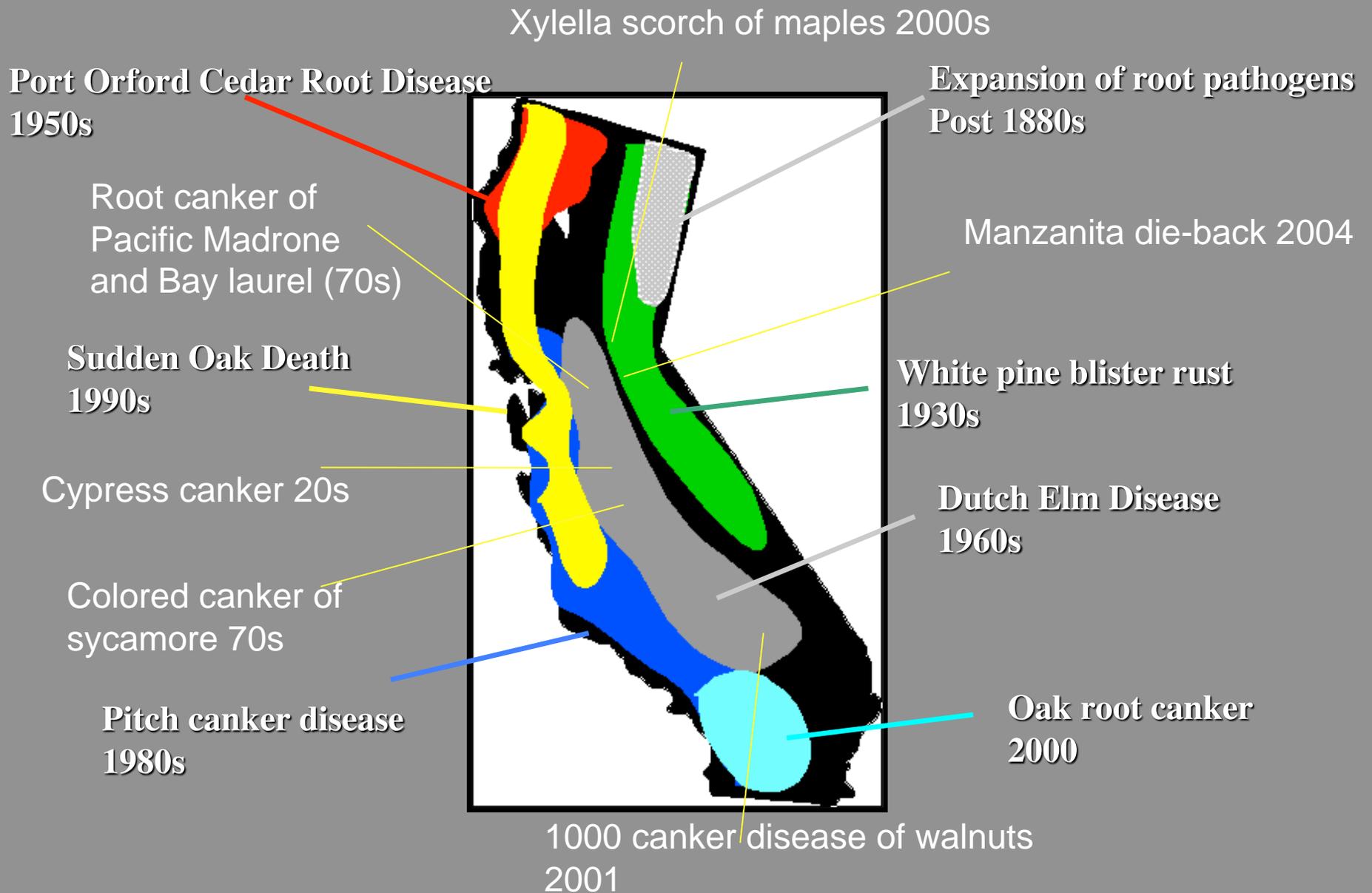


California invaded: 1849 A.D.



- 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)

Eastern White pines

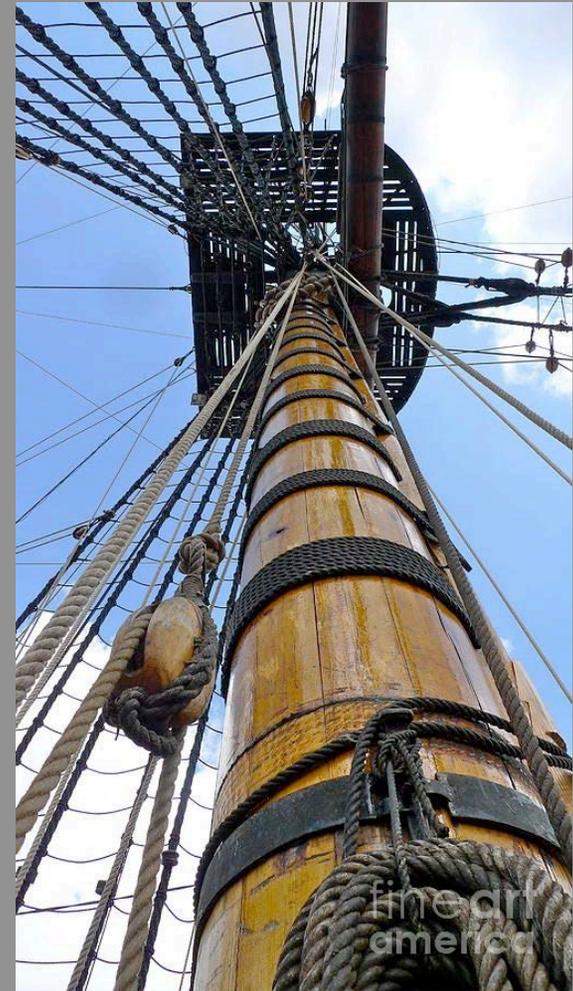
- Most valuable timber resource of Eastern North America
- Used especially in the shipbuilding industry to build masts
- Eastern white pine stands owned by the English crown and one of the main economic reasons for independence
- Planted in Europe where they failed and produced poor quality timber



White pines reserved for the British navy



Cone and leaves of
Pinus strobus



A ship mast made with white pine

In Western North America

- Nine species of white pines
- Eight are infected (*P. longaevea* 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

Blister rust cankers:
sugar pine



whitebark pine

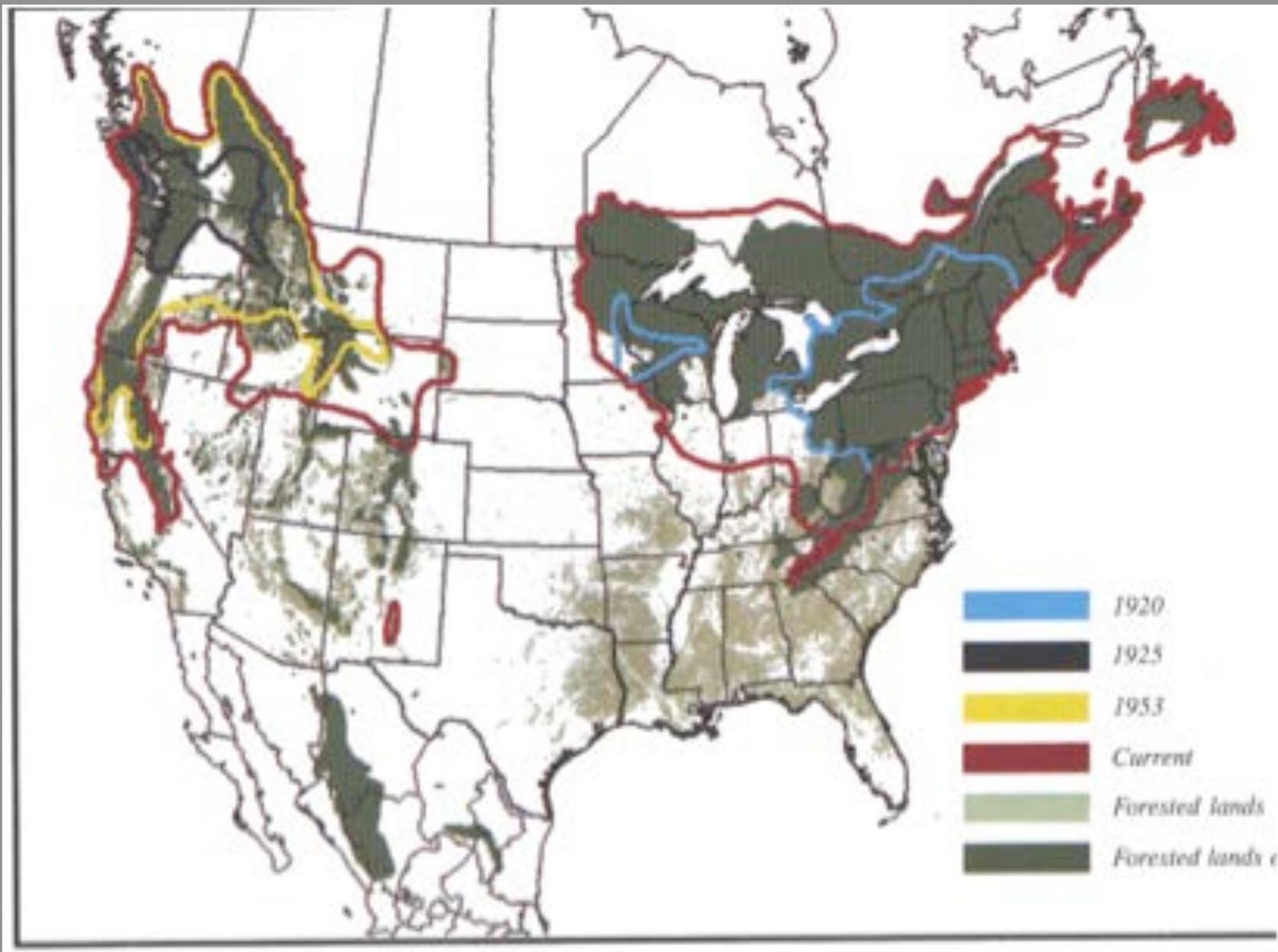


Top kill in whitebark pine



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

- Pre 1900 or 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 (gooseberry, currant) 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



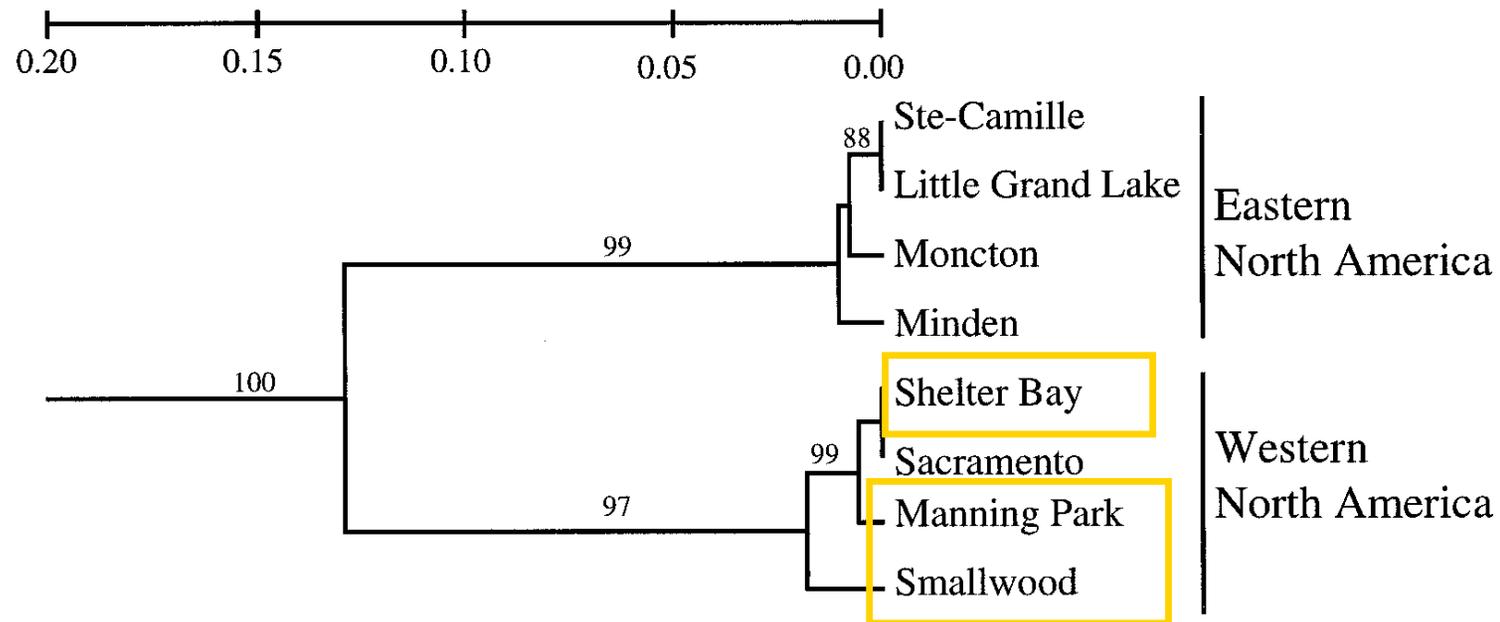
Results

- 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 ^a	Source	df	Variance components	ϕ -Statistics	Proportion of variance components (%)	P value ^b
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

^a NFLD = Newfoundland, QC = Quebec, ON = Ontario, NB = New Brunswick, BC = British Columbia, and NM = New Mexico.

^b 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 Revisited

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

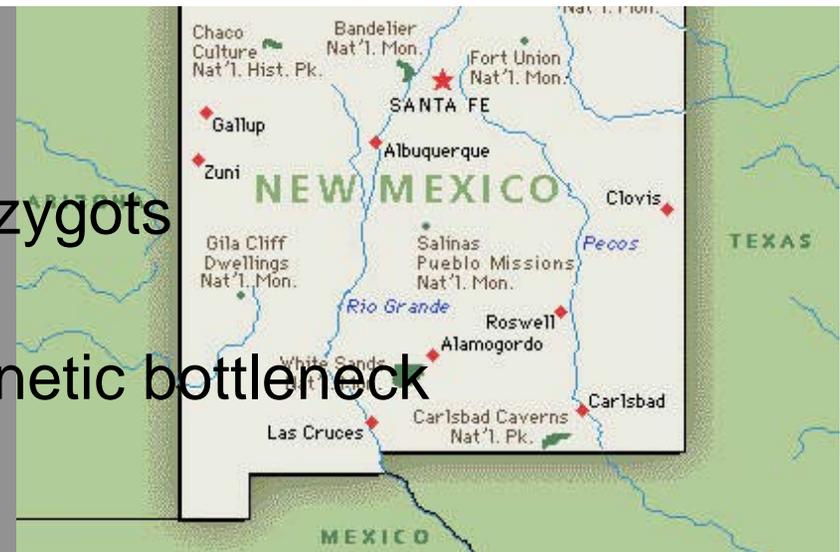
Population ^a	n ^b	Estimated allele frequency (q) ^c							H_e
		OPA01-1700	OPA01-2000	OPC08-750	OPC08-900	OPE15-1600	OPK19-2000	OPK11-500	
Manning Park (coastal BC)	55 (21)	0.963	1.000	1.000	1.000	1.000	0.203	0.000	0.069
Smallwood (interior BC)	37 (14)	1.000	0.987	1.000	1.000	0.987	0.410	0.000	0.077
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

^a 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.

^b Number of aecia and number of trees in parentheses.

^c 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

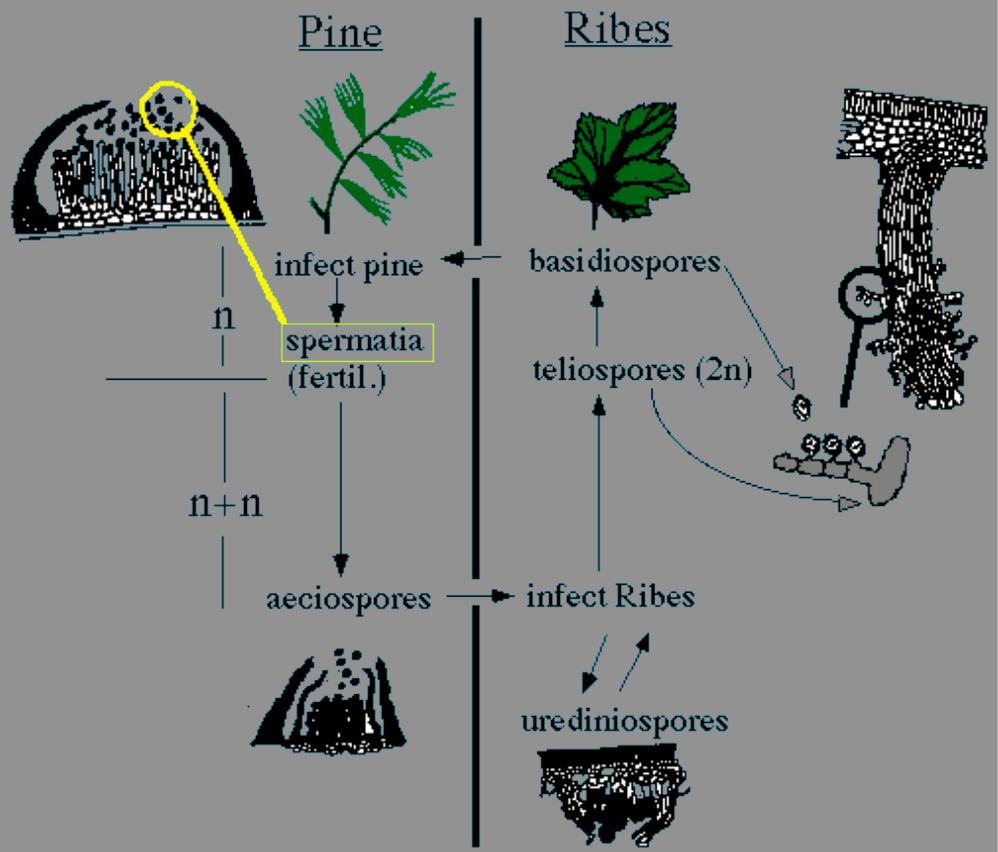
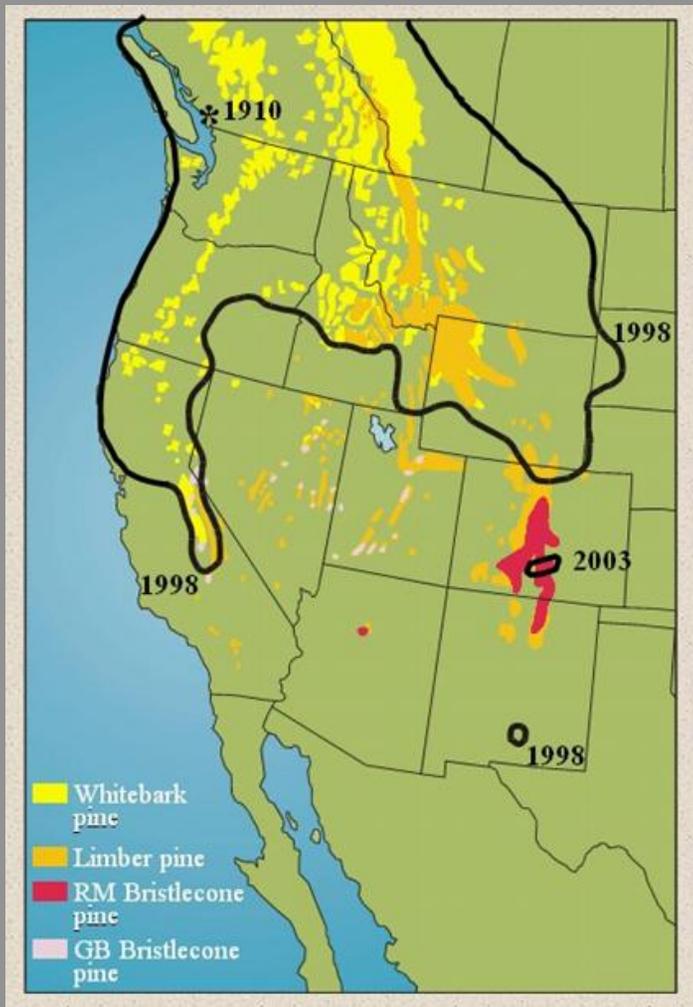


Conclusions

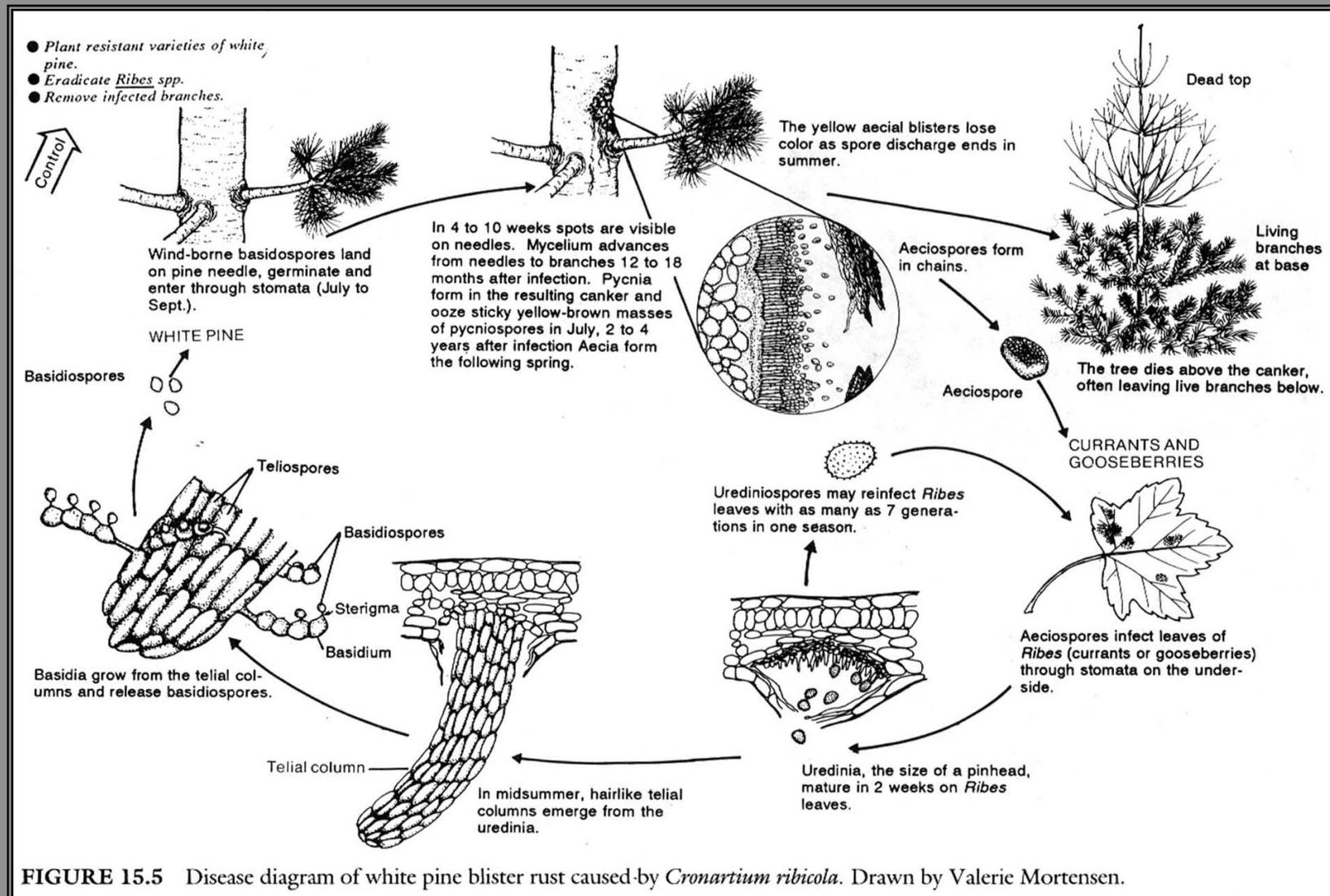
- Eastern and western populations are not panmictic
- Barrier to gene flow between eastern and western populations
 - Great Plains – intense agriculture
 - 100 km absence of aecial and telial hosts

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



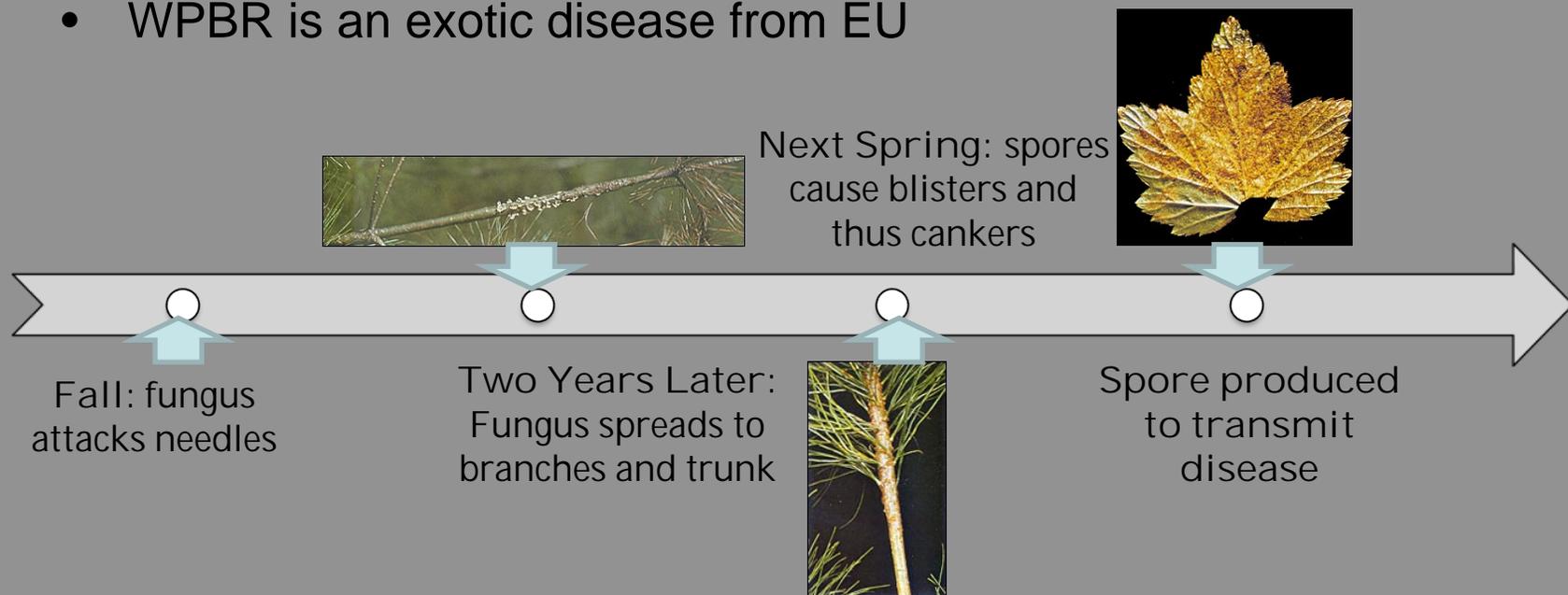
C. ribicola life cycle





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



Detwiler, S.B. 1923. American Forestry. p. 337.

- 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



...aft

er

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%

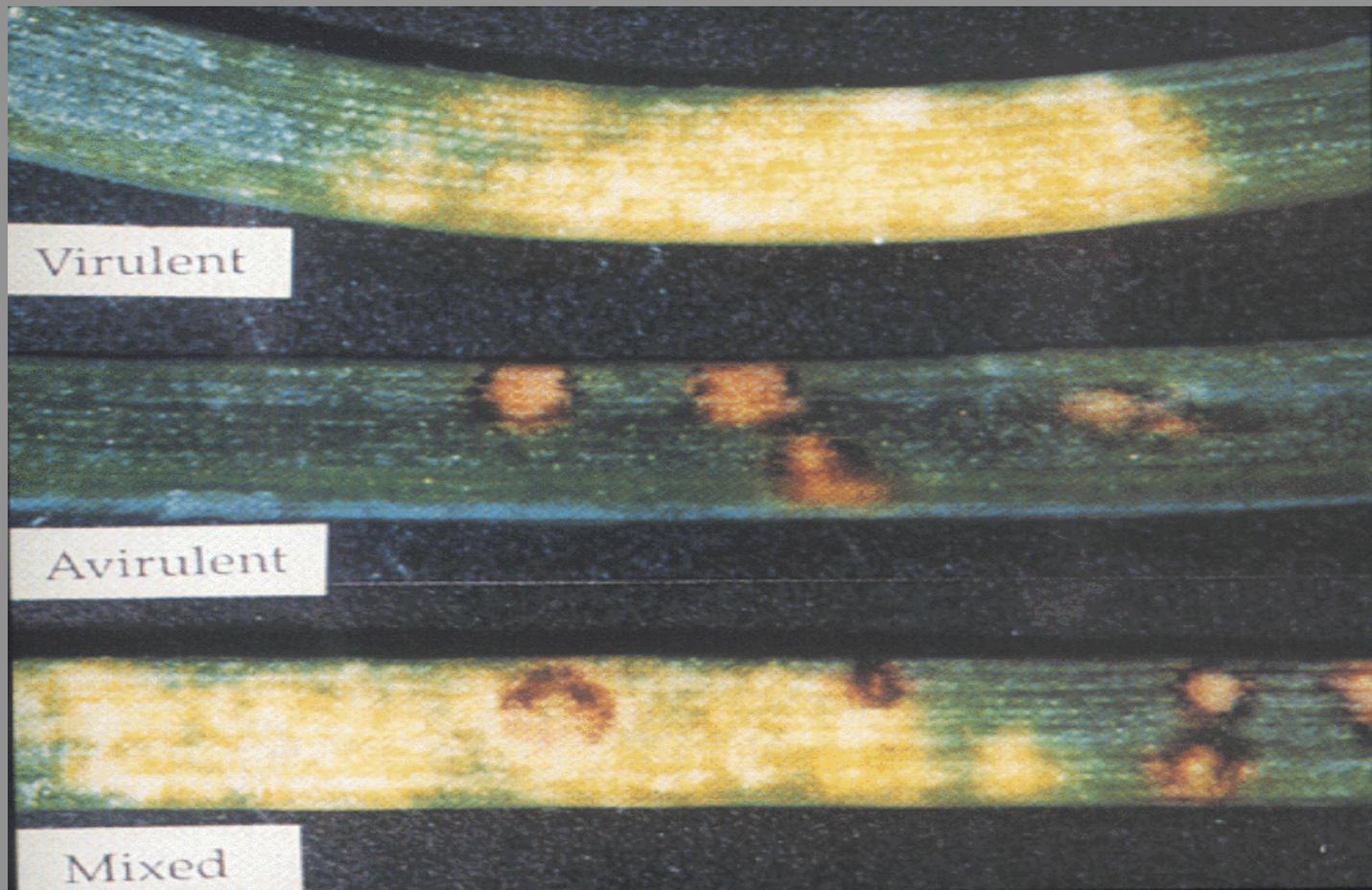
Tree resistance

- 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
VV	-	-	+
Vv	-	-	+
vv	+	+	+

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

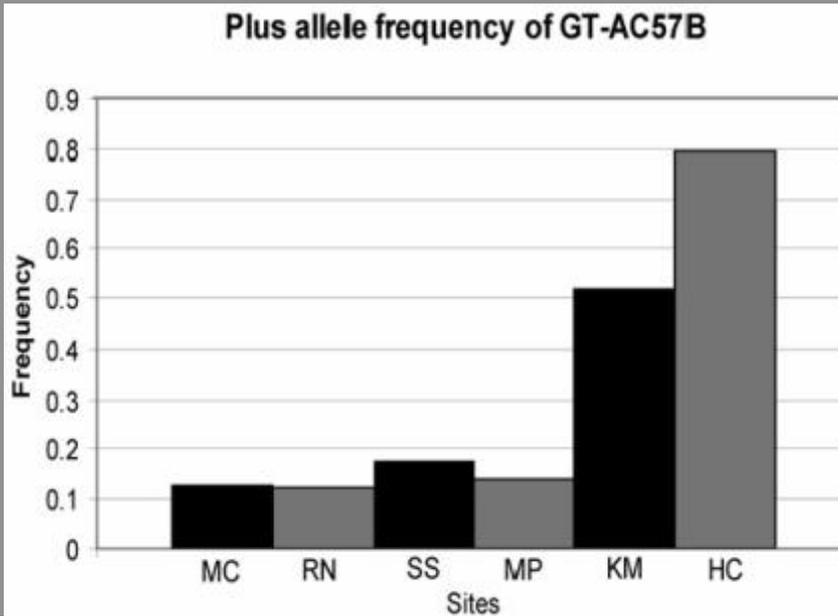
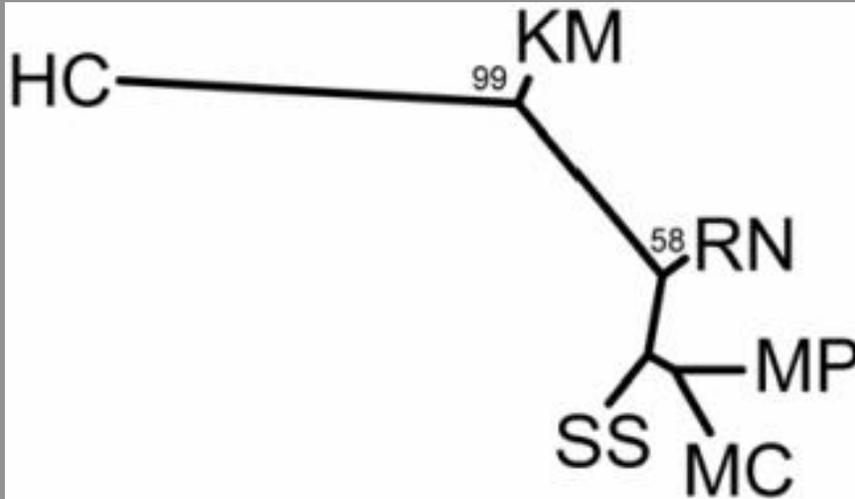
- 1) Examine genetic diversity within and among population of western N.America
- 2) Effects of host resistance on *C. ribicola*
- 3) Identify loci that behave as if linked to loci undergoing environmental selection

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

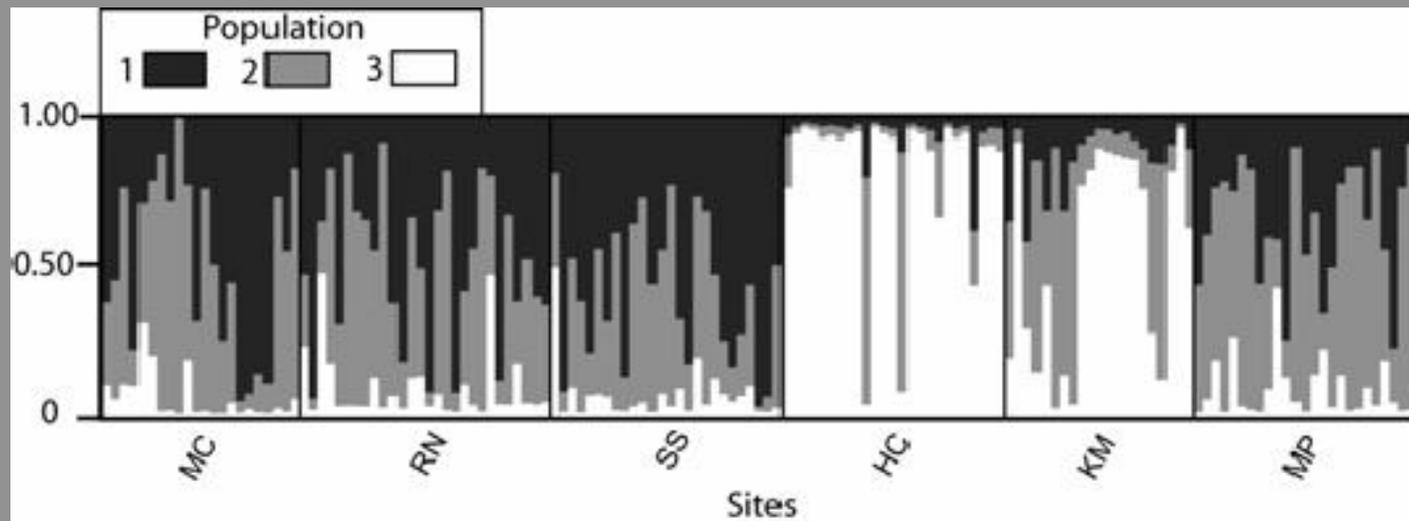
Happy Camp (major gene resistant trees):
had

lower heterozygosity

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

Results

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

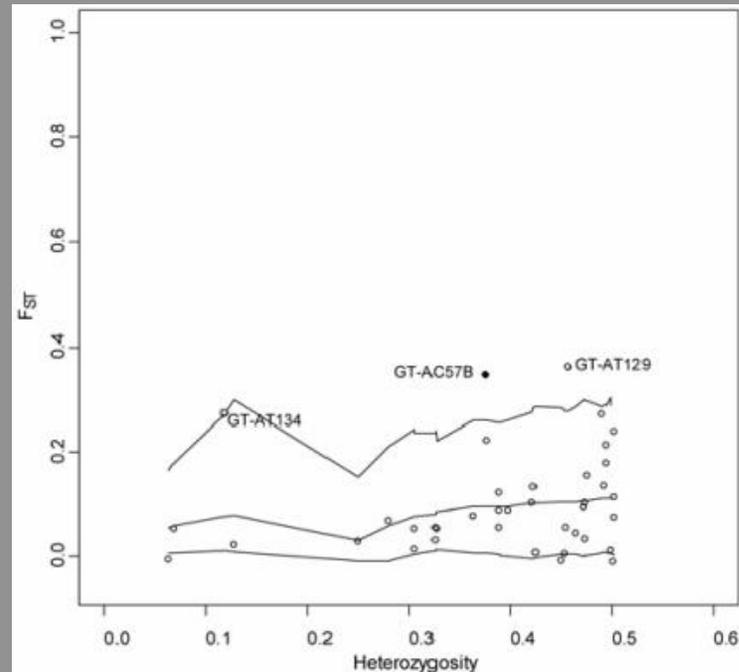


- Loci GFAC57B had very high F_{st} among different sites.

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."

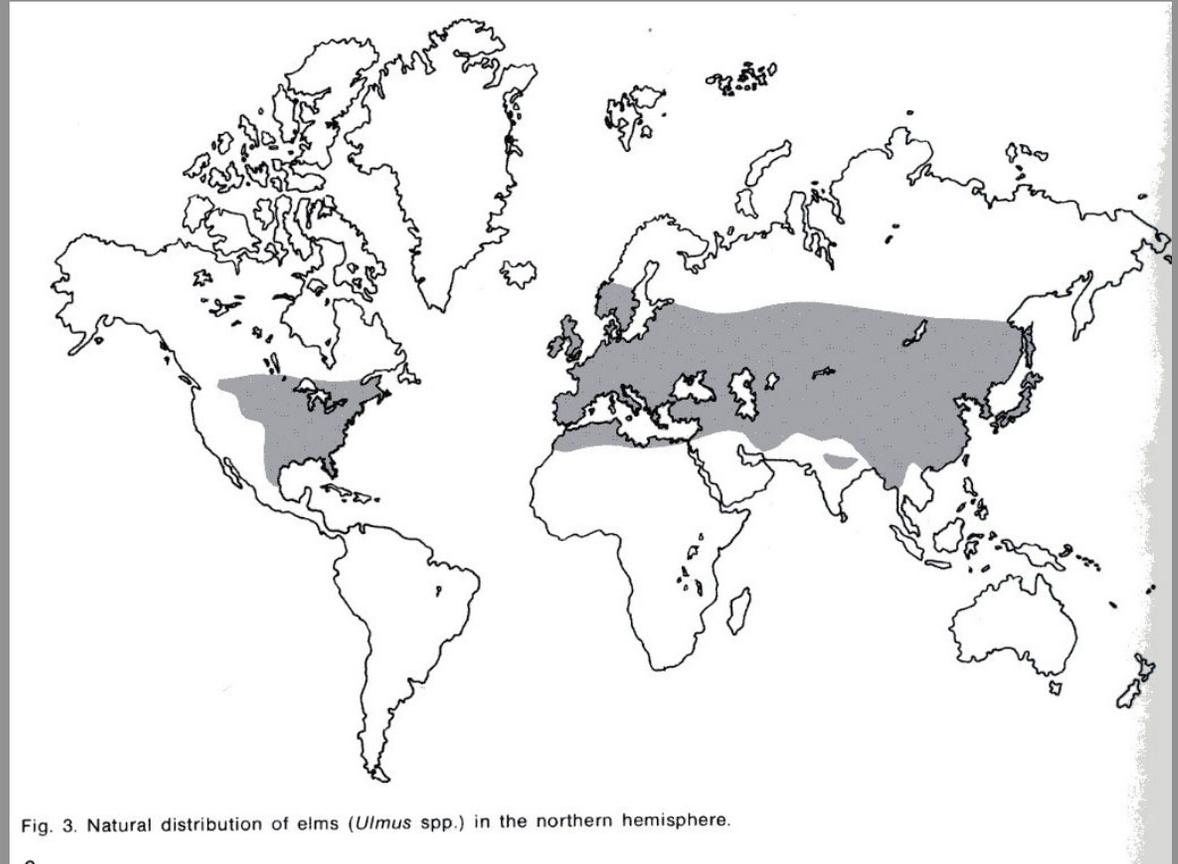
Robert F. Sharpf, U.S. Department of Agriculture Handbook 521 (p.85)

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



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)

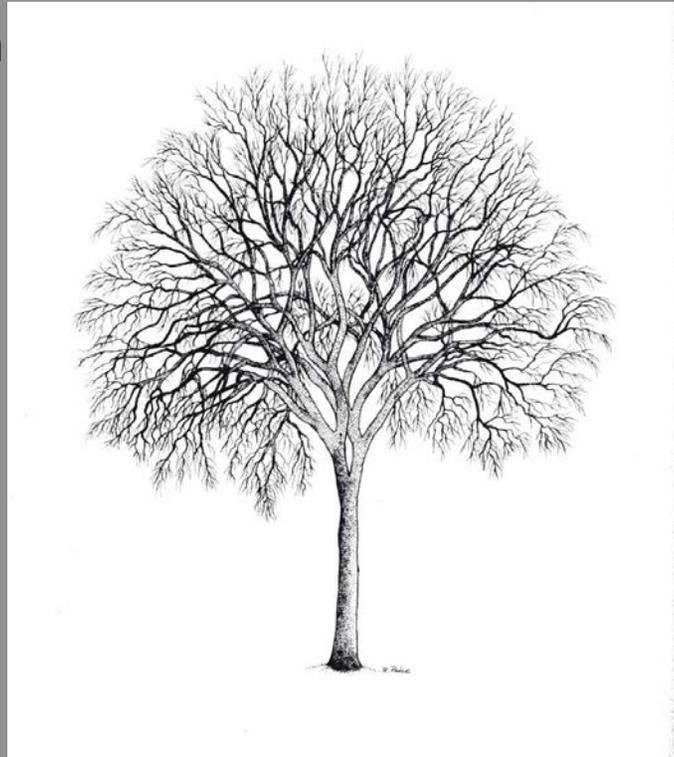


Fig. 25. Street scene of arching American elms in Evanston, Illinois (1976). (Courtesy E. B. Himelick)

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*)
- Vectored by beetles (family *Scolytidae*) and root graft

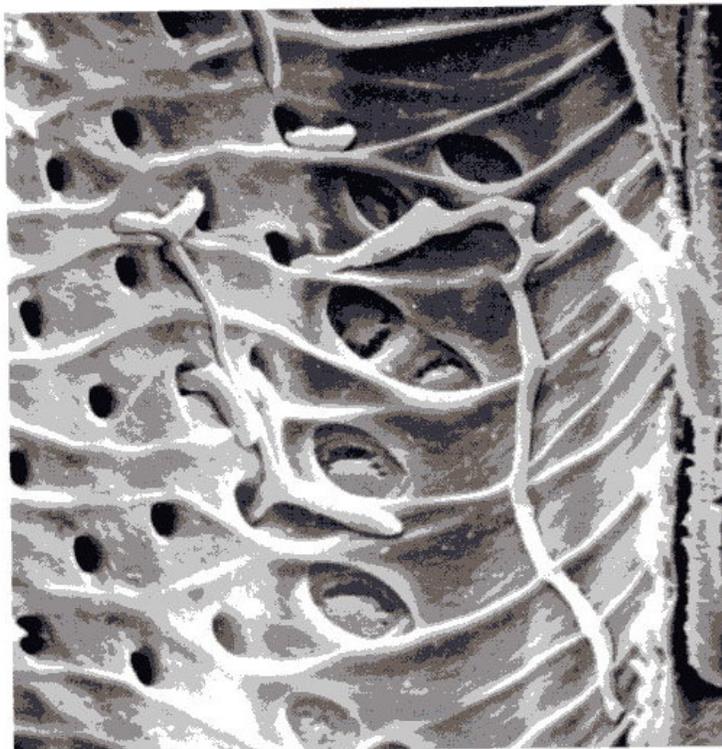
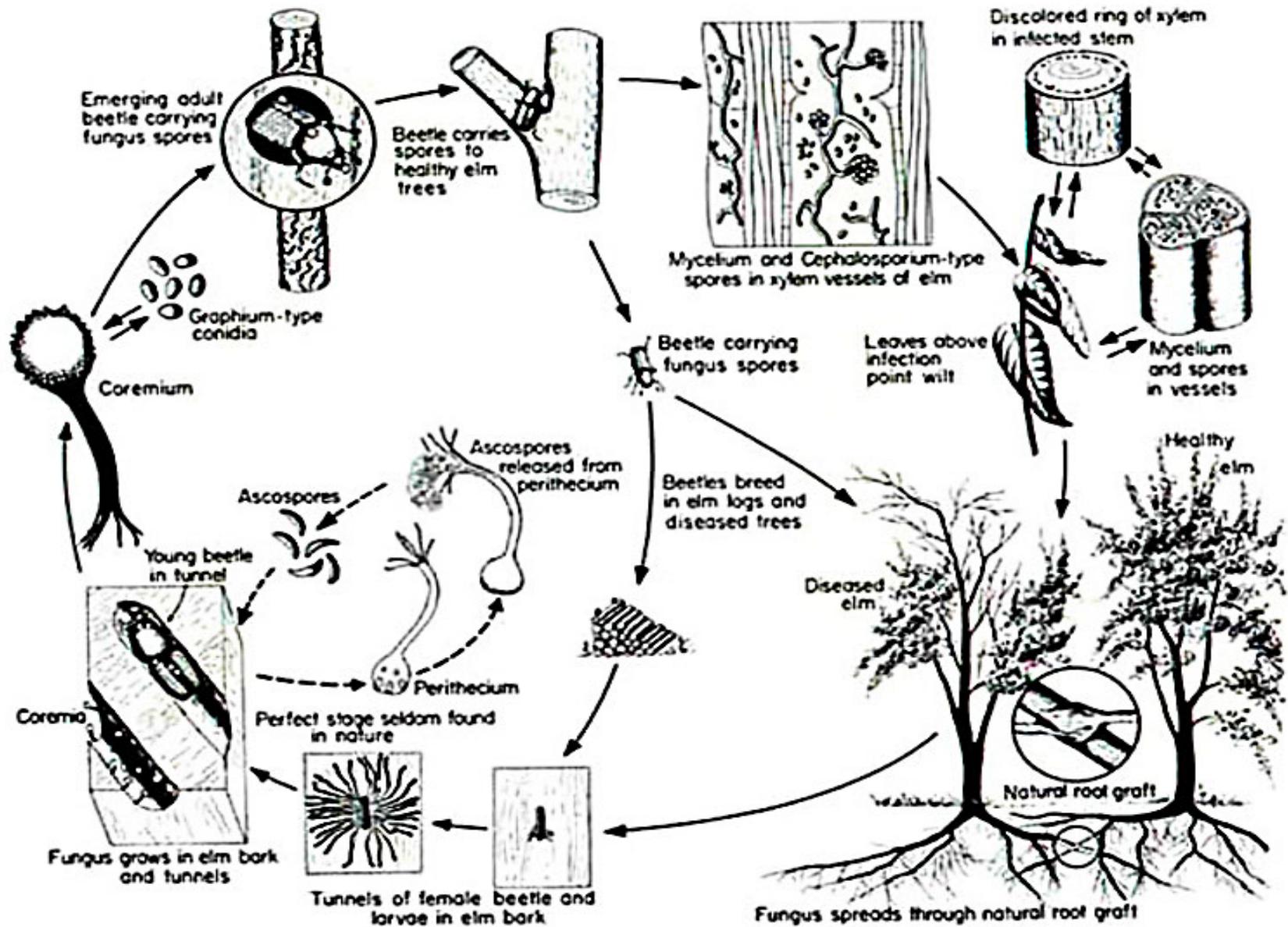


Fig. 22. Hyphae of *Ceratocystis ulmi* in diseased elm wood. Note passage of fungus through pit openings between vessels. (Courtesy D. M. Elgersma)

Life Cycle of *Ophiostoma ulmi*



Vectors of disease

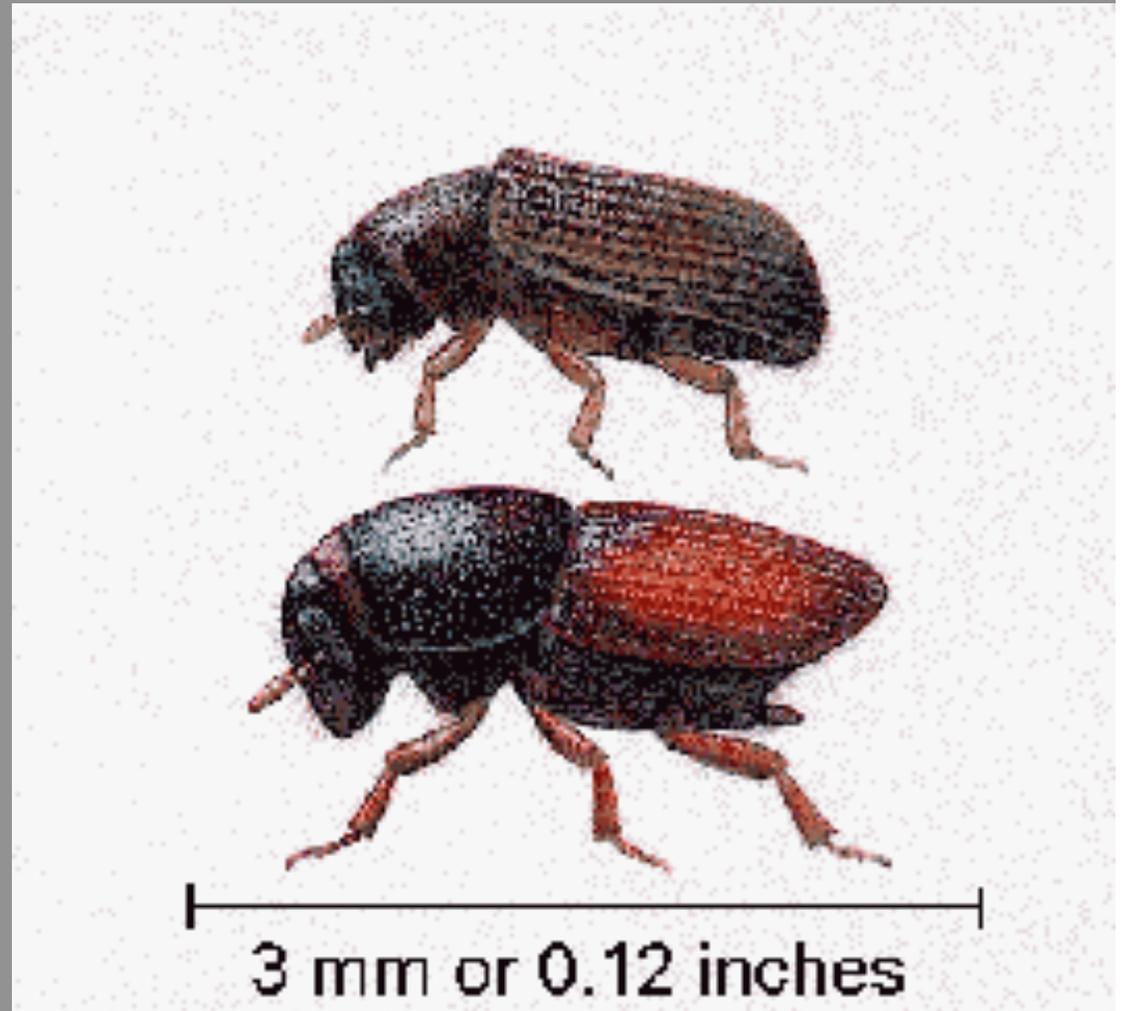
- Insects: 1) the native elm beetle 2) the smaller European elm beetle. The beetles can fly for several miles, allowing the disease to spread over a wide area
- Root grafts: when elms are within 50 feet of one another, their roots can grow together and disease passes easily along. Important in urban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

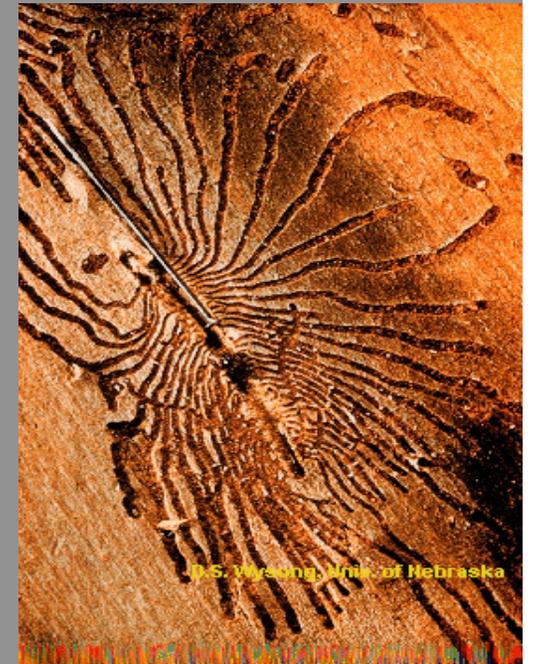
- Development of resistant hybrid elms
- Additional treatments: breaking up root grafts is commonly used and effective.
- Timing of pruning: wounded trees attract the bark beetle vectors of DED (Byers et al., 1980), so routine pruning should be done in the dormant season or during periods of beetle inactivity.

Dutch Elm Disease

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



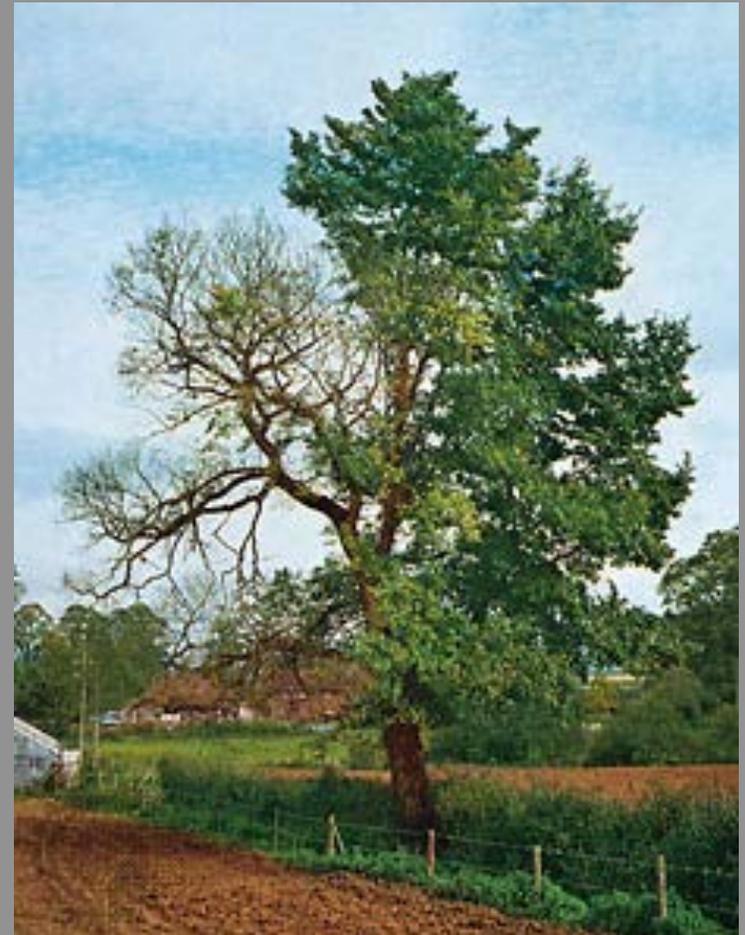
- Transmitted by *Scolytus* bark beetle
 - Beetle carves larval galleries in sapwood and carries fungus from tree to tree



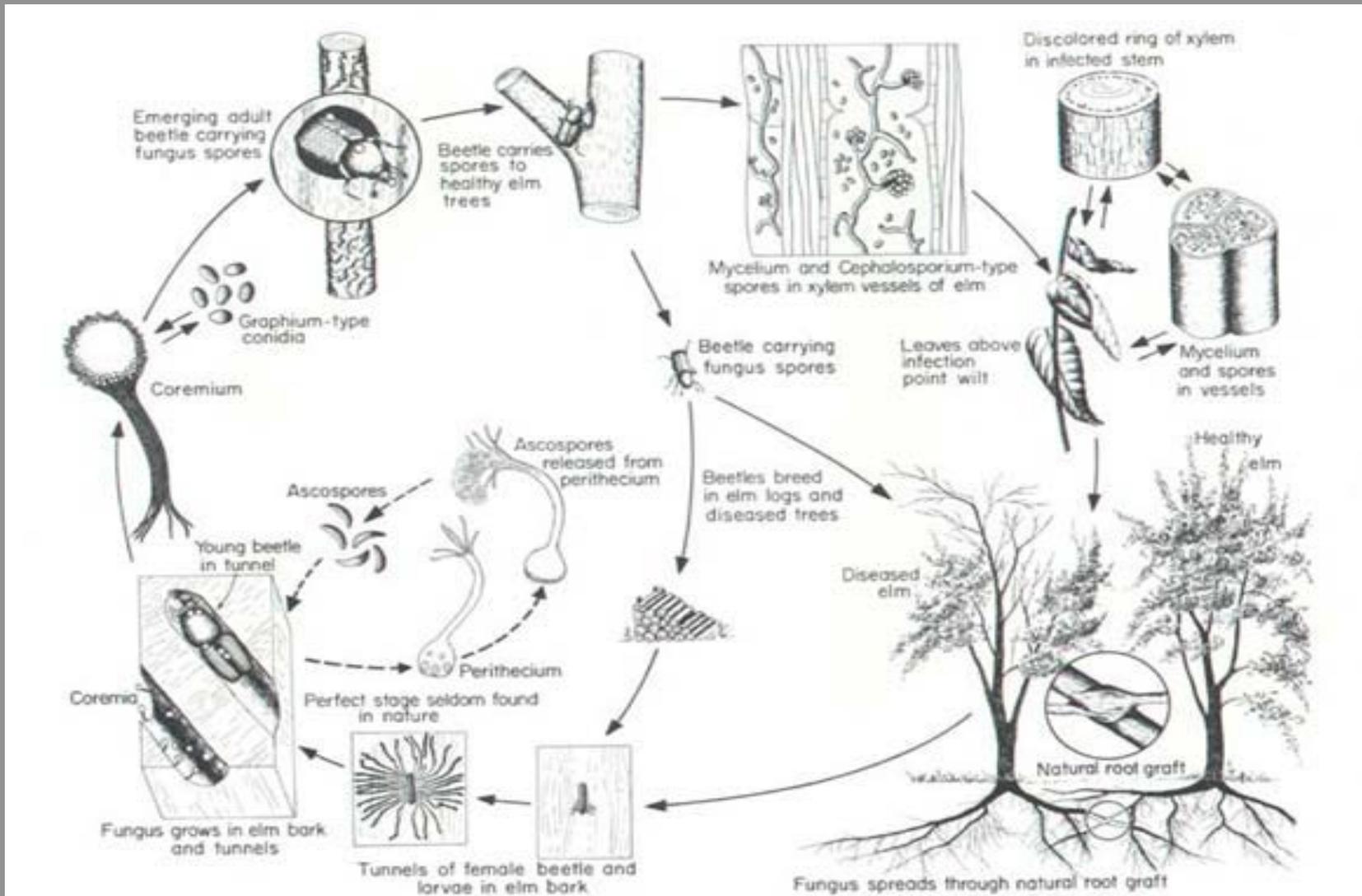
- Spreads through trees vascular system
- Tree tries to slow fungus by plugging its own xylem tissue with tyloses
- Plugged xylem causes branch dieback

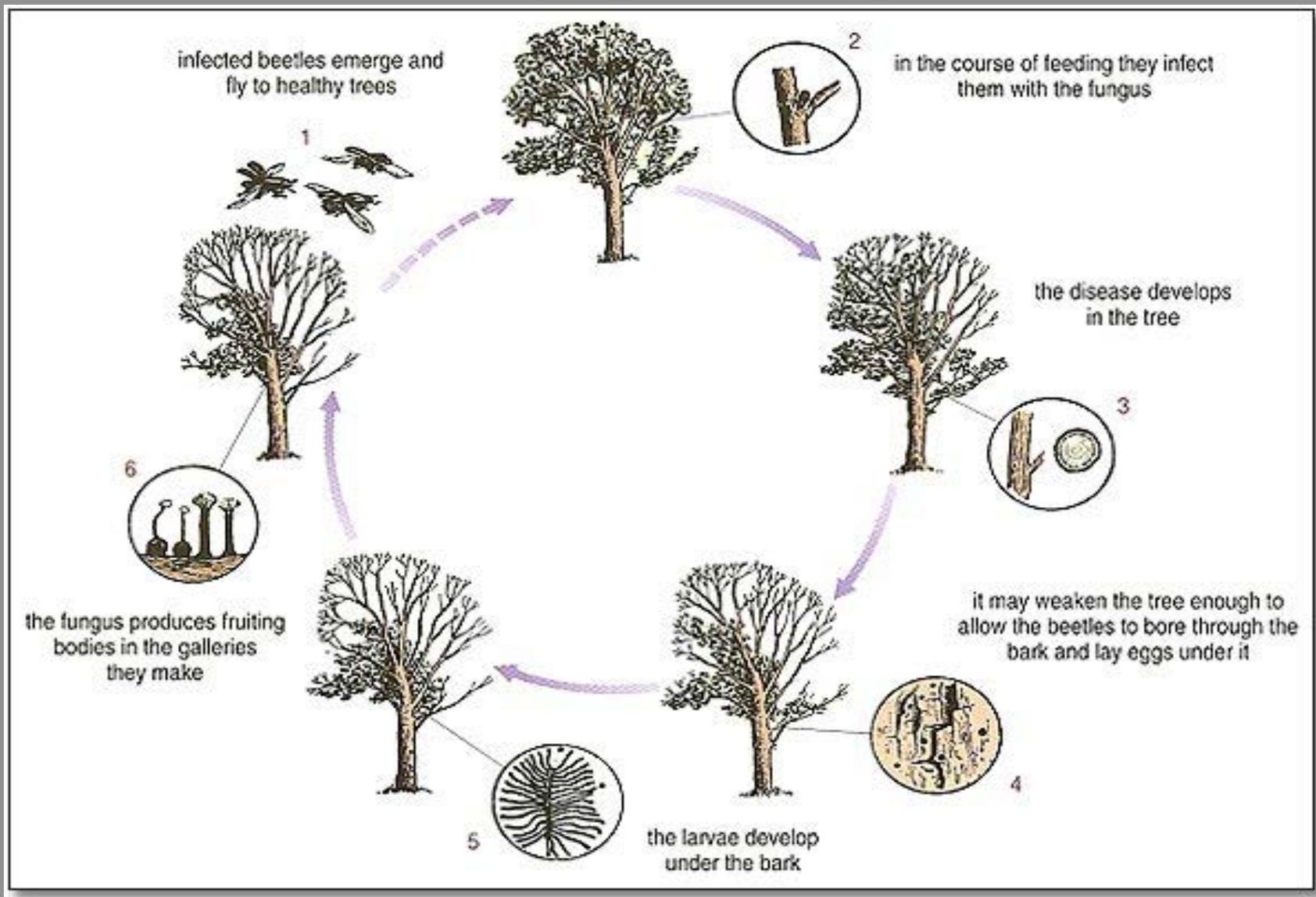


- Toxins force stomata to open
- Increased evapotranspiration causes desiccation and rapid death of tree

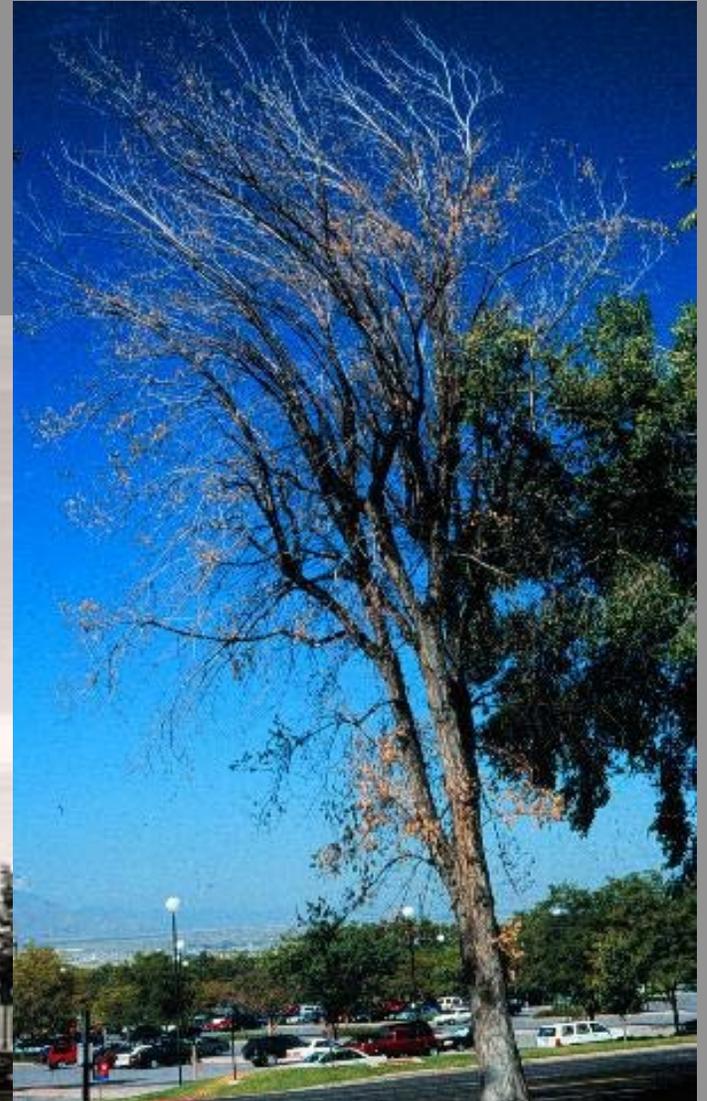


Life cycle with beetle vector

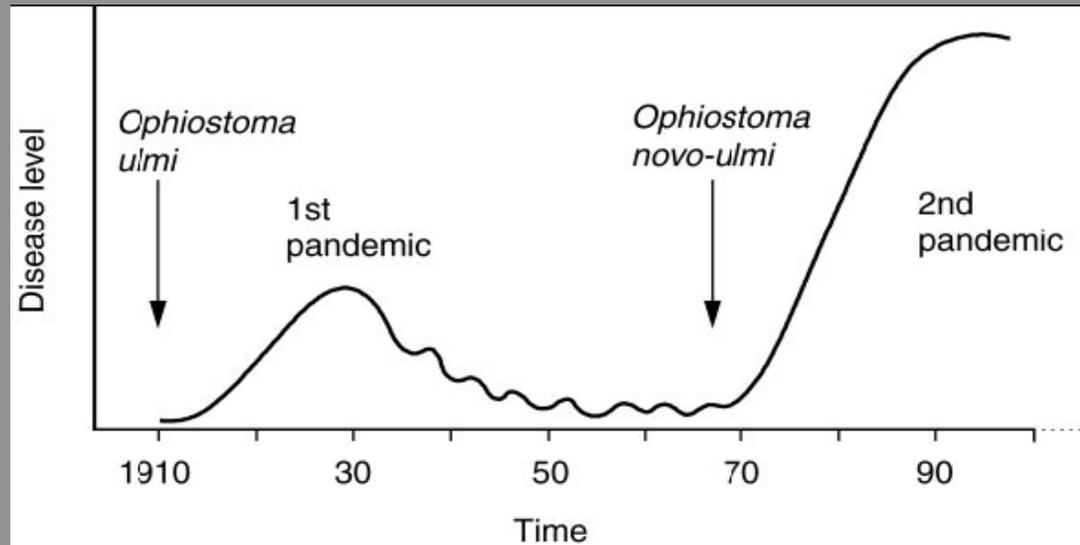




- Two separate pandemics caused by two different species
- *Ophiostomata ulmi*
- *Ophiostomata nova-ulmi*
- Origins still unknown



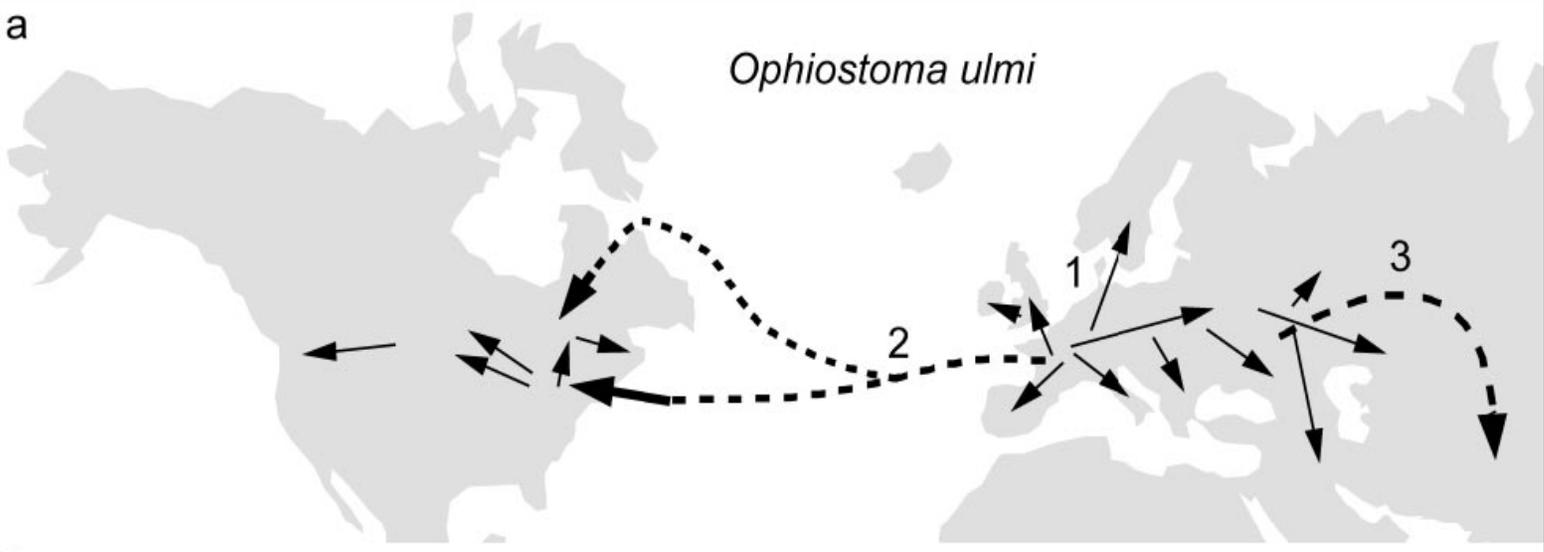
Two Pandemics



- *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

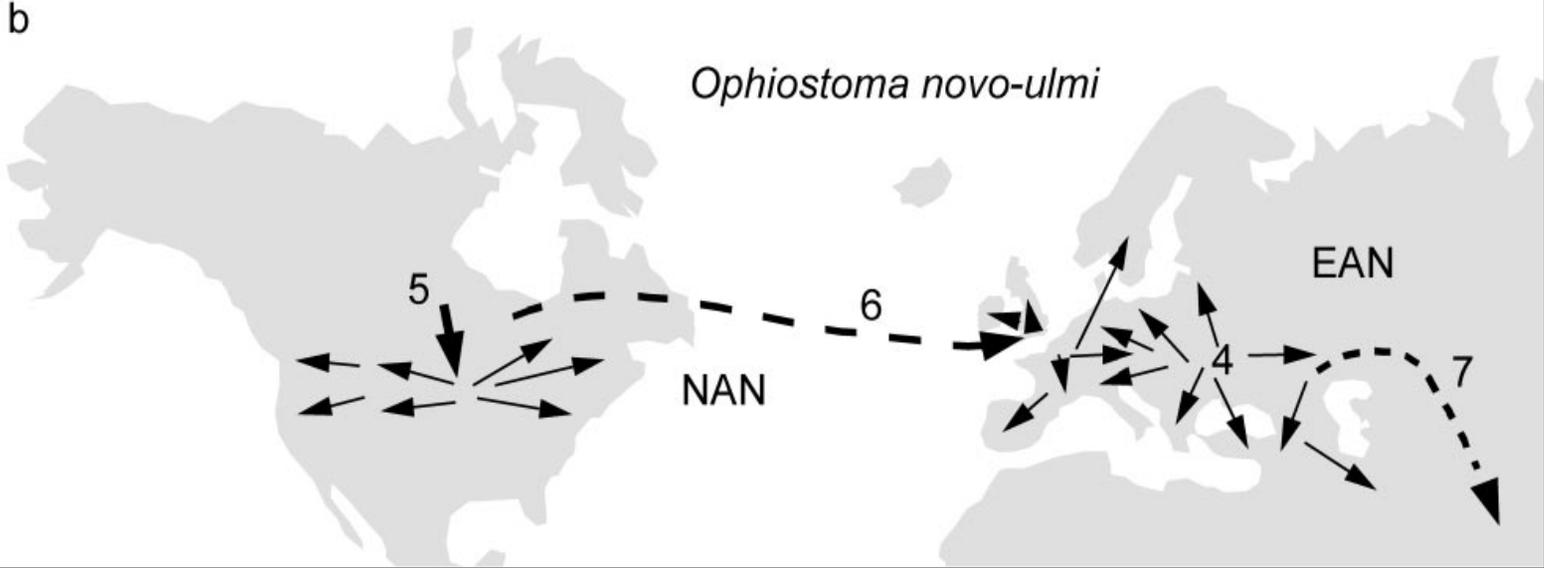
a

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

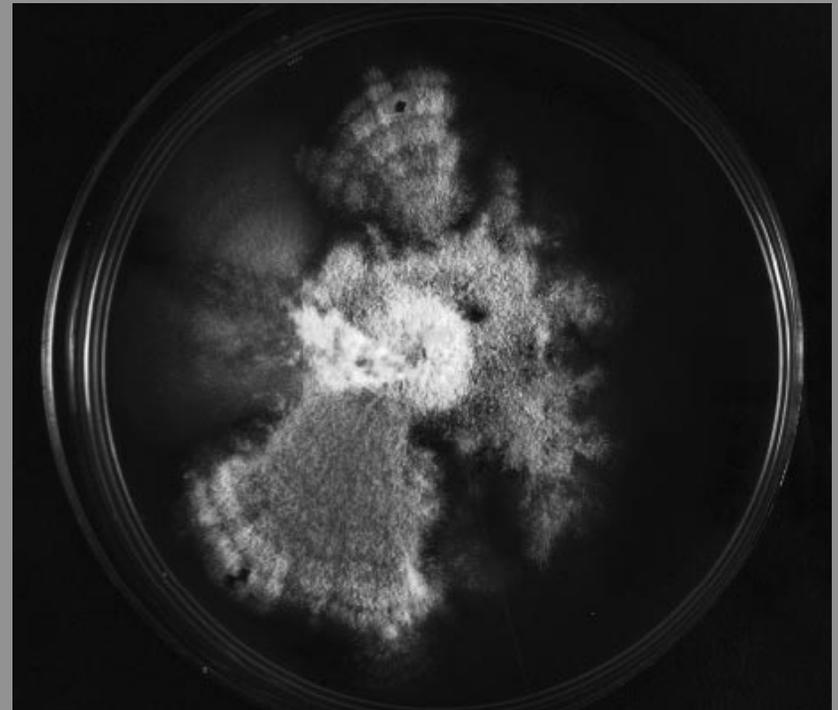
- DNA Fingerprinting
 - Probed with cloned dna fragments to distinguish between two species
 - Some *O. novo-ulmi* isolates had rare *O. ulmi* like polymorphisms
 - Acquired through introgression
 - 15 of 50 *O. novo-ulmi* isolates had *O. ulmi* gene
 - 1 had altered phenotype
 - Less aggressive
 - AFLP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

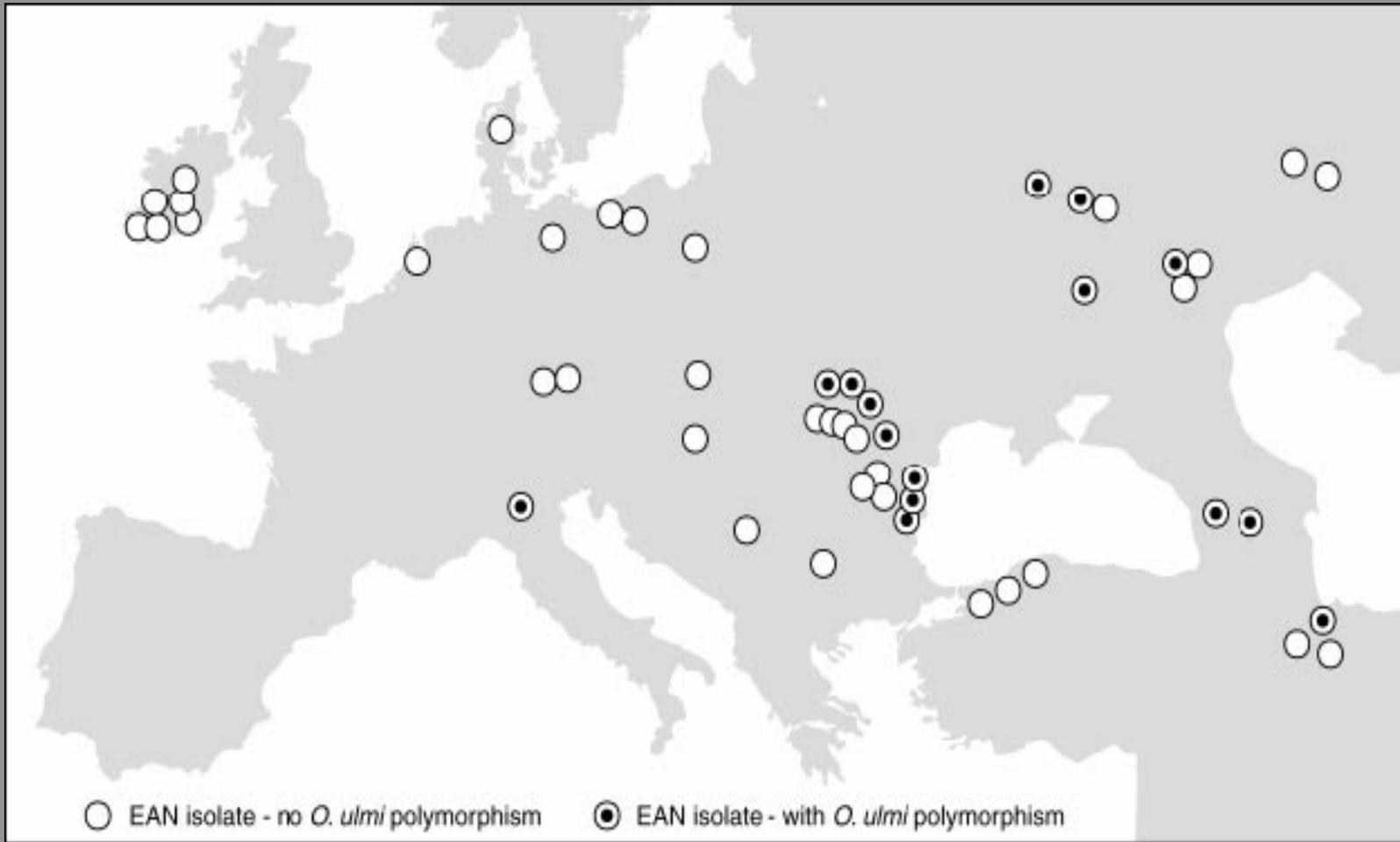
Europe

- 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?

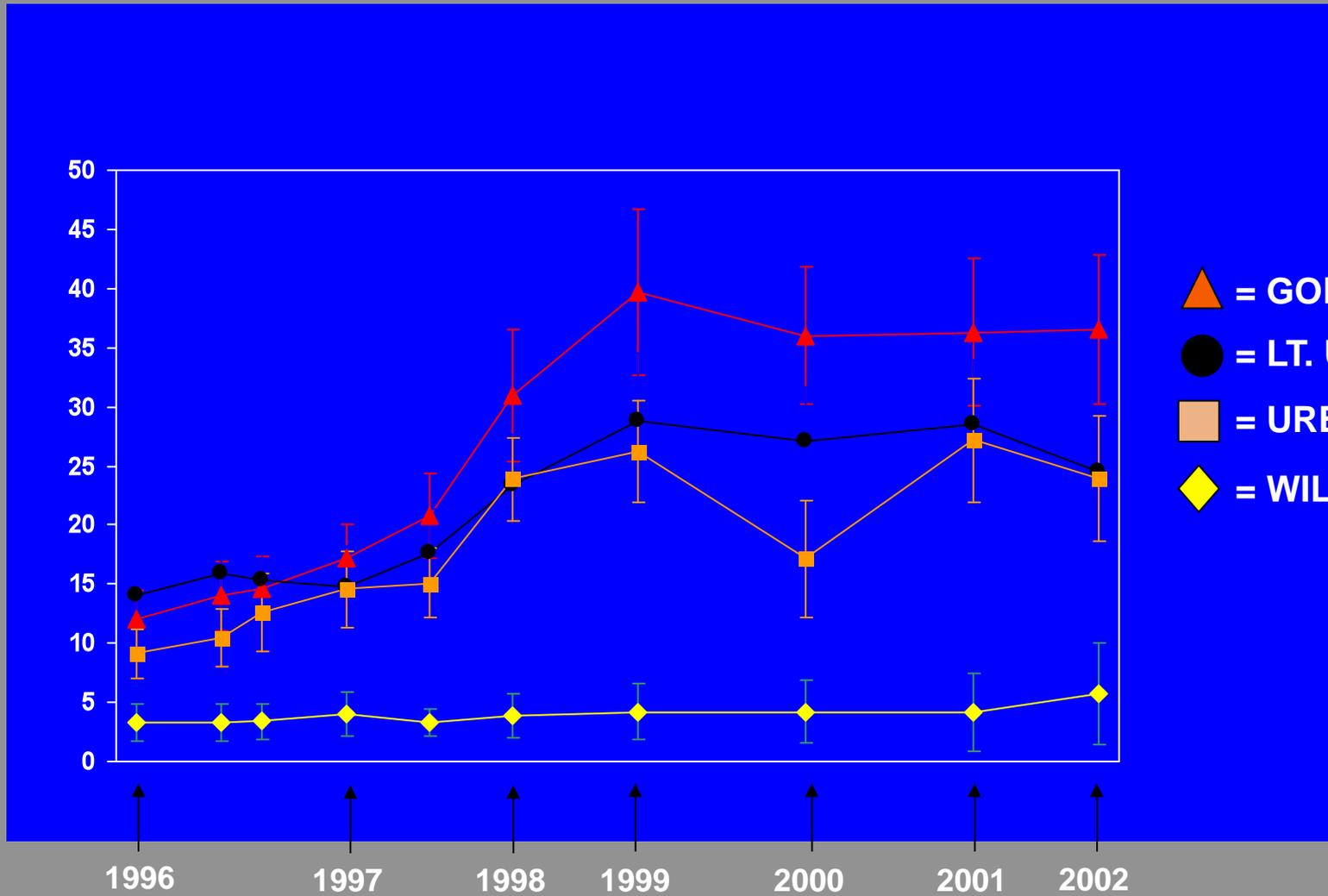
- Maximum genetic diversity found in Mexico
- East Coast of US has relatively high diversity, suggesting longer exposure
- South Africa, California and Japan have very low diversity, with California and Japan being quite similar presumably because infestations have the same source

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

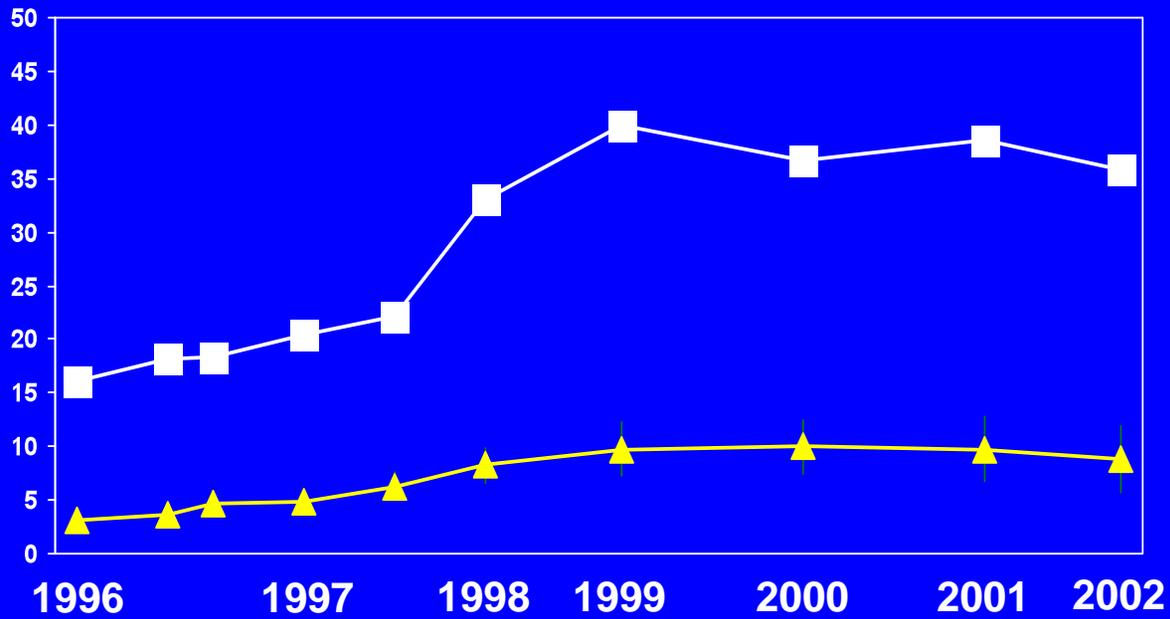
DISEASE SEVERITY



- ▲ = GOLF
- = LT. URBAN
- = URBAN
- ◆ = WILDLAND

DISEASE PROGRESS BY LOCATION

DISEASE SEVERITY



□ = INLAND
▲ = COASTAL

SURVEY DATE

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:

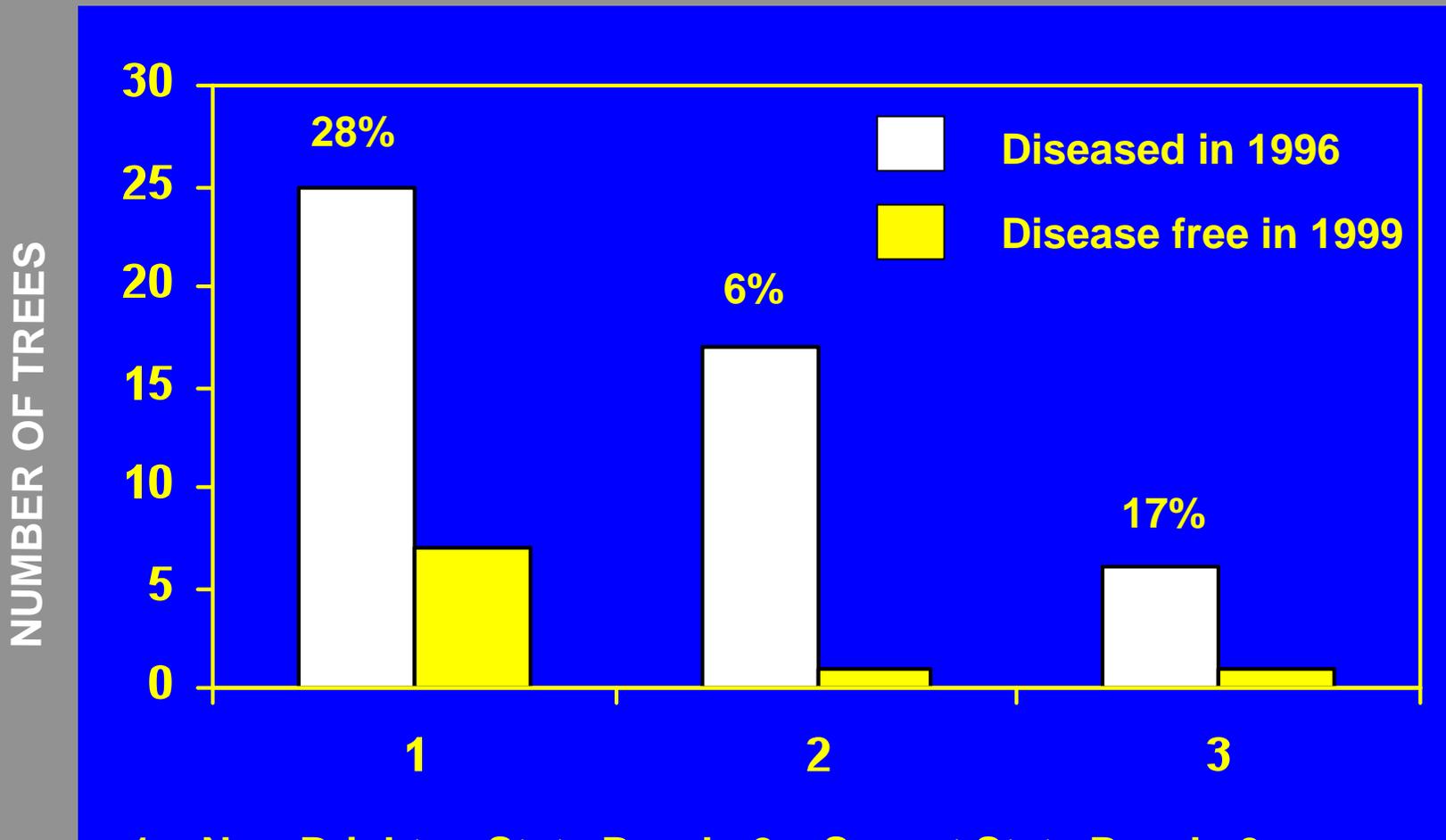
5

% MORTALITY DUE TO PITCH CANKER = 3.6%

LONG TERM IMPACTS



CHANGE IN DISEASE STATUS FROM 1996 TO 1999

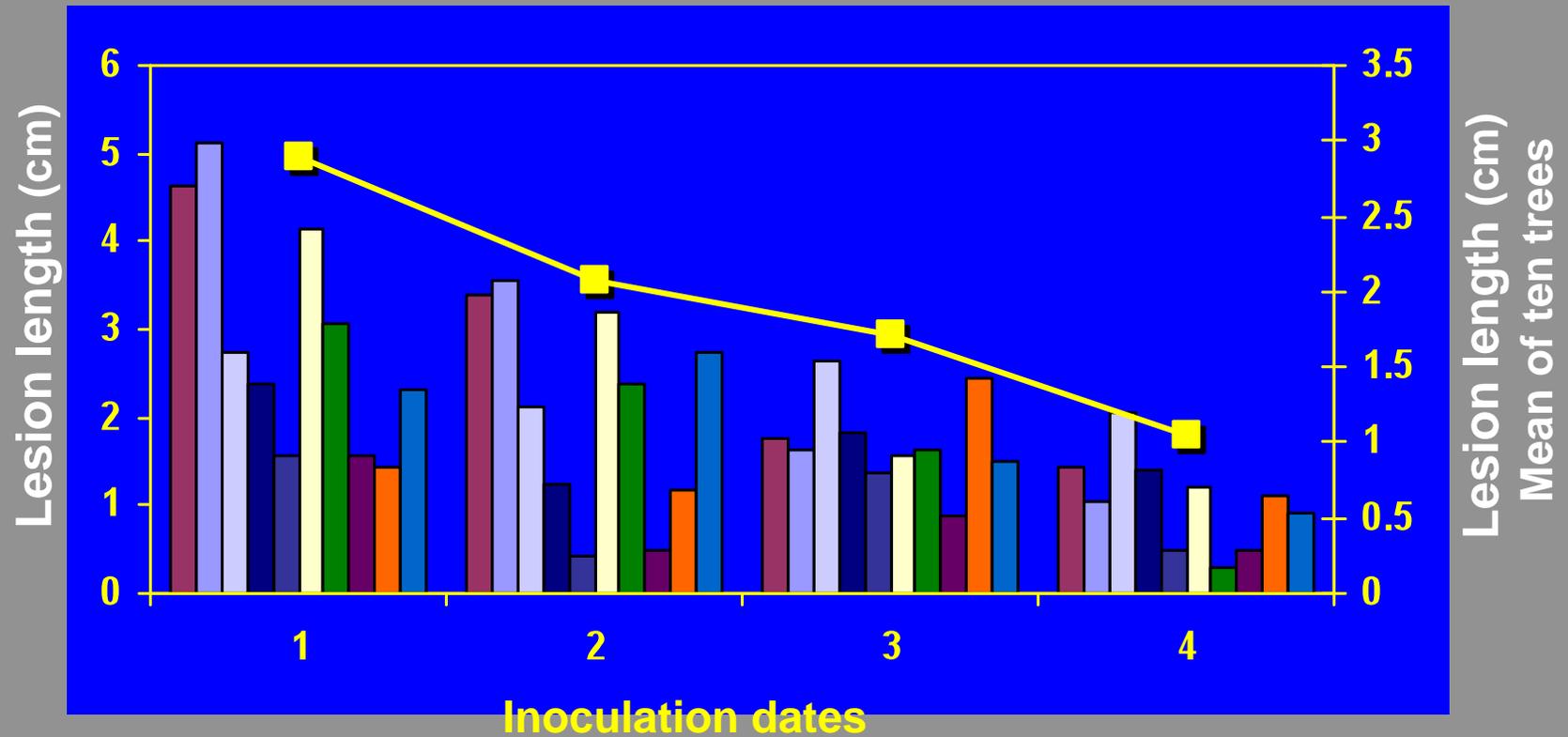


1 = New Brighton State Beach, 2 = Sunset State Beach, 3 = DeLaveaga golf course

**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:

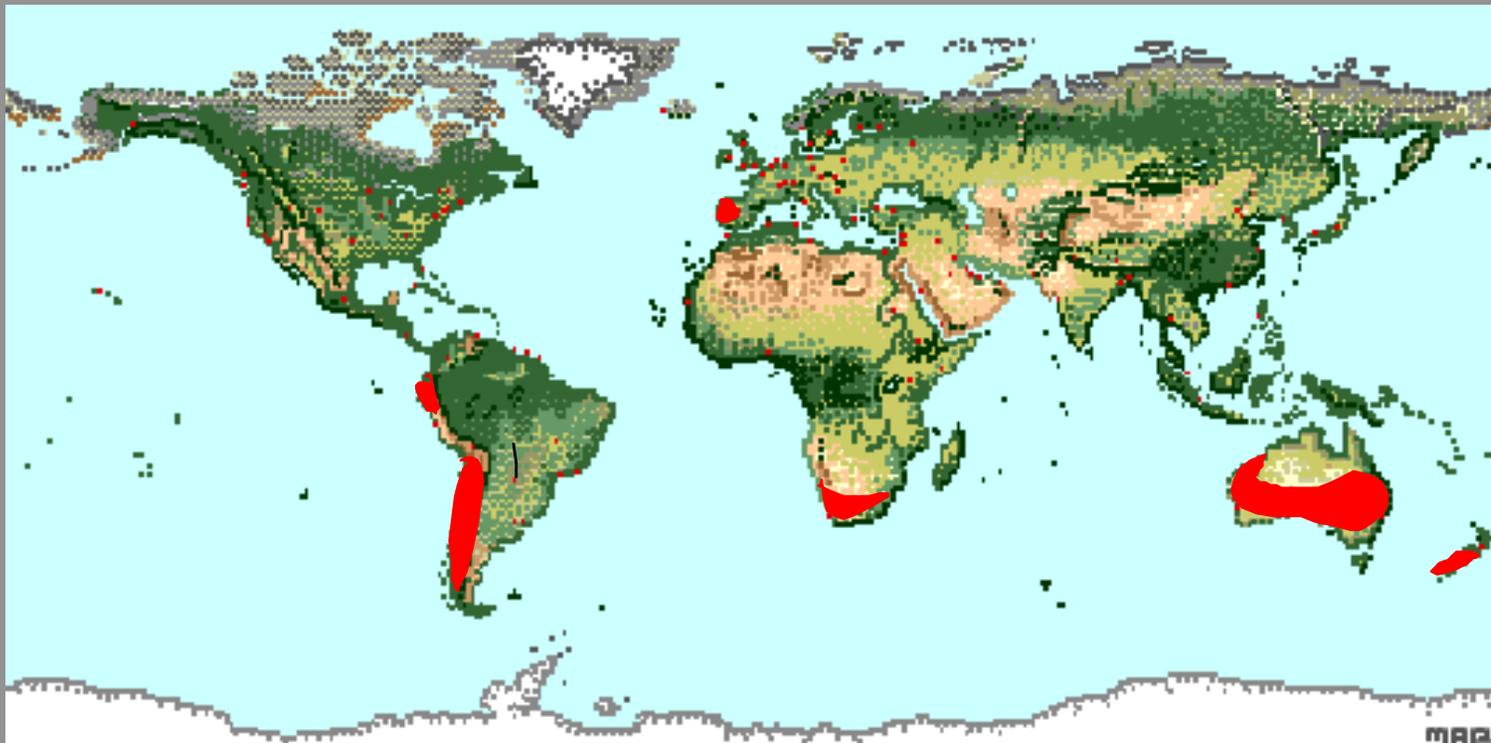
- Mortality rates may decrease**
- Mortality rates may increase**

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

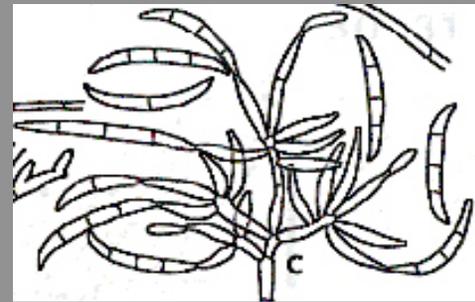


PITCH CANKER AS A SEEDLING DISEASE



Spreading of the disease

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



macro conidia

micro conidia



Unusual mortality of Aleppo pine in SoCal

First Report of *Fusarium circinatum*, Causal Agent of Pitch Canker Disease, from the Roots of Mature Aleppo Pines in California

M. Garbelotto and **W. Schweigkofler**, Department of ESPM-ES, 137 Mulford Hall, University of California, Berkeley 94720; and **D. Shaw**, and 5555 Overland Avenue, Suite 4101, University of California Cooperative Extension, San Diego 92123

Trees infected in nursery before being outplanted



Fig. 1. The chlorotic crown of an Aleppo pine (left) whose roots are colonized by *Fusarium circinatum*.



Fig. 2. Resin-soaked sapwood was observed under bark of primary-woody roots and root collars. *Fusarium circinatum* was always isolated from such lesions.

Fusarium sampling to monitor presence and quantify aerial spores

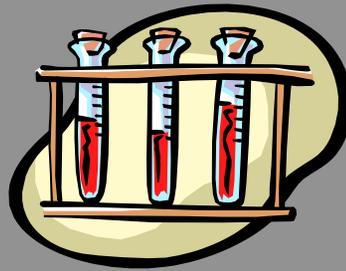


Real-time Quantification Working Scheme

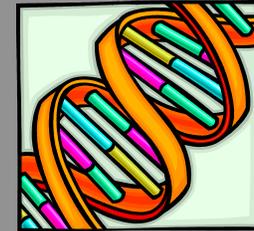


Spore trap with filter paper

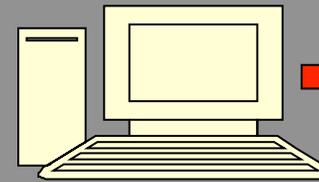
suspend in
20 ml 4X TE 65°C



Centrifugation
Spore concentration



DNA-extraction



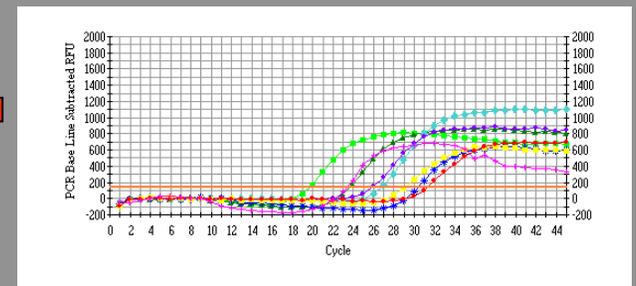
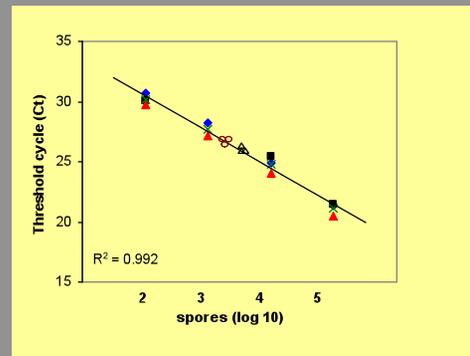
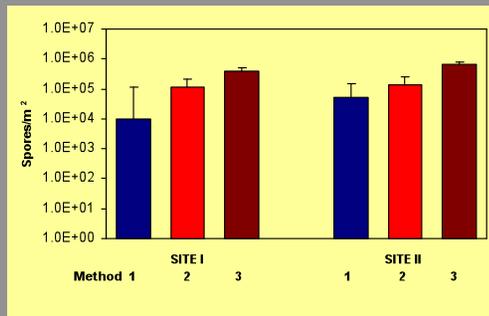
Mating type determination



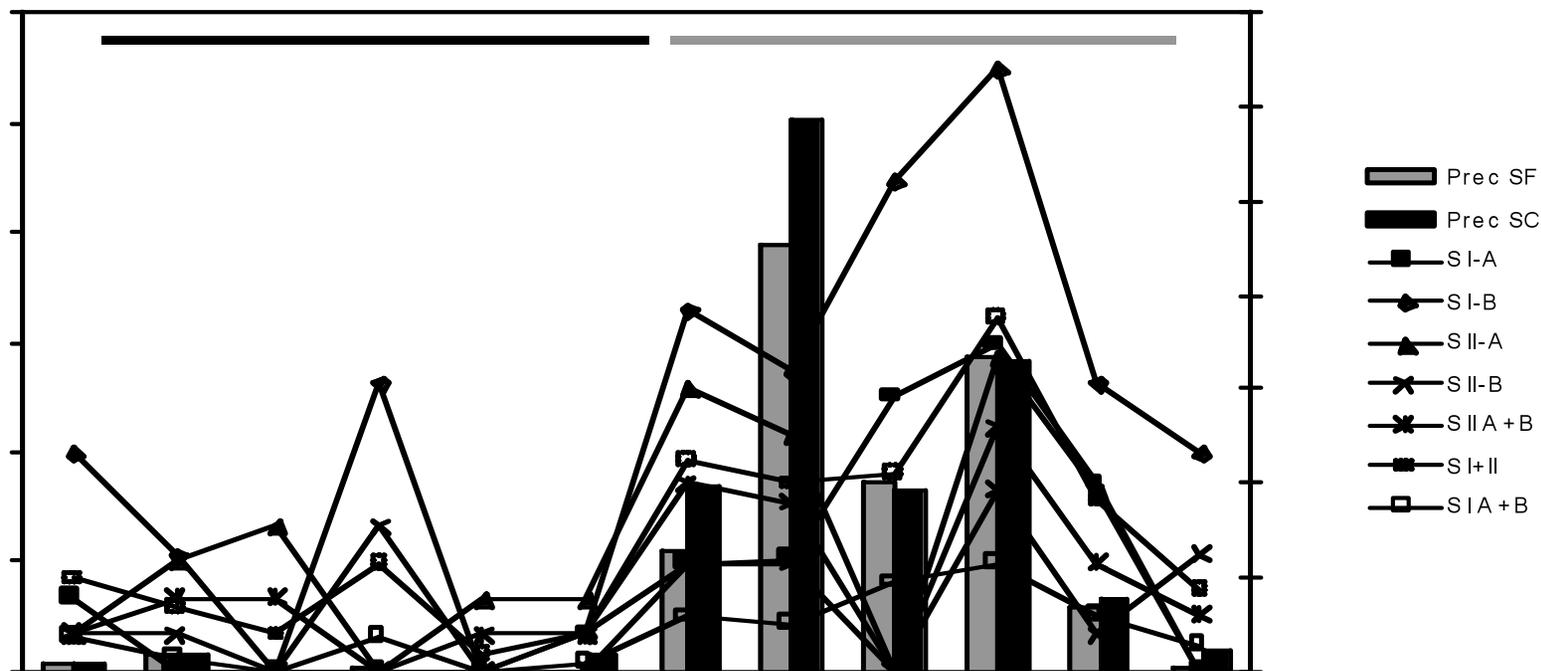
RT-PCR using
CIRC1A-4A

Calculate spores/m²

Compare with Spore (DNA) standard



Seasonal differences



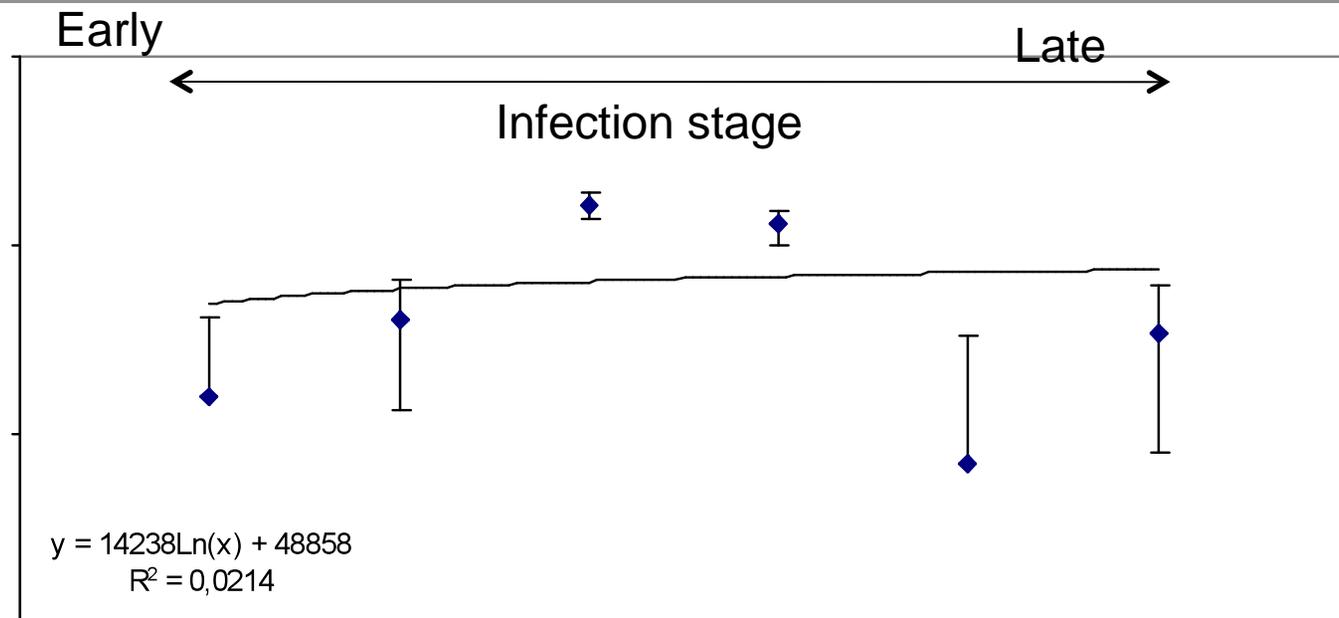
Dry season: May-October
Wet season: October-April

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



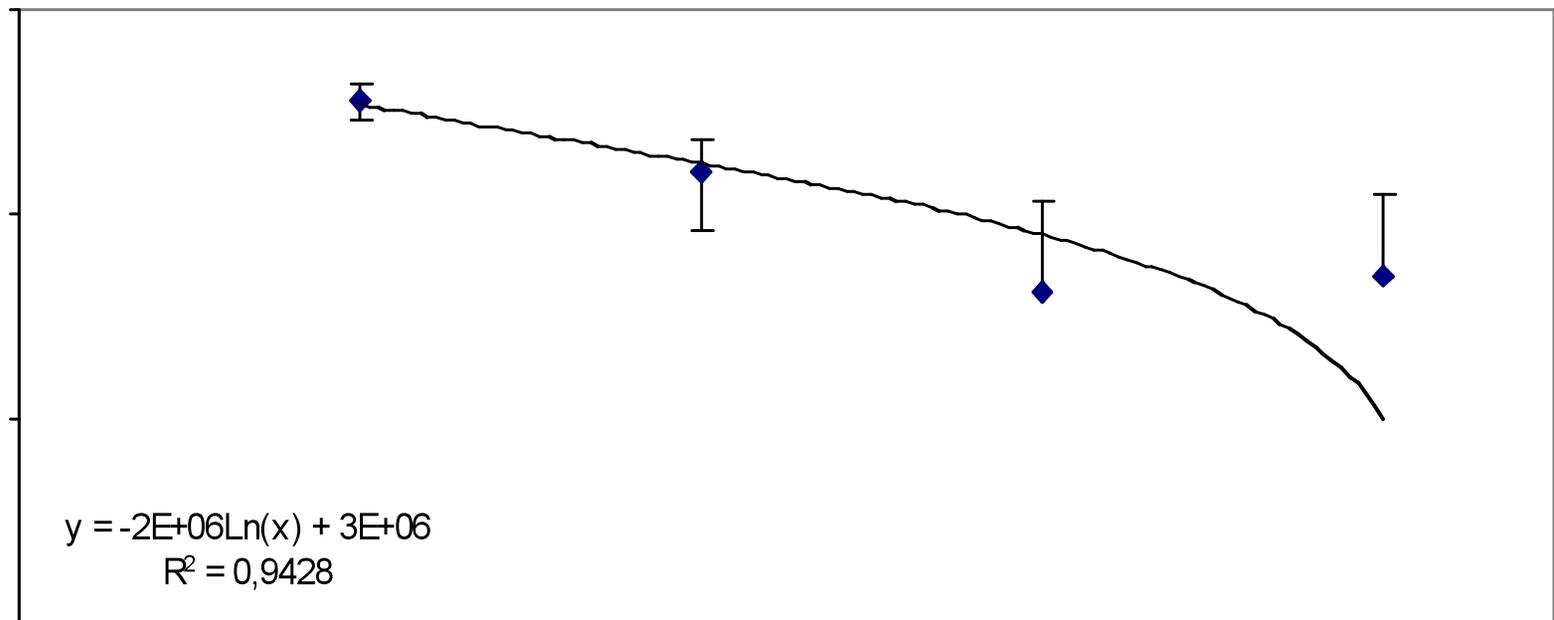
Epidemiology

- 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 (15 species so far!)

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

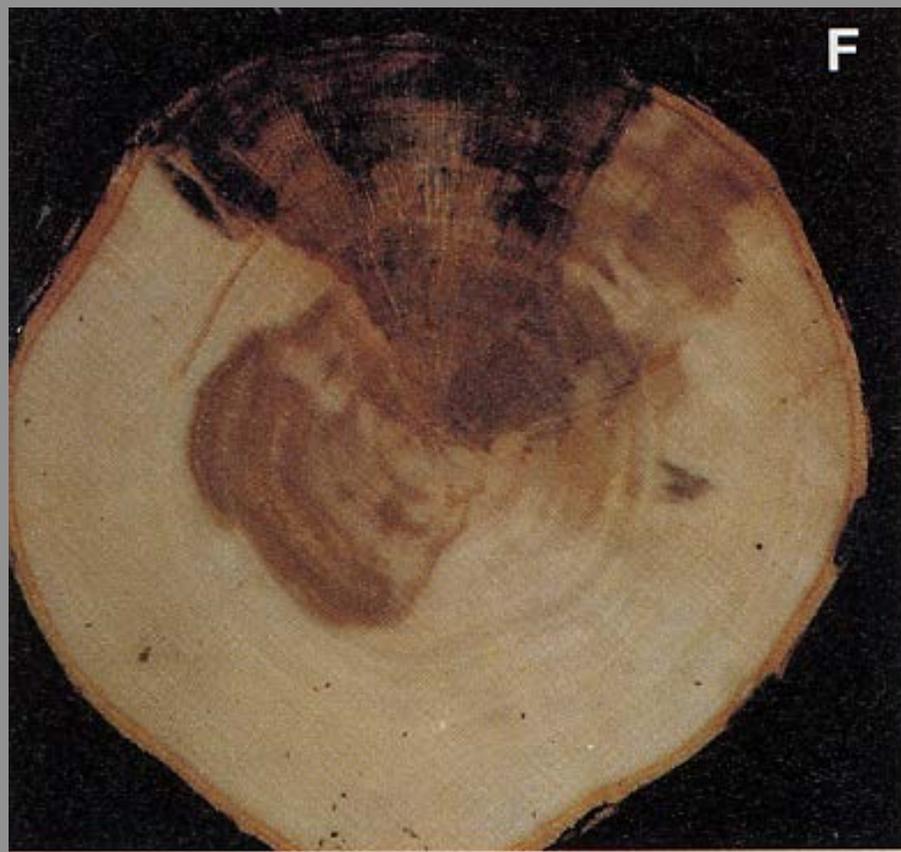
Visible symptoms: die-back



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



i cancri

Field diagnosis



Bark canker caused by *Fomitiporia punctata*

Lack of callus at the edges of the lesion

EPPO

PM 7/14(1)

Leaf chlorosis, dieback
Necrosis under the bark, cankers



Field diagnosis





Field diagnosis



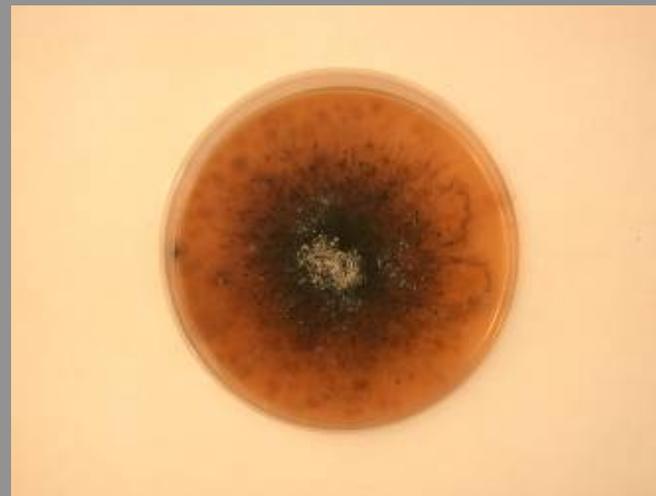
Field diagnosis



Diagnosis in the lab

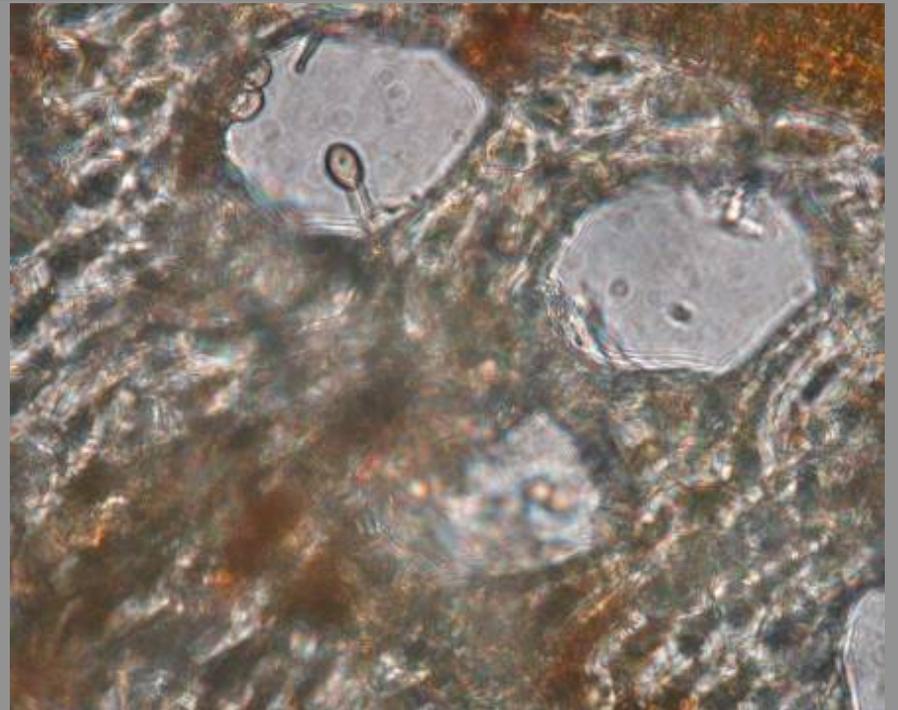
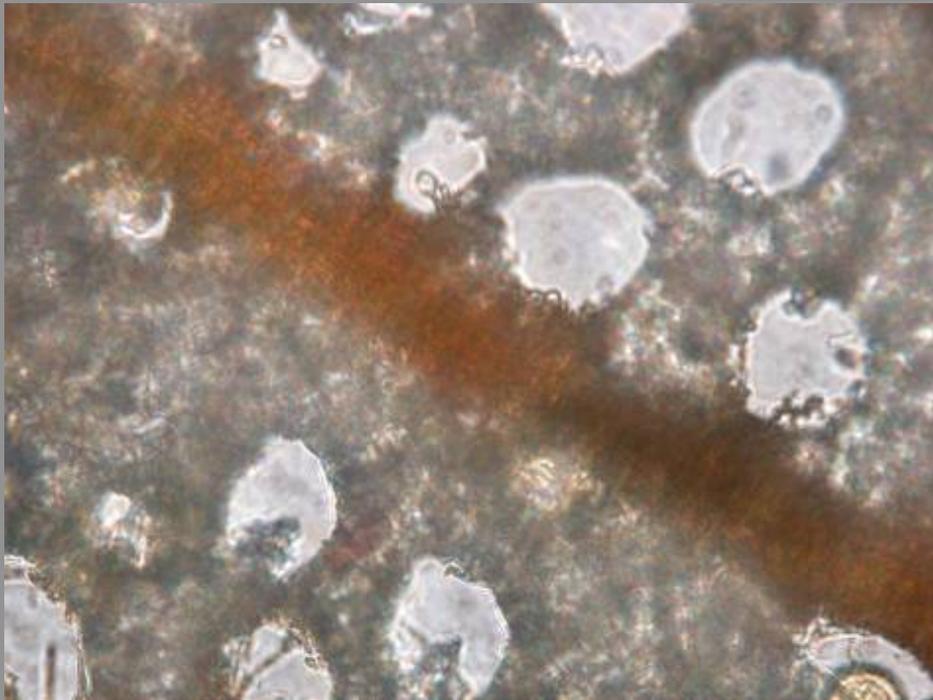
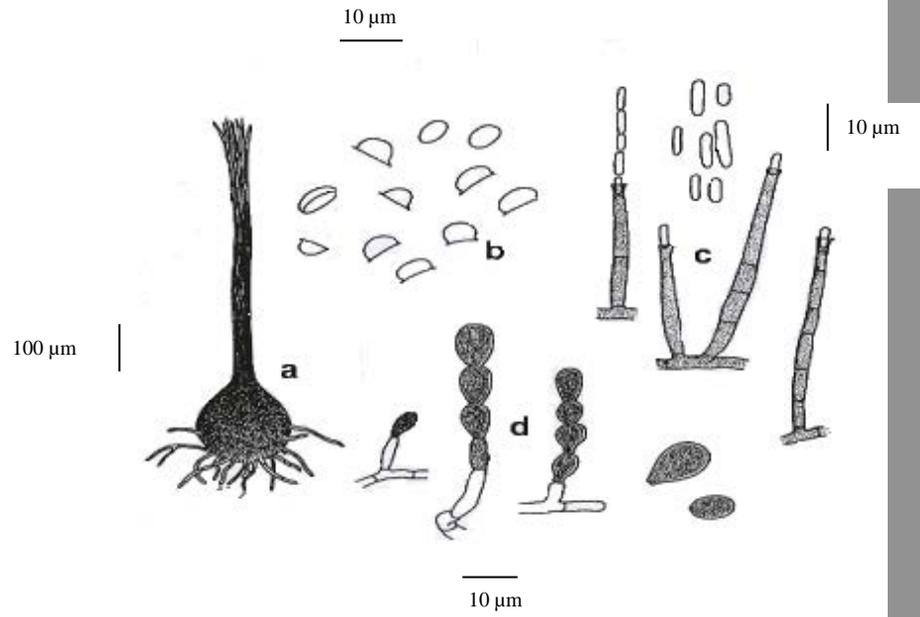


Inspection for the presence of perithecia after incubation in a damp room



Isolation

Diagnosis in the lab



Infection biology and epidemiology











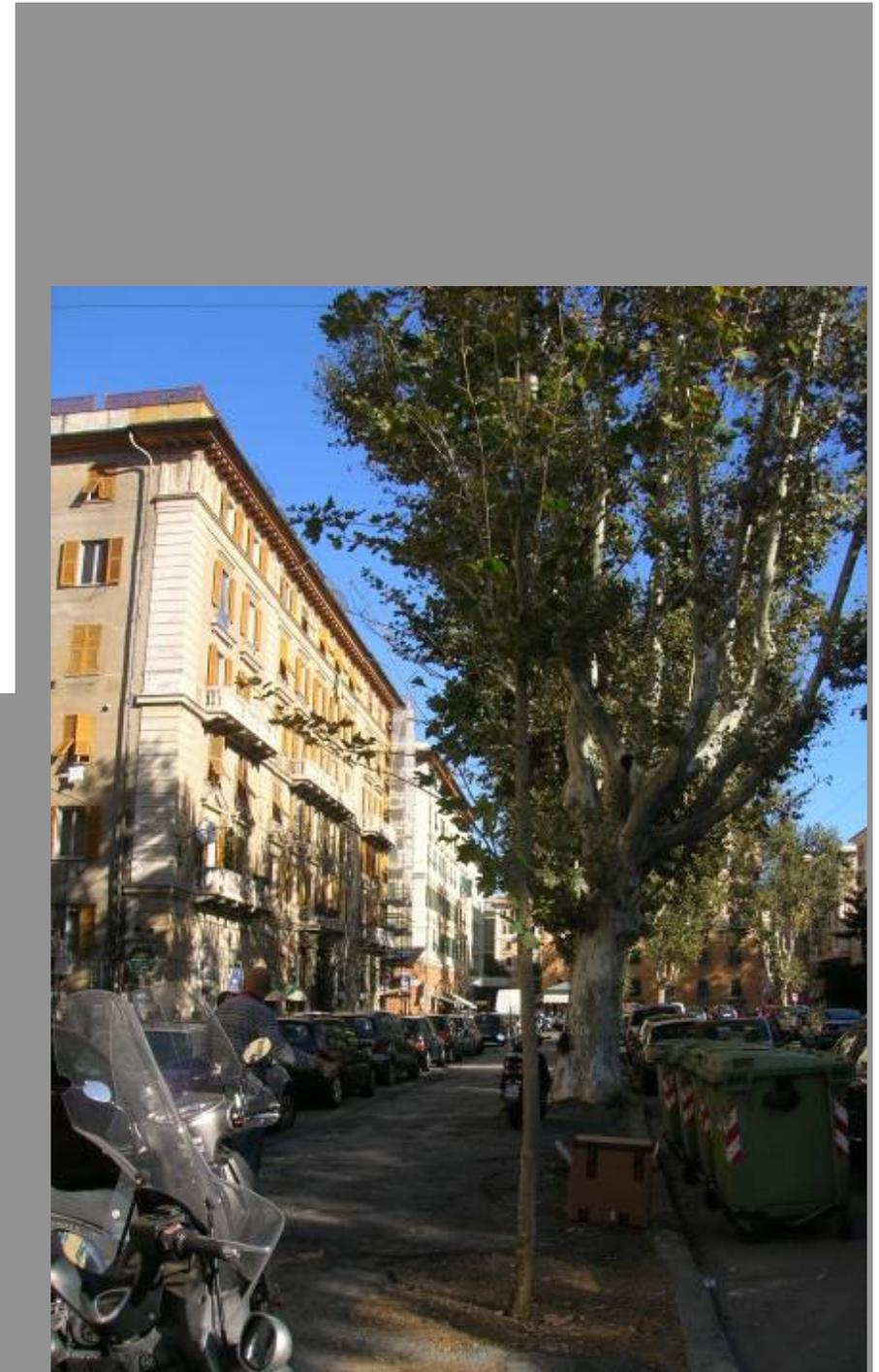
First hybrid plane trees to show resistance against canker stain (*Ceratocystis fimbriata* f. sp. *platani*)

By A. VIGOUROUX¹ and R. OLIVIER²

¹Laboratoire, INRA, UMR BGPI de Pathologie Végétale, Montpellier, France (Presently in Avignon, as n°2) E-mail: andre.vigouroux@avignon.inra.fr; ²Station 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 'Valis clausa' is described in detail.



Thousand canker disease of walnuts

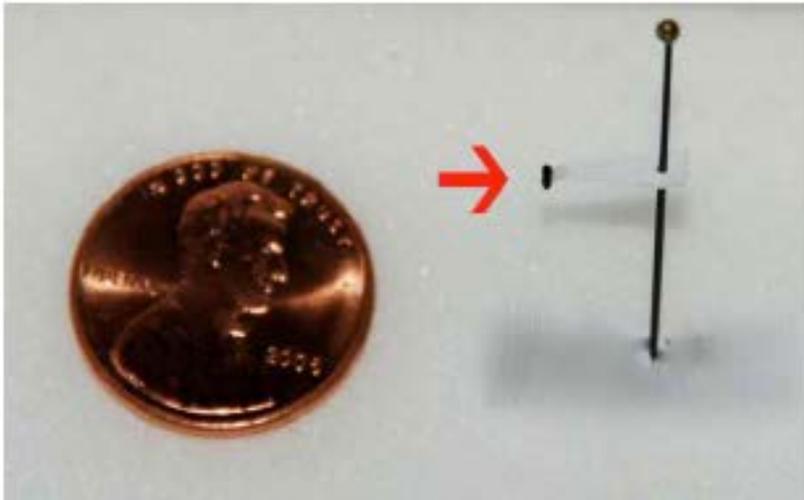
Geosmithia morbida: fungus

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).*

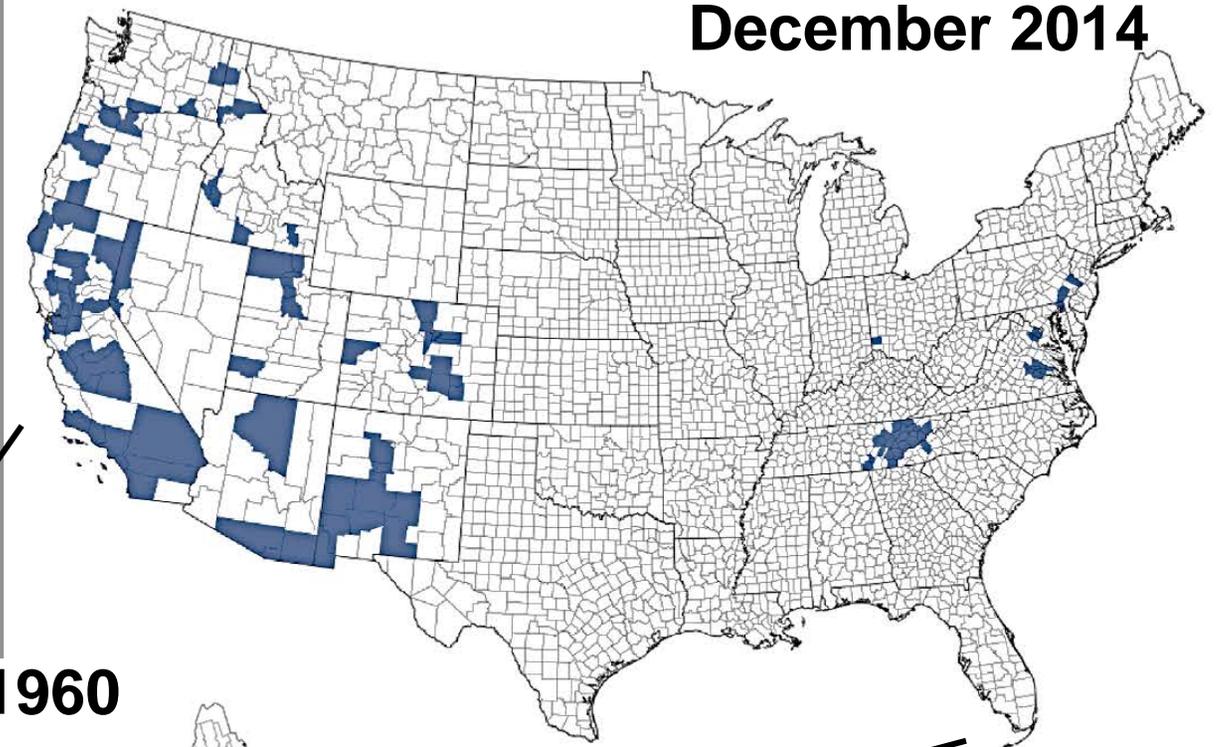


*The walnut twig beetle, *Pityophthorus juglandis* (photo by Eric Day).*

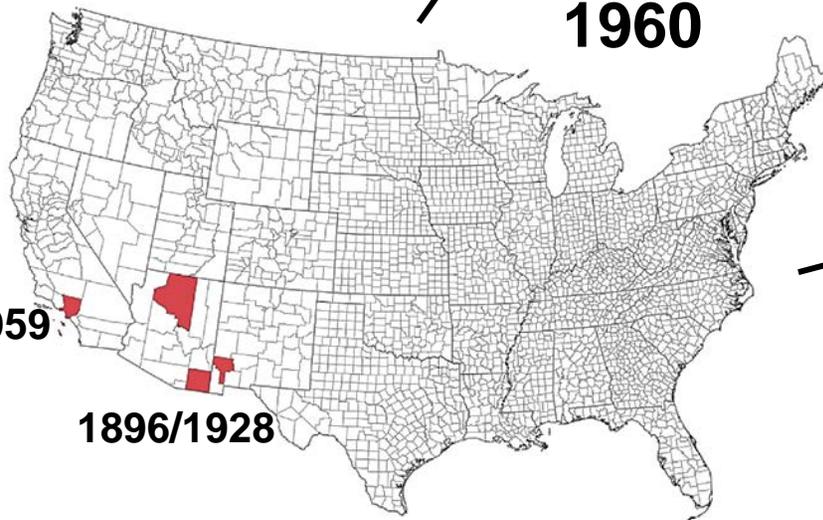


WTB distribution in the USA

December 2014



1960

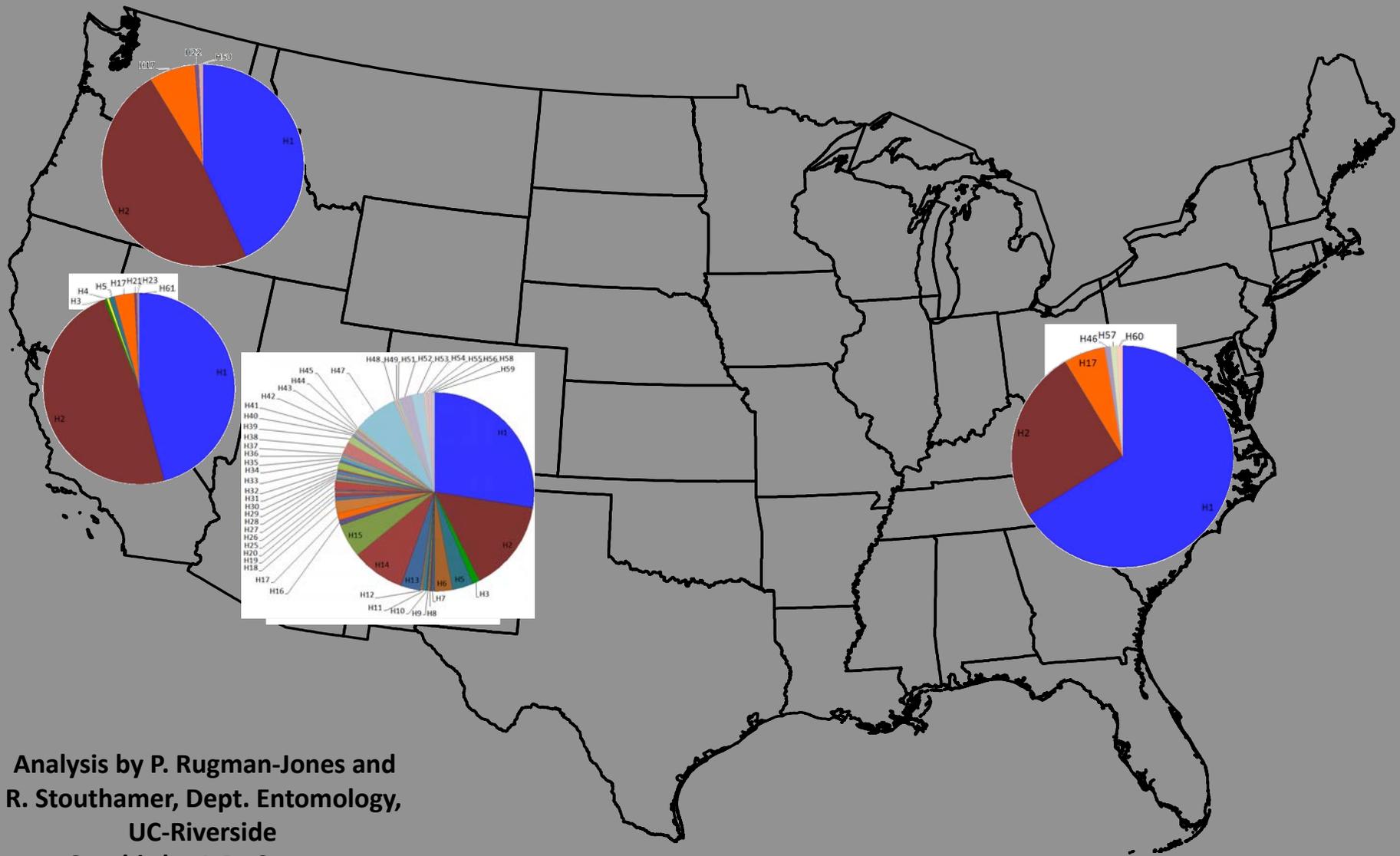


1959

1896/1928

from Rugman-Jones et al. (2015) PLoS ONE 10(2):e0118264

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

Rugman-Jones et al. (2015) PLoS ONE 10(2):e0118264

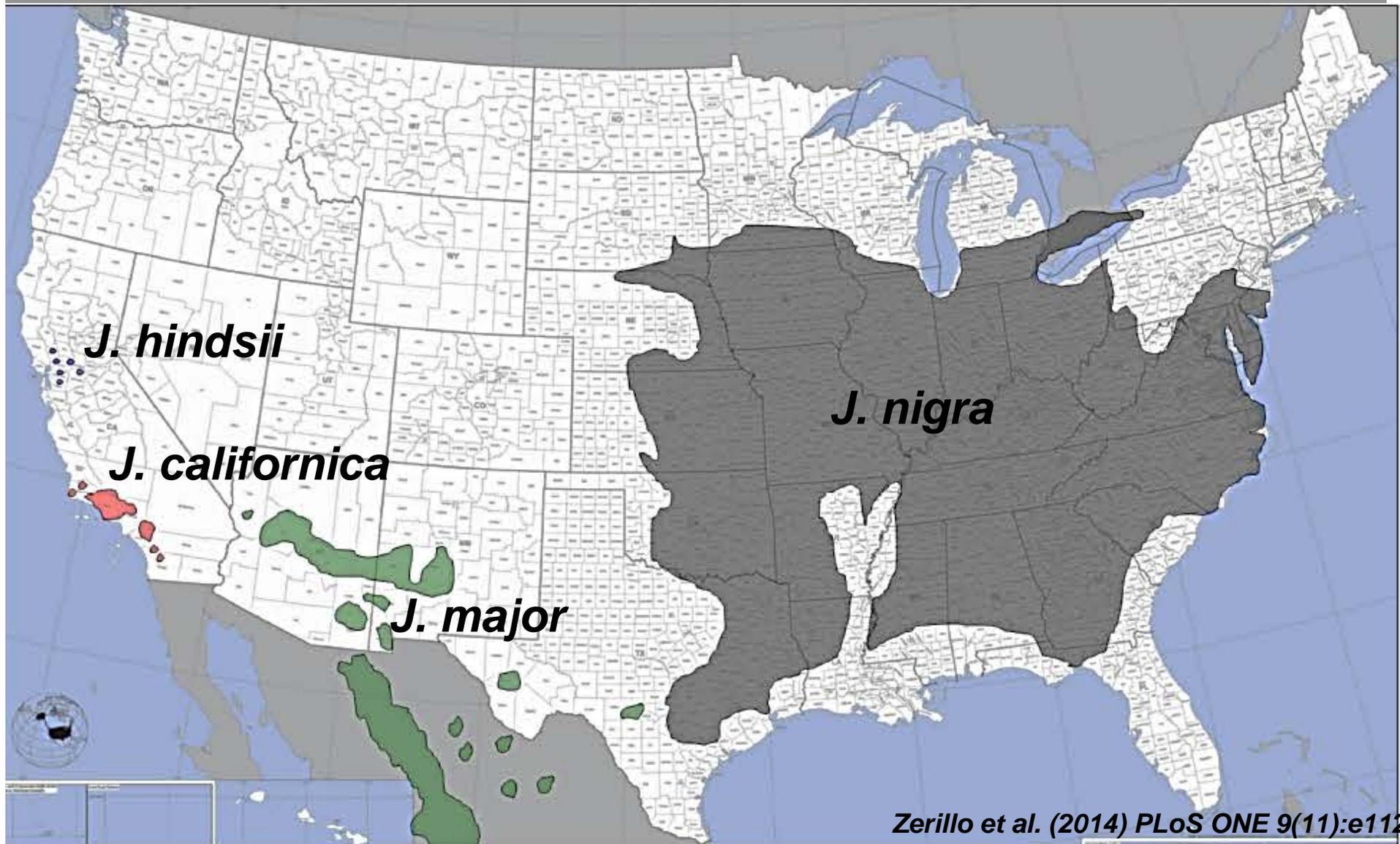
[Phylogram](#)

WTB range expansion and spread of TCD?



Zerillo et al. (2014) *PLoS ONE* 9(11):e112847
Rugman-Jones et al. (2015) *PLoS ONE* 10(2):e0118264

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



Comparative TCD Symptoms



Photo by Stacy Hishinuma

Wolfskill NCGR
Solano Co., CA



Juglans major
(southern NM)

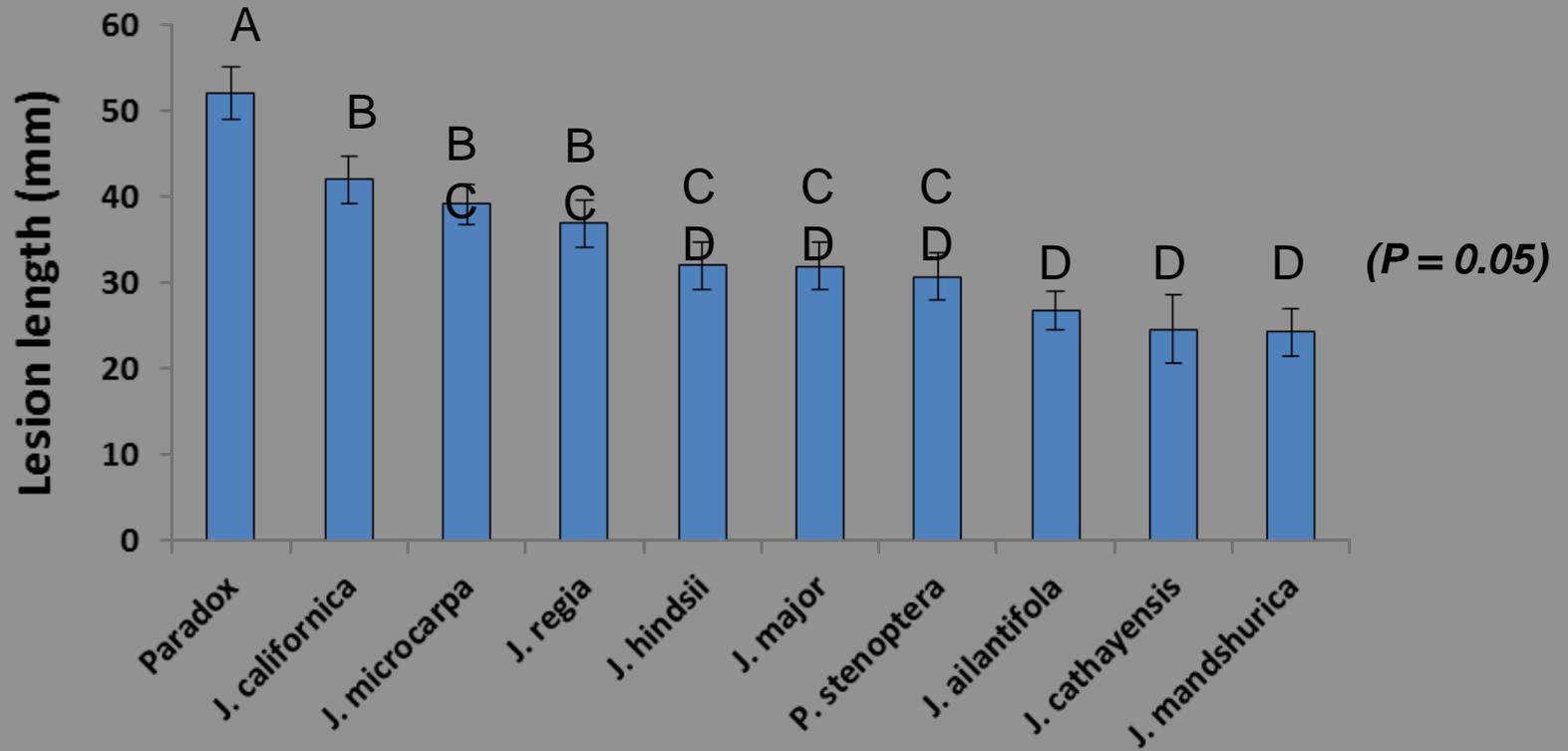
Sporadic
cankers,
minimal staining
on bark surface



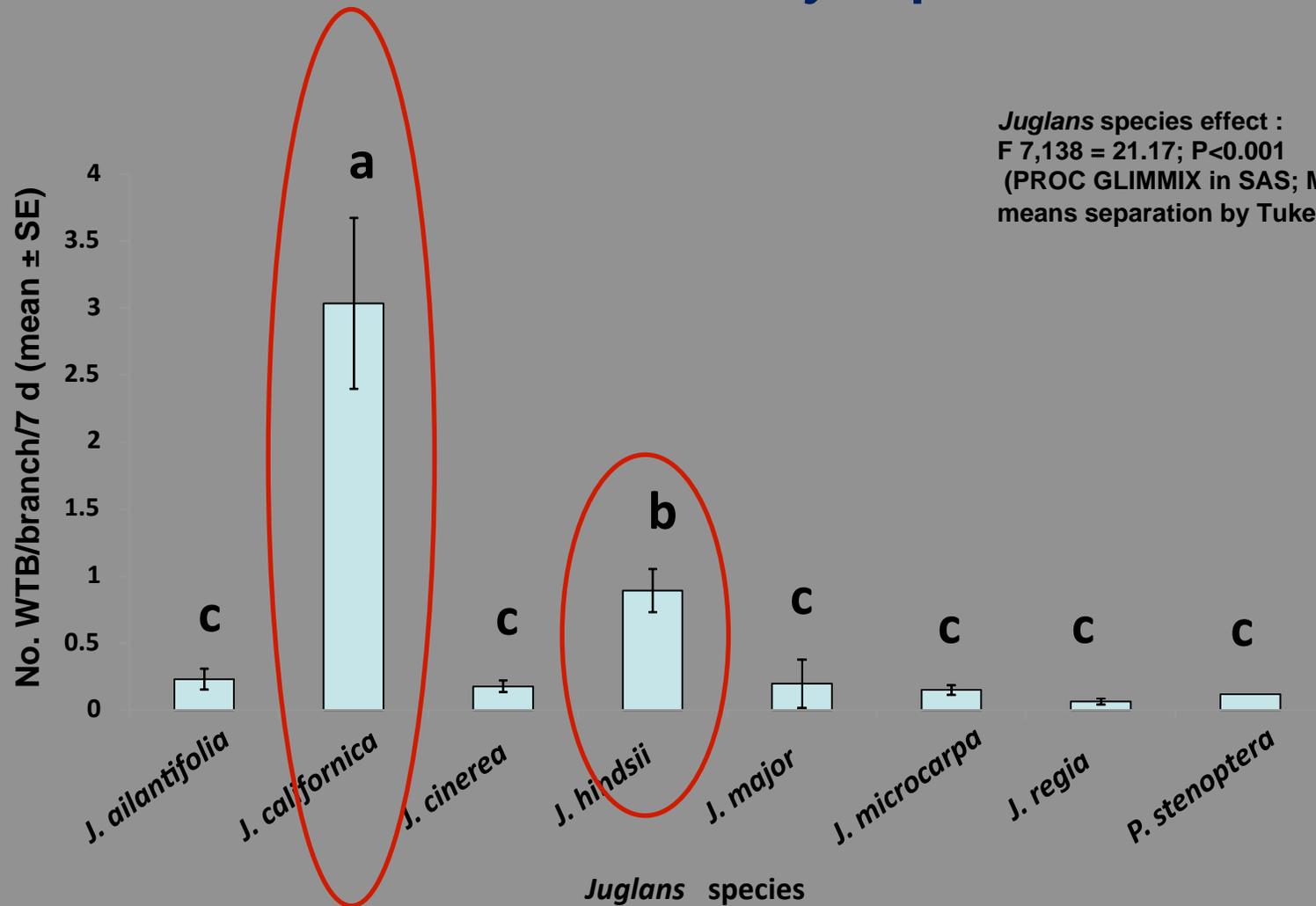
Juglans californica
(in northern CA)

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



Juglans species effect :
F 7,138 = 21.17; P<0.001
(PROC GLIMMIX in SAS; Multiple
means separation by Tukey-Kramer)

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



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

Close-up symptoms



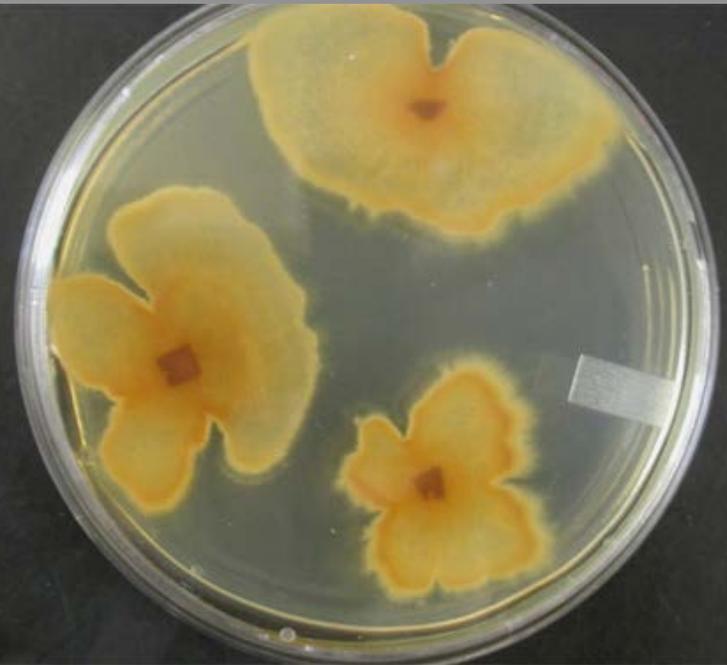
Mortality caused by coalescence of multiple cankers

Colonies of Gm on both sides

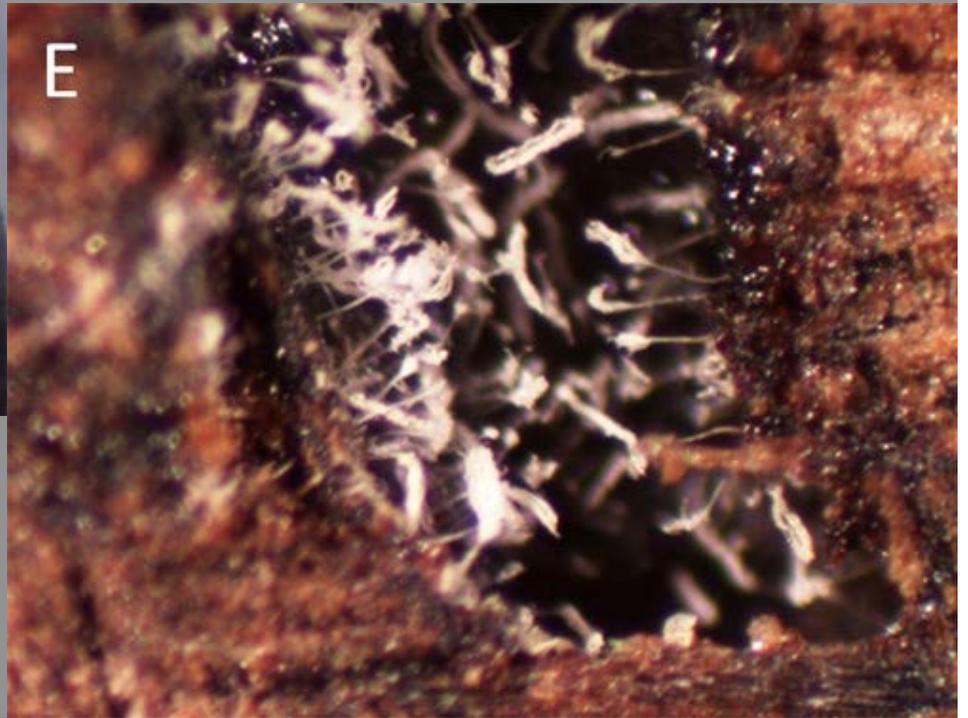
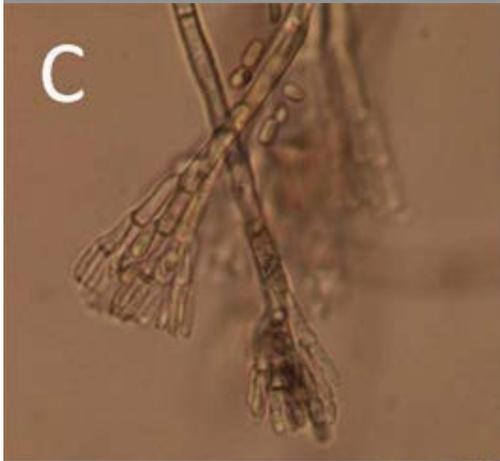
A



B



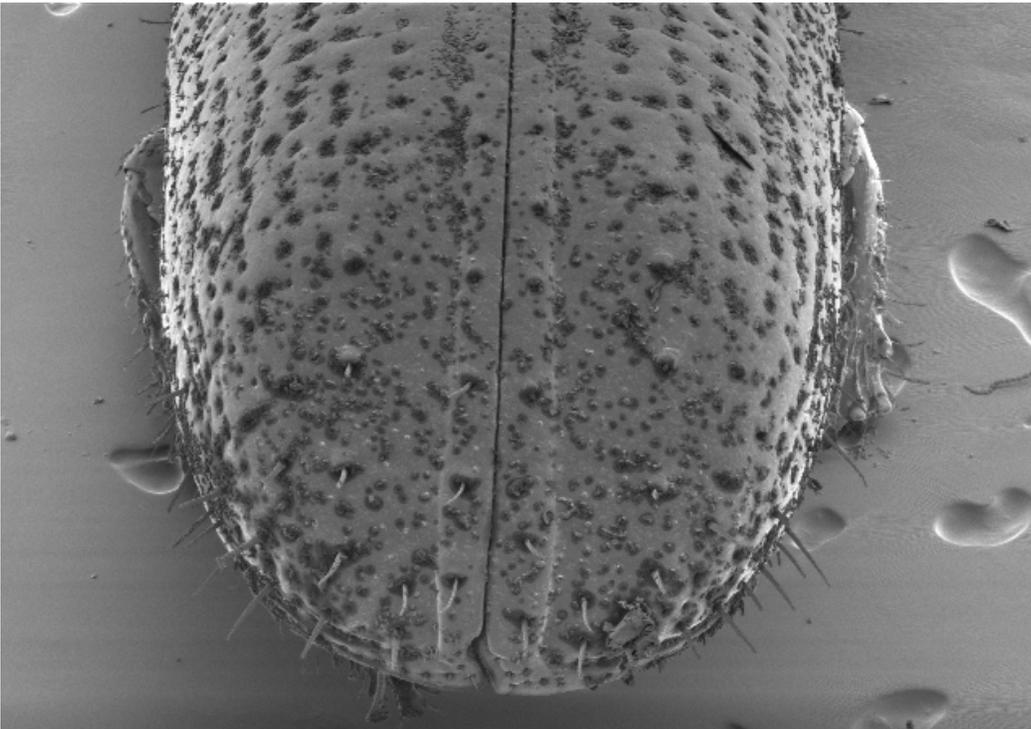
Fungus sporulating on beetle and in beetle gallery



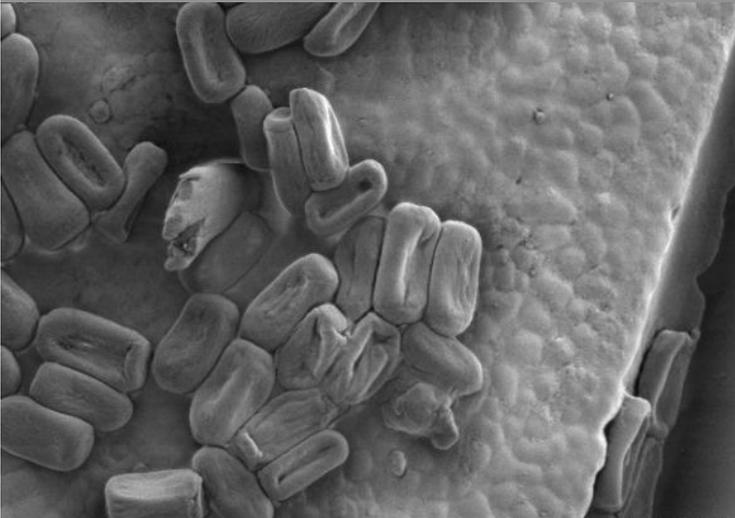
The vector + the fungus

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

Photos by Andrew Graves & Steve Seybold, USDA Forest Service

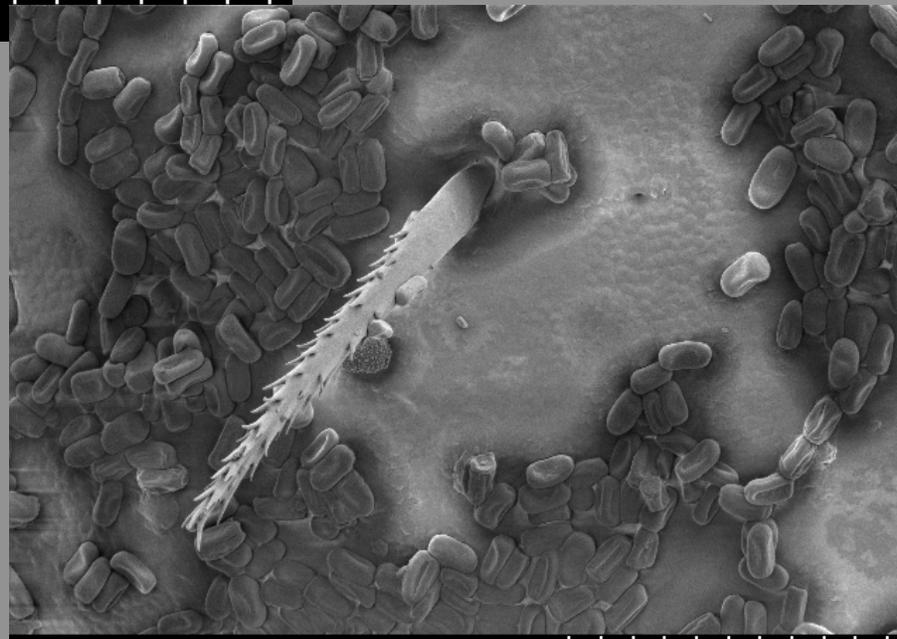


Beetles 2.0kV 21.1mm x130 6/24/08 14:08



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