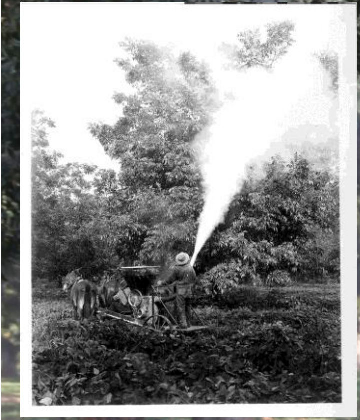
Pecan Scab – A Retrospective, Current Status and Future Management



Clive H. Bock

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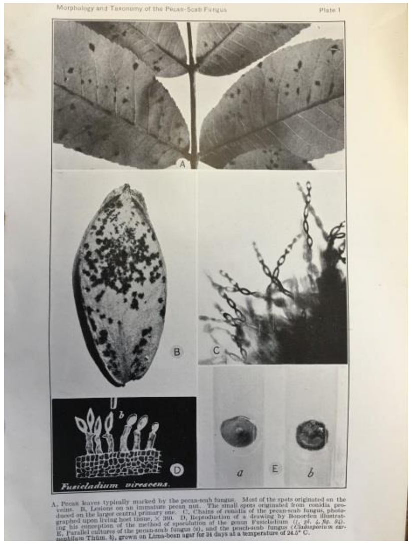
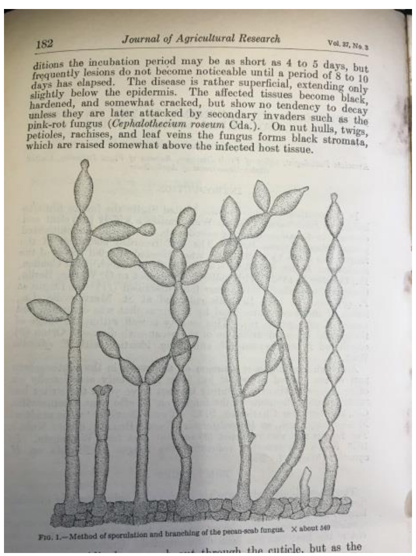
A new disease of hickory species….1882





ditions the incubation period may be as short as 4 to 5 days, but frequently lesions do not become noticeable until a period of 8 to 10 days has elapsed. The disease is rather superficial, extending only slightly below the epidermis. The affected tissues become black, hardened, and somewhat cracked, but show no tendency to decay unless they are later attacked by secondary invaders such as the pink-rot fungus (*Cephalothecium roseum* Cda.). On nut hulls, twigs, petioles, rachises, and leaf veins the fungus forms black stromata, which are raised somewhat above the infected host tissue.

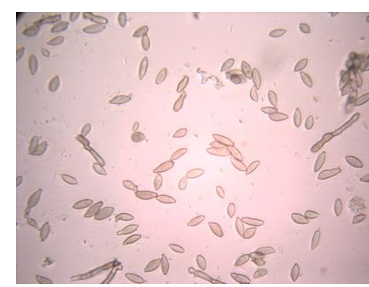
FIG. 1.—Method of sporulation and branching of the pecan-scab fungus.

- The first report: F.S. Earle found symptoms of scab on mockernut (*Carya alba = C. tomentosa*) in Illinois
- He sent samples to G. Winter in Berlin, for identification
- The fungus was subsequently described as *Fusicladium effusum*
- In 1888 Langlois collected a fungus on pecan (*Carya illinoinensis*) that was described as *F. caryigenium*
- Orton later considered these two species to be the same. *Fusicladium effusum = F. caryigenium*
- The pathogen has gone through various name permutations since, but is now firmly *Fusicladium effusum*
- The pecan industry was underway at this stage, having been rapidly expanding since the late eighteen-hundreds

Scab symptoms on foliage

Scab symptoms on fruit

Spores of *Fusicladium effusum*



Demaree, J. B. (1928). Morphology and taxonomy of the pecan-scab fungus, *Cladosporium effusum* wint. USDA Agricultural Research, (37), 181-187

As acreage increased in the Southeast
- an emerging issue for early pecan growers

- By 1924, Demaree noted that "…, scab caused by *Fusicladium effusum* Wint., is preeminently the most important pecan disease."
- In regard to the development of scab as a widespread issue he observed "This condition is thought to be due largely to the increased number of plantings and to the frequent summer rains of the past few years, which have favored the rapid spread of the disease."

Cultivars become susceptible, epidemics develop….

- Several cultivars grown by the early industry in the southeast tended to be very susceptible, including cv Georgia
- Many trees were top worked with alternative cultivars thought to be resistant
- Unfortunately, many of these rapidly become susceptible as the pathogen adapted (e.g. Delmas, Schley, Van Deman)

Demaree, J. B. (1928). Morphology and taxonomy of the pecan-scab fungus, *Cladosporium effusum* wint. USDA Agricultural Research, (37), 181-187

New cultivars are developed and planted

- Other new cultivars were added to the list of potentials, emphasizing various agronomic benefits, including resistance to pecan scab (grower selections, USDA & University Breeding Program releases)
- Cv. Desirable (~1915) was initially immune in at least some locations, but succumbed to scab as it became widely planted (Sparks, 1992)
- Other older cultivars with promise against scab included Stuart and Elliott. There are several others
- Pathogenic variability and adaptability in the pathogen makes it difficult to predict ‘durability’ of resistance

Loss of resistance of pecan cultivars to pecan scab between 1910 and 1956 (Goff et al., 1996) S=susceptible, R=resistant, VR=very resistant.

Cultivar	1910	1920	1931	1940	1954	1956
San Saba	S	S	S	S	S	S
Delmas	R	S	S	S	S	S
Schley	R	S	S	S	S	S
Alley	R	R	S	S	S	S
Success	VR	R	S	S	S	S
Teche	VR	VR	R	S	S	S
Frotscher	VR	VR	R	S	S	S
Moneymaker	VR	VR	R	R	S	S
Stuart	VR	VR	VR	VR	VR	S

Sparks, D. 1992. Pecan cultivars - The orchard’s foundation. Pecan Production Innovations. Watkinsville, GA.
Goff, W.D., McVay, J.R. and Gazaway, W.S. 1996. Pecan production in the Southeast. Alabama Cooperative Extension System Circular ANR-459, University, Auburn, AL. P222.

Spraying fungicides to control scab

- Waite (1911) showed Bordeaux mixture to have some efficacy controlling scab on pecan. Many different chemistries were tested, including sulfur and copper
- Demaree (1924) "Spraying is now being considered somewhat more seriously as a means of reducing the loss by scab, but as yet most of the growers seem to prefer top-working to the more pains-taking and labor involving operation of spraying."
- Other researchers refined the use of Bordeaux mixture, with sprays at 2-3 week intervals recommended
- Problems identified included canopy penetration and tree height – only high powered sprayers and spray guns could reach ~40 ft. "It is hoped that horticulturalists will devise some system of pruning that will tend to reduce the height of the trees and open up their centers so as to facilitate spraying operations." (Demaree, 1924)

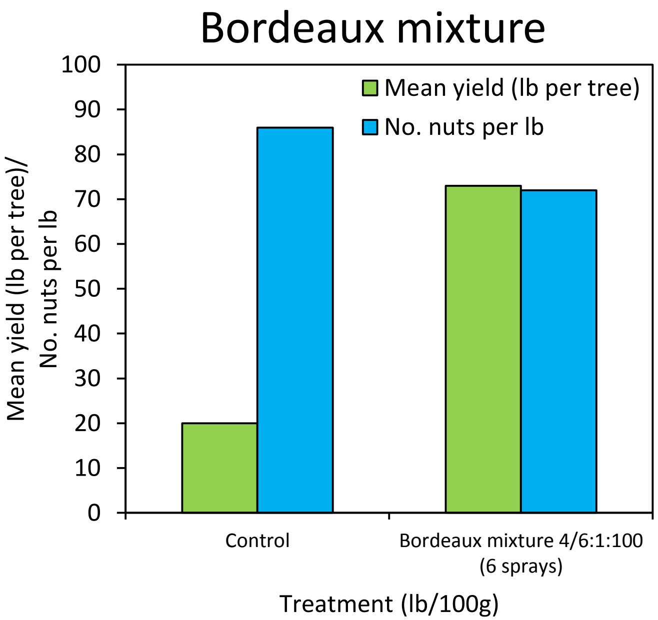
Waite, M. B. 1911. Pecan scab [Fusicladium effusum]. Science 33: 77-78.

Demaree, J. B. 1928. Morphology and taxonomy of the pecan-scab fungus, *Cladosporium effusum* wint. USDA Agricultural Research, 37: 181-187.

Bordeaux mixture is the earliest material that showed promise

- And that status quo remained the same from the 1920s to the late 1940s (Cole, 1951)
- To this day Bordeaux mixture remains a viable option for scab control
- In the late 1940s pathologists experimented with other new fungicides including dithiocarbmates Fermate A, Zerlate, Zineb and Ziram
- Efficacy was similar to Bordeaux mixture, and often were recommended in combination

Cv. Schley



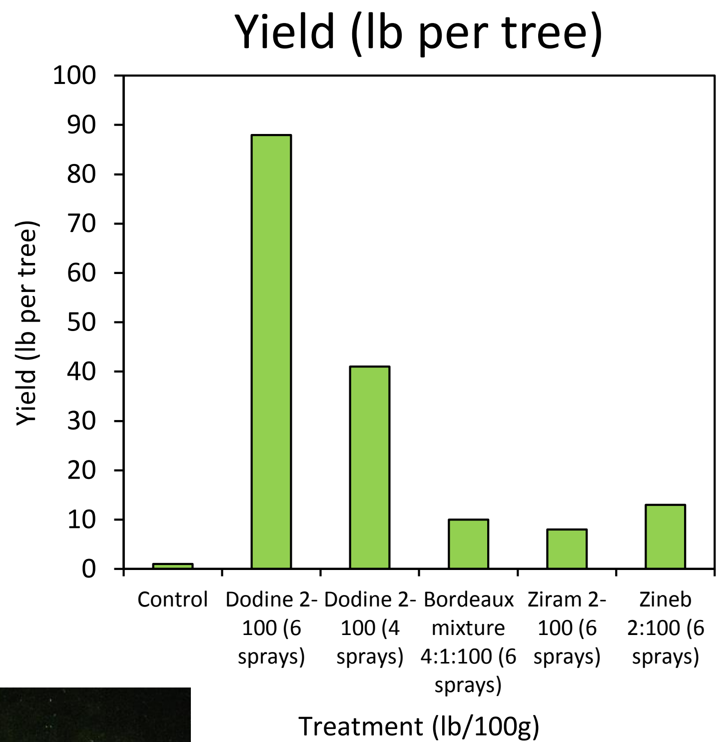
Although Bordeaux mixture was effective at reducing disease in many cases, there were some risks of phytotoxicity. Low lime formulations reduced this risk

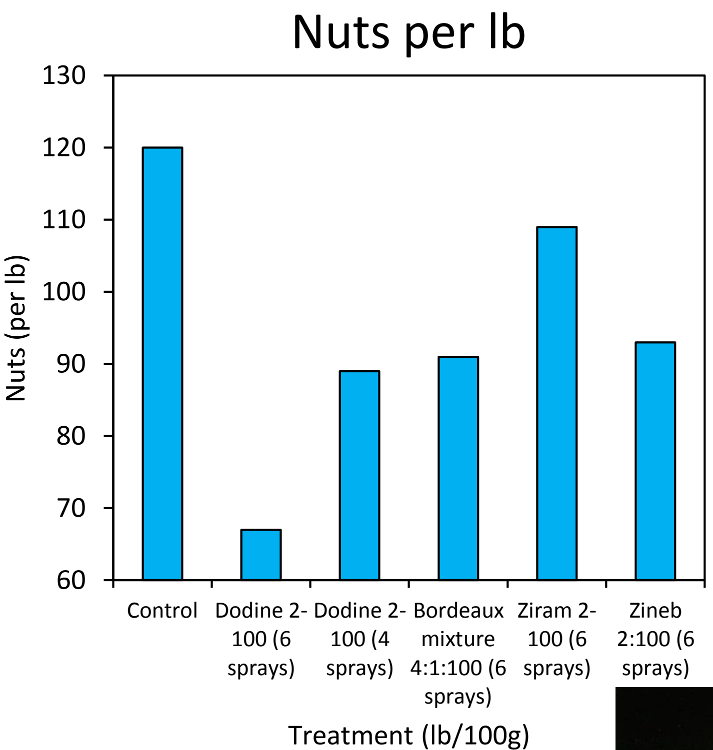
Healthier, larger fruit

Cole, J. R. 1951. Comparative results of applying fungicides with hydraulic and mist sprayers to control pecan scab. Southeastern Pecan Growers Association 44: 41-48.

Modern fungicides arrive – the late 1950s and early 1960s

- Dodine was first tested in 1958 (Cole, 1960), and provided outstanding control of scab compared to all previously tested fungicides
- In an experiment on cv. Schley, Fort Valley, GA, 1959:

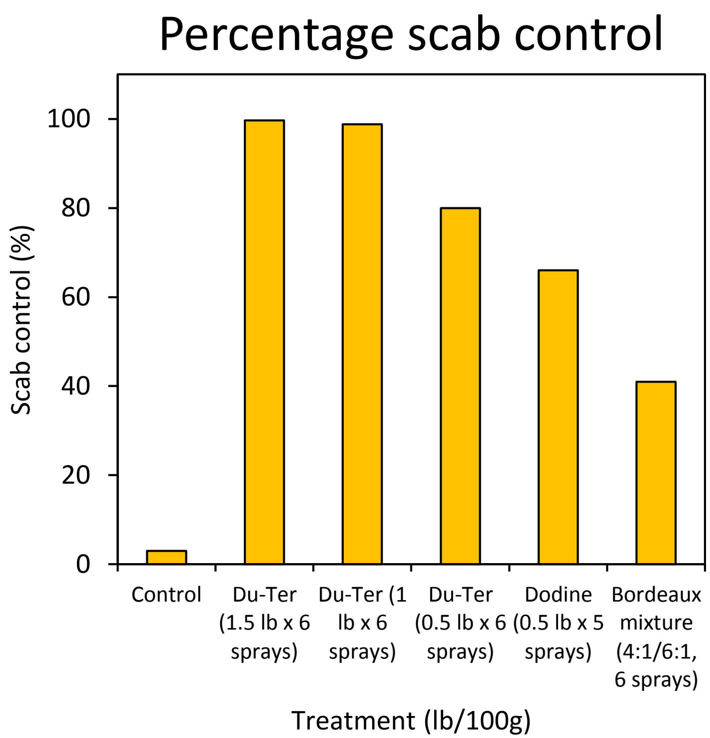




Cole, J. R. 1960. Dodine, an outstanding fungicde for the control of pecan scab. Southeastern Pecan Growers Association 53: 34-35.

More modern fungicides arrive – the late 1950s and early 1960s

- The organo-tin, triphenyltin hydroxide was first tested in 1963 (Large, 1965), as 'Du-Ter', and also provided outstanding control of scab
- In an experiment on cv. Moore, Monticello, FL, 1964:



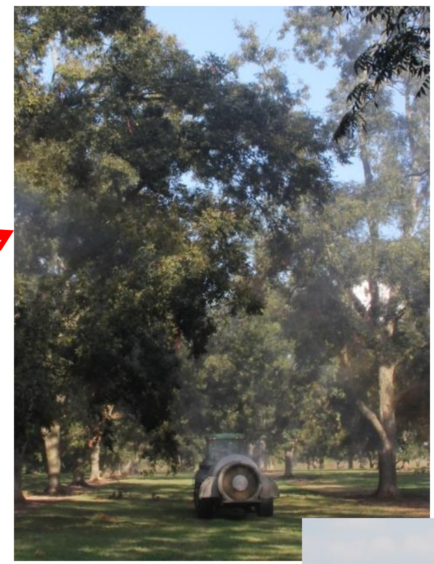
- Many other conventional fungicides subsequently became available in the following 3 deacdes including benzimidazoles, and strobilurins
- Most recently phosphites have been added to the list of efficacious fungicides
- All give disease control approaching or equal to that offered by supertin, but all require similarly frequent application
- Strengths of some may be on foliage or fruit

Large, J. R. 1965. Results of two years spraying with Du-Ter (tri phenol tin hydroxide) compared with other fungicides for the control of pecan scab. Southeastern Pecan Growers Association 58: 55-59.

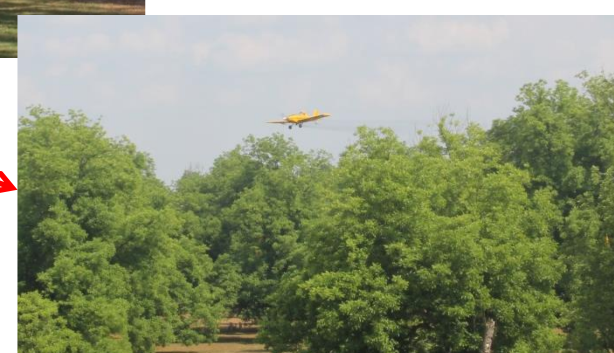
Fungicide sprayers improve

- In the early part of last century hand gun sprayers were common
- By the late 1940s air-blast speed sprayers were developed, and provided much better coverage
- Today there are powerful air-blast sprayers that provide good spray coverage to 40+ ft
- Aircraft/helicopter application also appeared to provide useful control in certain situations (tall trees)

Then



Now



Fungicide insensitivity surfaces

- The first fungicide to lose efficacy against pecan scab was benlate (benzimidazole), reported in 1976 (Littrell, 1976)
- Unfortunately it did not end there, and other FRAC classes have recently been reported with reduced sensitivity
- Reduced sensitivity now exits in to the DMIs, organo-tin (the backbone of most scab control programs for the last 50 y) and thiophanate-methyl

Fungicides available to manage pecan scab (GA Pest Management Handbook)

Fungicide group	FRAC code	Common name	Trade names	Relative risk of resistance
MBCs (benzimidazoles)	1	thiophanate-methyl	Topsin-M	HIGH
DMIs (sterol inhibitors)	3	propiconazole	Orbit	MEDIUM
			Propimax	
			Bumper	
			Quilt*	
			Quilt Xcel*	
		fenbuconazole	Enable	
		tebuconazole	Folicur	
			Tebuzole	
			Monsoon	
			Orius	
			Toledo	
			Absolute*	
		metconazole	Quash	
		difenconazole	Quadris Top*	
QoIs (strobilurins)	11	kresoxim-methyl	Sovran	HIGH
		azoxystrobin	Abound	
			Azaka	
			Quilt*	
			Quilt Xcel*	
			Quadris Top*	
		pyraclostrobin	Headline	
		trifloxystrobin	Absolute*	
Guanadines	U12	dodine	Elast	LOW to MEDIUM
Organotins	30	fentin hydroxide	Super Tin	LOW to MEDIUM
Phosphonates (phosphites)	33	phosphorous acid	Phostrol	LOW
			Prophyt	
			Fungiphyte	
			Reliant	
			Viathon**	
Ziram	48H/55D	ziram	ziram	LOW

Insensitivity has developed in the past. High risk

Single site mode of action. Reduced sensitivity to DMIs now on the record

Single site mode of action. High risk

No insensitivity

Reduced sensitivity now on the record

No insensitivity

No insensitivity

*Formulated mixture of DMI+QoI
**Formulated mixture of phosphorous acid+tebuconazole

- Single site mode-of –action fungicides are particularly susceptible to a fungus developing insensitivity
- These include benzimidazoles, DMIs and strobilurins
- When reduced sensitivity develops, the result is:

Loss of disease control

Littrell, R.H., 1976. Resistant pecan scab strains to Benlate and pecan fungicide management. Pecan South 3, 335-337.
Modified from: GA Pest Management Handbook. http://www.ent.uga.edu/pmh/

Current challenges

- There remain several fungicides with good efficacy against pecan scab
    - But fungicide insensitivity is threatening this defense
- There are resistant cultivars available with a range of other favorable agronomic traits
    - But the fungus had demonstrated historically that it can adapt to resistance (particularly as they become more widely planted and selection for virulence becomes greater)
- Many older, tall trees
    - Challenging to manage disease in upper canopies. Also, more selection on the pathogen to adapt….
- The weather is unknown
    - Unnecessary spray wastes money, and also exposes the pathogen to selection. Good advisories exist (do they provide enough window for growers to apply fungicide?)

The present - "rules" for applying fungicides in the era of fungicide insensitivity

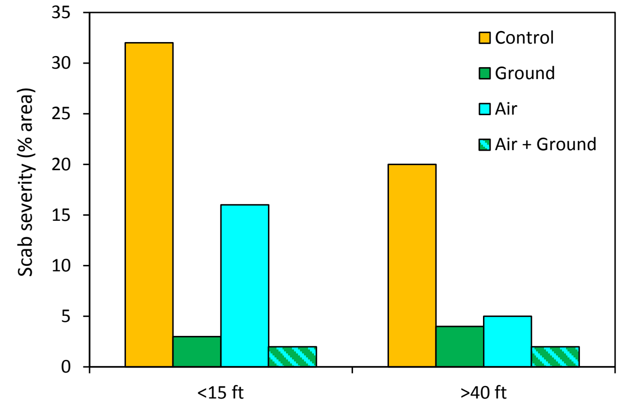
- ***R***otate between different fungicide modes of action
- ***U***se labeled rates – fungicide labels often provide a range of rates: use the upper range for high disease pressure and the lower range for low disease pressure
- ***L***imit use of single-site mode of action fungicide to one or two per growing season
- ***E***ducate – learn about the mode of action, spectrum of activity, recommended rates, etc.
- ***S***tart a fungicide spray program with a multi-site mode of action fungicide, pre-mixture, or tank mixture to reduce the total fungal population that is exposed to any single-site mode of action fungicide used later in a sequence of fungicide applications

NOTE: Don't use single-site mode of action fungicides when high levels of disease are present. The risk of selecting fungicide insensitive individuals is greater when there is a large population being exposed to the fungicide selection

The present – managing scab in tall trees

- Tall trees are difficult to control disease.
  - Scab can be better controlled by aerial application (Bertrand and Brenneman, 2001). Number and timing of sprays will be critical
  - Hedging - a means to control disease more uniformly
  - Volutes?

Scab severity depending on height and application method, cv Schley
(Bertrand and Brenneman, 2001)

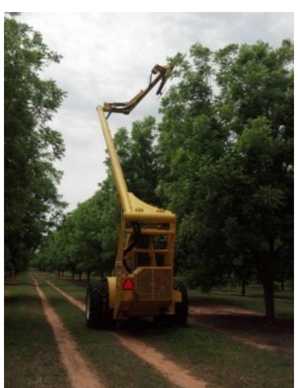


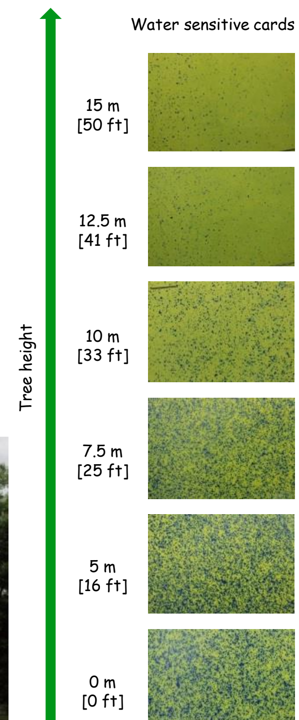
Bertrand P, Brenneman TB. 2001. Aerial and weather based application for pecan scab control. Proceedings of the Southeastern Pecan Growers Association Annual Meeting 94: 62-69.

Spray coverage (demonstrated using kaolin)

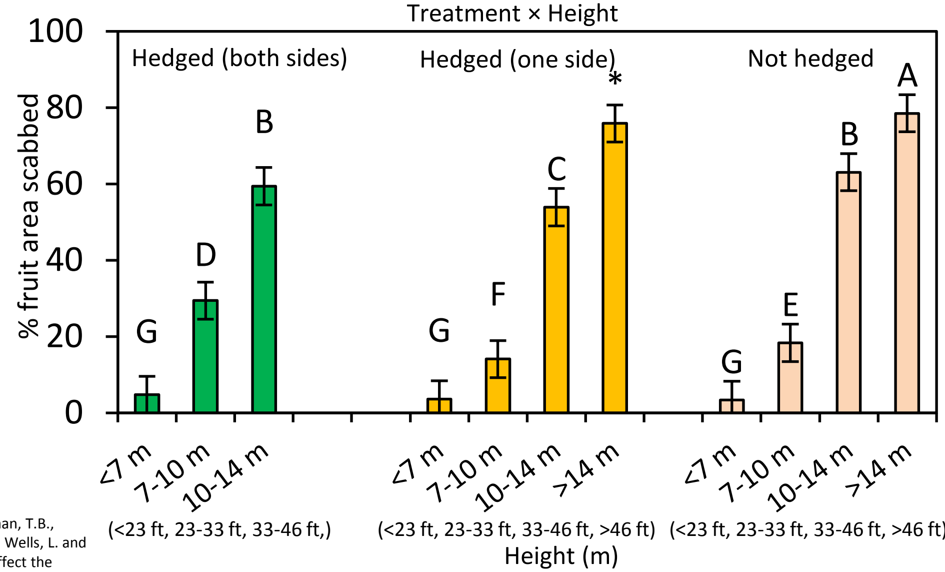
Volutes might increase coverage high up

Hedging = more of the tree accessible to spray



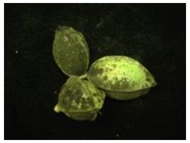


Scab severity on fruit in hedged and non-hedged trees cv Desirable, 2013
Treatment × Height



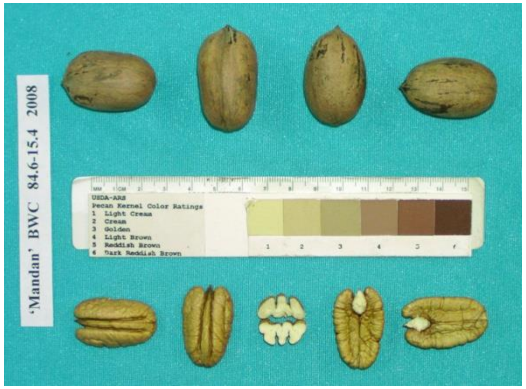
Bock, C. H., Hotchkiss, M. W., Brenneman, T.B., Stevenson, K.L., Goff, W., Smith, M.W., Wells, L. and Wood, B. W. 2014. Hedging – does it affect the severity of pecan scab? The Pecan Grower 26: 46-58.

* = non-estimable



The present - resistant cultivars

- Relies on traditional methods of breeding, crossing and trait recognition
- There are several cultivars available that are apparently resistant to scab (some have ‘durable’ resistance)
  - Both from breeding programs and grower selections
  - Elliott (1912), Excel, Mandan
- The disadvantage is traditional breeding takes a long time….and at the moment we can never be sure that the resistance therein is ‘durable’
  - No knowledge of the number or diversity of resistance genes (or the avirulence genes in the pathogen)

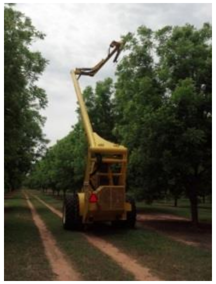


http://cgru.usda.gov/carya/pecans/pecalph.htm
http://www.caes.uga.edu/commodities/fruits/pecanbreeding/cultivars/cultivar_list.html

Summary of actions to ameliorate scab

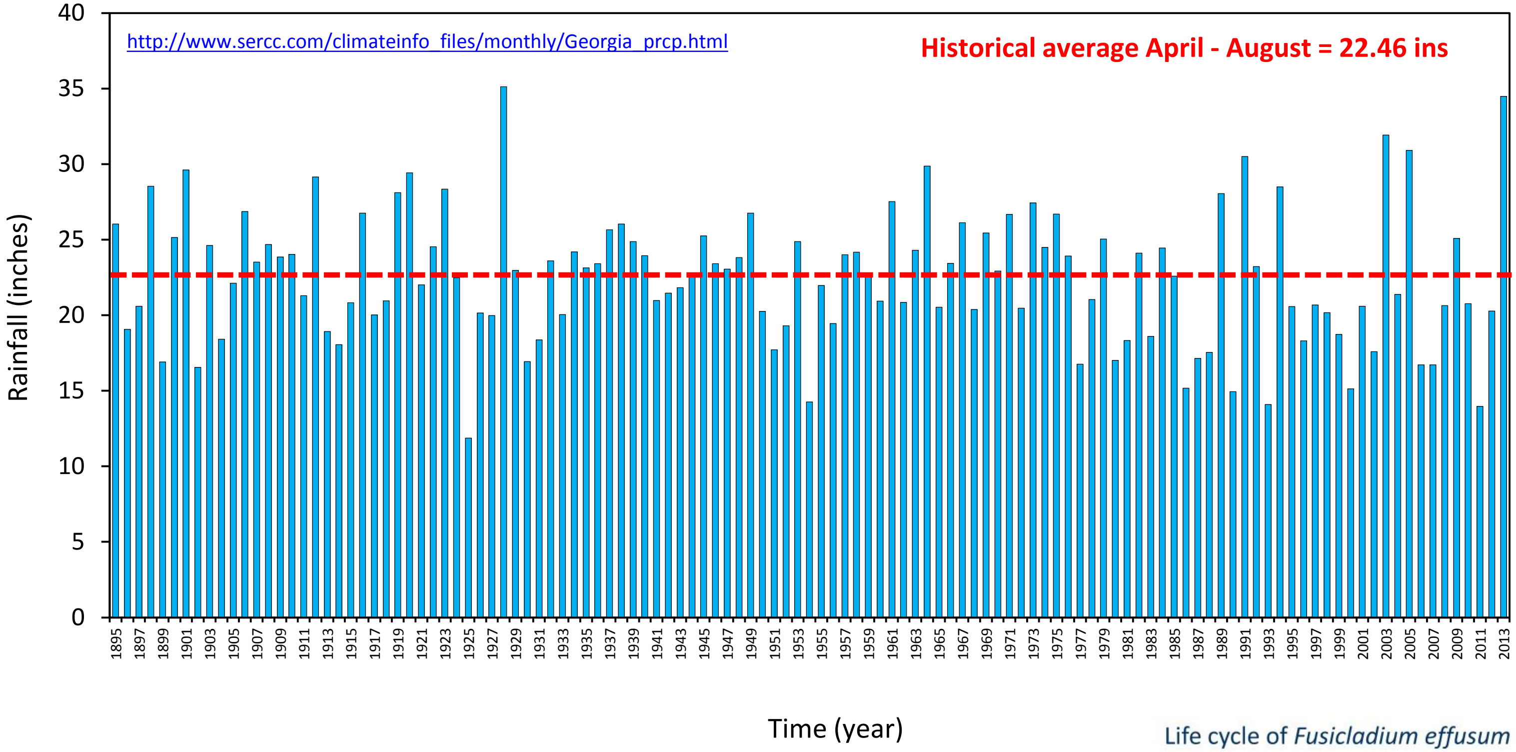
- Choose resistant cultivars whenever possible
- Use fungicides appropriately and the correct equipment to apply them to maximize coverage
- Hedge trees if possible (and adjust planting density accordingly)
- Do not overcrowd trees (tree spacing does affect scab)
- Wherever possible, select locations that are not conducive to scab
- Follow the scab advisories to minimize costs and fungicide use
- (Dormant season sprays? Trash removal?)

- *Yet the challenges that remain are substantial and scab is not about to go away…..the arms race will continue*

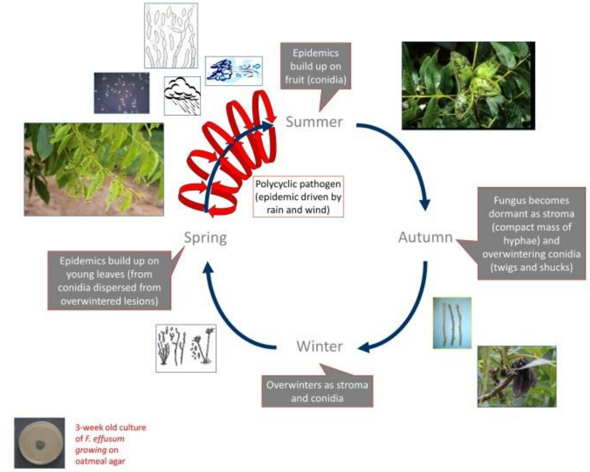


Demaree, J. B. (1928). Morphology and taxonomy of the pecan-scab fungus, *Cladosporium effusum* wint. USDA Agricultural Research, (37), 181-187

GA historic rainfall (April to August, 1895 to 2013)



Life cycle of *Fusicladium effusum* (pecan scab)



- A total of 119 years of rainfall records for the state of GA
- Of these, 61 had above average (22.46 ins) rainfall (51.26% of years)
- Average rainfall is known to result in scab requiring substantial effort to control
- Thus, historically 1 in every 2 years is a scab-prone year, which means continued vigilance and use of existing and novel methods of control are required

For the future….

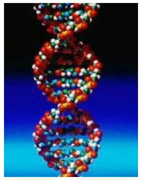
- New fungicides will doubtless become available, but existing ones will need to be managed (phosphites are one recent example….)
- Improved spray application equipment? UAVs (e.g. the air mule with a half-ton payload capacity). UAVs are already used in several other crops to apply pesticides
- Conventionally bred cultivars from breeding programs will continue to become available, as will grower selections
- Orchard management techniques
- And the promise of a genomics-based solution……

Fungicides and spraying – UAV? (the air mule)

http://www.urbanaero.com/category/airmule

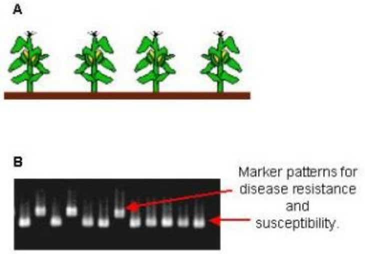
Cultivars and tree/orchard management

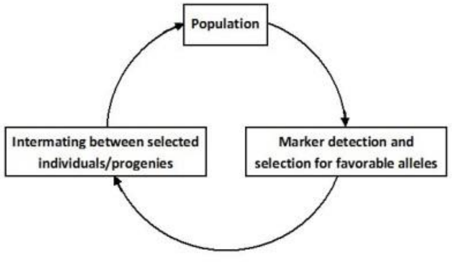
New technologies (biotechnology/genomics)



Pathogen and host diversity, genomics and breeding for disease resistance

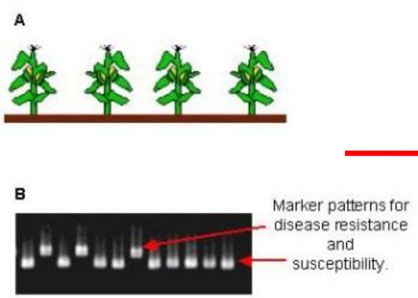
- Genome: the complete set of DNA within a single cell of an organism
- Genomics is a discipline in genetics that applies recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble, and analyze the function and structure of genomes
- Aids in identifying loci on chromosomes which can be used for marker assisted selection (MAS), and to ascertain the gene activities
- A draft genome of pecan has been sequenced (NMSU/USDA-ARS)
- Also, a draft genome of *F. effusum* is sequenced and published (USDA-ARS)
- An understanding of the host and pathogen population genetics and diversity is needed to gauge number and frequencies of different genes in the populations
- This information will offer a powerful tool for developing 'durable' resistant pecan
- Genetic engineering/transgenic approaches are also possible



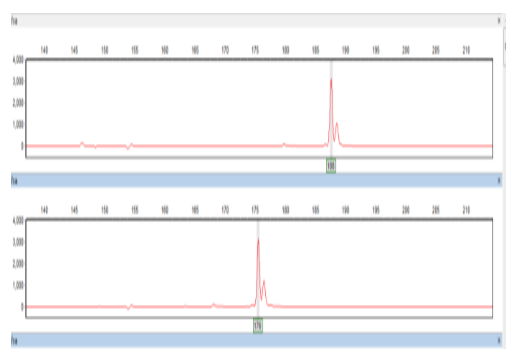


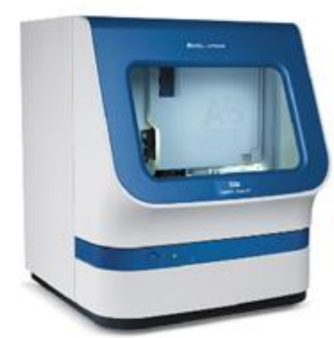
The application of genomics – marker assisted selection (MAS) for disease resistance genes

- MAS has been successfully used to introduce disease resistance into several varieties of annual crops including rice, wheat and tomato etc.
- And has been used for pyramiding of genes to ensure durable resistance (where different genes for resistance are identified and incorporated into a single variety)
- Knowledge of these genomes is far advanced
- Use in woody perennials has some unique issues (generation time), but genomic approaches allow accurate and early identification and following of resistance markers – saving time



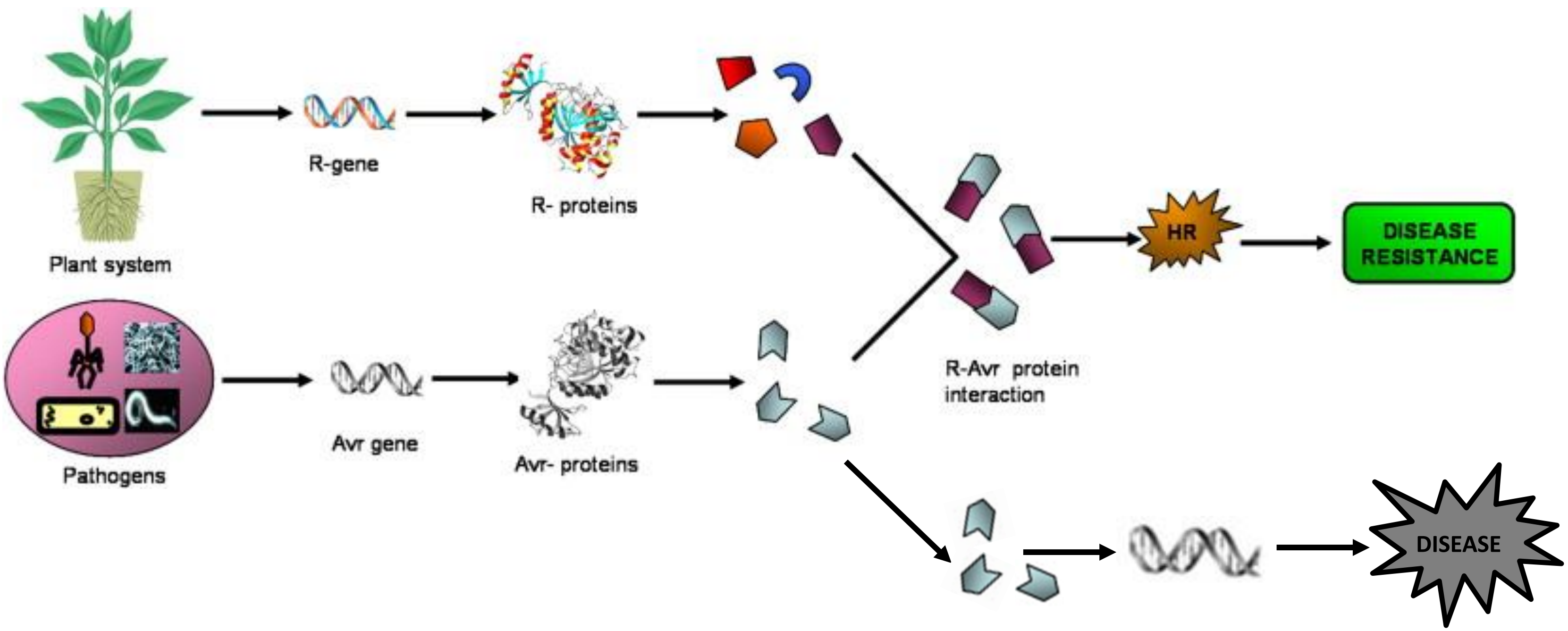
Automated systems allow rapid identification of markers on numerous samples





The application of genomics - the host pathogen interaction

Plants respond to infection through a cascade of molecular activity



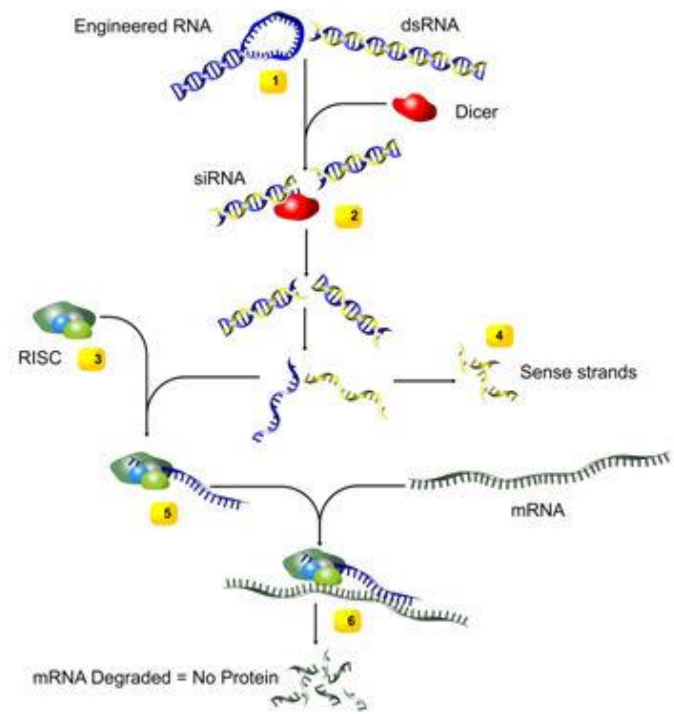
- Genomics provides the basis for locating and understanding gene expression of both the host and pathogen
- It also provides the basis for an understanding of the genetic diversity in both the host and pathogen
- And thus for utilizing existing resistance genes, tracking transgenes and developing gene silencing approaches

Modified from: Gururani, M.A., Venkatesh, J., Upadhyaya, C.P. et al. 2012. Plant disease resistance genes: Current status and future directions *Physiological and Molecular Plant Pathology* 78: 51-65.

The application of genomics - RNA-mediated gene silencing

- Gene silencing is a natural phenomenon
- Functional genomics harnesses gene silencing for controlling gene activity through genetic engineering
- This is a process where the gene is made inaccessible or the mRNA is destroyed preventing the pathogen from causing infection
- This system has been harnessed and demonstrated to work in several crop-pathogen systems

1. The entry of long double stranded RNA, such as an introduced transgene, a rogue genetic element or a viral intruder, triggers the RNAi pathway of cells. This results in the recruitment of the enzyme Dicer.
2. Dicer cleaves the dsRNA into short, 20-25 basepair long, fragments, called small interfering RNA (siRNA).
3. An RNA-induced silencing complex (RISC) then distinguishes between the two siRNA strands as either sense or antisense. The sense strands (with exactly the same sequence as the target gene) are degraded.
4. The antisense strands on the other hand are incorporated to the RISC.
5. These are used as guide to target messenger RNAs (mRNA) in a sequence-specific manner.
6. Messenger RNAs (mRNA), which codes for amino acids, are cleaved by RISC. The activated RISC can repeatedly participate in mRNA degradation, inhibiting protein synthesis.



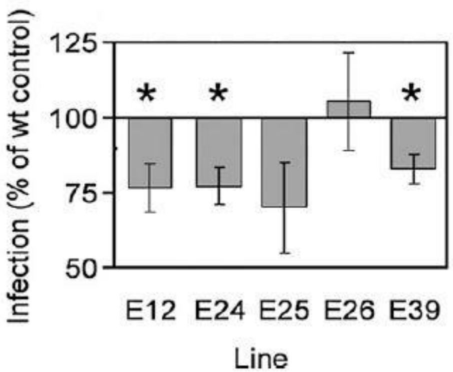
http://www.isaaa.org/resources/publications/pocketk/34/default.asp

The application of genomics – disease resistance

Host/virus-induced gene silencing (HIGS and VIGS) of pathogen genes

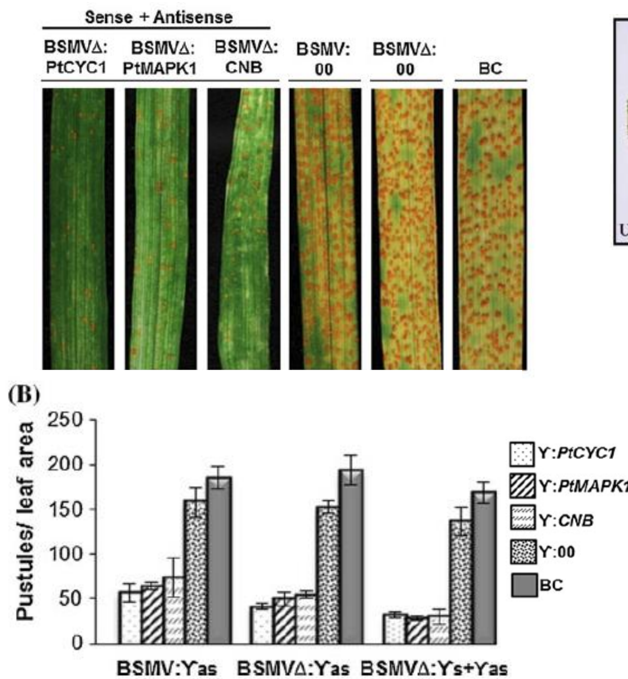
Resistance to barley powdery mildew

- Reduced disease severity by *B. graminis* on transgenic barley
- Plants carry an RNAi construct against *GTF1* (E12, 24, 25 and 39)
- E26 that had lost the hairpin RNAi cassette construct and was as susceptible as wild-type control plants



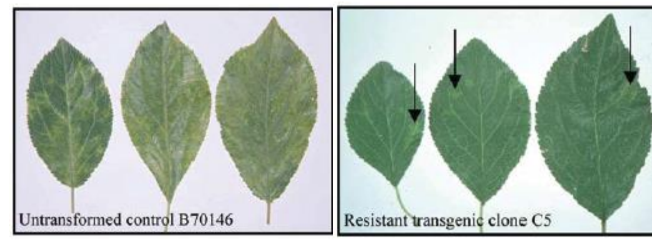
Resistance to wheat leaf rust

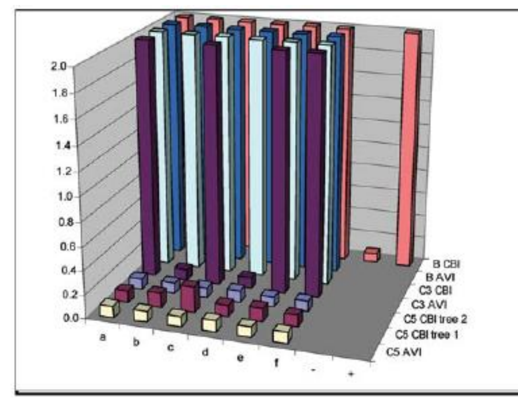
- Expression of target Pt gene fragments in wheat using a virus constructs results in siRNA
- siRNA molecules trigger RNA silencing of the corresponding genes in colonizing fungi, resulting in disease suppression



Resistance to plum pox virus

- 'HoneySweet' plum (in the process of release)
- PPV protection by RNA interference (RNAi)
- Highly effective, stable, durable, and heritable as a dominant trait





- Gene silencing has been used in several crop plant species against viruses and insect. The example of PPV transgenic resistance is the first in a woody perennial
- Gene silencing in regard to fungal pathogens is becoming better understood and will doubtless be used more widely in the future

Nowara, D., Gay, A., Lacomme, C., Shaw, J., Ridout, C., Douchkov, D., … Schweizer, P. (2010). HIGS: Host-Induced Gene Silencing in the Obligate Biotrophic Fungal Pathogen *Blumeria graminis*. *The Plant Cell*, *22*(9), 3130–3141. http://doi.org/10.1105/tpc.110.077040
Panwar, V., McCallum, B.D., and Bakkeren, G. (2013). Host-induced gene silencing of wheat leaf rust fungus *Puccinia triticina* pathogenicity genes mediated by the *Barley stripe mosaic virus*., *Plant Molecular Biology, 81*(6), pp. 595-608
Hily JM, Scorza R, Malinowski T, Zawadska B, Ravelonandro M (2004) Stability of gene silencing-based resistance to Plum pox virus in transgenic plum (Prunus domestica L.) under field conditions. Transgenic Res 13:427–436

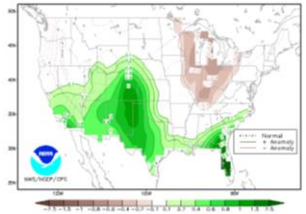
So what hope do these and future genomic tools hold for scab?

- Resistance will doubtless be improved by marker assisted selection (MAS)
- Genomics and genetic engineering tools are increasing knowledge of the genetics and molecular determinants of plant resistance and pathogen virulence, leading to strategies to enhance resistance durability
- The rationale for these strategies involves slowing the evolution of virulent variants by maximizing the evolutionary hurdle required for the pathogen to overcome the resistance
- Pyramiding non-redundant R genes through use of cassettes of native and transgenes, including gene silencing technologies
- There is a lot of work to do, and much of it is quite fundamental
- ‘Durable’ disease resistance has no one genetic or molecular basis, and the success of a strategy can be judged only in retrospect

Rainfall outlook for the 2016 season

Coming out of an El Niño pattern (wet winter-early spring)

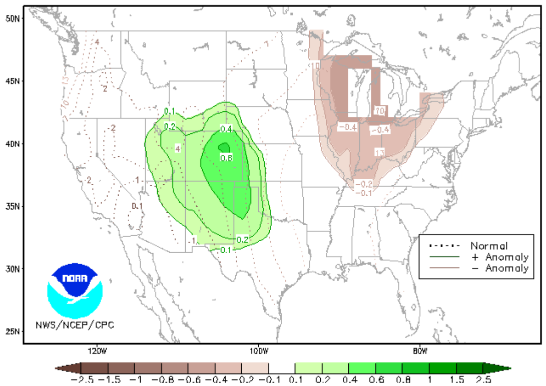
Mar-Apr-May



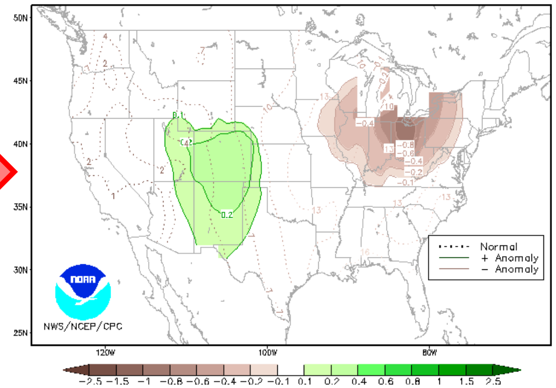
http://www.cpc.ncep.noaa.gov/products/predictions/long_range/

Outlook for +/- average precipitation this season. Non colored areas indicate there is an equal chance of there being more or less rain compared to the long-term average.

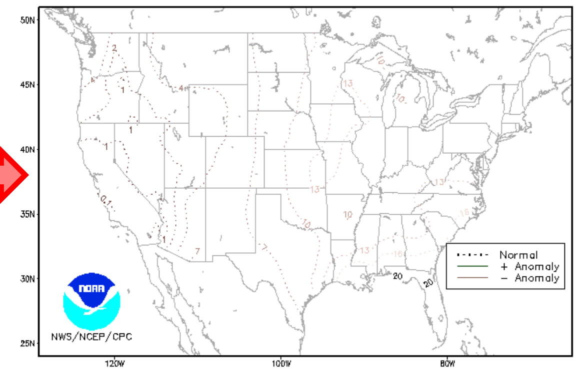
Apr-May-Jun



May-Jun-Jul



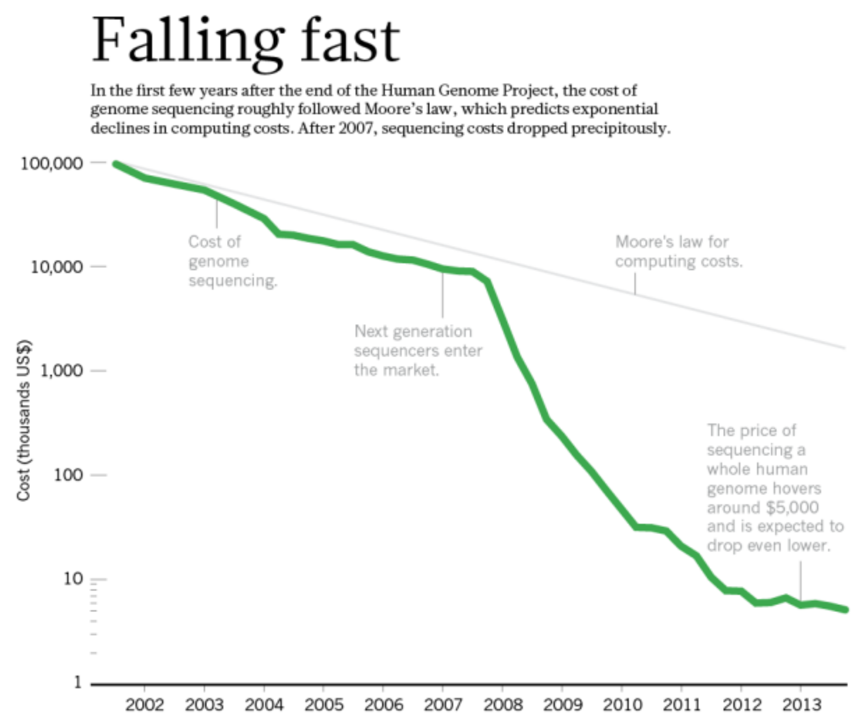
Jun-Jul-Aug



- The national weather service includes a comprehensive disclaimer on these data
- We are coming out of a strong El Niño which usually heralds a wet winter and early spring (which it has been in parts of the Southeast)
- Based on the models the 2016 growing season has an equal chance of ‘average’ rainfall in the Southeast
- Average rainfall will support significant epidemics of scab in susceptible cultivars, particularly in scab favorable locations

Conclusions

- Scab has been the major biotic constraint to yield in pecan in the Southeast and will continue have impact for the foreseeable future
- We will need to use existing technologies for many years to come (resistant cultivars, fungicides, sprayers and orchard management tools)
- But it is important to be proactive and embrace what new biotechnology tools offer:
  - Sequencing the first human genome cost ~$2.7 billion (3 billion base pairs). 2001
  - Sequencing the scab genome cost ~$8,000 (0.4 billion base pairs). 2015
- Progress will continue to accelerate
  - "Moore's law" is the observation that the number of transistors in a dense integrated circuit doubles approximately every two years - exponential
  - "Carlson curve" Doubling time of DNA sequencing and associated technologies (measured by cost and performance) – "hyper-exponential"
- Understanding host and pathogen diversity and gene purpose and activity is crucial to improving pecan using these tools



http://www.nature.com/news/technology-the-1-000-genome-1.14901#/falling

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