

# California invaded: 1849 A.D.

Xylella scorch of maples 2000s

Port Orford Cedar Root Disease  
1950s

Expansion of root pathogens  
Post 1880s

Root canker of  
Pacific Madrone  
and Bay laurel (70s)

Manzanita die-back 2004

Sudden Oak Death  
1990s

White pine blister rust  
1930s

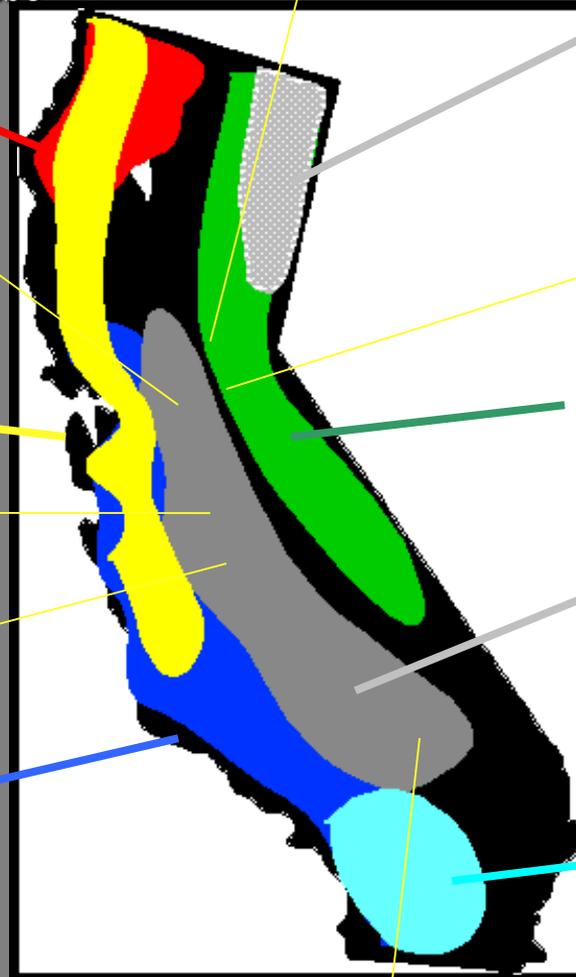
Cypress canker 20s

Dutch Elm Disease  
1960s

Colored canker of  
sycamore 70s

Pitch canker disease  
1980s

Oak root canker  
2000



1000 canker disease of walnuts  
2001

- Port Orford Cedar root disease; exotic agent= *Phytophthora lateralis* (East Asia); first found in a nursery in Oregon
- Sudden Oak Death; exotic agent *Phytophthora ramorum* (origin unknown) introduced late '80s multiple times by infected ornamental plants
- Colored canker of sycamore, exotic agent *Ceratocystis platani* from East coast, introduced through wood packaging or untreated wood
- Pine pitch canker, exotic agent *Fusarium circinatum* introduced in the 80s on pine seed and pine seedlings, origin: Mexico
- Oak root canker caused by exotic *Phytophthora cinnamomi* introduced from Papua New Guinea via orchard stock probably after World War II. Same pathogen causes manzanita die-offs (Sierra Nevada Foothills) and decline of Bay Laurel and Pacific Madrone (greater bay area)
- Cypress canker outbreaks caused by native *Seiridium cardinale* on trees planted off site or on artificial crosses
- Dutch Elm Disease first caused by exotic *Ophiostoma ulmi* then replaced by more aggressive *O. novo-ulmi* in the 60s's. From Asia via Europe via infected wood and vectoring insects (one European and one North American)
- 1000 canker disease caused by fungus *Geosmithia morbida* (exotic to Ca) vectored by native walnut twig beetle (post 2003)
- White pine blister rust caused by *Cronartium ribicola* introduced from Asia via France on infected western white pine in 1914 in Vancouver island
- Native *Heterobasidion* on pines, junipers, sequoias and true firs increased by change in tree species composition, logging and fire exclusion
- *Xylella*= Pierce's disease via Mexico/Southern California

# White pine blister rust:

An emergent disease caused  
by an introduced pathogen

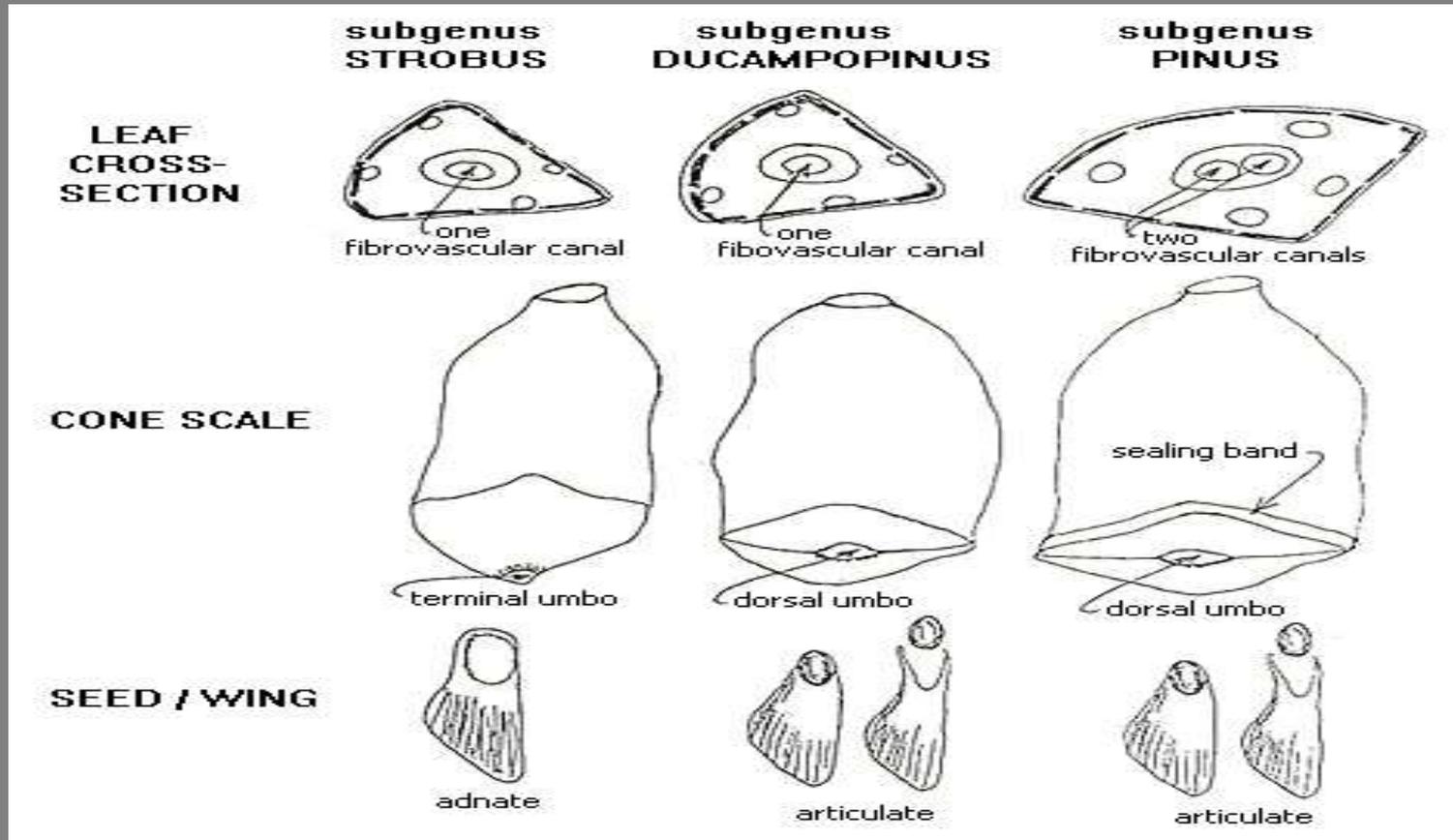
# The tree host: white pines

- Genus *Pinus*
- Hapoxylon subgroup
- Five-needled
- Eastern and western white pines, whitebark, sugar, limber, southwestern white, foxtail, bristlecone pines
- Whitebark is closely related to European stone pines, where rust is endemic (but there are questions on whether it is the same species)

# In Western North America

- Nine species of white pines
- Eight are infected (*P. longaeva* is the only one without a report)
- Incidence of disease is not same across all species. E. g.: western white pine less resistant than Sugar pine. SP require wave years for infection to occur, that is years where Fall conditions have mild temperatures and rainfall

# Some details on *Pinus*



# Blister rust cankers: sugar pine      whitebark pine

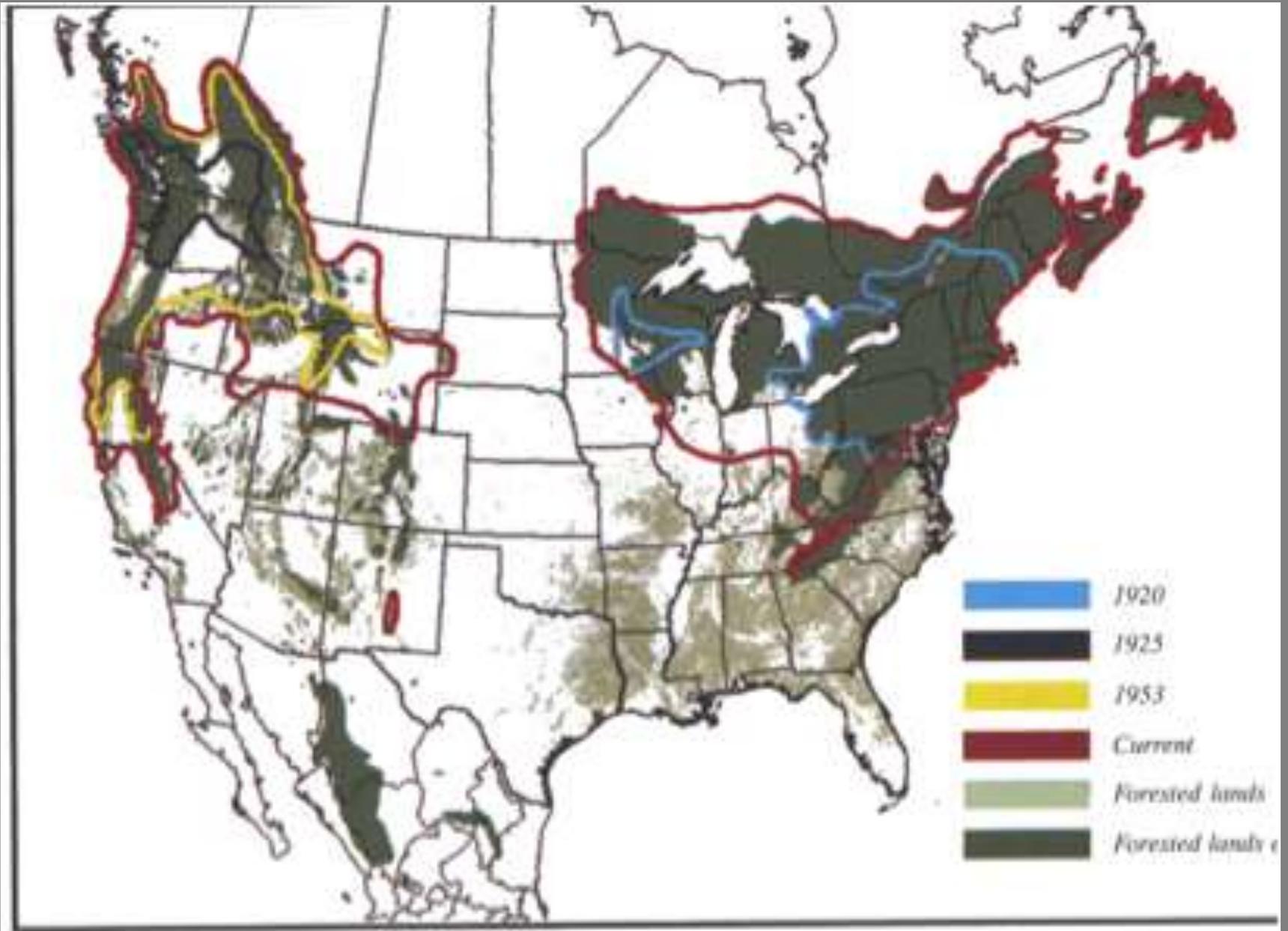


# Top kill in whitebark pine



# *Cronartium ribicola*: the causal agent

- Complex system involving 5 spore stages and two hosts
  - *Pinus* and *Ribes*
- Introduced into North America around 1900 on infected eastern white pine stock; separate introductions on east and west coasts
- Native to Asia



# Some details about introduction

- 1906 on East Coast, but there are records of many shipments from Germany and Holland, in multiple locations including the midwest
- 1910, Vancouver BC, One shipment documented from France but most reconstructions suggest more than a single introduction occurred
- Ribes also imported from Europe, but most ribes lose their foliage in fall, Introduction most likely to have happened through pines

# Methods

- 4 collection sites of a single aecidia in West
  - 2 coastal sites (Manning Park, BC; Sacramento, NM)
  - 2 interior sites (Smallwood, BC; Shelter Bay, BC)
- 4 collection sites in the East
  - Ste-Camille, Quebec; Minden, Ontario; Little Grand Lake, NFLD; Moncton, New Brunswick



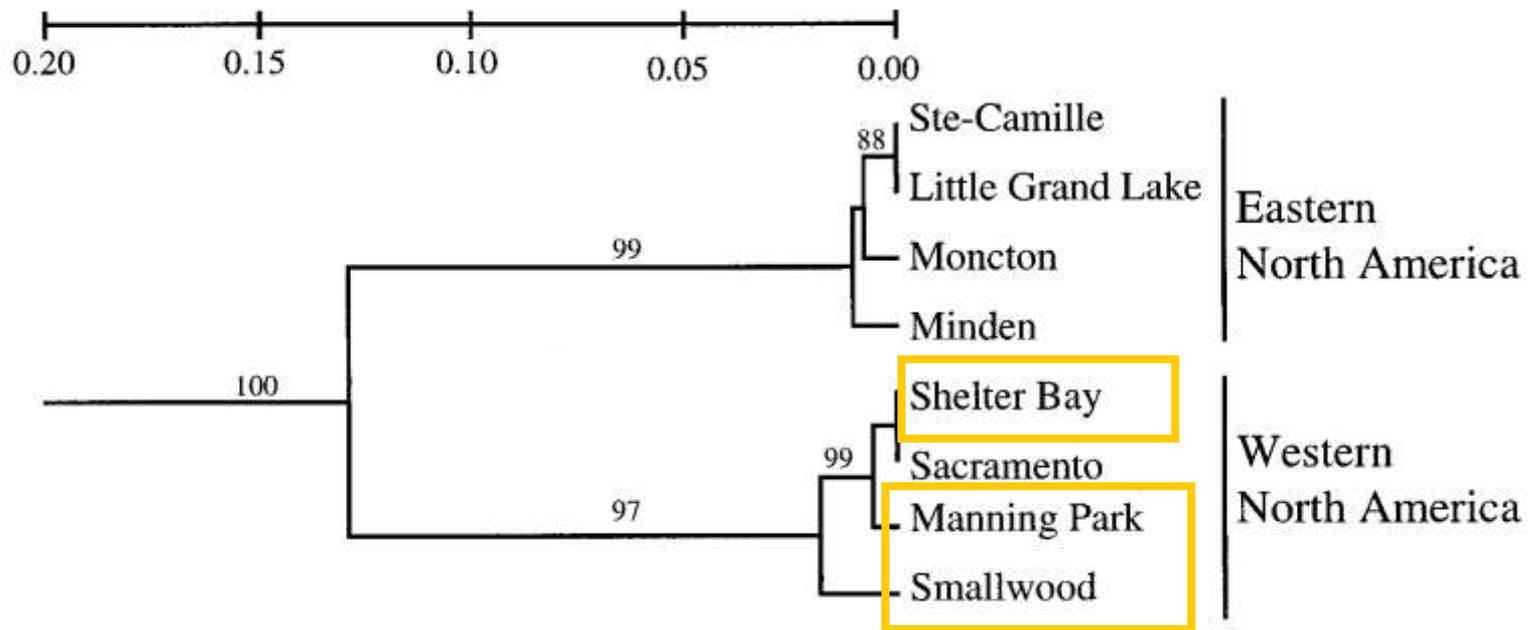
# Results

- Among regions: West v. East (0.605)
  - Migration between east and west extremely low, less than 1 migrant per generation
- Among populations in regions (but markers were imperfect and fail to detect differences among western populations)
  - East (0.00 – 0.02)
  - West (0.00- 0.02)
- In Populations (0.493), a lot of sexual reproduction > No surprise because of rust cycle

Hierarchical structure <sup>a</sup>	Source	df	Variance components	$\phi$ -Statistics	Proportion of variance components (%)	<i>P</i> value <sup>b</sup>
East (NFLD, QC, ON, NB) vs West (BC, NM)	Among regions	1	0.605	0.546	54.58	<0.001
	Among populations in regions	6	0.011	0.021	0.97	0.076
	In populations	262	0.493	0.556	44.45	<0.001
Interior BC vs Coastal BC	Among regions	1	0.000	0.000	0.00	0.78
	Among populations in regions	1	0.008	0.044	4.49	0.20
	In populations	105	0.174	0.032	95.51	<0.001

<sup>a</sup> NFLD = Newfoundland, QC = Quebec, ON = Ontario, NB = New Brunswick, BC = British Columbia, and NM = New Mexico.

<sup>b</sup> Probability of obtaining equal or larger value determined by 1,000 random permutations of the treatments.



Shelter Bay = interior BC  
 Manning Park = interior BC  
 Smallwood = coastal BC

# New Mexico Revisted

TABLE 1. Estimated frequency of random amplified polymorphic DNA (RAPD) markers and expected heterozygosity ( $H_j$ ) for populations of acidia of *Cronartium ribicola*

Population <sup>a</sup>	n <sup>b</sup>	Estimated allele frequency ( $q$ ) <sup>c</sup>							$H_j$
		OPA01-1700	OPA01-2000	OPC08-750	OPC08-900	OPE15-1600	OPK19-2000	OPK11-500	
Manning Park (coastal BC)	55 (21)	0.963	1.000	1.000	1.000	1.000	0.203	0.000	0.069
Smallwood (interior BC)	37 (14)	1.000	0.987	1.000	1.000	0.987	0.410	0.000	0.077
Shelter Bay (interior BC)	16 (6)	1.000	1.000	1.000	0.969	1.000	0.000	0.000	0.009
Sacramento (NM)	45 (15)	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.000
Ste-Camille (Quebec)	30 (10)	0.417	1.000	0.966	0.610	0.733	0.375	0.490	0.347
Minden (Ontario)	30 (10)	0.522	1.000	0.983	0.522	0.582	0.455	0.553	0.369
Little Grand Lake (NFLD)	30 (10)	0.417	1.000	1.000	0.610	0.832	0.329	0.417	0.319
Moncton (New Brunswick)	30 (10)	0.564	0.969	0.985	0.641	0.655	0.440	0.313	0.344

<sup>a</sup> BC = British Columbia; NM = New Mexico; NFLD = Newfoundland. Quebec, Ontario, NFLD, and New Brunswick reproduced from Et-touil et al. (2); expected heterozygosity may differ from Et-touil et al. because two of the markers used in that study were not used here.

<sup>b</sup> Number of acia and number of trees in parentheses.

<sup>c</sup> Allele frequency for dominant RAPD markers was estimated according to the method of Lynch and Milligan (27); frequencies are those of the null allele.

- Sacramento, NM
- All fixed loci, absence of heterozygots
- Founder's effect
- Low genetic differentiation = genetic bottleneck

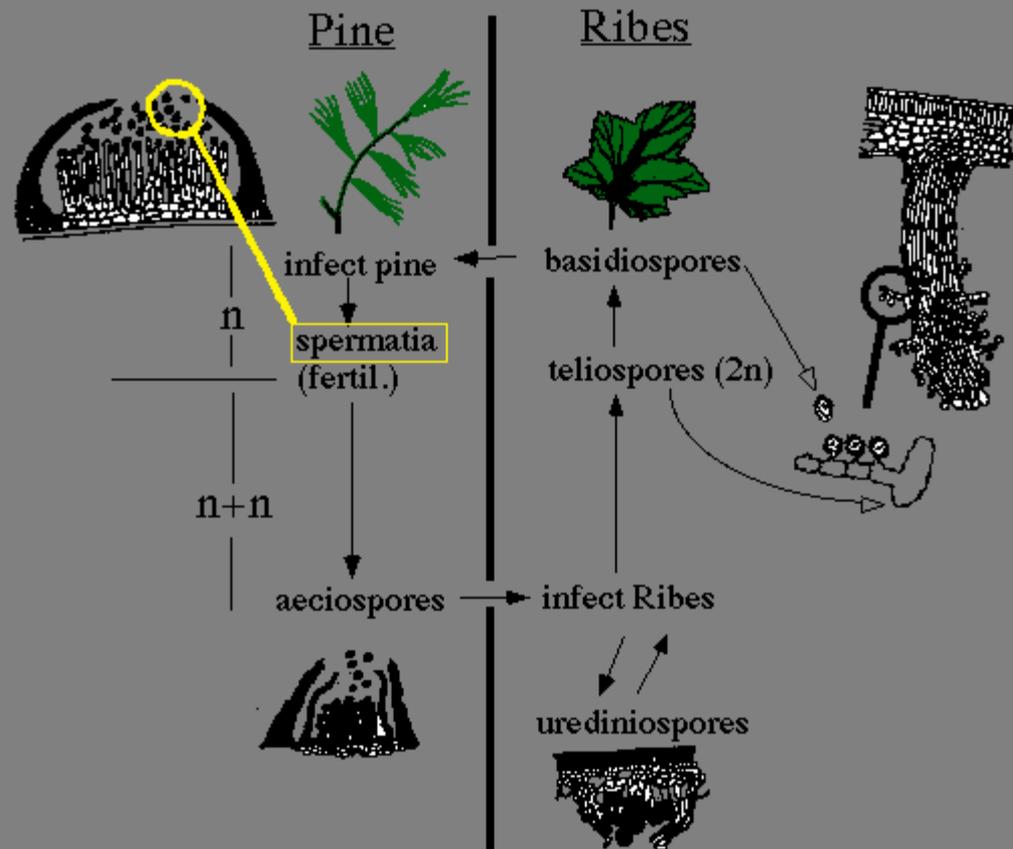
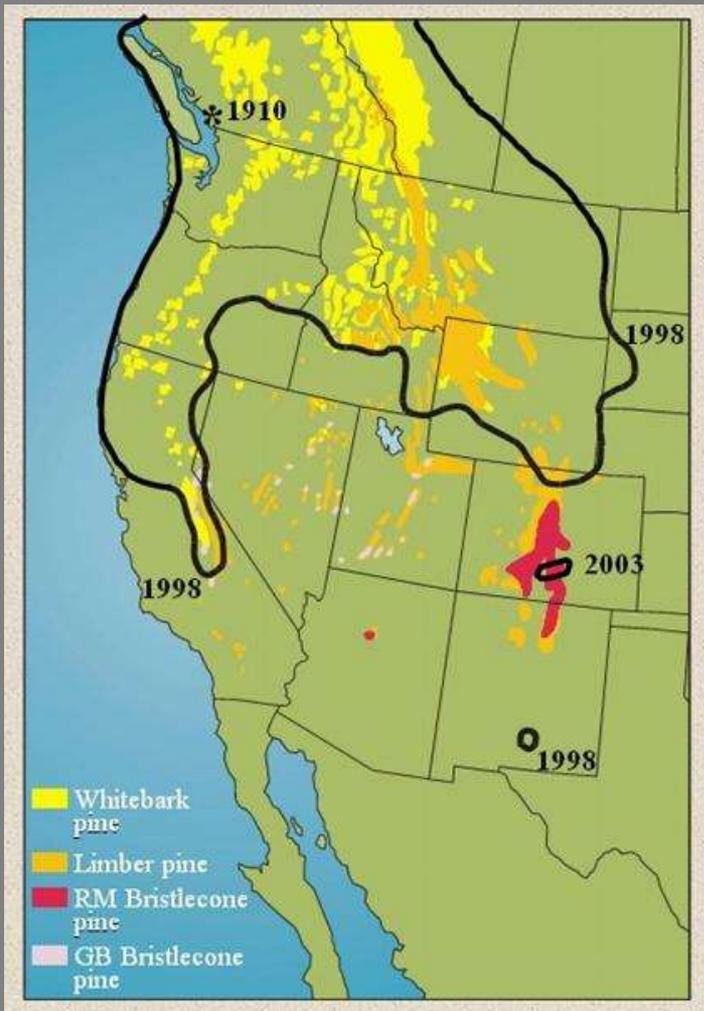


# Conclusions

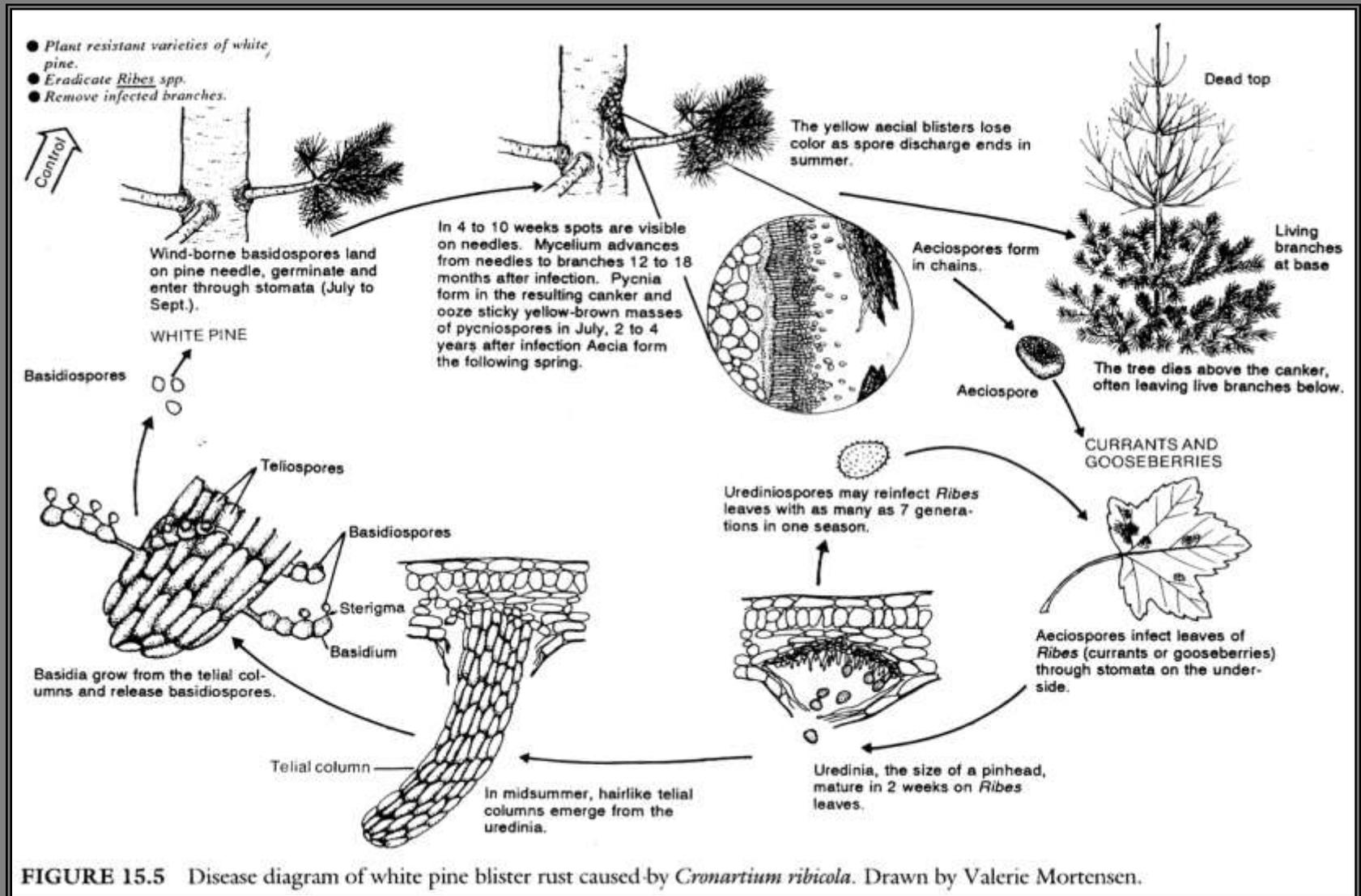
- Eastern and western populations are not panmictic
- Barrier to gene flow between eastern and western populations
  - Great Plains – intense agriculture
  - 100 km absence of aecial and telial hosts

# Factors affecting spread

- Founder population of pathogen (Germany vs. France)
- Number of introductions
- Species of white pines
- Amount of obligate alternate host *Ribes* and their distance from pines
- Climate: as it gets colder infection less successful (for instance moving inland and east in Eastern North America)
- Weather: pine infection requires rainfall and moderate temperature in Fall, as we move South in the West these conditions happen only rarely, when they do we speak of WAVE YEAR
- Topography: the more rugged the more difficult the spread. Although while basidiospores only travel a few hundred yards or a few km in the presence of currents induced by lakes in mountainous areas, aeciospores may be able to travel over 1000km
- Amount of resistance within species For instance in Sugar pine resistance is 1% in North and 8% in South CA, maybe due to resistance to *C. occidentale*, a rust of pinyon pines



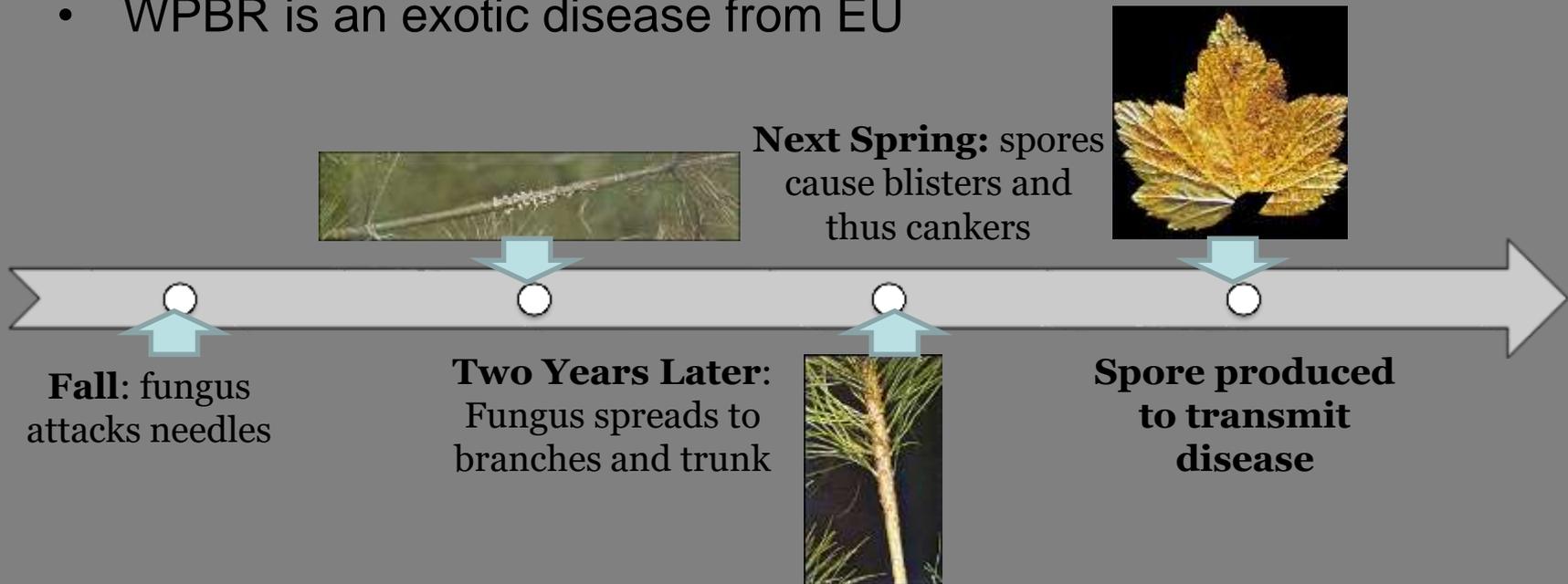
# *C. ribicola* life cycle





# *Cronartium ribicola*—Causal Agent of White Pine Blister Rust

- WPBR is an exotic disease from EU



- Leaves above the canker die, causing branch/stem to break
- Opens site for decay fungus

# A Few Pathogen Details

- Infection occurs through stomata of needles of all age, if needle is on stem then infection directly leads to tree girdling. If needle on branch, it will cause branch death and then if it moves into stem it will cause stem girdling, if stem does not die before pathogen gets to stem...
- Because pathogen is obligate biotroph
- Overall Low genetic diversity in N.A. Sign of introduced disease
  - Diversity between subpopulations is greater in West because of rugged topography
  - Indicative of frequent founder events and little gene flow
- Genetic center: Asia
- To infect white pines: 48 hours <68 F, 100% relative humidity

# Attempts to control WPBR

- Ribes eradication
  - More successful in East than West
- Use of Risk Zones for planting and management
  - potential pitfalls: must also account for airflow patterns
- Pruning
  - Can be successful if infection caught 12 inches from main stem; costly; may need repeated entries; probably would not work in whitebark
- Genetics: probably most successful method
  - Sugar and western white pines
  - Whitebark pine work in progress

# Ribes Eradication



- In East:
  - effective
  - well supported
  - easy
- In Lake States:
  - variable results
- In West:
  - difficult

# Civilian Conservation Camps during the Depression,



# Widespread mortality in western white pine



# Why mortality appears in clusters if pine to pine infection does not occur ?

- 1- Threshold of inoculum necessary for infections low in western white pine, so a single source can infect trees at various distance because dilution effects with distance are less pronounced
- 2- Resistance very infrequent (1 in a thousand)
- 3- Compounding effect of Mountain Pine Beetle

# Pruning research in sugar pine before...



# Pruning research in sugar pine



...afte

# Eastern white pine (*P. strobus*)

- Largely cut over prior to rust, so loss due to rust minimal, but regenerating difficult
- Only tree where *Ribes* control was mildly successful
- Most land managers won't risk it in high risk zones

# Whitebark pine (*P. albicaulis*)

- High elevations in the western US and Canada
- Keystone species; slow growth
- Mutualistic relationship with Clark's nutcracker
- Wildlife dependence on nuts
- Restoration treatments: a helping hand for a tree with a bleak future



# Western white pine (*P. monticola*)

- Largely disappeared from the Inland Northwest, where it was once most valuable timber species
- Like eastern wp, avoided in plantings
- Changing species comp. and structure made forest more susceptible to fire, insects and other pathogens

# Sugar pine (*P. lambertiana*)

- CA and PNW
- Tree of largest stature in mixed-conifer forests
- Few native pests, none causing such widespread mortality
- Also avoided in some planted settings
- Resistance 1% to 8%

# Tree resistance

- Major gene for resistance
- Found in sugar, western white, and southwestern white so far
  - Thought to be gene-for-gene (because virulent race of pathogen neutralizes this gene)
  - Gene-for-gene typically indicates a pathosystem in which the host and pathogen have evolved over long time periods- so what is going on in this system?

# A quick review of gene-for-gene resistance

Pathogen  
genotype

Host  
genotype

RR

Rr

rr

WW

-

-

+

Vv

-

-

+

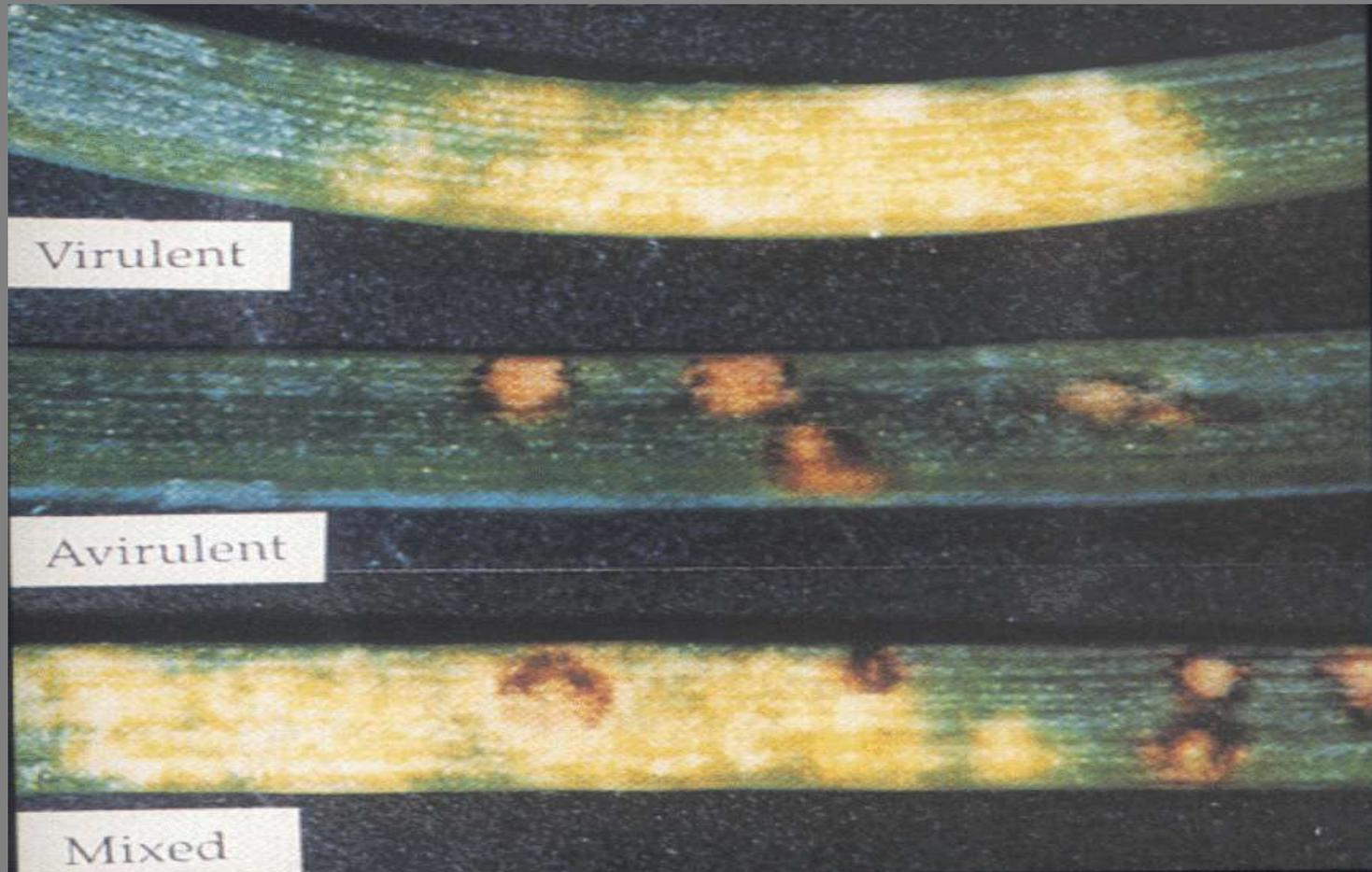
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# Lesion types: sugar pine



# Additional types of tree resistance

- Sugar pine
  - Slow rusting resistance - many components of resistance combined into a single phenotypic expression, exhibited as amount and type of infection with moderately strong inheritance and independently inherited expressions (low infection # and high infection abortion)
  - Ontogenetic resistance - another phenotypic expression that develops as the tree ages; under genetic controls; offspring may be fully susceptible

# Additional types of tree resistance, cont'd

- Western white pine
  - Slow canker growth - non race specific trait; produces abnormally small cankers; may reduce pruning necessity (due to success)
  - Reduced needle lesion frequency - also non race specific trait; few individual infection sites per seedling; may only be juvenile trait (seen in cotyledons)

# Evaluation of longevity of control practices

- Race of pathogen able to overcome major gene resistance in Sugar pine already present. Slow resistance or combination of two may be more durable approach

# Influence of Host Resistance on the Genetic Structure of White Pine Blister Rust Fungus in the Western United States

Richardson, Klopfenstein, Zambino, McDonald, Geils,  
Carris



# Purpose

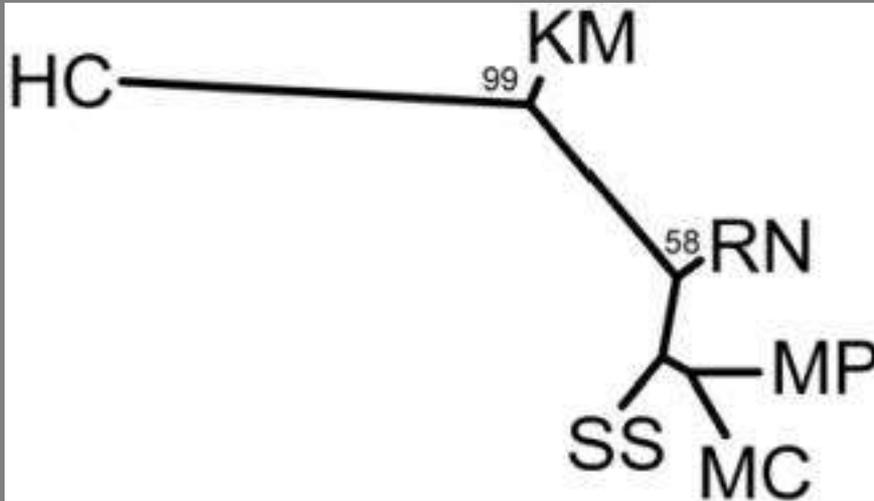
- 1) Examine genetic diversity within and among population of western N.America
- 2) Effects of host resistance on *C. ribicola*
- 3) Identify loci that behave as if linked to loci undergoing environmental selection

# Material + Methods



- Sampling of isolates from 6 sites
- B= merry creek: multigenic resistant, D= happy camp : major gene resistant

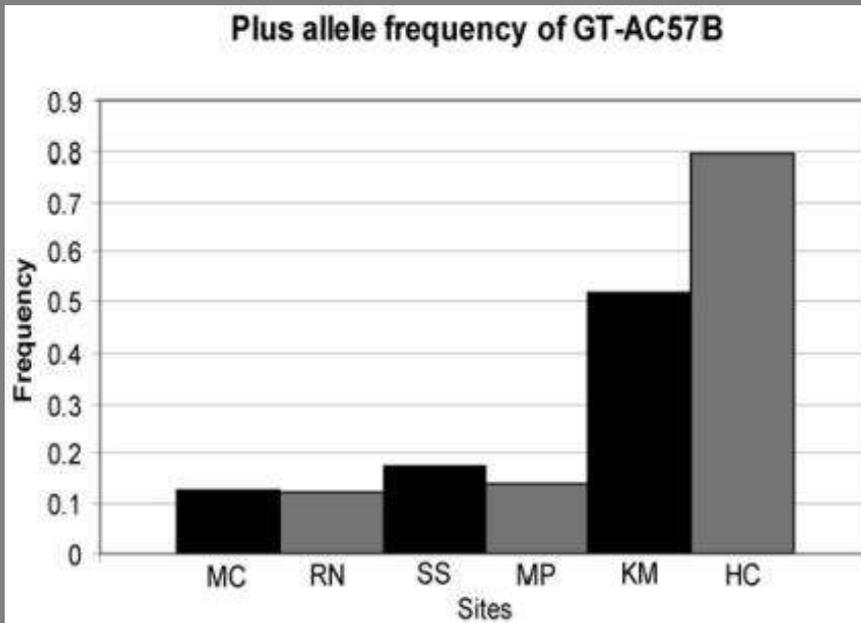
# Results



- Low number of polymorphic loci among 148 *C. ribicola* isolates

- Heterozygosity
  - Highest at MC
  - Lowest at HC

- $F_{st} = 0.082$  among sites, significant



# Discussion

## Effects of host resistance on *C. ribicola*

Merry Creek (multigenic resistant trees):  
had highest heterozygosity

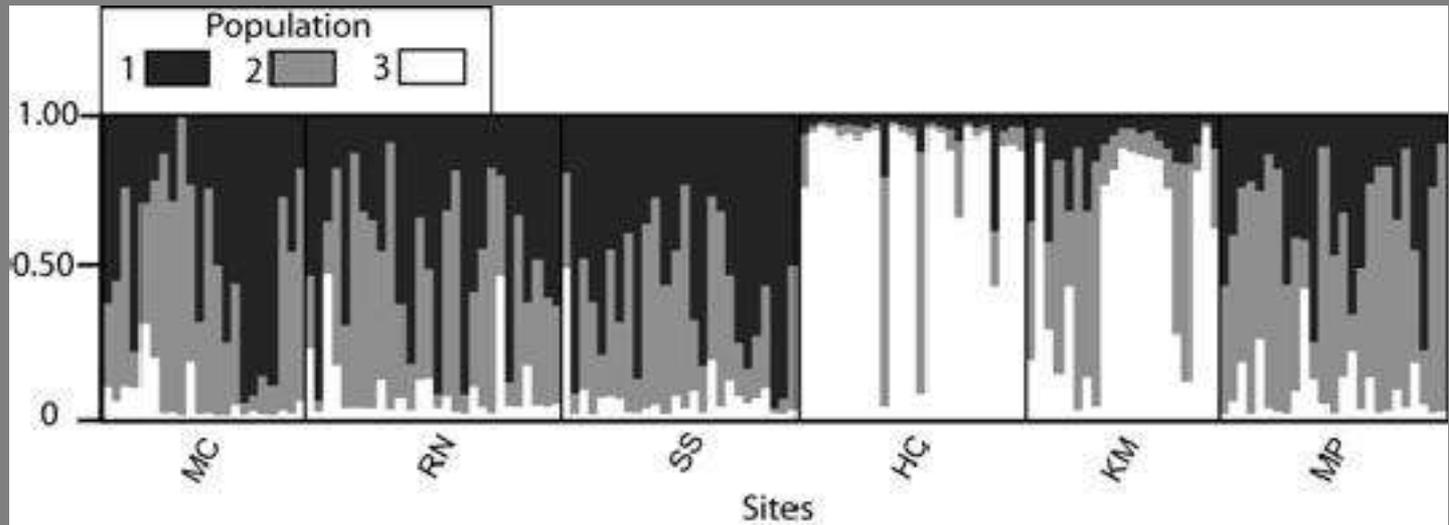
Happy Camp (major gene resistant trees):  
had

lower heterozygosity

- Selection for rust isolates carrying *vc1*  
because all trees have *cr1*.

# Results

- Possibly 3 populations of *C. Ribicola* in Western US

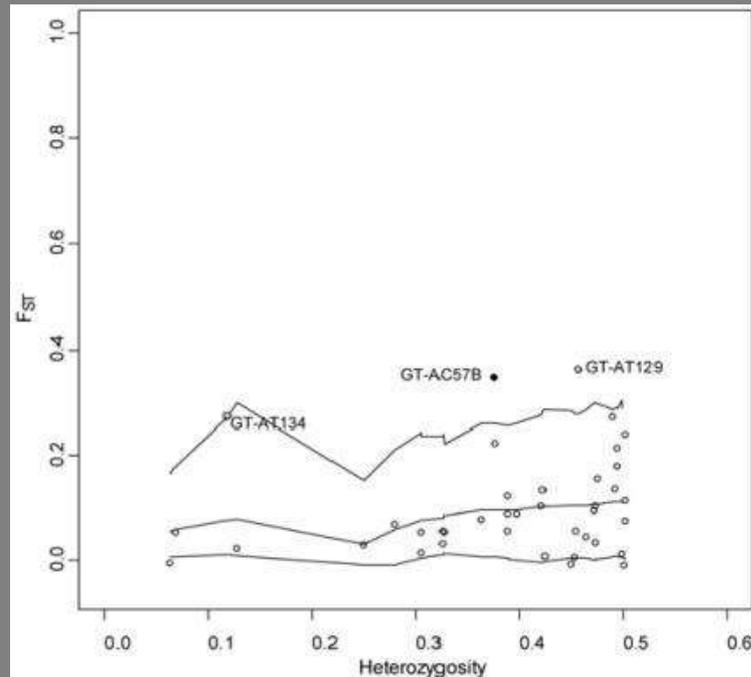


- Loci GFAC57B had very high  $F_{st}$  among different sites.

# Discussion

Identify loci undergoing environmental selection

- GTAC57B could be linked to genes under selection.



# Mortality and decline of white pine not only due to WPBR

- Fire suppression: most wp species like open spaces created by fire and are fire-adapted. With lack of fire, sites are encroached by shade tolerant species and white pine regeneration is limited
- Insect (mountain pine beetle) outbreaks. When populations of this insect become large they attack healthy trees as well. Effect of WPBT and mountain pine beetle is more than the sum of the two
- Dothistroma needle blight can cause outbreaks, however both Dothistroma and insect outbreaks may be cyclical and natural
- Global warming

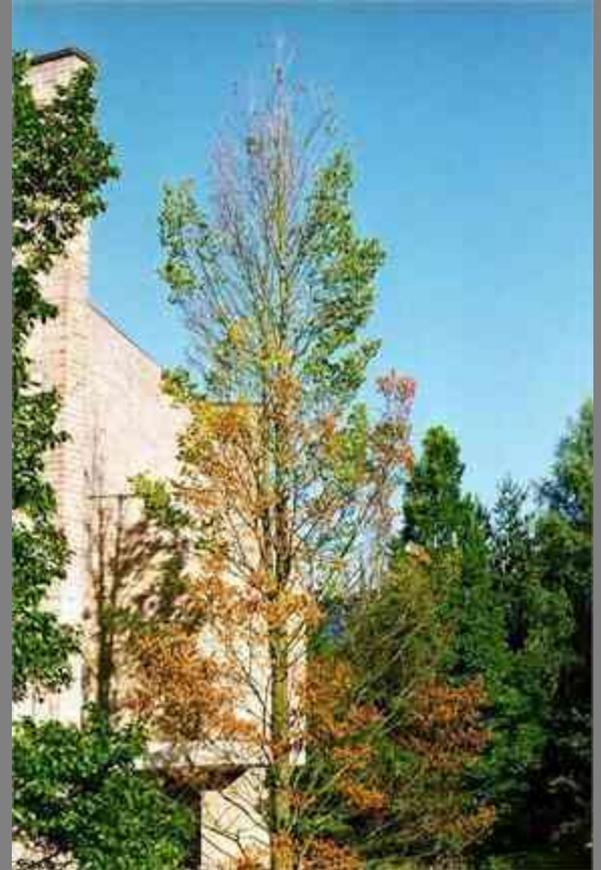
# Consequences of wp mortality

- Group of species that is extremely adaptable, and that in western North America, depending on latitude, goes from sea level to tree-line
- High market value: white pines timber is king. In past times it was the best timber to build ships' masts. One of the reasons for the secession of American territories
- It includes the oldest living organism on earth (Bristlecone pine)
- In the Rockies it is essential for survival of Clark's nutcracker and Grizzly bears. In the West, white pines are diversity hotspots

"In North America, white pine blister rust has caused more damage and costs more to control than any other conifer disease. Since the 1920's, millions of dollars have been spent on the eradication of the alternate host, *Ribes*, and thousands of white pine stands have been severely damaged. In the western United States and Canada, some stands have been completely destroyed. When the main stem of a tree is invaded, death is only a question of time."

Robert F. Sharp, U.S. Department of Agriculture Handbook 521 (p.85)

# Dutch Elm Disease



# Host: the Elms (genus *Ulmus*)

- >30 species in genus. Europe has 5; N. America 8; Asia has 23 or more
- 6 species native to the northeastern U.S., including *Ulmus americana*, the American elm
- New species are still being found in China, the center of diversity

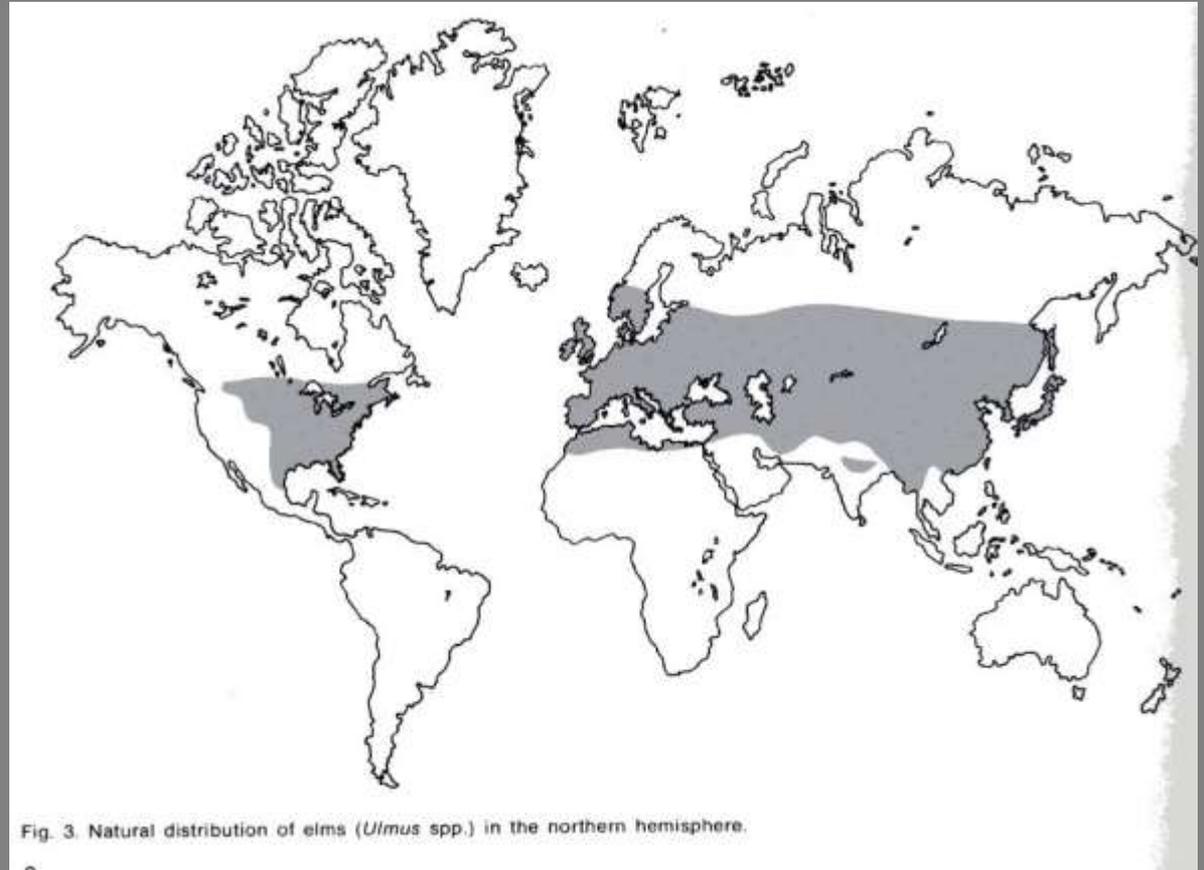


Fig. 3. Natural distribution of elms (*Ulmus* spp.) in the northern hemisphere.

# Elms: the perfect shade tree

- Used as street-liners
- Fast-growing, easily transported, tolerant of soil compaction and different soil types
- Shade trees, with branches high above ground. When planted in rows, they overhang the street forming a Gothic-style arch. Good for windbreaks
- #1 urban tree in U.S east of the Rockies, and in large parts of Europe and Asia (Heybroek, 1993)

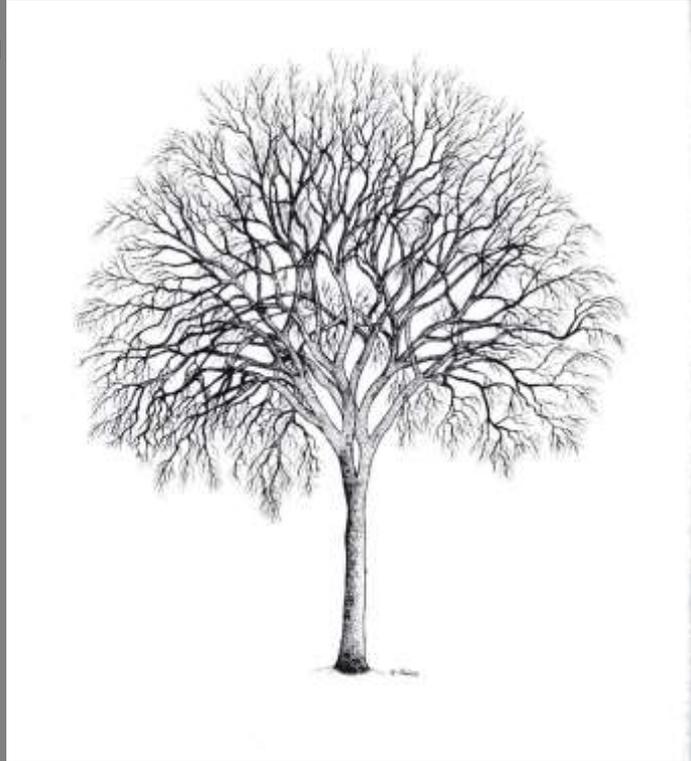


Fig. 25. Street scene of arching American elms in Evanston, Illinois (1976). (Courtesy E. B. Himelick)

# Elms: rural and natural Settings

In rural settings:

- In coastal western Europe, used as windbreaks
- The Siberian Elm was planted as “shelterbelts” to prevent erosion during the Dustbowl in the 30’s in the U.S.



In Natural Settings:

- A generally riparian, river bottom group that can survive periods of anoxia, explaining tolerance to over-watering and soil compaction

# Overview: Dutch Elm Disease

- Why “Dutch”? First isolated in 1920 by a Dr. Schwarz in the Netherlands
- Wilt disease that attacks elm (*Ulmus* spp) and spreads through the vascular system
- 
- Caused by ascomycete fungi (genus *Ophiostoma*)
- Vected by beetles (family *Scolytidae*) and root graft

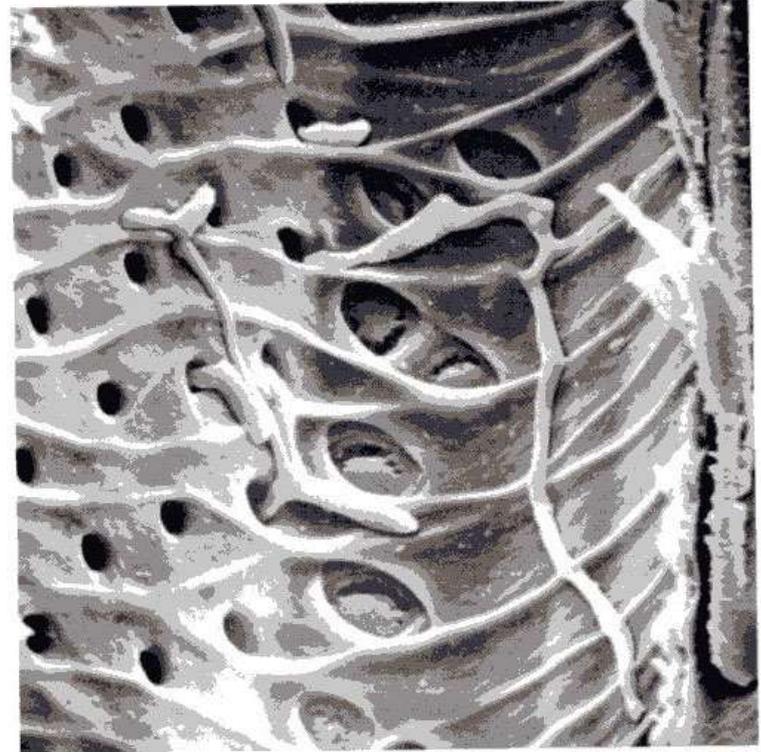
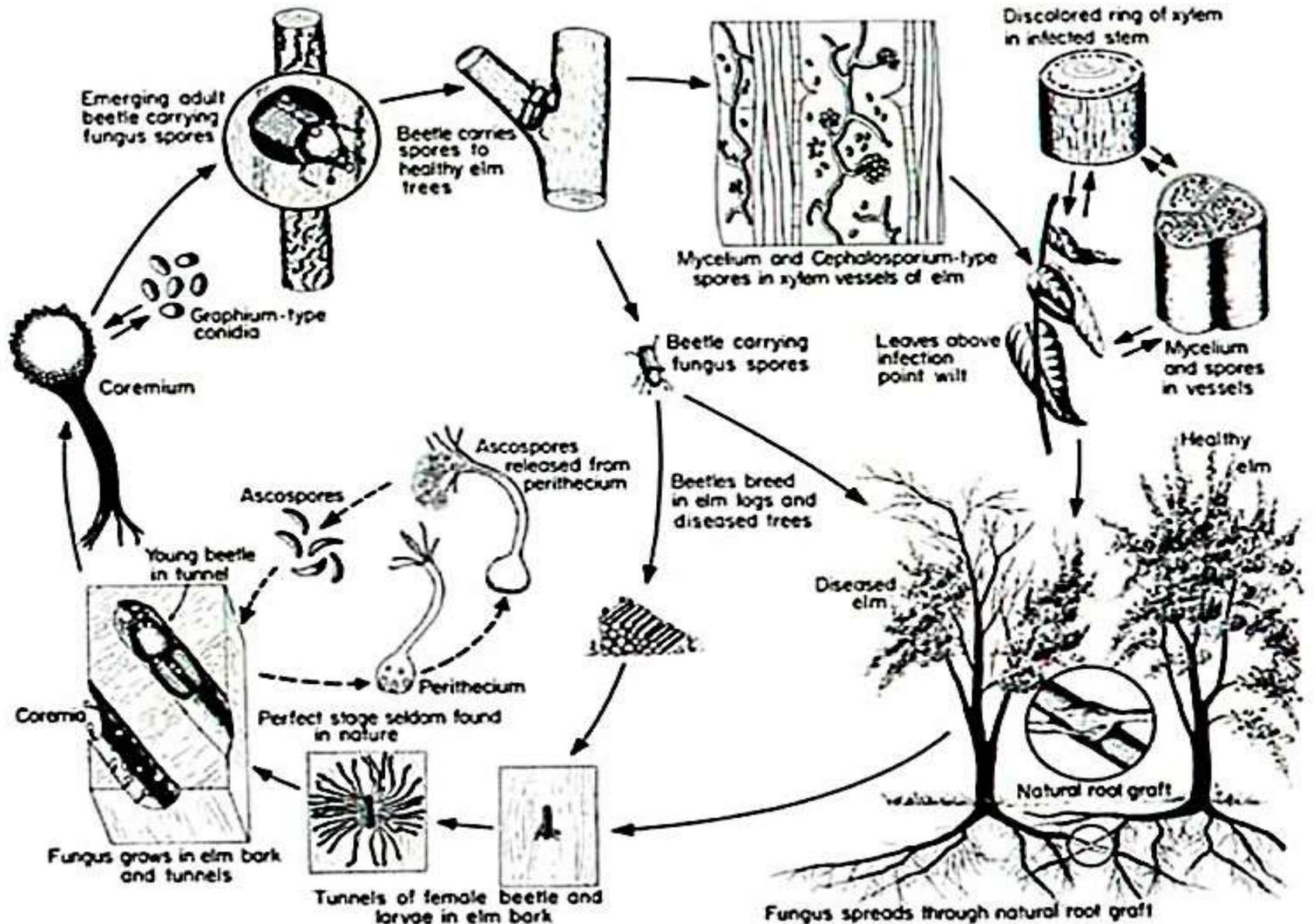


Fig. 22. Hyphae of *Ceratocystis ulmi* in diseased elm wood. Note passage of fungus through pit openings between vessels. (Courtesy D. M. Elgersma)

# Life Cycle of *Ophiostoma ulmi*



# Vectors of disease

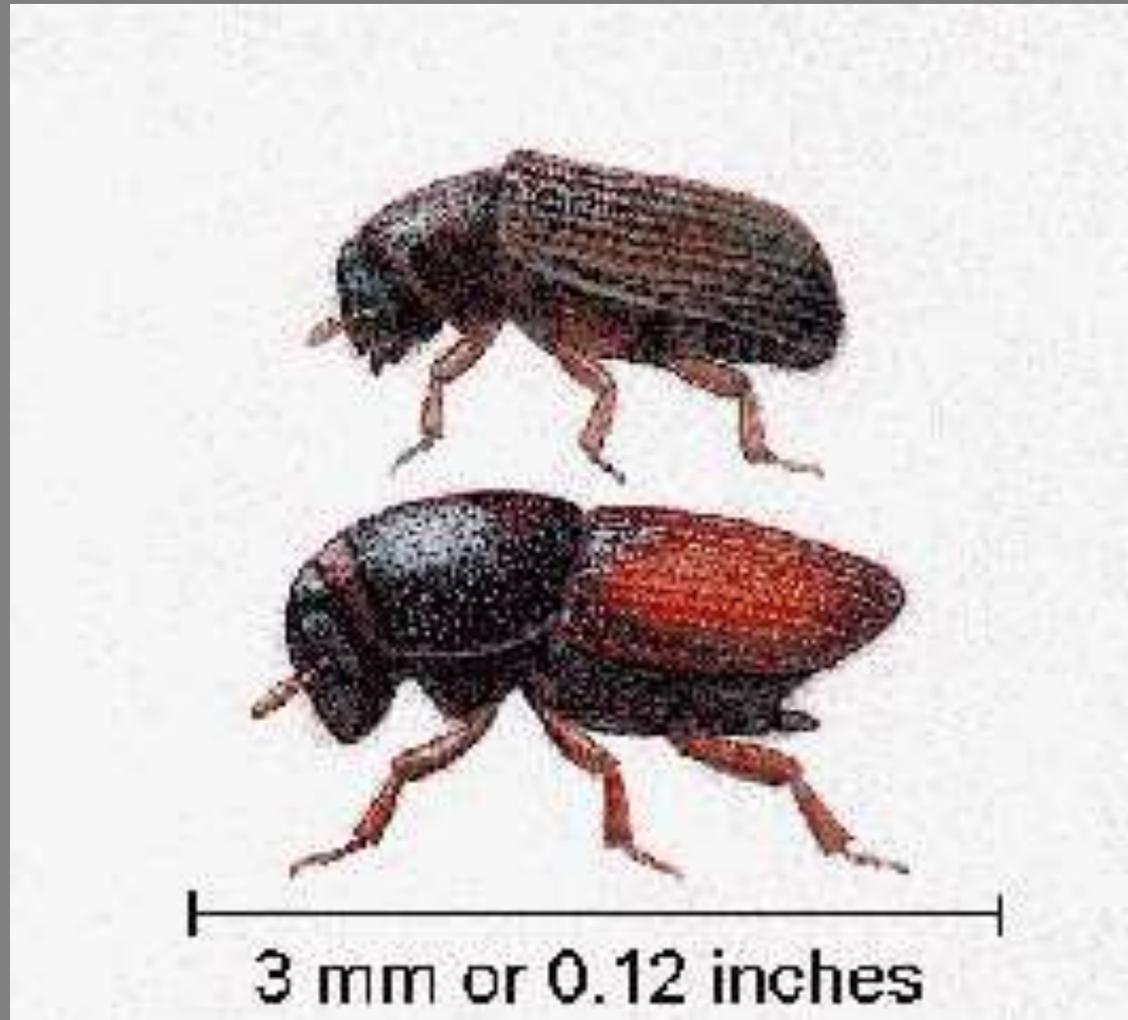
- Insects: 1) the native elm beetle 2) the smaller European elm beetle. The beetles can fly for several miles, allowing the disease to spread over a wide area
- Root grafts: when elms are within 50 feet of one another, their roots can grow together and disease passes easily along. Important in urban settings
- Infected logs: Often transferred long distances

# Beetles: key disease vector

1) Native elm bark beetle  
(*Hylurgopinus rufipes*) (above) is the primary vector in parts of the northern United States, New England, and all of Canada.

However, temperatures below -6F kill the larvae.

2) European elm bark beetle  
(*Scolytus multistriatus* Marsh.)  
(below) is the major vector of the disease.

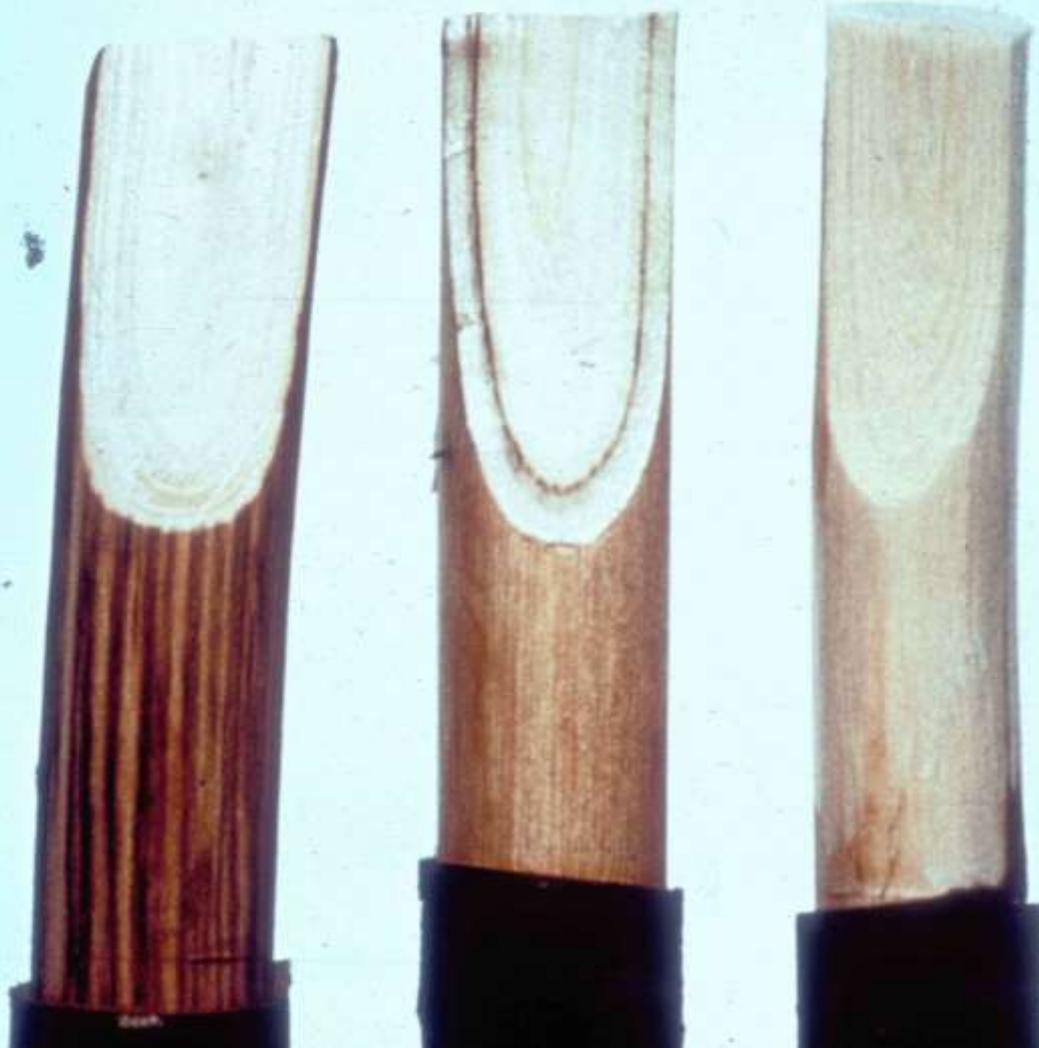




# Dutch elm disease – crown symptoms



# Dutch elm disease – vascular discoloration



# Elm root grafts



**Elm bark beetle galleries**



**Maturation feeding**



1970



1976



1977



1978



1991



# History of the Disease

- Disease was unknown in Europe and N. America before 1900
- Since 1910, two pandemics
- Pandemics caused by two different species:
  - 1) *Ophiostoma ulm*
  - 2) *Ophiostoma novo-ulmi*
- In both cases, geographic origins are still unknown (probably Asia)

# Management: Sanitation

- Includes removing bark from elm logs which are being stored for use as fuel and/or covering or burning all downed wood (so that beetles can't get in it). AND, removing dead or diseased branches of standing trees (again because of the beetles).
- Needs to be community-wide, and coupled w/fungicide use.
- Thought of as the most effective way of curbing DED.

# Management: injections

- Systemic fungicides labeled for preventative control, injected into root flares. Effective on trees showing < 5-10% crown symptoms.
- Need new injections every 3 years, expensive.

# Management: Spraying

- Best when coupled w/sanitation methods.
- Timing of spraying is important

# Other Management Methods

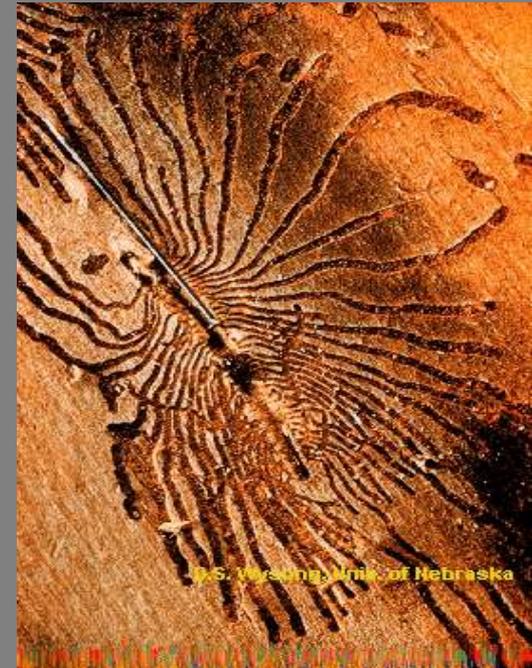
- Development of resistant hybrid elms
- Additional treatments: breaking up root grafts is commonly used and effective.
- Timing of pruning: wounded trees attract the bark beetle vectors of DED (Byers et al., 1980), so routine pruning should be done in the dormant season or during periods of beetle inactivity.

# Dutch Elm Disease

- Wilt disease caused by ascomycete fungus in the genus *Ophiostoma*



- Transmitted by *Scolytus* bark beetle
  - Beetle carves larval galleries in sapwood and carries fungus from tree to tree



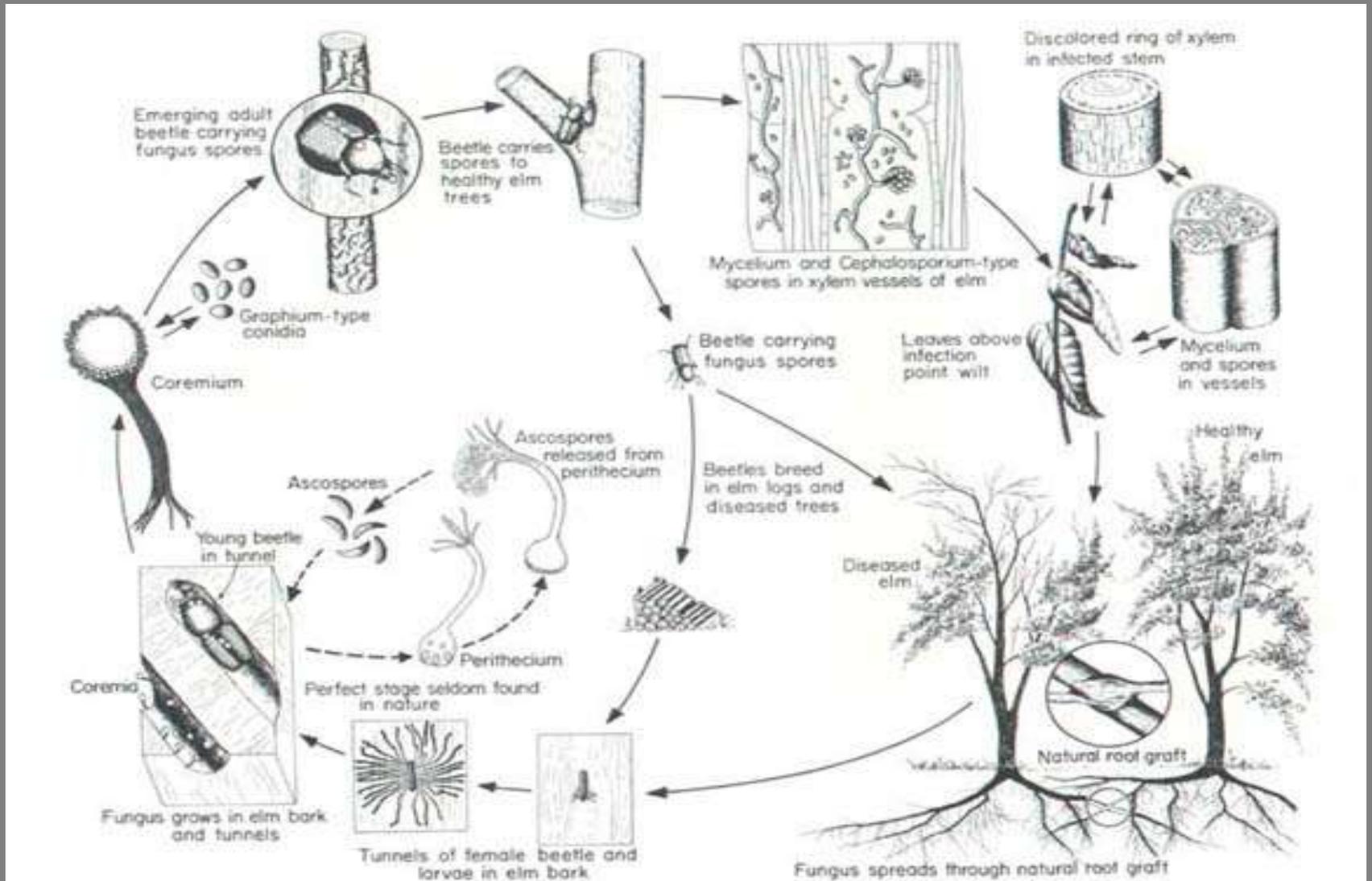
- Spreads through trees vascular system
- Tree tries to slow fungus by plugging its own xylem tissue with tyloses
- Plugged xylem causes branch dieback

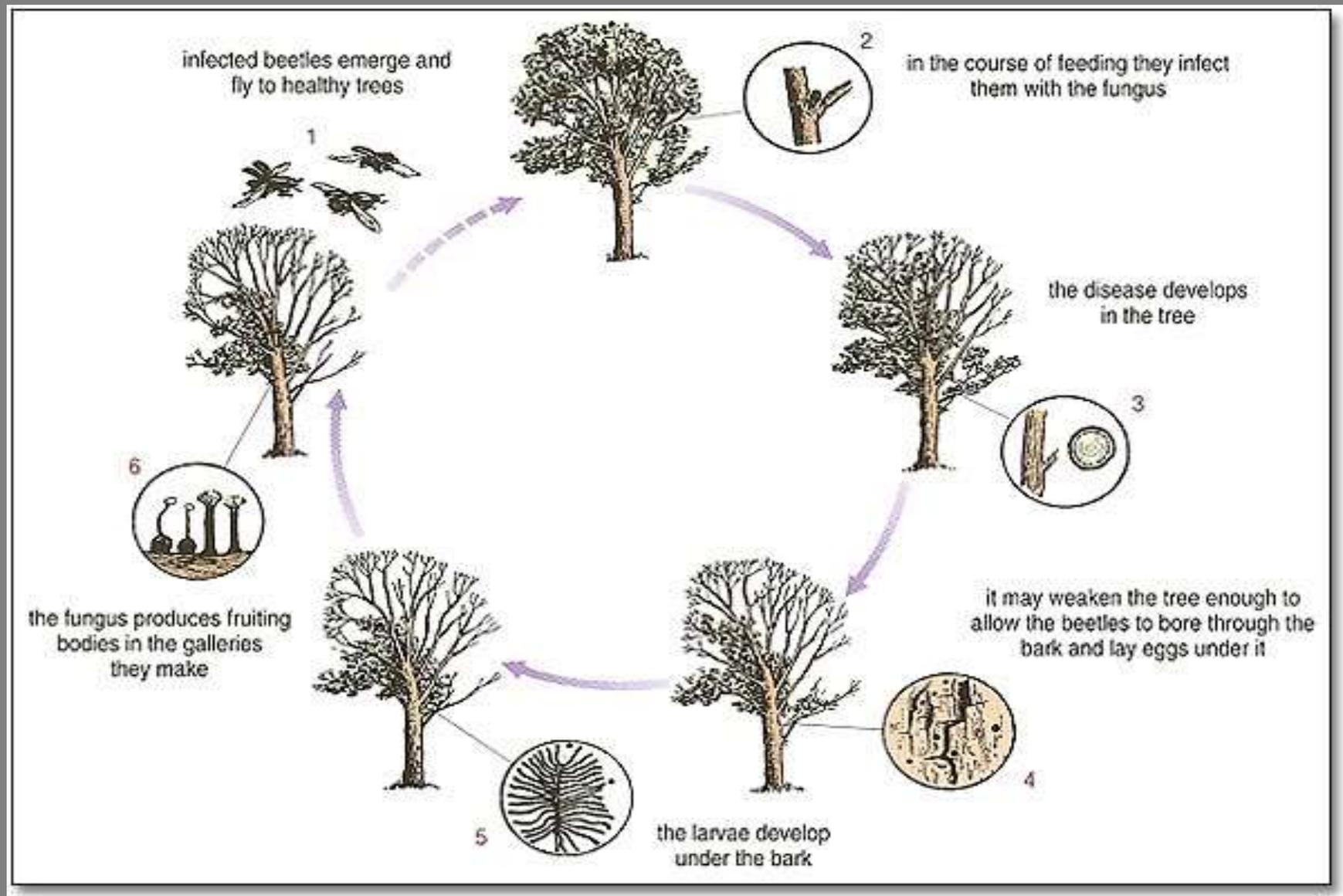


- Toxins force stomata to open
- Increased evapotranspiration causes desiccation and rapid death of tree

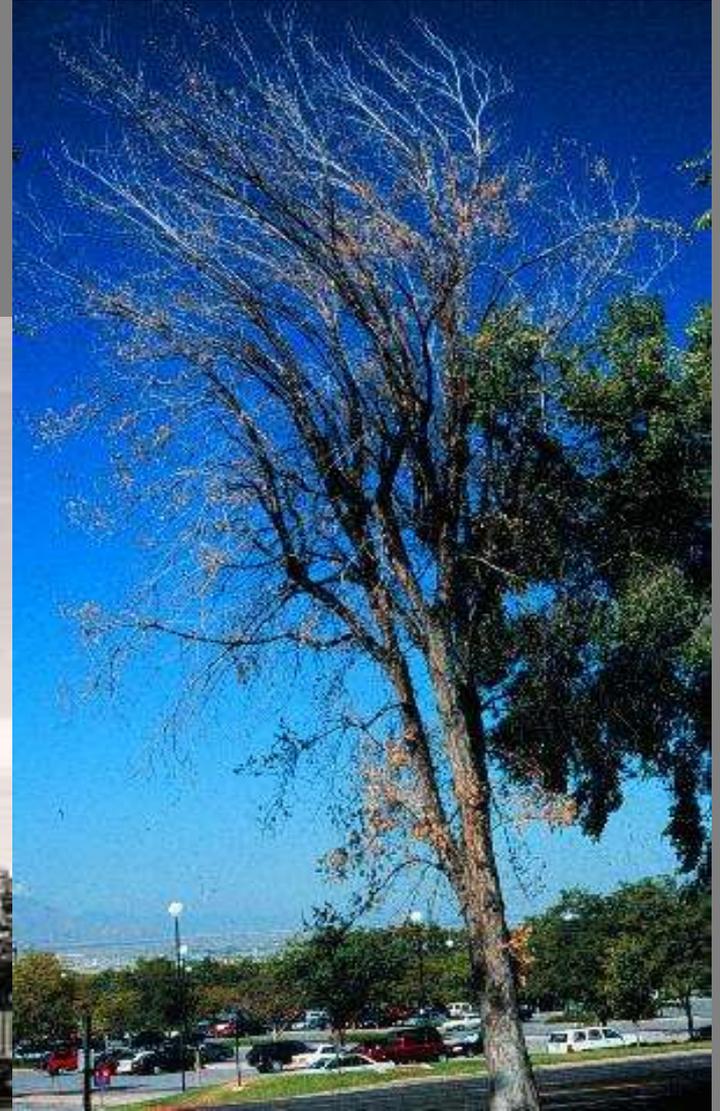


# Life cycle with beetle vector

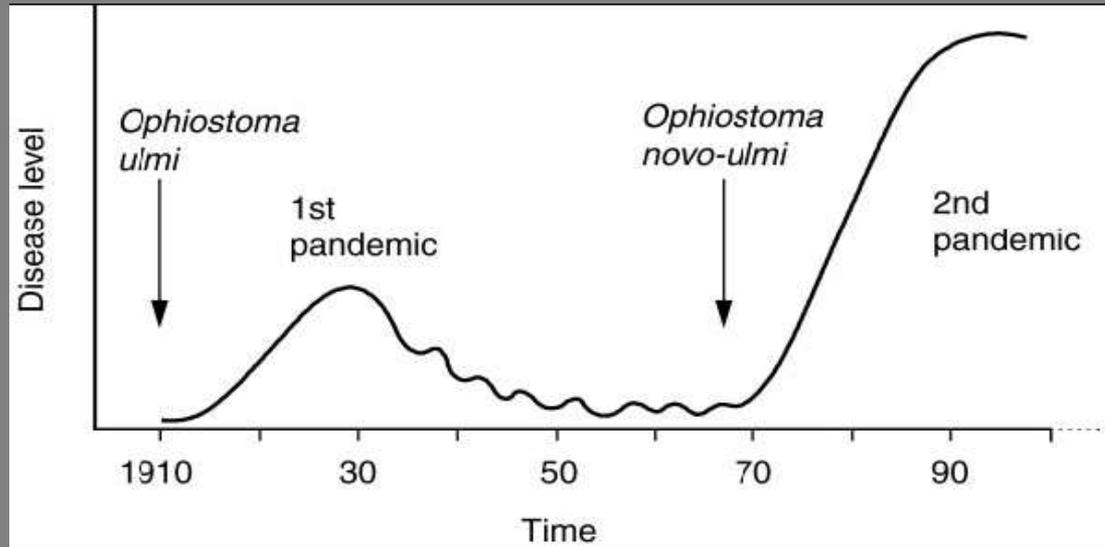




- Two separate pandemics caused by two different species
- *Ophiostomata ulmi*
- *Ophiostomata nova-ulmi*
- Origins still unknown



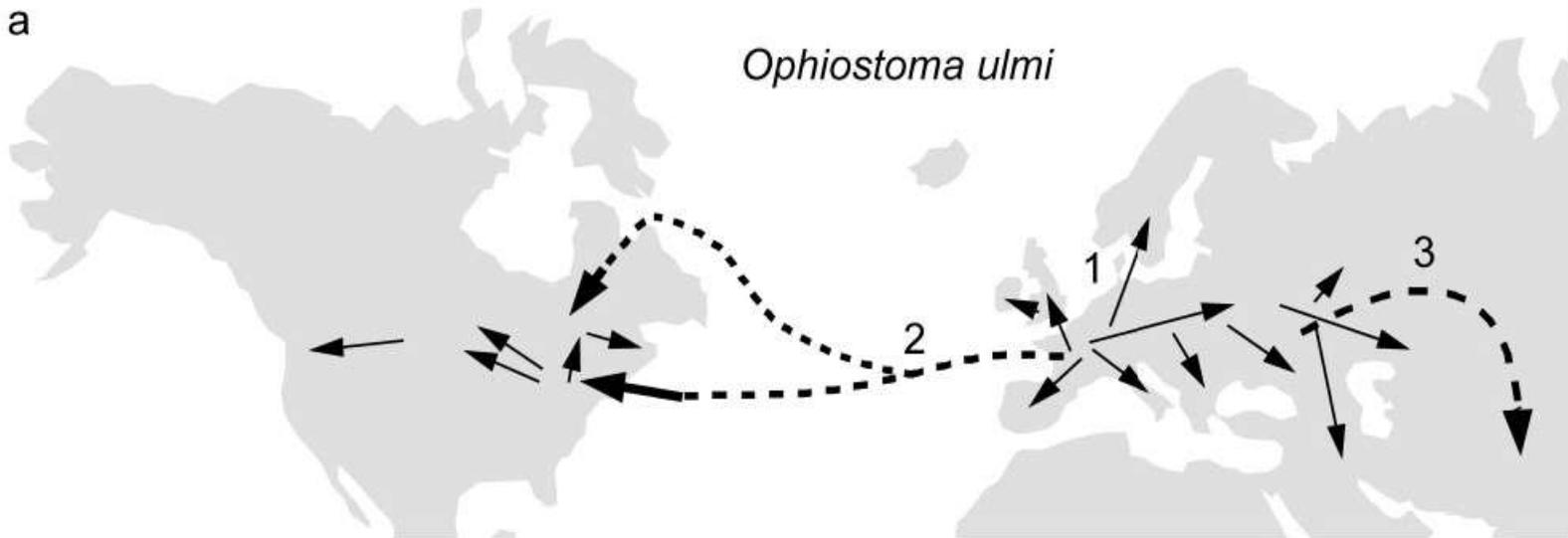
# Two Pandemics



- *O. ulmi* arrives in Europe and expands outward on infected timber, kills 10-40% elms then stops...Virus!
- *O. novo-ulmi* strains introduced in both Europe and N. America-radiates further
- Now both species overlap in Europe

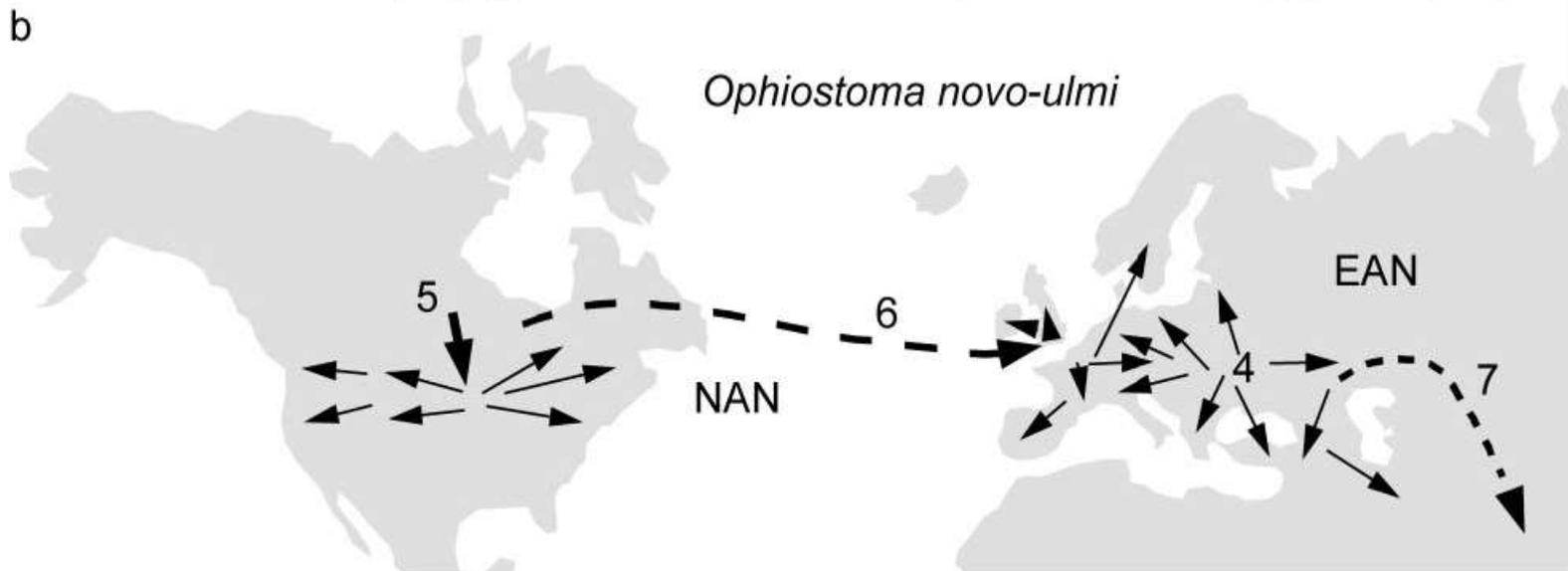
a

*Ophiostoma ulmi*



b

*Ophiostoma novo-ulmi*



# Two species differ in...

- Optimal growing temp
  - *O. ulmi* 28 C subtropical origins
  - *O. novo-ulmi* 22 C temperate origins
- Colony morphology
- Molecular fingerprint
- Pathogenicity to elms
  - *O. ulmi* moderately aggressive
  - *O. novo-ulmi* highly aggressive



# Reproductively Isolated?

- Not completely...
- Both species have two mating types and crosses within species are fertile
- Between species...
  - *O. ulmi* can not fertilize *O. novo-ulmi*
  - *O. novo-ulmi* CAN fertilize *O. ulmi*
- Progeny include
  - Sterile females
  - Offspring with low vigor and fitness
    - Basically out-competed by stronger parent species

# When the two species meet...

- *O. ulmi* usually present when *O. novo-ulmi* arrives and is quickly replaced
- Two species meet in bark surrounding beetle galleries
- Chance for interspecific genetic exchange
- Hybrids don't survive or are transient  
BUT...
- Act as **GENETIC BRIDGE**

# Definitions

- Interspecific hybrid
  - Mating between two species of same genus produces a sterile or transient progeny to prevent movement of genes and thus keeps species separate
- Introgression
  - Backcrossing of an interspecific hybrid with one of its parents leading to movement of genes in between species

# Methods of Detecting Gene Flow

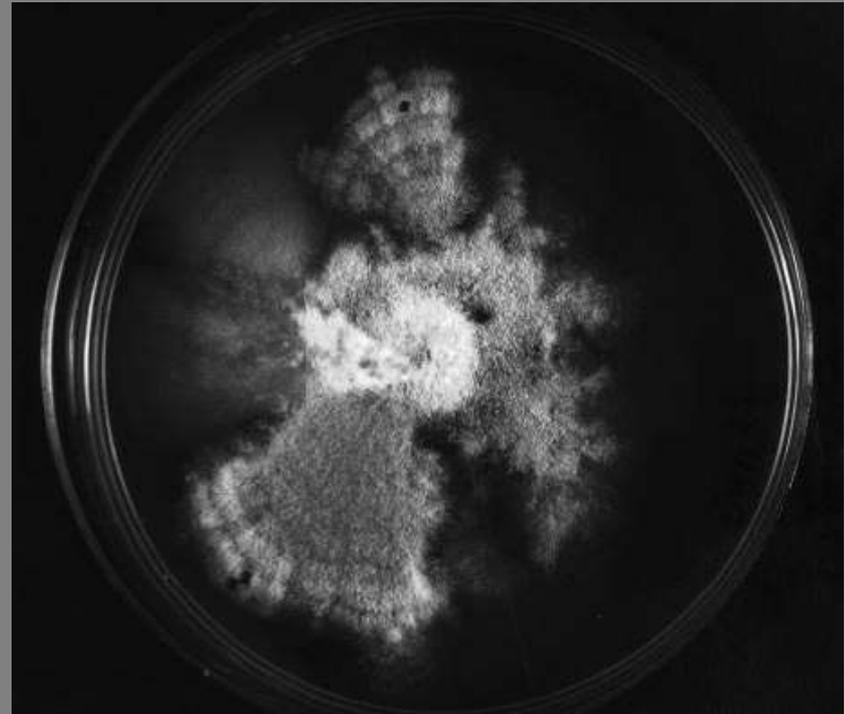
- DNA Fingerprinting
  - Probed with cloned dna fragments to distinguish between two species
  - Some *O. novo-ulmi* isolates had rare *O. ulmi* like polymorphisms
    - Acquired through introgression
  - 15 of 50 *O. novo-ulmi* isolates had *O. ulmi* gene
  - 1 had altered phenotype
    - Less aggressive
    - AFLP showed gene was involved with pathogenicity

# Gene flow? More clues...

- Sudden increases of Vegetative Compatibility (VC) types
  - Necessary to prevent spread of “cooties” (viral factors) between adjacent colonies
  - Controlled by many genes with many alleles; only isolates that have the same alleles at all VC genes fuse their hyphae because they recognize each other as selves
  - Viruses will spread through fused cells of same VC types

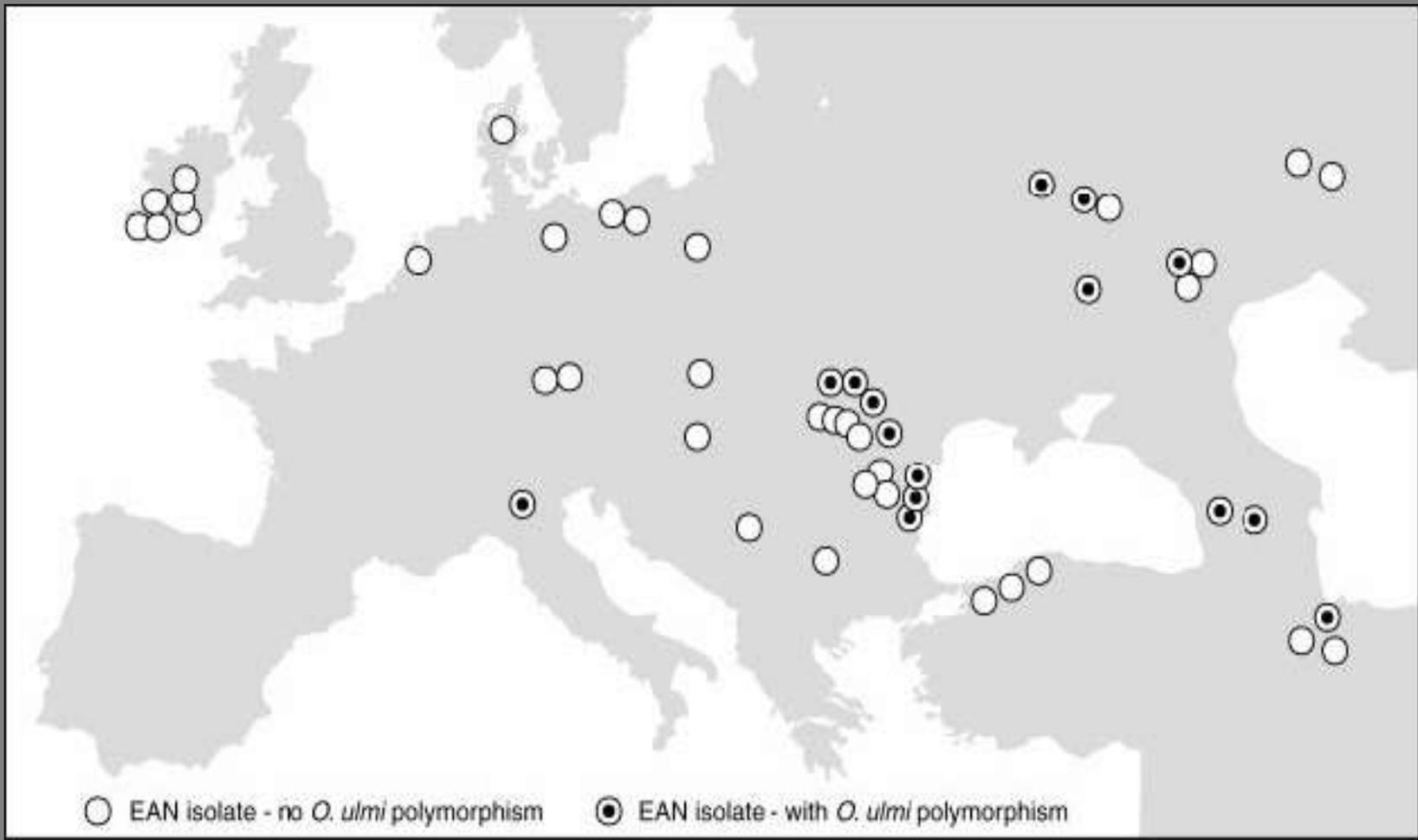
# Europe

- Single clones of VC types with single mating type introduced and spread through Europe
  - High rate of viral spread through pop
- After a few years population diversifies in VC type and mating type
  - Spread of viruses declines
- Effect slower in North America due to less pressure from virus



# Conclusions

- *O. novo-ulmi* VC clones diversify only where *O. ulmi* was already present
- Only when virus activity is high will clones diversify rapidly
- Novel VC genes are acquired by *O. novo-ulmi* from *O. ulmi*
- Selection pressure from viruses favors novel VC types



*O. novo-ulmi* with single VC type-black  
As it changes to many VC types in grey

# WOW!!!

- *O. novo-ulmi* outcompeted *O. ulmi* in Europe
- *O. novo-ulmi* caught virus from *O. ulmi* that would have killed it off BUT.....
- At the same time *O. novo-ulmi* acquired VC genes from *O. ulmi* that made it less susceptible to virus

# *O. Himal-ulmi*

- Found in Himalayas while searching for origins of pathogens in Asia
- Appears to be in natural balance with elms and bark beetles
- Very aggressive on European Elms
- Importance of regulating timber trade



# The End



# Pitch canker disease of pines

- Threat to native populations of Monterey pine
- Threat to exotic plantations overseas



# THE PRINCIPAL HOST TO PITCH CANKER IN CALIFORNIA



*PINUS RADIATA* (MONTEREY PINE)



Just in case you were wondering why it is called pine pitch canker...



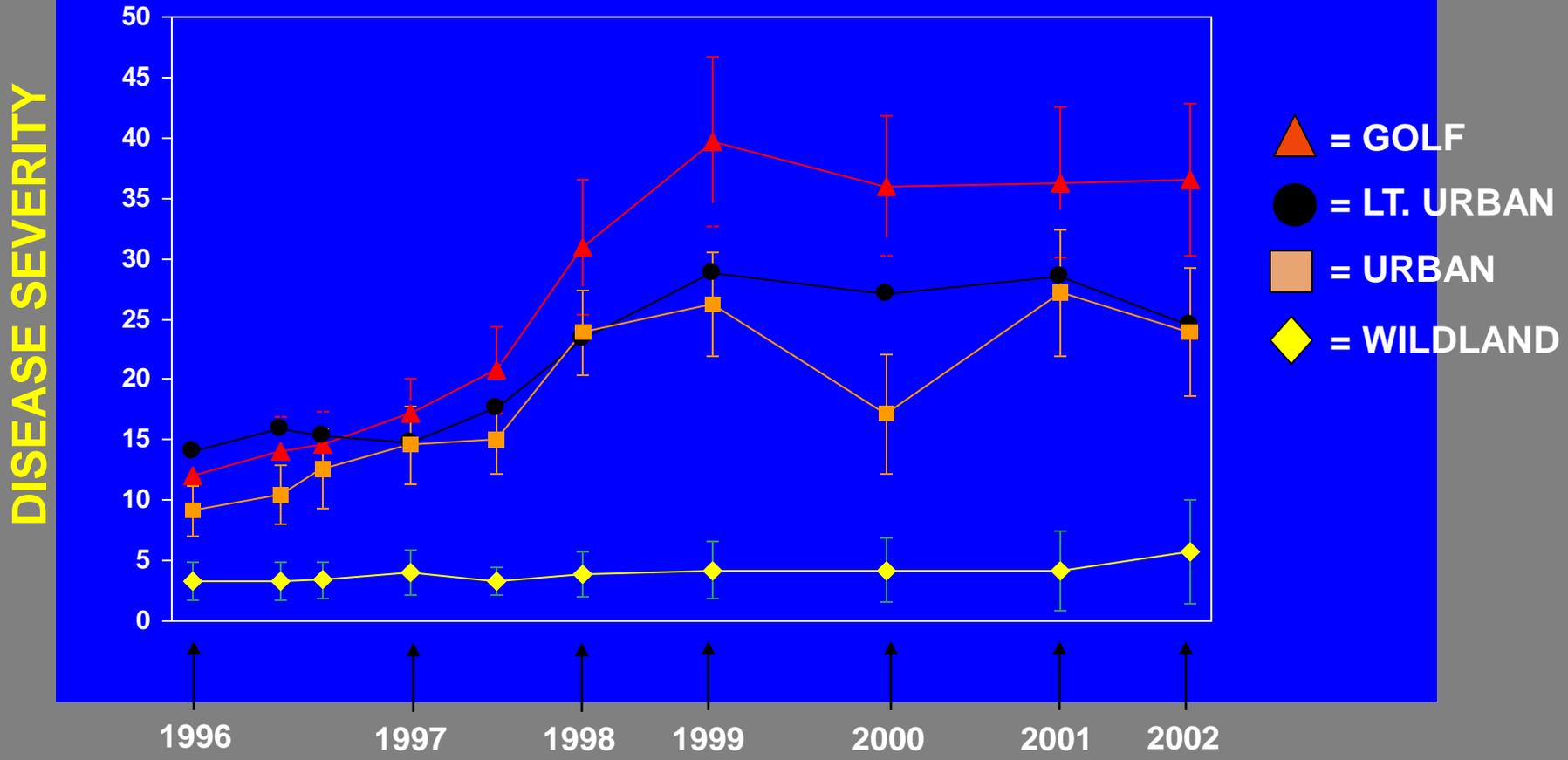
# Where does it come from?

- Maximum genetic diversity found in Mexico
- East Coast of US has relatively high diversity, suggesting longer exposure
- South Africa, California and Japan have very low diversity, with California and Japan being quite similar presumably because infestations have the same source

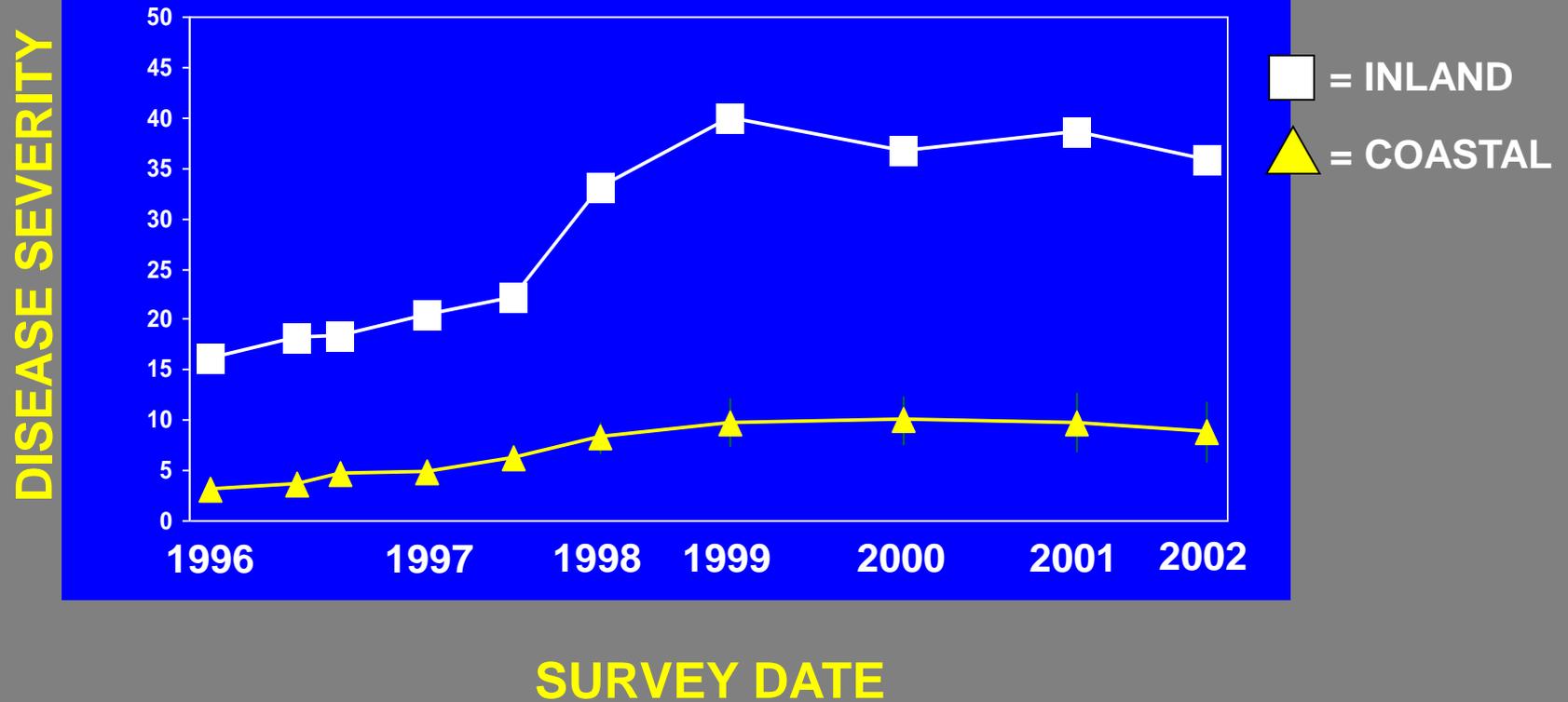
# Careful when interpreting data:

- Genetic similarity between two sites does not necessarily imply a source-sink relationship, but can indicate a third location was the source for both
- Observational correlations, especially in new systems (like new host-pathogen combinations) may be misleading. The pathogen was found on many insect species but this was an accidental not a causal correlation, meaning that insects were not vectoring the disease, but rather they were accidentally contaminated in correlation with the significant outbreaks due to the novelty of the association between Monterey pine and *Fusarium circinatum*

# DISEASE PROGRESS BY LANDSCAPE TYPE



# DISEASE PROGRESS BY LOCATION



# HOW MANY TREES WILL DIE?



# MORTALITY DUE TO PITCH CANKER IN A PLANTED STAND OF MONTEREY PINE

**1989:** 8% OF TREES INFECTED (N =50)

**1993:** 96% OF TREES INFECTED

**1999:** 14 TREES WERE DEAD, NEARLY DEAD OR  
HAD BEEN REMOVED

**MORTALITY RATE = 28%**

# MORTALITY RECORDS FROM MONTEREY PENINSULA PLOTS

TOTAL NUMBER OF TREES LOST SINCE 1996:

138

CONFIRMED PITCH CANKER DEATHS:

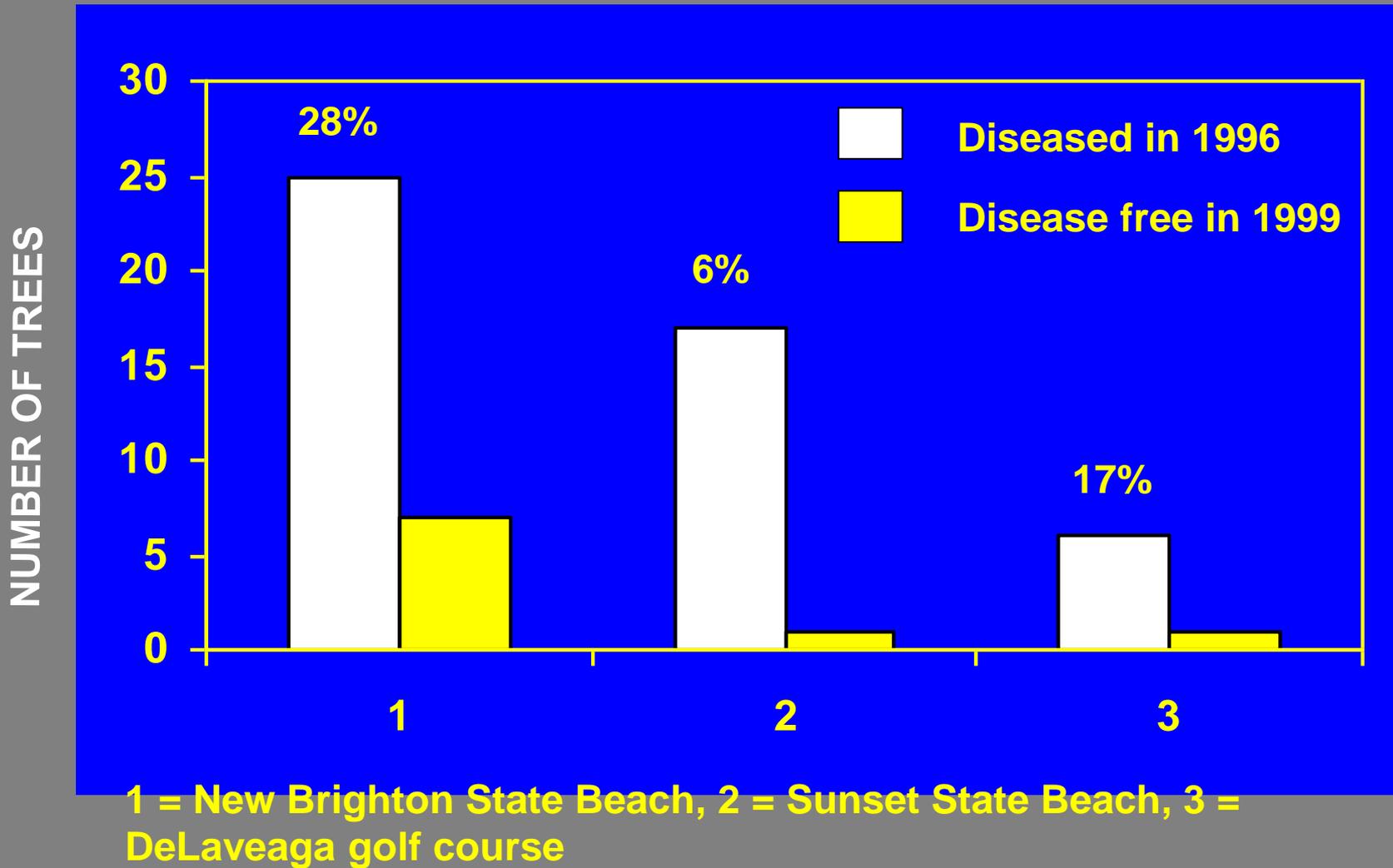
5

% MORTaIITY DUE TO PITCH CANKER = 3.6%

# LONG TERM IMPACTS



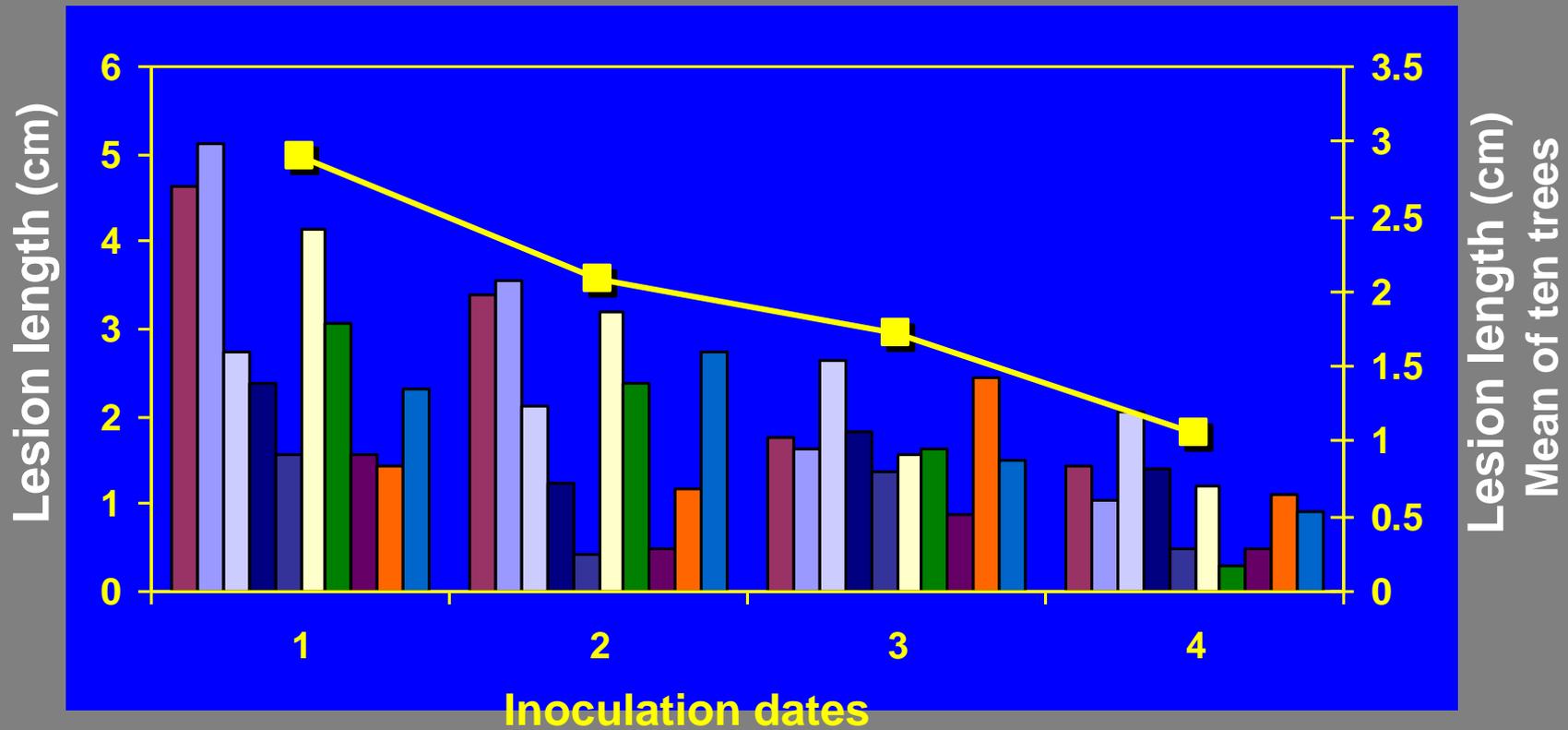
# CHANGE IN DISEASE STATUS FROM 1996 TO 1999



**DISEASE REMISSION COULD BE DUE  
TO ELEVATED RESISTANCE  
RESULTING FROM REPEATED  
INFECTIONS**

**= INDUCED RESISTANCE**

# EFFECT OF REPEATED INOCULATIONS ON SUSCEPTIBILITY



## **PITCH CANKER IN OTHER SPECIES:**

**At this point significant only in Bishop pine: however, in order to understand impact we need to ensure exposure has been long enough, and in areas with significant host density. With time:**

- Mortality rates may decrease**
- Mortality rates may increase**

**BISHOP PINE**

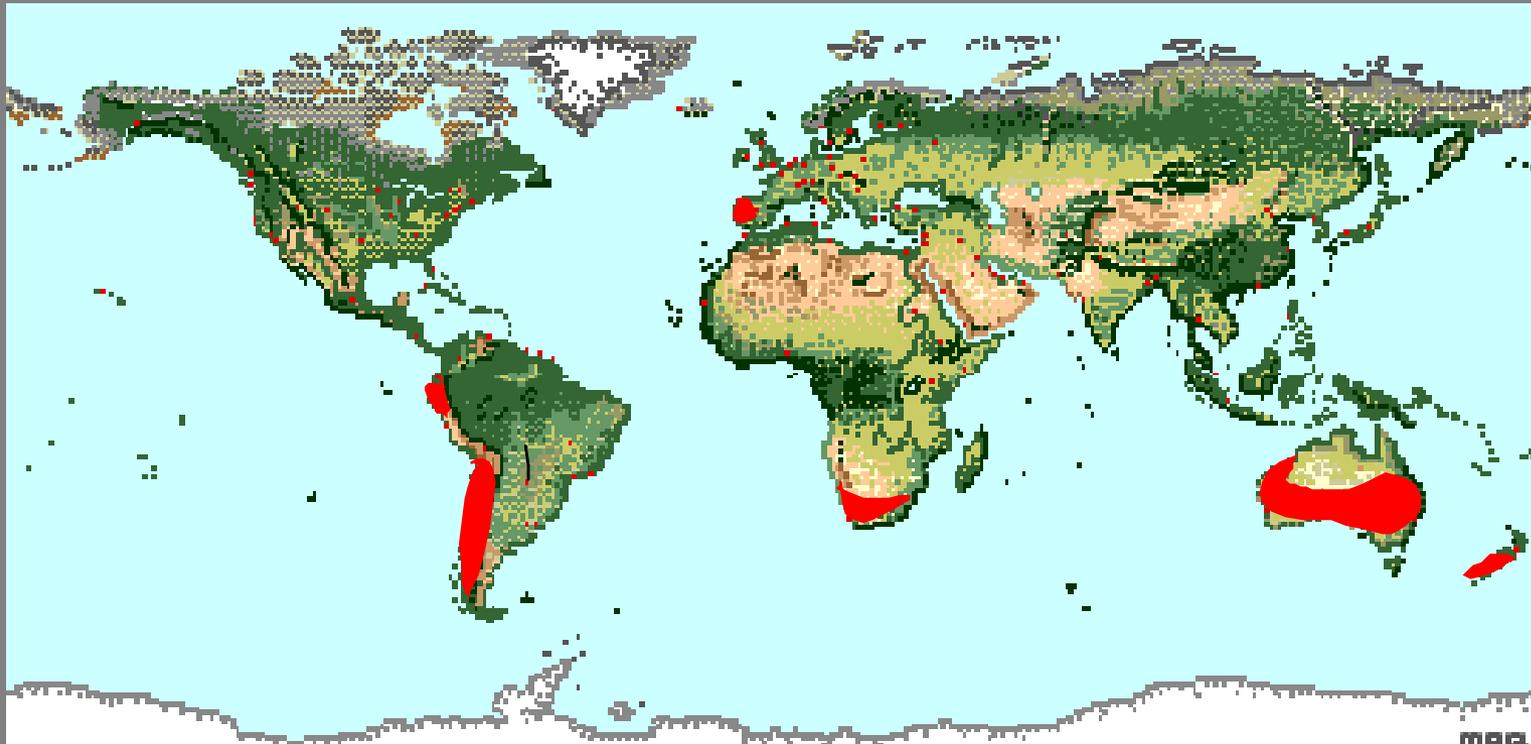
**KNOBCONE PINE**

**DOUGLAS-FIR**

# Pitch canker disease of pines

- Introduced in California
- Spread around by Christmas tree trade

**Regions in red are characterized by significant presence of Monterey pine plantations**

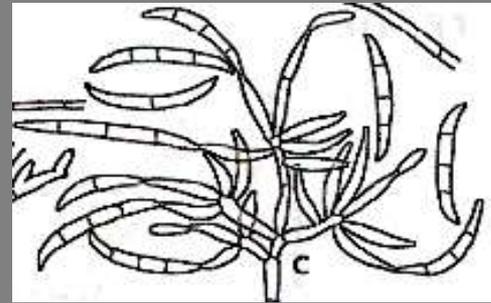


## PITCH CANKER AS A SEEDLING DISEASE



## Spreading of the disease

- Vectors ?
- Infected nursery material ?
- Aerial spores ?



macro conidia

micro conidia



# Fusarium sampling,

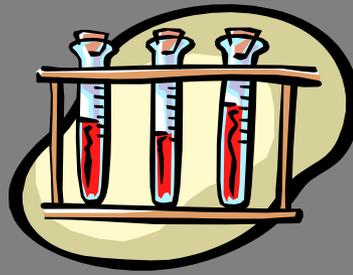


# Real-time Quantification Working Schema

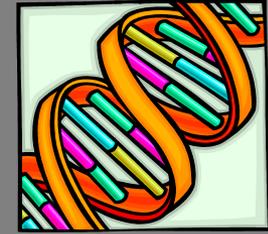


Spore trap with filter paper

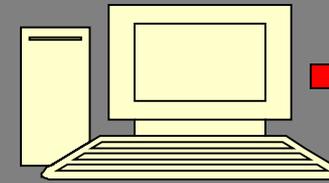
suspend in  
20 ml 4X TE 65°C



Centrifugation  
Spore concentration



DNA-extraction



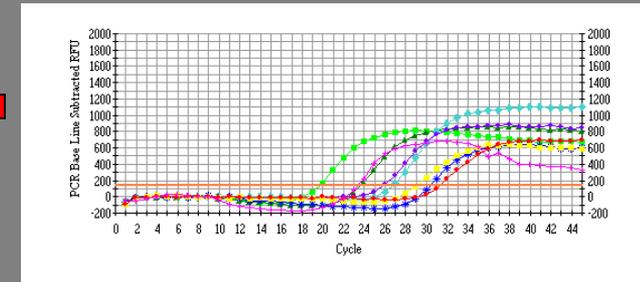
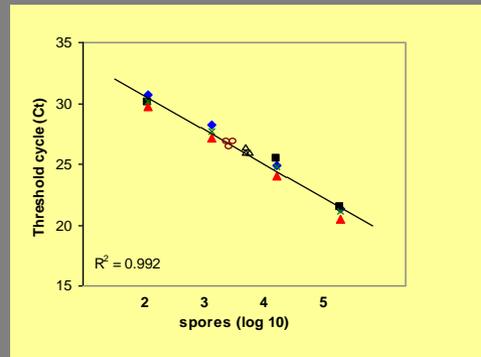
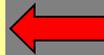
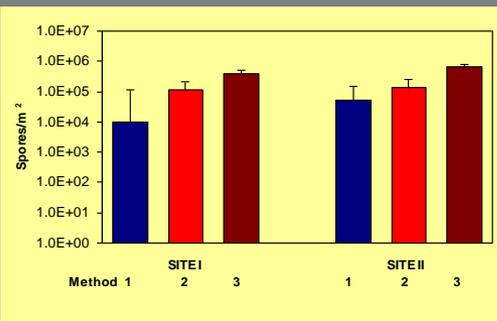
Mating type determination



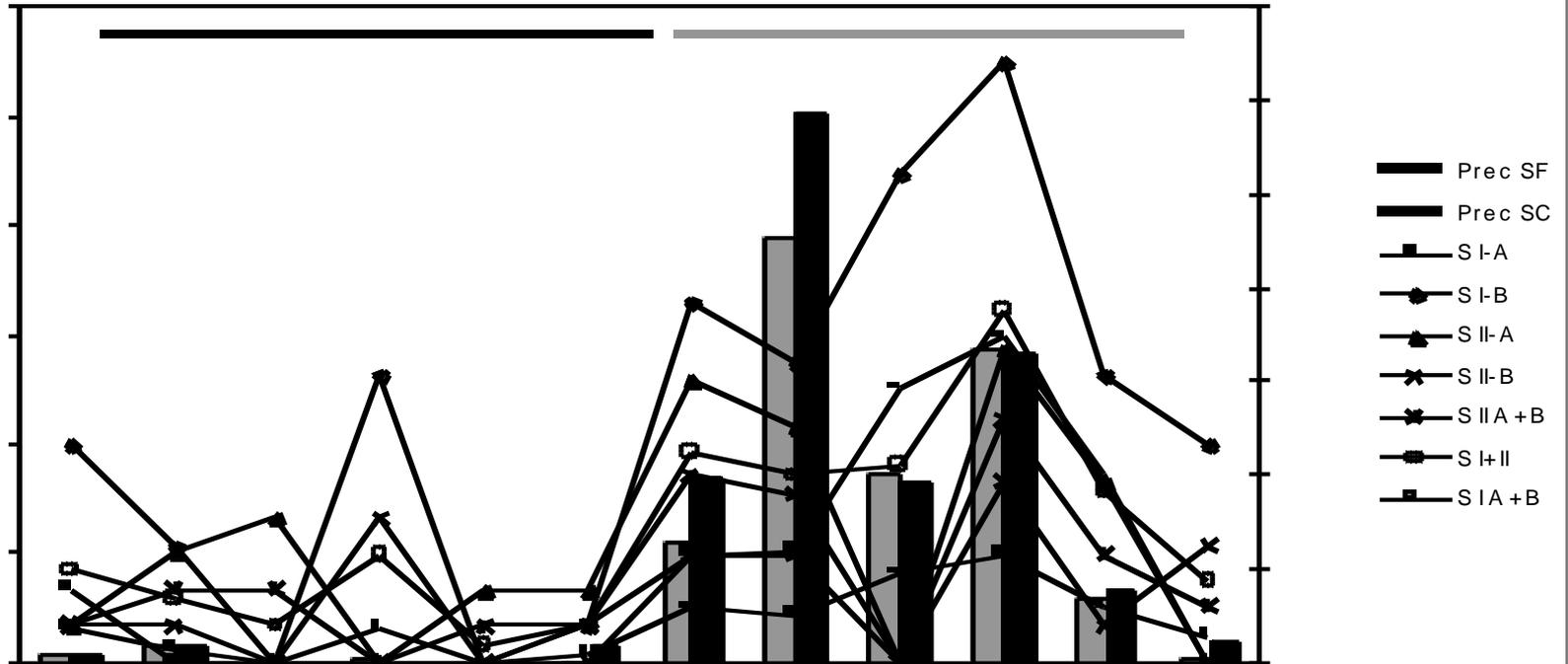
RT-PCR using  
CIRC1A-4A

Calculate spores/m<sup>2</sup>

Compare with Spore (DNA) standard



# Seasonal differences



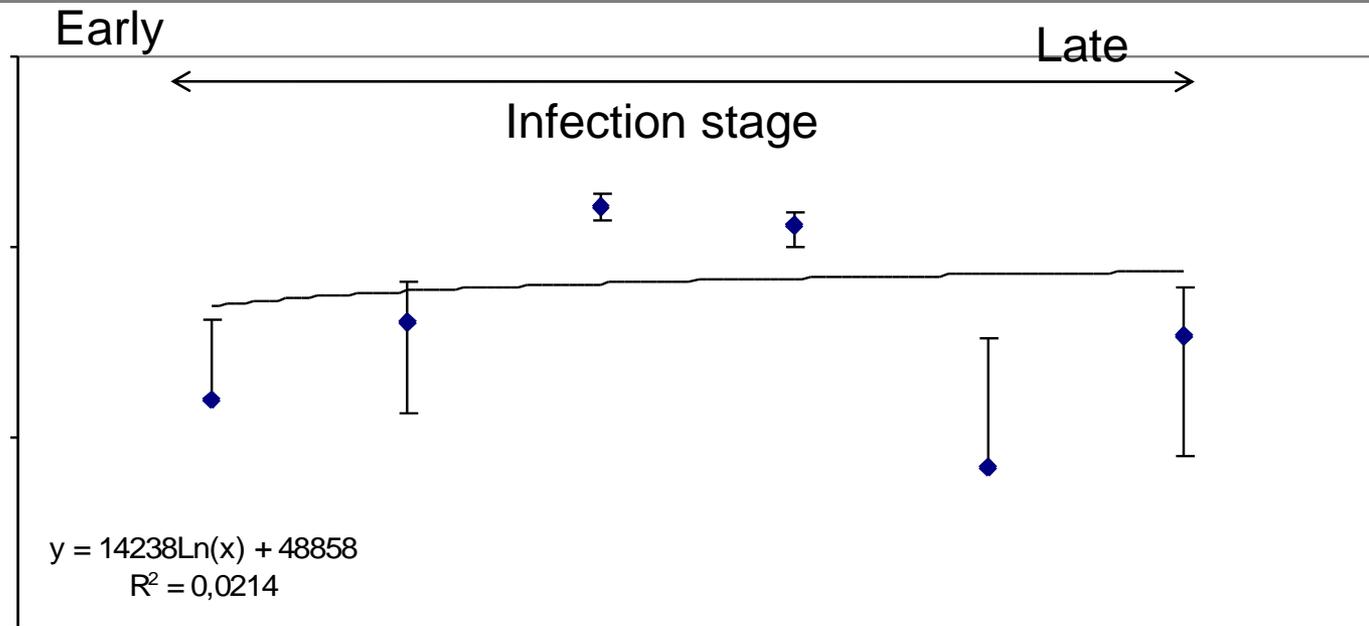
Dry season: May-October  
Wet season: October-April

PCR+ : Wet:Dry = 3:1

# What do trapping results tell us:

- Precipitation or high fog levels are both conducive to sporulation
- Warmer temperatures seem to favor sporulation: rainfall in late spring generate the best spreading conditions. Places with rainy summers may be very conducive
- When temperatures approach the zero, sporulation is completely interrupted (not a good Sierra pathogen). This insight was gained by comparing higher elevation and colder SC plots with lower elevation SF plots

# Correlation between symptoms level and inoculum load



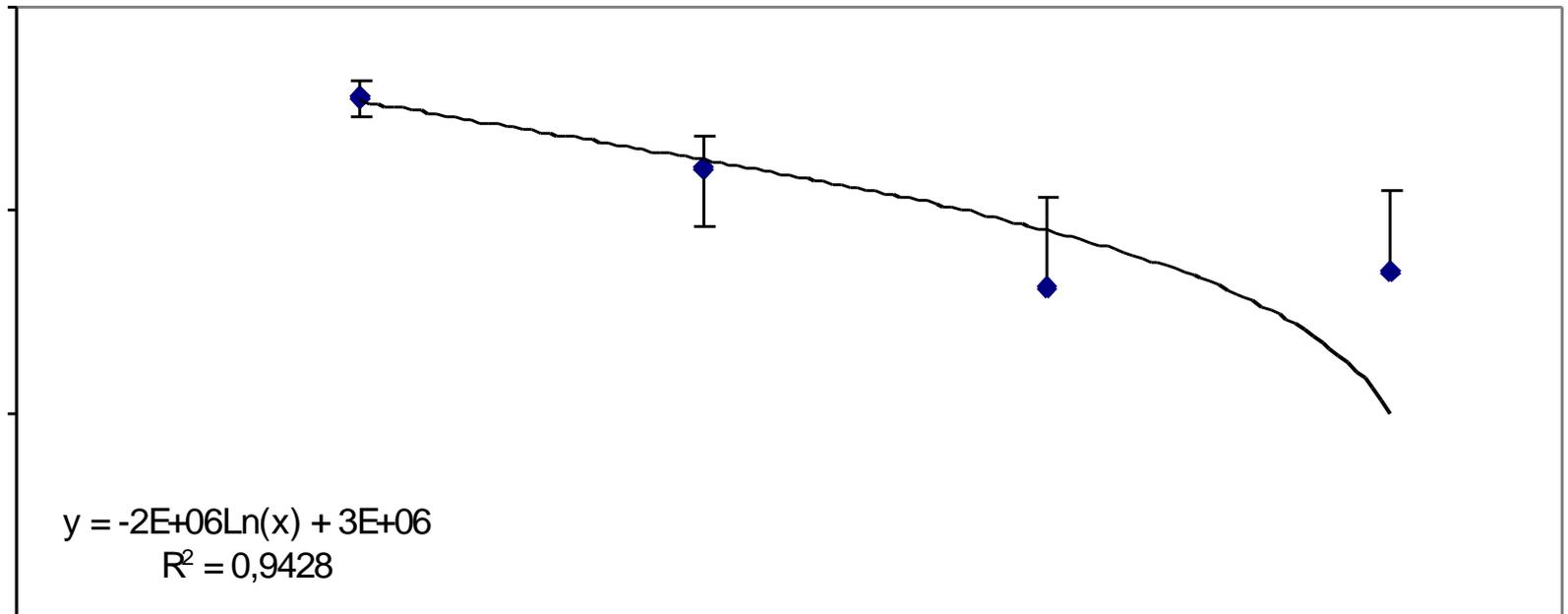
# Epidemiology

- Artificial movement through plant material
- Spores are sticky and long lived (tools, insects), tools can be infectious even if they “look” clean
- Insect vectoring (facultative), it seems to be particularly important in association with cone-insects on Monterey pines
- Wounding? Insect feeding increases infection rates
- Airborne relatively long distance, each year we see an advancing disease front in Northern California, North-South movement favored by frequency of hosts and by warmer temperatures, as opposed to East-West movement

# CURRENT DISTRIBUTION OF PITCH CANKER IN CALIFORNIA



# Inoculum dilution analysis



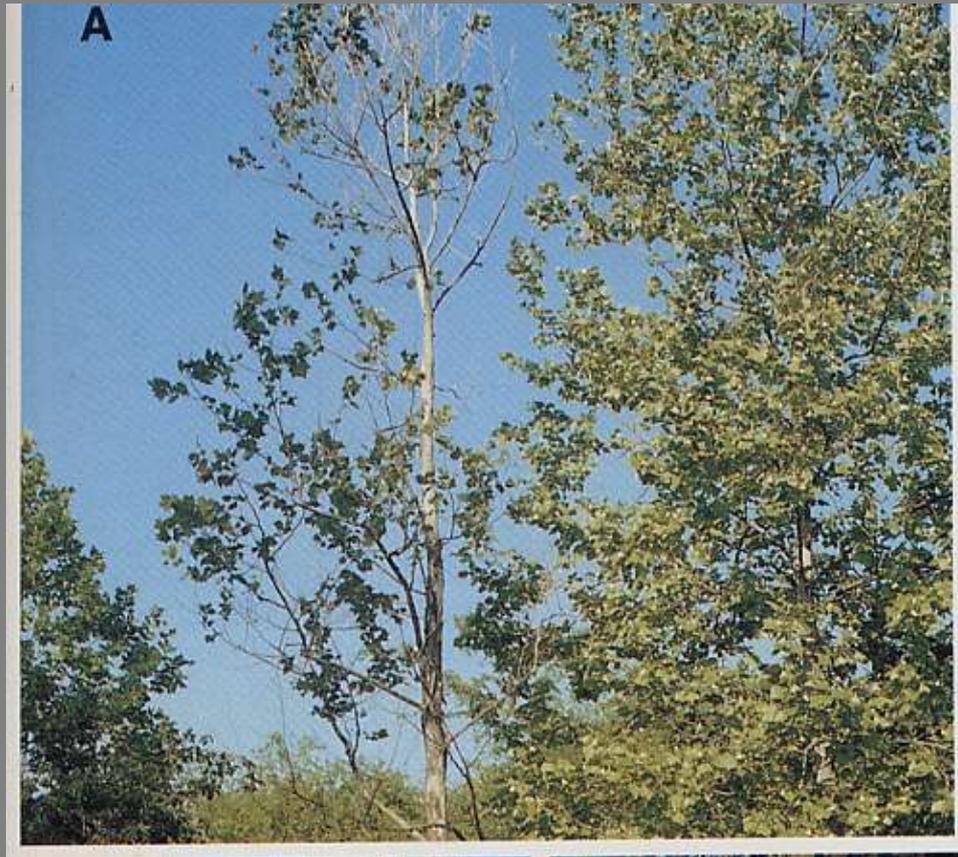
# Surprising “late” findings:

- Pathogen reported in roots of mature Aleppo pines in Southern California
- Pathogen transported to New Zealand on Douglas fir seedlings from Placerville (CA)
- Recently, second mating type introduced in California. How=?
- Found as an endophyte of grasses

# Canker stain of sycamore

- Pathogen is a vascular fungus called *Ceratocystis platani*
- Native to Eastern USA, moved to Italy and California via infected wood
- In both places two serious epidemics started
- Introduced in the Stockton area, it killed all sycamores there

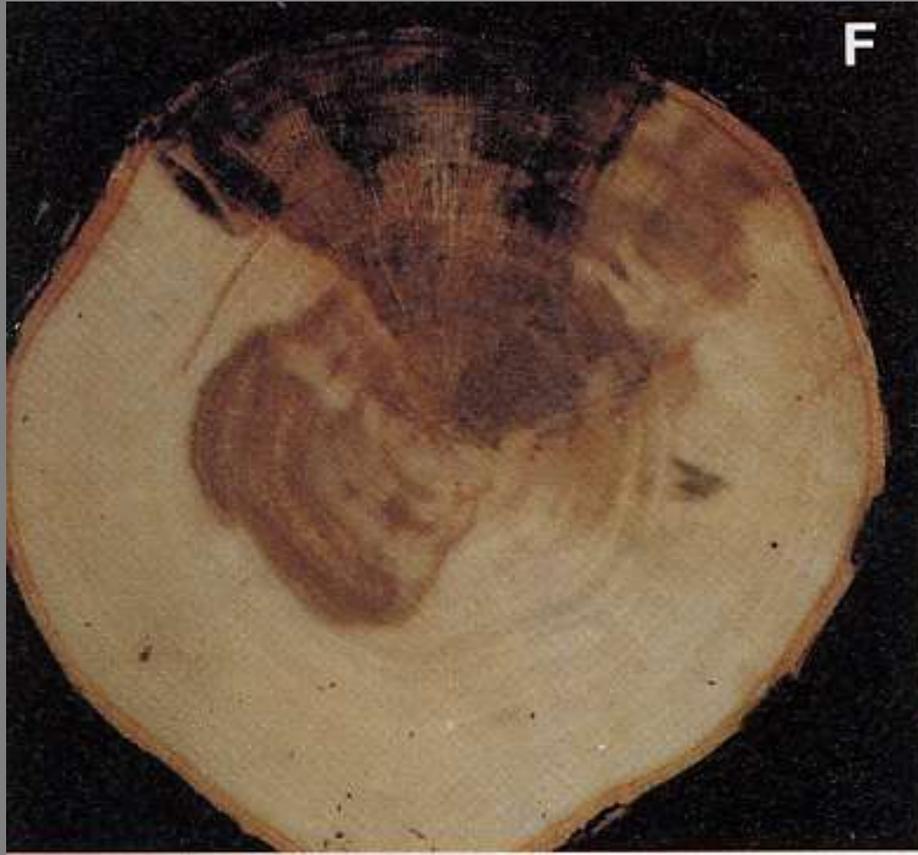
# Visible symptoms: die-back



# Sunken lesions



# Wood staining



# Epidemiology and control

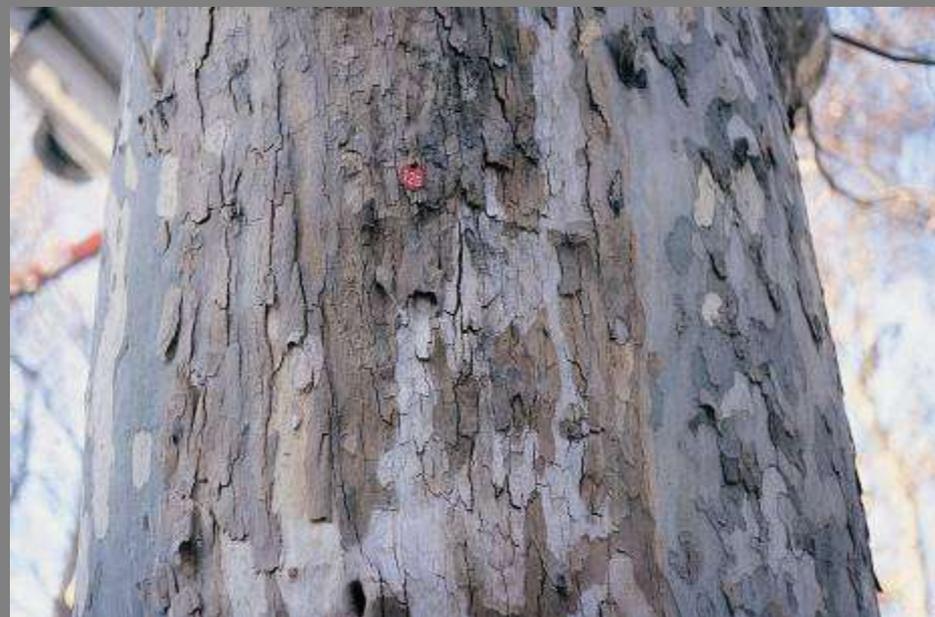
- Infected wood and tools spread disease
- Possible association with nititulidae beetles
- Spreads through root grafts
- Controlled by sanitation of tools and severing root grafts

## Canker stain of plane trees by *Ceratocystis platani*



Tree death within 3-7 years  
(EPPO/CABI, 1997)

# Symptoms



## Field diagnosis

EPPO

PM 7/14(1)



Leaf chlorosis, dieback  
Necrosis under the bark, cankers



Bark canker caused by *Fomitiporia punctata*

Lack of callus at the edges of the lesion



## Field diagnosis





**Field diagnosis**



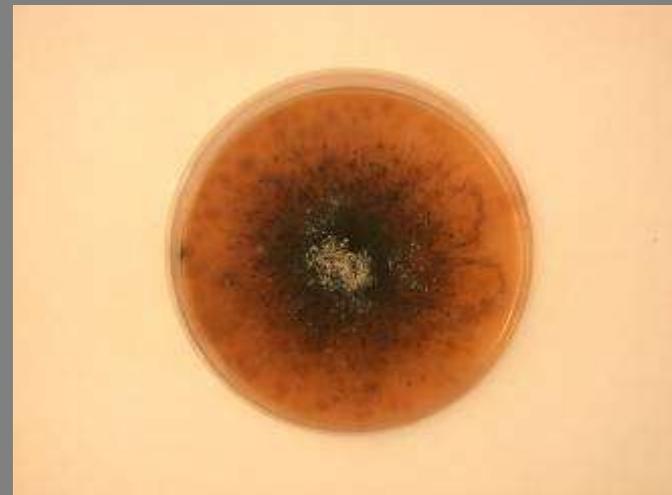
## Field diagnosis



## Diagnosis in the lab

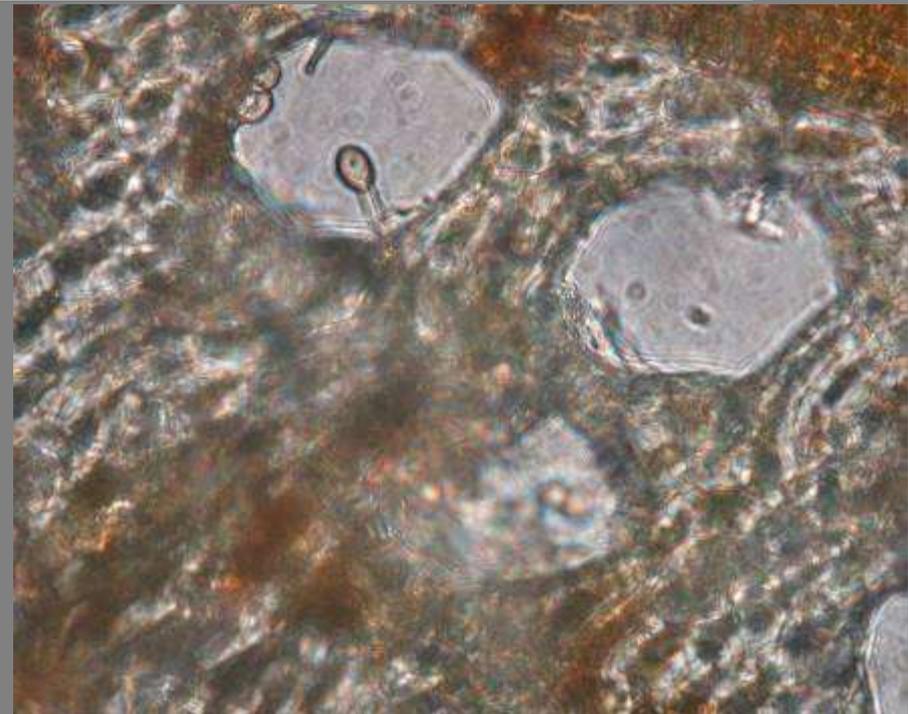
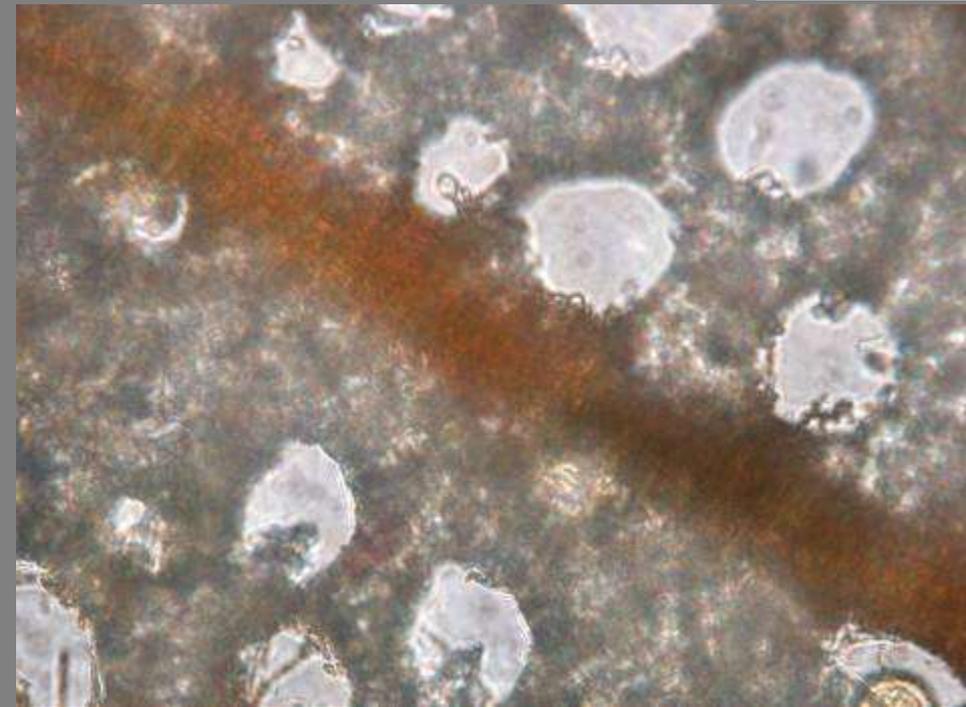
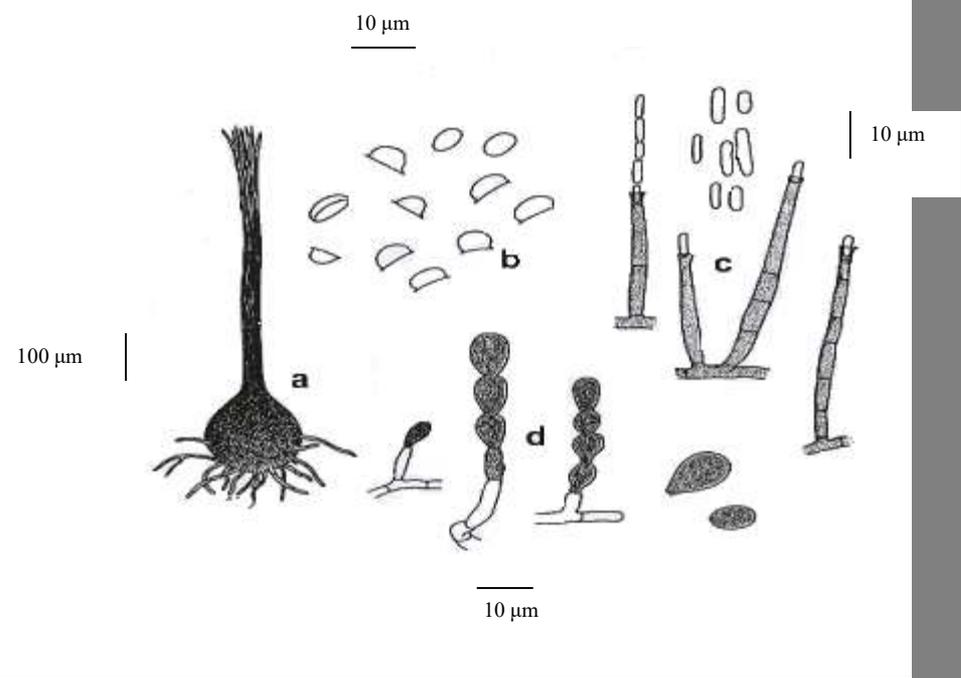


Inspection for the presence of perithecia after incubation in a damp room



Isolation

# Diagnosis in the lab



# Infection biology and epidemiology











## First hybrid plane trees to show resistance against canker stain (*Ceratocystis fimbriata* f. sp. *platani*)

By A. VIGOUROUX<sup>1</sup> and R. OLIVIER<sup>2</sup>

<sup>1</sup>Laboratoire, INRA, UMR BGPI de Pathologie Végétale, Montpellier, France (Presently in Avignon, as n°2) E-mail: andre.vigouroux@avignon.inra.fr; <sup>2</sup>Station de Pathologie Végétale, INRA, 84 140 Avignon, Montfavet, France

### Summary

Canker stain, caused by the ascomycete *Ceratocystis fimbriata* f. sp. *platani*, is a major threat to plane trees in Europe. The American plane tree (*Platanus occidentalis*) carries some genetic resistance, but this species is not adapted to the climatic conditions in Europe. Therefore, hybrids between susceptible oriental plane trees (*Platanus orientalis*) and resistant *P. occidentalis* were screened for disease resistance in France. Among 960 hybrids, we found 18 individuals which survived two successive inoculations. These putatively resistant trees were cut back and, after 18 months, on each tree 12 of the newly produced shoots as well as two roots, were inoculated again. From these trees, only one showed complete and another one partial resistance. This selection process resulting in the resistant clone 'Vallis clausa' is described in detail.



# Thousand canker disease of walnuts

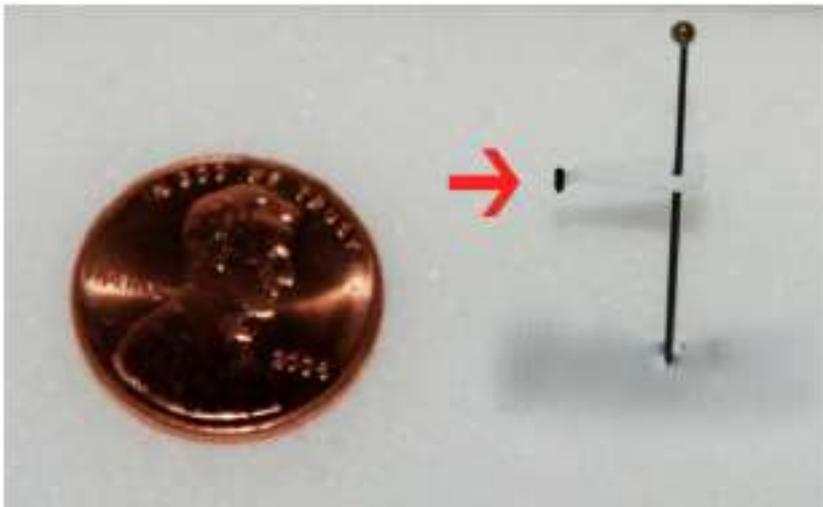
*Geosmithia morbida*: fungus

*Pityophthorus juglandis*: insect  
vector

# Some facts about TCD

- Insect is native
- Fungus apparently is not native
- Association between insect and fungus happened in the Southwest (1990s?)
- Disease started in Southwest, moved to California, from California elsewhere in the USA
- Insect of the genus *Pityophthorus* normally attack small sized portions of trees, not this one
- Mortality is due to coalescence of large numbers of cankers, and not to spread of a single lesion
- Black walnuts are the only hosts: *J. californica*, *J. hindsii*, and their hybrids with *J. nigra* (are all susceptible

# Walnut twig beetle



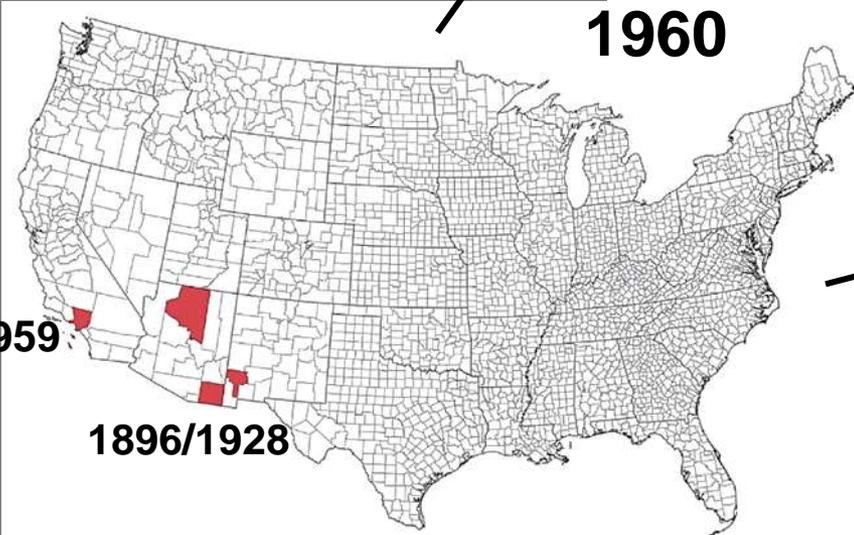
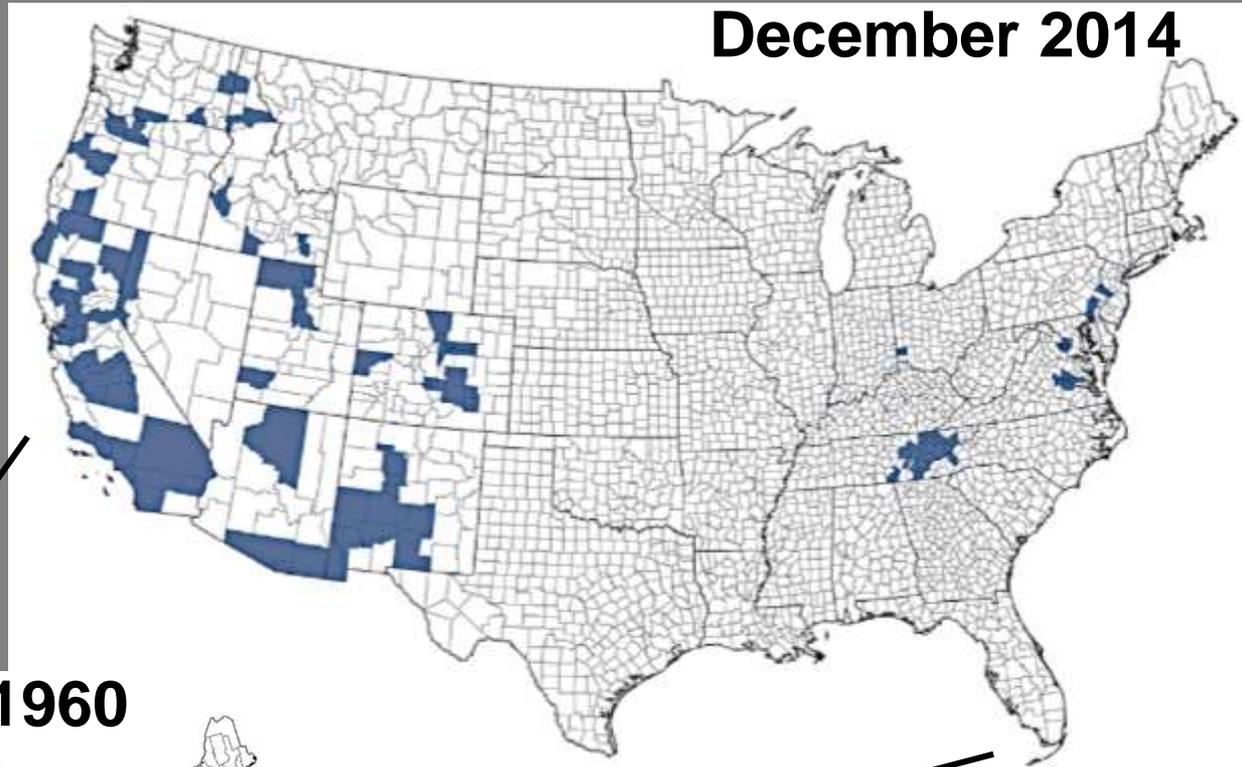
*Small size of walnut twig beetle, *Pityophthorus juglandis* (photo by Eric Day).*



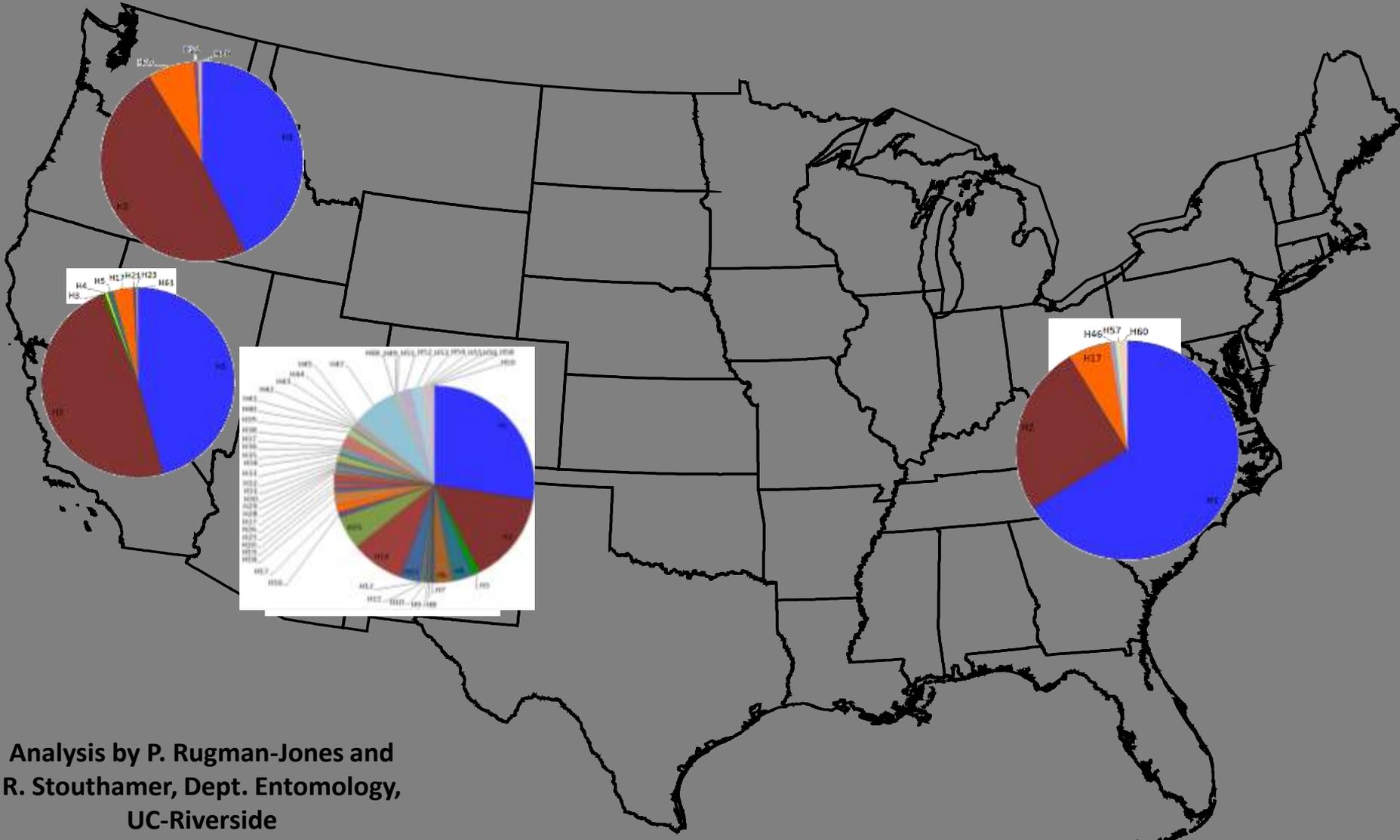
*The walnut twig beetle, *Pityophthorus juglandis* (photo by Eric Day).*



# WTB distribution in the USA



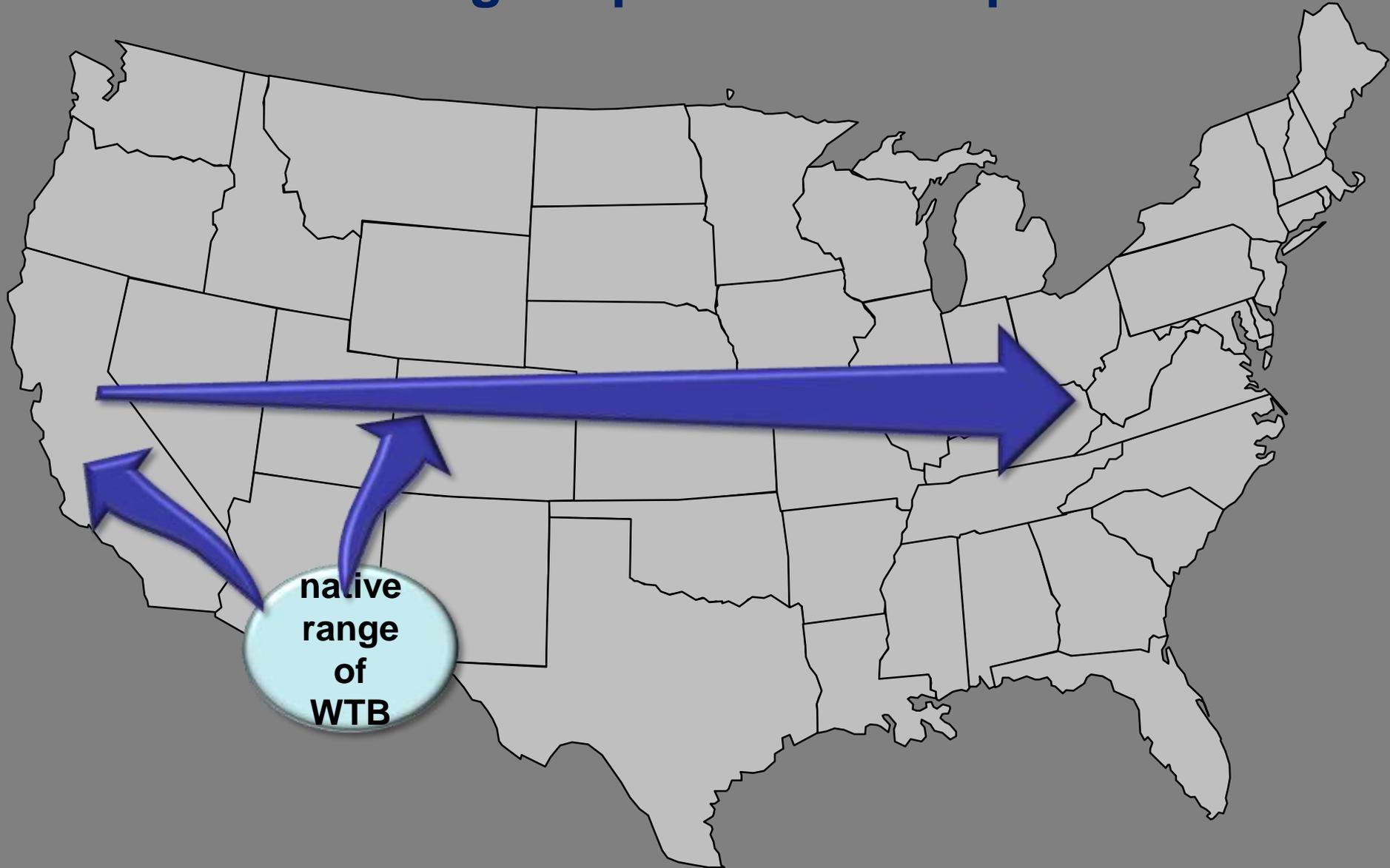
# Genetic analyses of WTB mitochondrial haplotypes suggest migrations from southwest U.S. to other regions



Analysis by P. Rugman-Jones and R. Stouthamer, Dept. Entomology, UC-Riverside

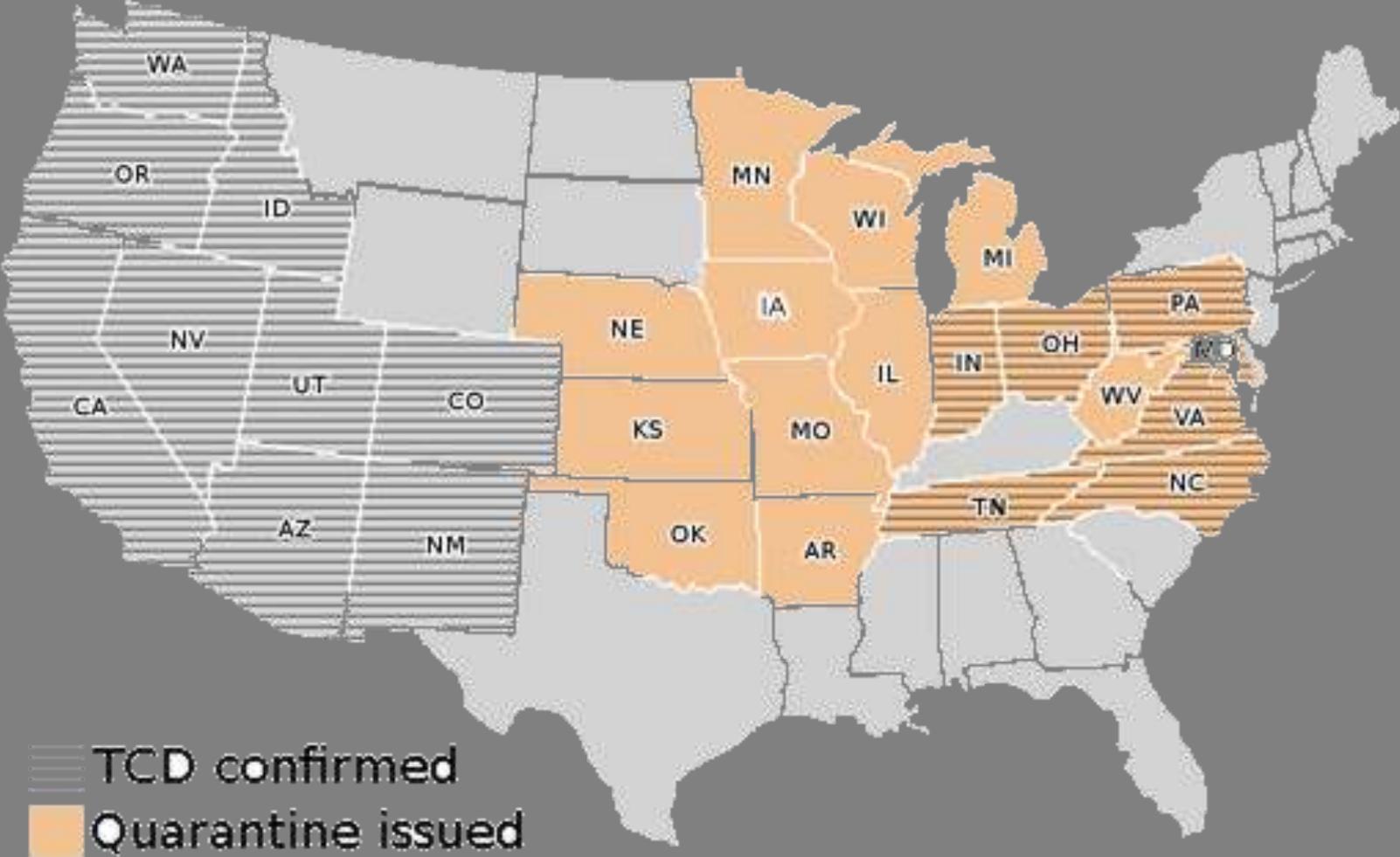
Graphic by A.D. Graves, USDA FS FHP, Albuquerque

# WTB range expansion and spread of TCD?

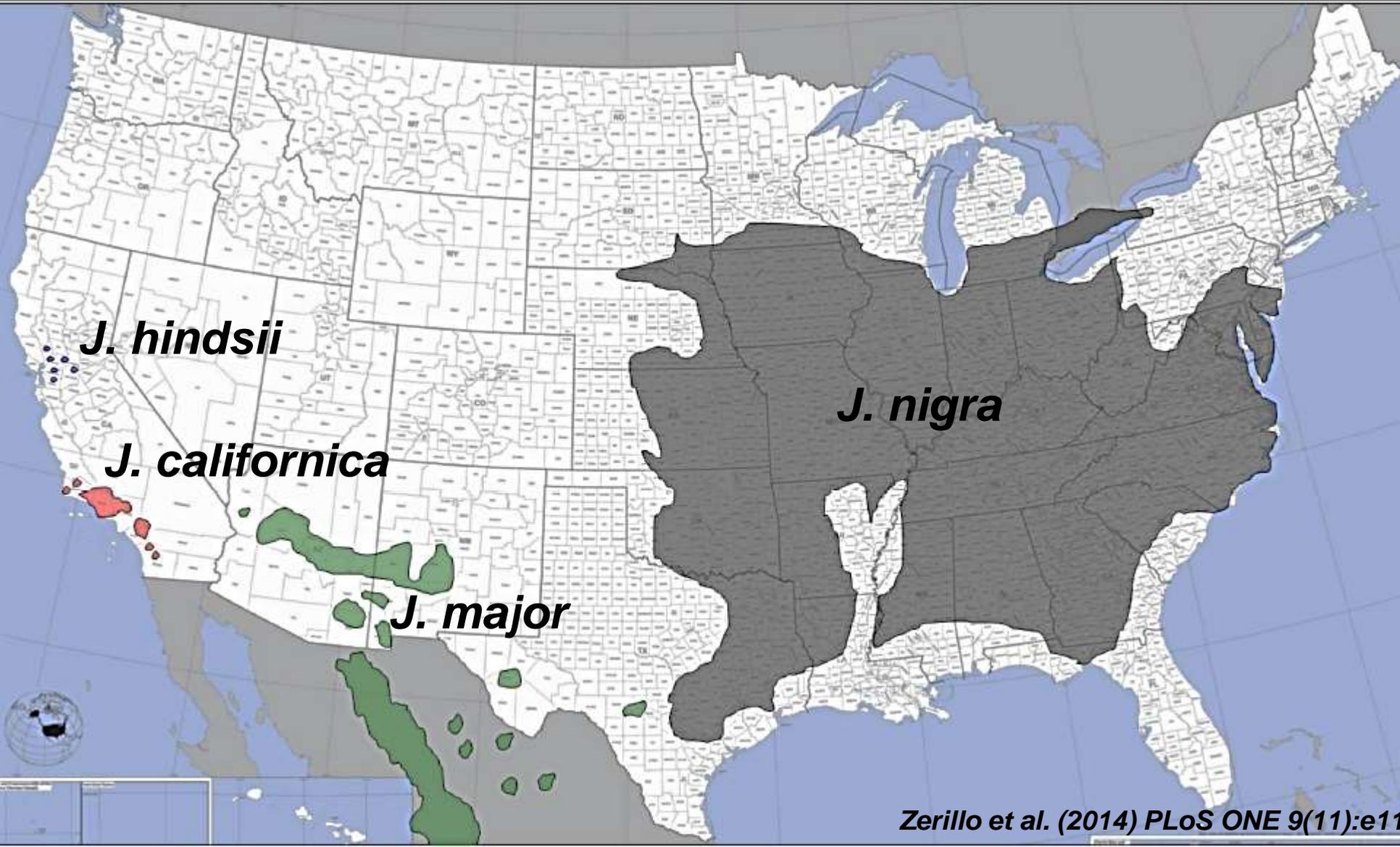


# Distribution of TCD in the United States

## October, 2015



# Natural distribution of some native *Juglans* species in the



# Comparative TCD Symptoms



Photo by Stacy Hishinuma

Wolfskill NCGR  
Solano Co., CA



*Juglans major*  
(southern NM)

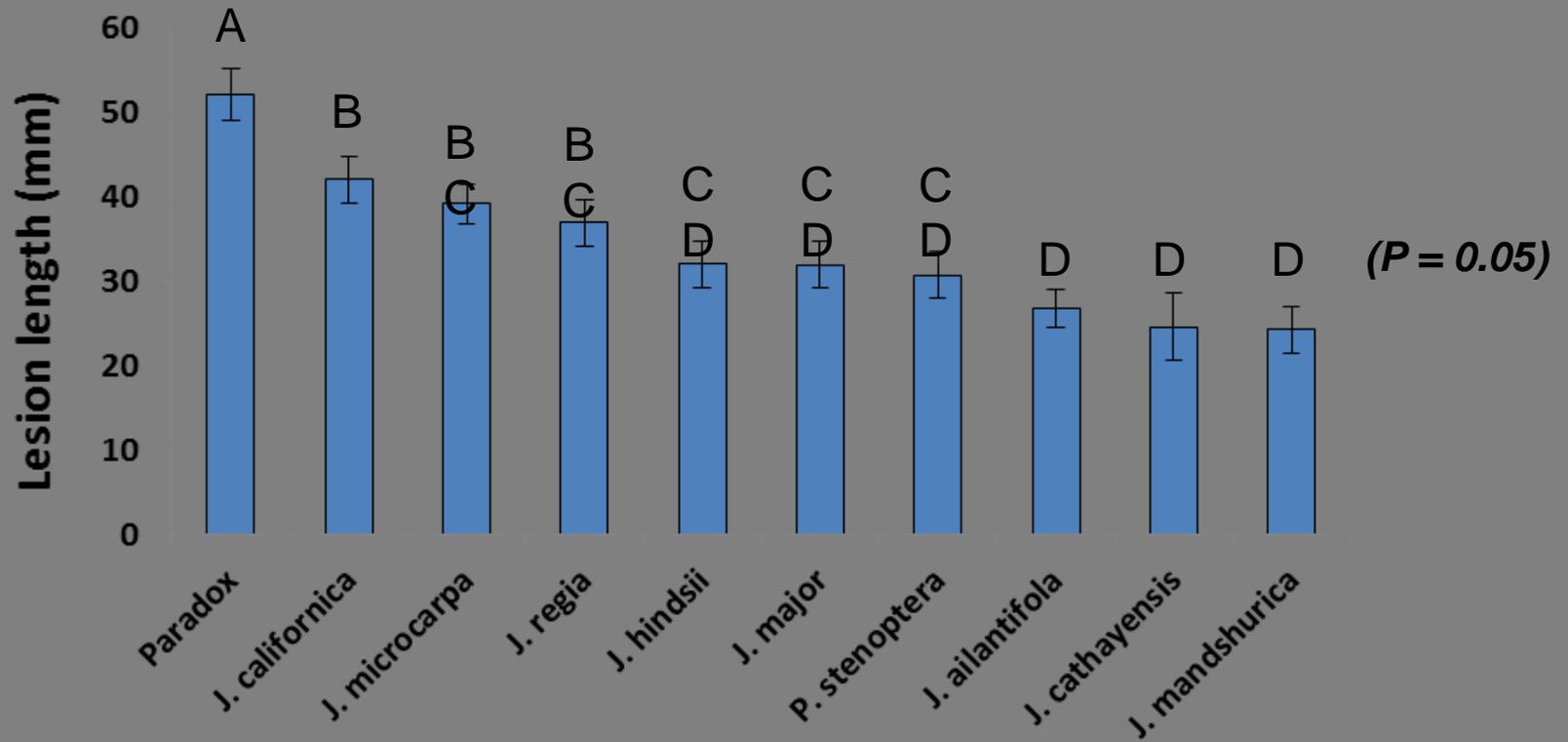
Sporadic  
cankers,  
minimal staining  
on bark surface



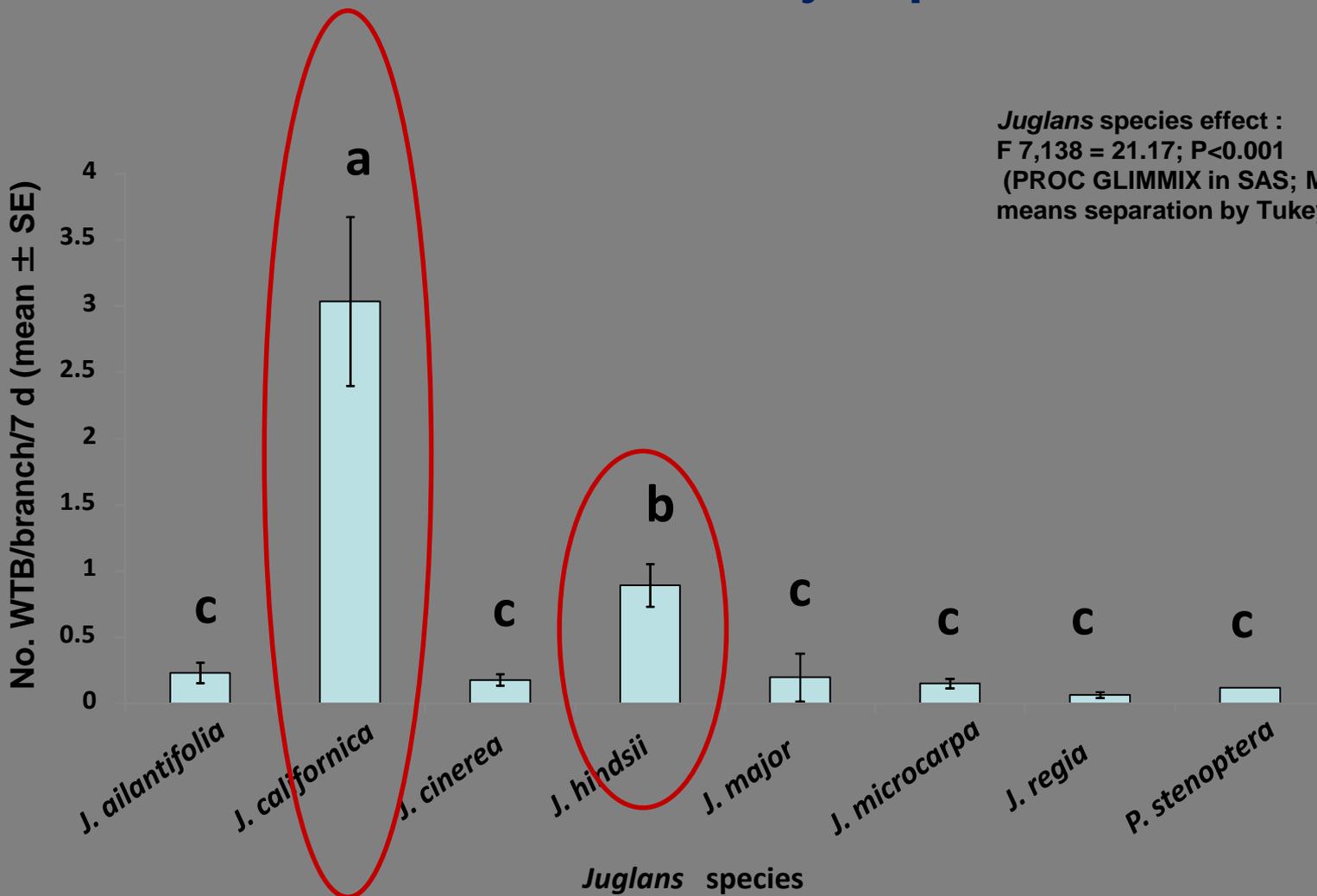
*Juglans californica*  
(in northern CA)

Numerous cankers,  
prolific staining on  
bark surface

# *Juglans* species show differences in susceptibility to *Geosmithia morbida*



# Landing rate of WTB on branches of six species of *Juglans* and one *Pterocarya* species



from Stacy Hishinuma

# Hypotheses on spread

- The most likely pathway for movement is raw wood (logs, burls, stumps, firewood, wood packaging material(WPM)). Other potential pathways include nursery stock, scion wood for grafting, and natural spread. The beetle/pathogen complex is likely to enter the east with each entrance event, as follows:
  - Movement of untreated walnut (logs, burls, stumps, firewood) across the country from the west into eastern states appears limited but it does occur and it is rarely documented. Low grade walnut maybe utilized if bark is attached this could be an important pathway. Raw wood is the most critical pathway.
  - Campsites and sawmills in the Great Plains states may facilitate the eastern movement of Thousand Canker Disease.
  - To date there have been no reports of infected trees in walnut production nurseries; however, if nurseries do become infected, this could become an important pathway.
  - Natural spread along riparian corridors is likely to occur.

# Symptoms from a distance

- Early=flagging  
Late=dead canopy with



# Close-up symptoms



Pinhead sized entry hole of beetle

# Close-up symptoms



UC Statewide IPM Program  
© 2010 Regents, University of California

Larvae tunnels produced along the grain, and so do the lesions

# Close-up symptoms



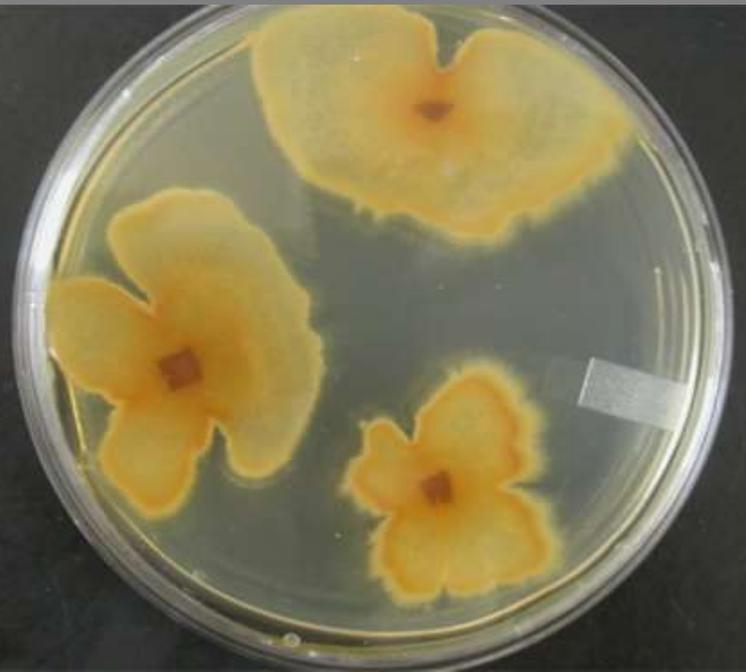
Mortality caused by coalescence of multiple cankers

# Colonies of Gm on both sides

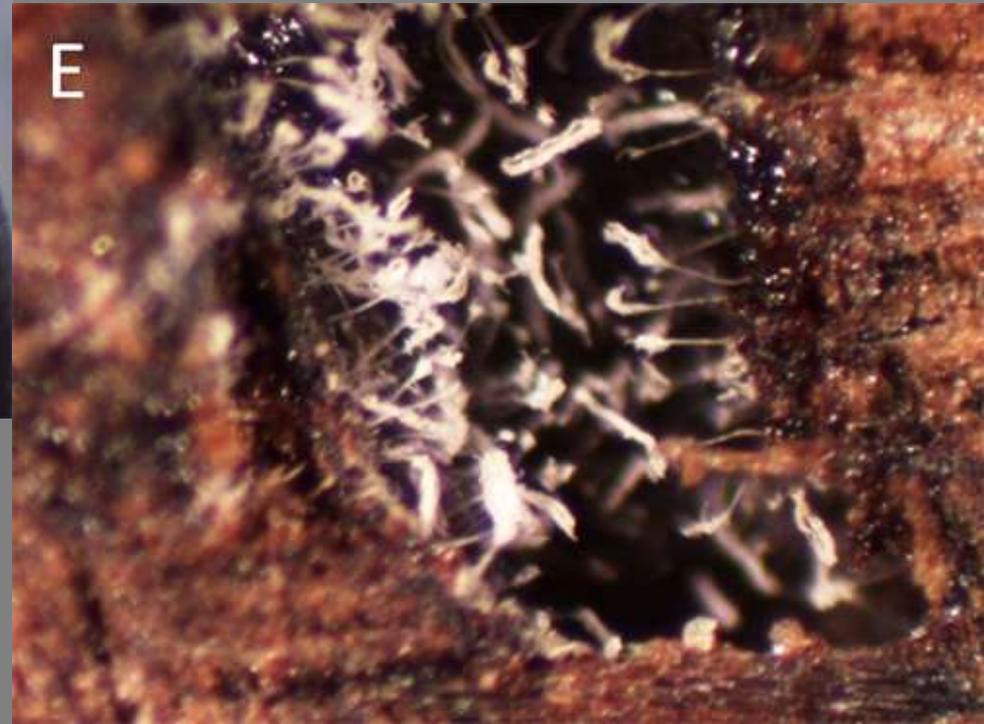
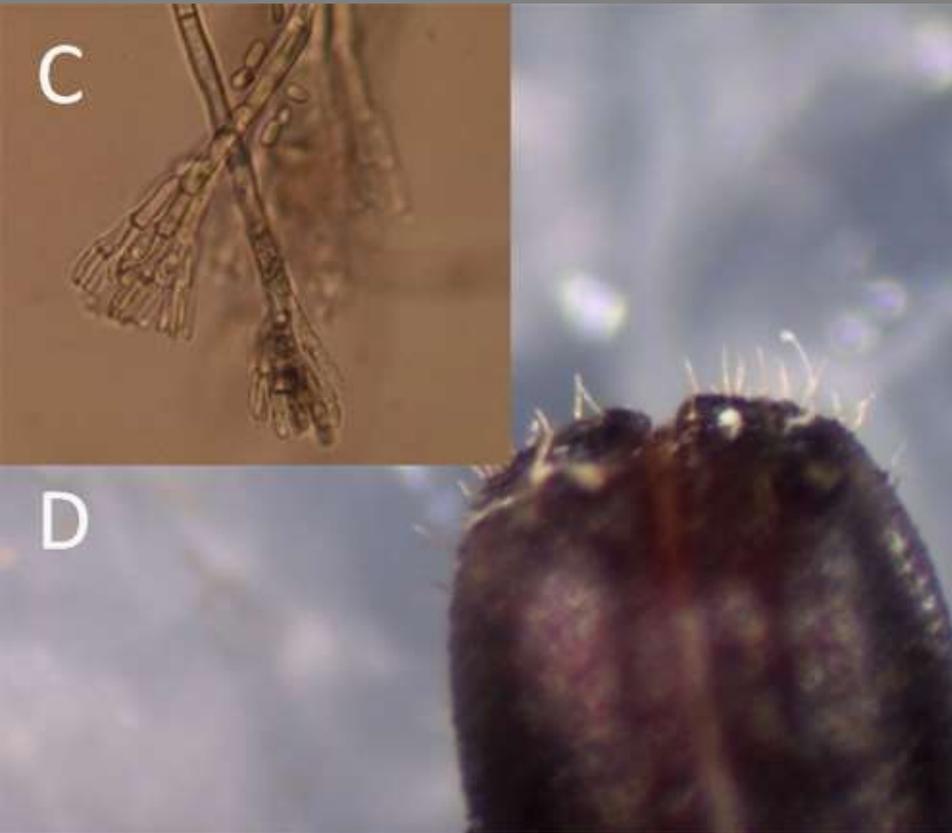
A



B



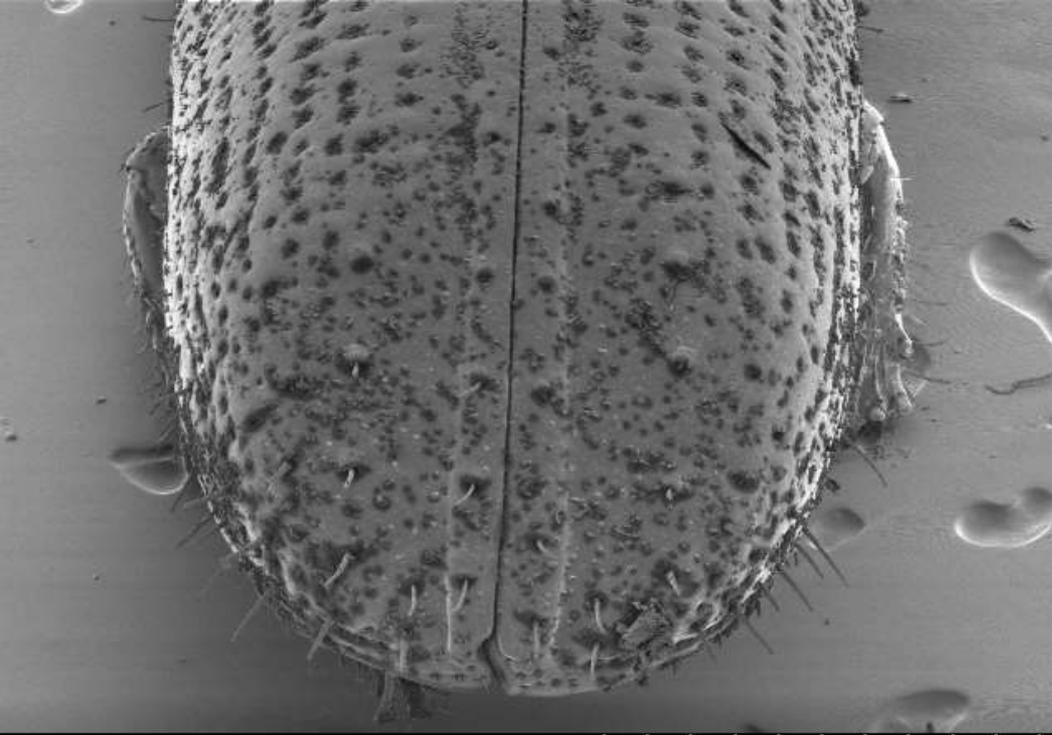
# Fungus sporulating on beetle and in beetle gallery



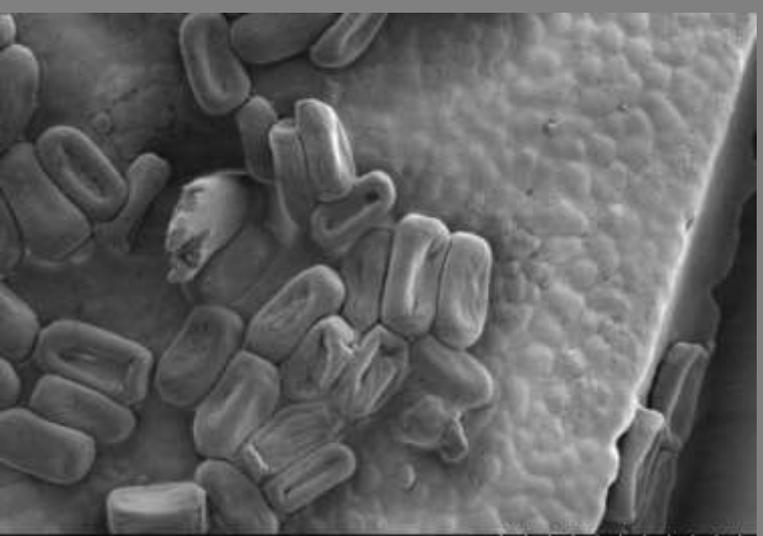
# The vector + the fungus

WTB carries  
the spores of the  
fungus on its surface  
(no specialized structures)

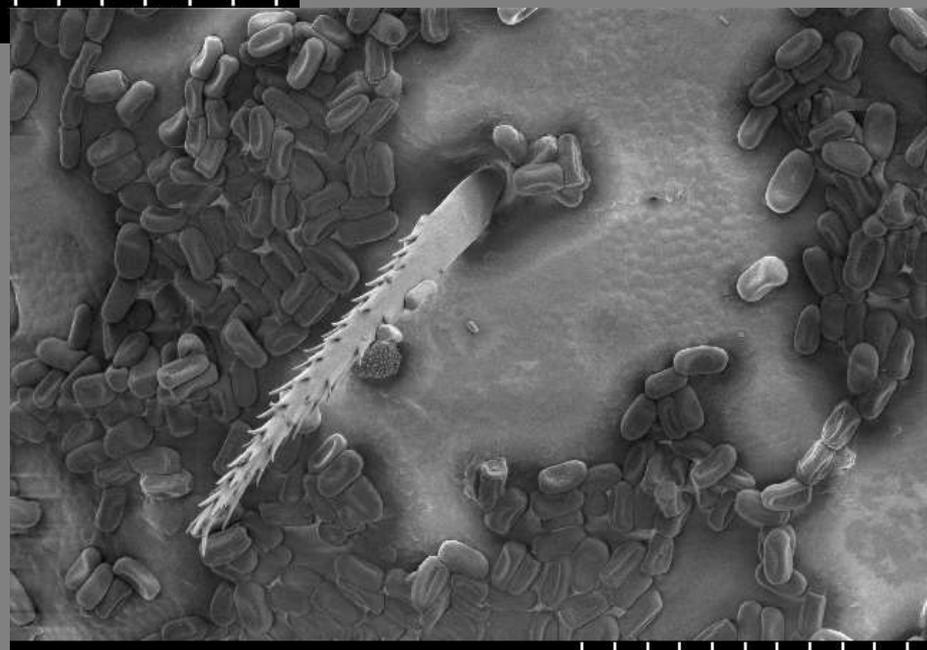
*Photos by Andrew Graves & Steve Seybold, USDA Forest Service*



Beetles 2.0kV 21.1mm x130 6/24/08 14:08



Beetles 2.0kV 20.2mm x4.00k 6/24/08 14:24 10.0um



Beetles 2.0kV 20.3mm x1.50k 6/24/08 14:29 30.0um

WTB is believed to have 2 to 3 generations a year in California. Adults emerge for an initial flight period in April and May followed by a longer second generation flight period in mid-July to mid-September. After flying, male beetles initiate brood galleries on branches often near leaf scars or lenticels.

Males produce a pheromone and attract 2 to 3 females, which attract additional beetles to the tree. Females deposit eggs in galleries (tunnels) that are directed against the grain and constructed in the phloem and xylem (wood) surfaces. The gallery imprint is left on the wood surface. Small white C-shaped larvae hatch and create feeding mines that extend from the egg galleries. These mines are contained in the phloem and filled with dark brown to black-colored boring dust.

Larvae complete development in the mines and subsequently pupate within a single pupal cell. Adults emerge and either remain at the original tree or fly to other trees to mate and reproduce. WTB does not appear to be attracted to stressed or injured branches or trees.

Beetles are believed to inoculate the *Geosmithia* sp. fungus into the phloem during construction of feeding or reproductive galleries. The fungal pathogen colonizes and kills the phloem. Dead tissue is limited to the phloem and cambium and the fungus does not penetrate woody tissues. Secondary saprophytic fungi may opportunistically colonize the wood beneath cankers

# Control?

- Bark application and drenches with insecticides apparently not effective
- Prevention is the best option
- Some efficacy reported with injections of insecticides, fungicides, and fertilizers