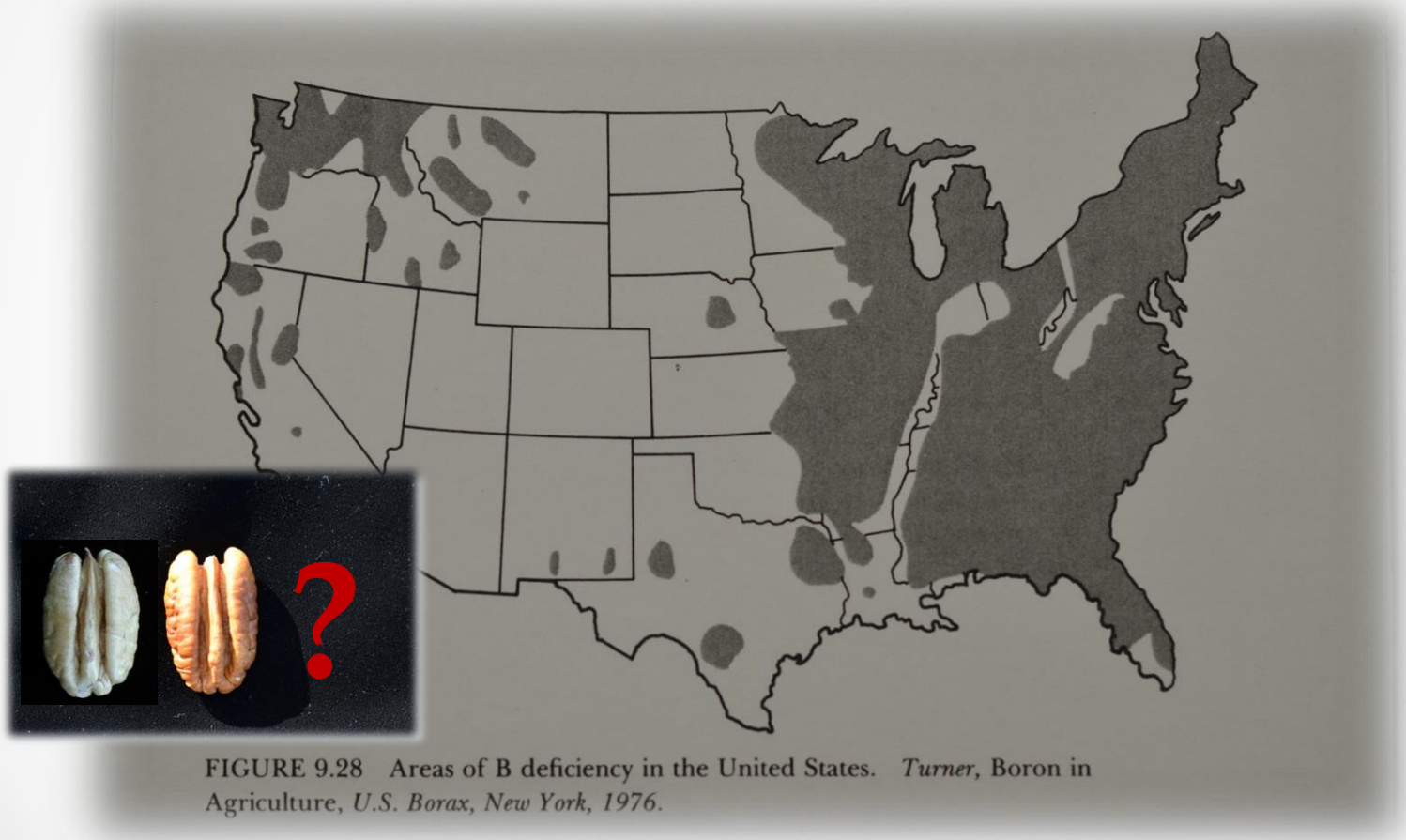


FIGURE 9.28 Areas of B deficiency in the United States. *Turner*, Boron in Agriculture, *U.S. Borax, New York, 1976.*

Kernel skin color: 'Light', 'Light Amber', 'Amber' vs. 'Dark Amber'

The Black Kernel Malady:

Prevent By Improving Boron/Water/Transition Metal Management ?

--The bacteria seems to be non pathogenic and therefore incidental--

Thanks