During which decade did DNA fingerprinting develop and begin to be used as a forensic genetic analysis technique? Give an example of how DNA fingerprinting technology is used.

What is the short name for Deoxyribonucleic acid (one word)? What is its composition (name the 4 building blocks)?

What is the main component of chromosomes mentioned in class (one word)? And what types of living organisms have chromosomes?

Give two examples of molecular markers, briefly describe how they differ, and how each is used.

California invaded: 1849 A.D.

Xylella scorch of maples 2000s

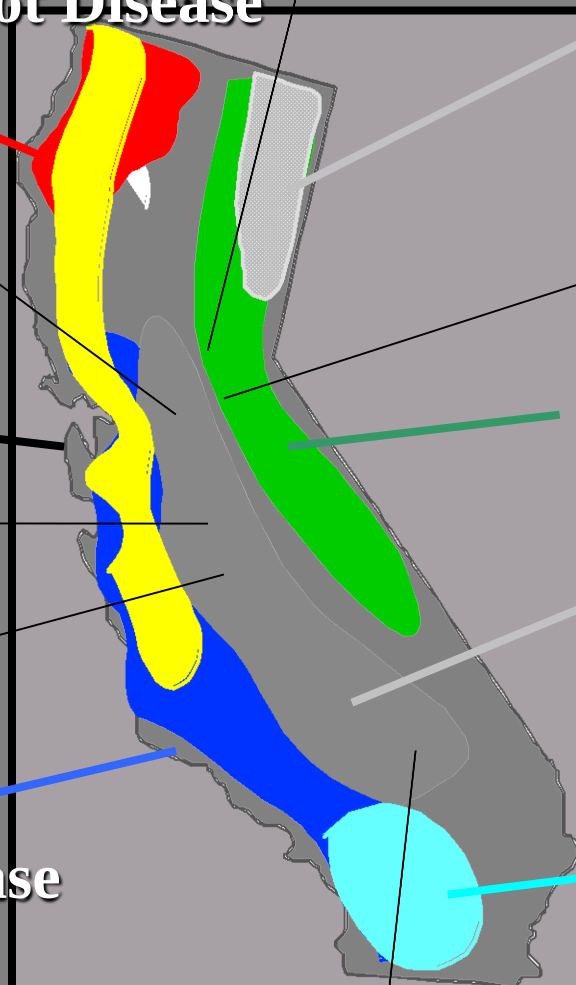
Port Orford Cedar Root Disease 1950s

Expansion of root patho

Post 1880s

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

Sudden Oak Death 1990s



Manzanita die-back 2004

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 *Ceratocystsis 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 prbably 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 the introduced basidiomycete

Cronartium ribicola

White pine blister rust

- Introduced in the West Coast on seedlings
- Efficiently airborne
- Needs alternate host: gooseberry
- Genetic resistance program

Spring

Fall

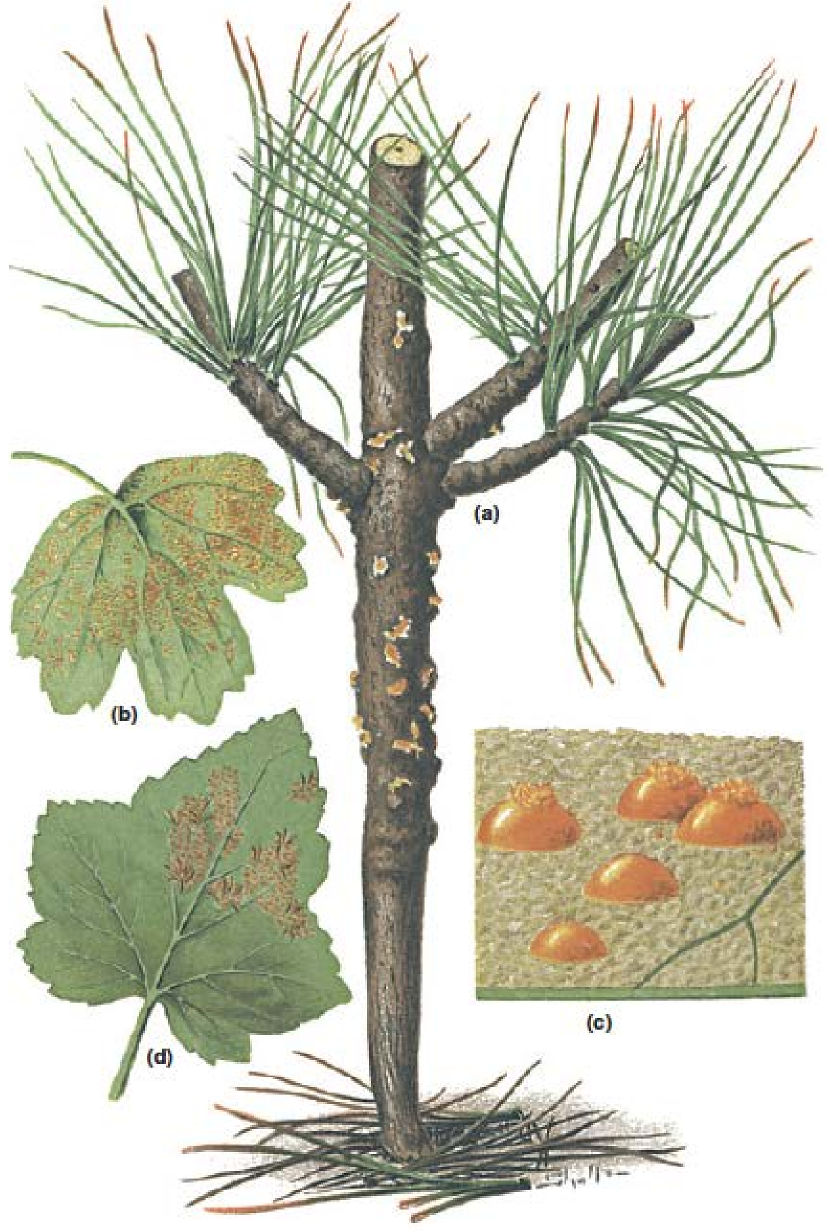
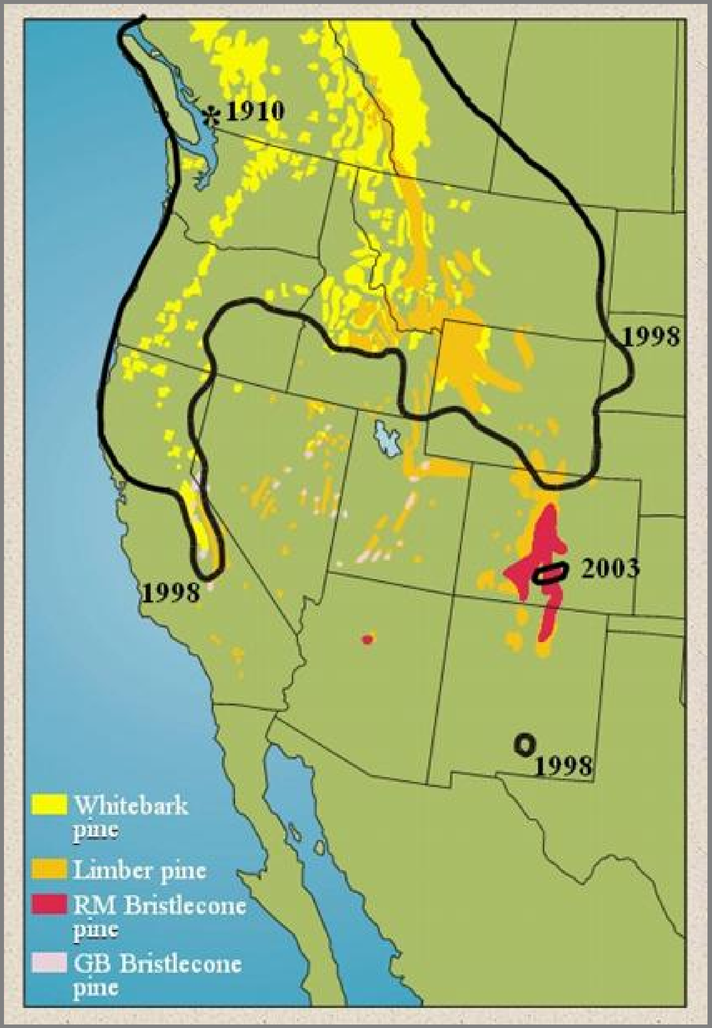
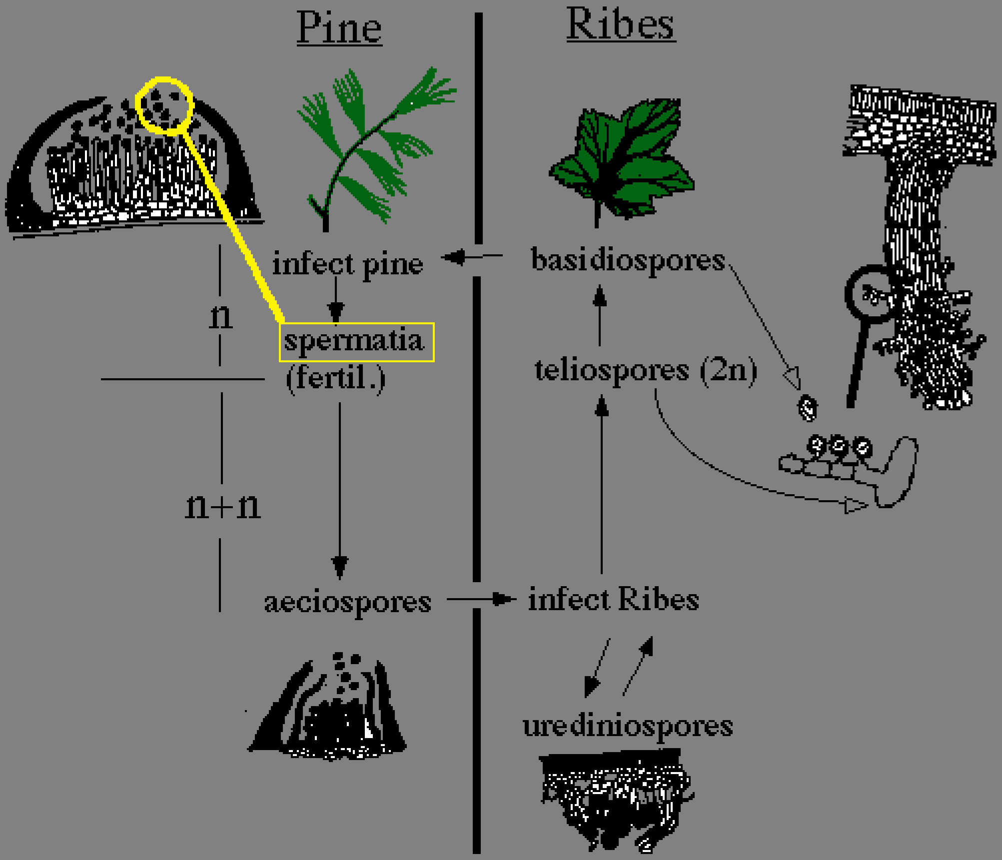


Fig. 1. Cronartium ribicola on *Pinus strobus* and *Ribes.* Reprinted Plate I (Frontispiece.) from Bulletin No. 206, *The Blister Rust Of White Pine* by SPAULDING (1911). From original description: (a) A 4-year-old white-pine tree diseased with blister rust. Note the slight swelling of the stem in the area bearing the yellow spore pustules. (b) Leaf of *Ribes aureum* bearing many pustules of uredospores (urediniospores). (c) Enlarged detail of uredo (uredinial) pustules. (d) Leaf of *Ribes americanum* with a number of the hair-like growths of the teleutospore (teliospore) stage. These spores are capable of infecting pine, while the first two forms cannot, but can infect the leaves of *Ribes*.'


1910
1998
1998
2003
1998
Whitebark pine
Limber pine
RM Bristlecone pine
GB Bristlecone pine


Pine
Ribes
infect pine
basidiospores
n
spermatia
(fertil.)
teliospores (2n)
n+n
aeciospores
infect Ribes
urediniospores

C. ribicola life cycle

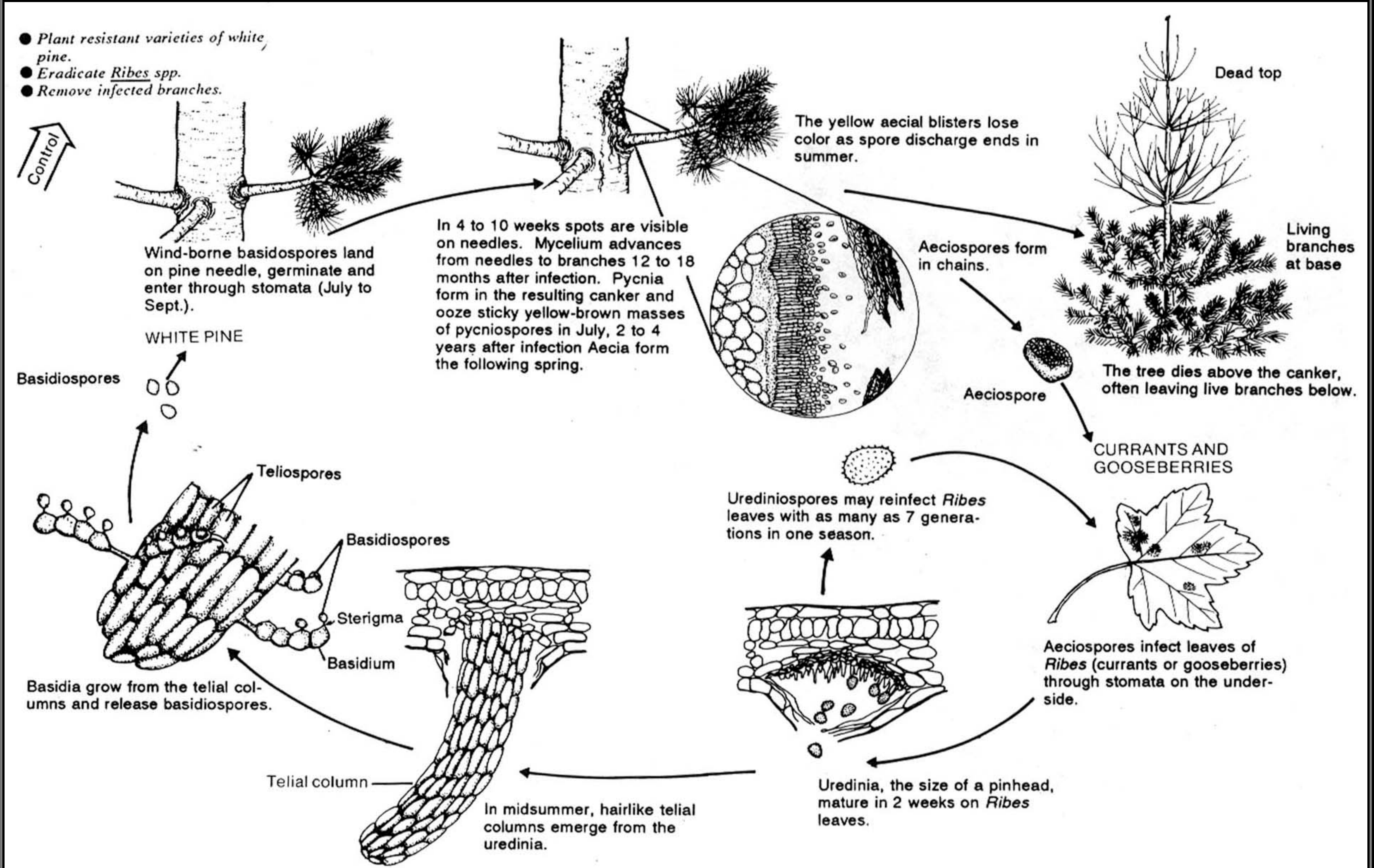
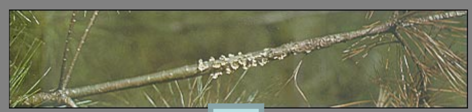


FIGURE 15.5 Disease diagram of white pine blister rust caused by *Cronartium ribicola*. Drawn by Valerie Mortensen.

Cronartium ribicola—Causal Agent of White Pine Blister Rust



Next Spring: spores cause blisters and thus cankers

Fall: fungus attacks needles

Two Years Later: Fungus spreads to branches and trunk

Spore produced to transmit disease

- 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, 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…

- 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

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

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

…after

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 (Grizzlies) dependence on nu

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%

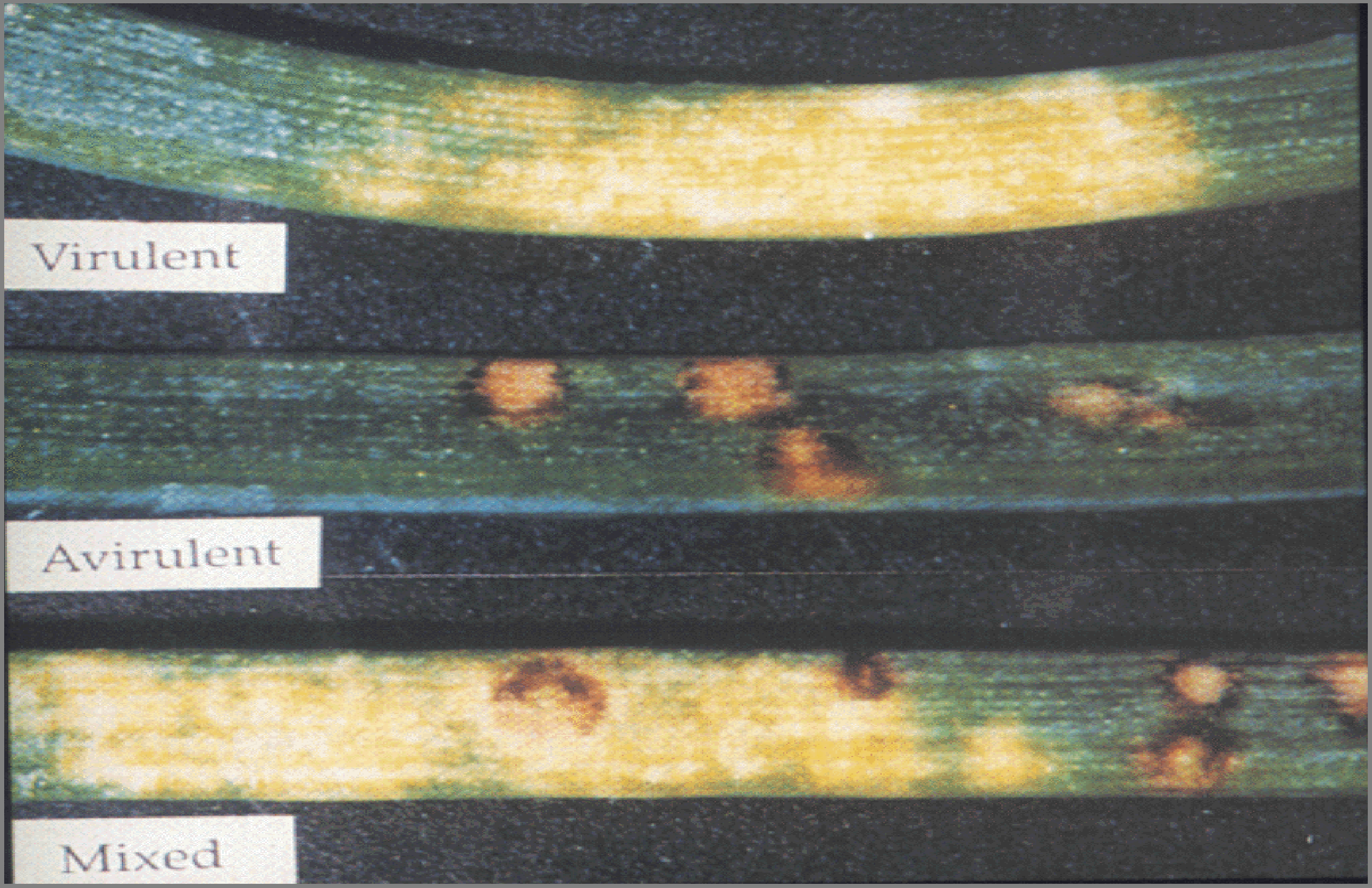
Tree resistance

- 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

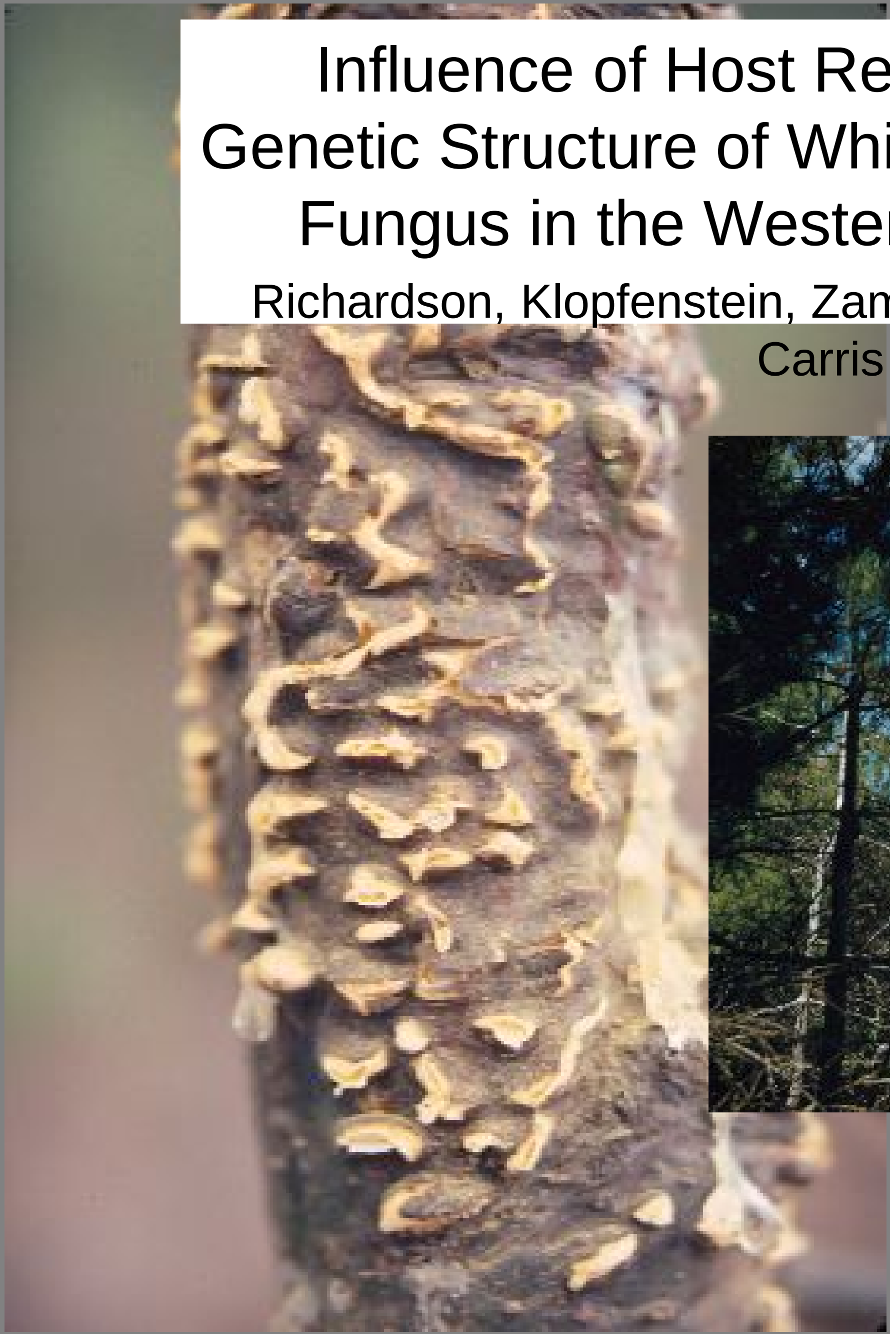
- Western white pine
  - Slow canker growth - non race specific trait; produces abnormally small cankers; may reduce pruning necessity (due to success)
  - Reduced needle lesion frequency - also non race specific trait; few individual infection sites per seedling; may only be juvenile trait (seen in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

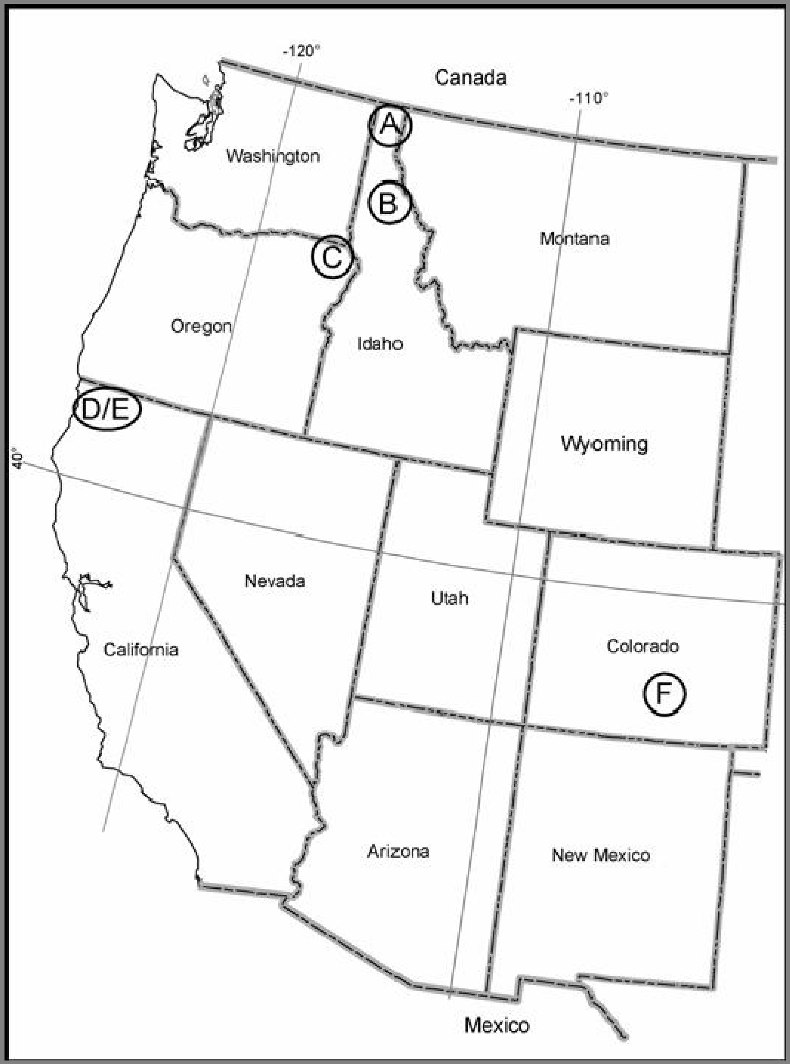


Purpose

1) Examine genetic diversity within and among population of western N.America

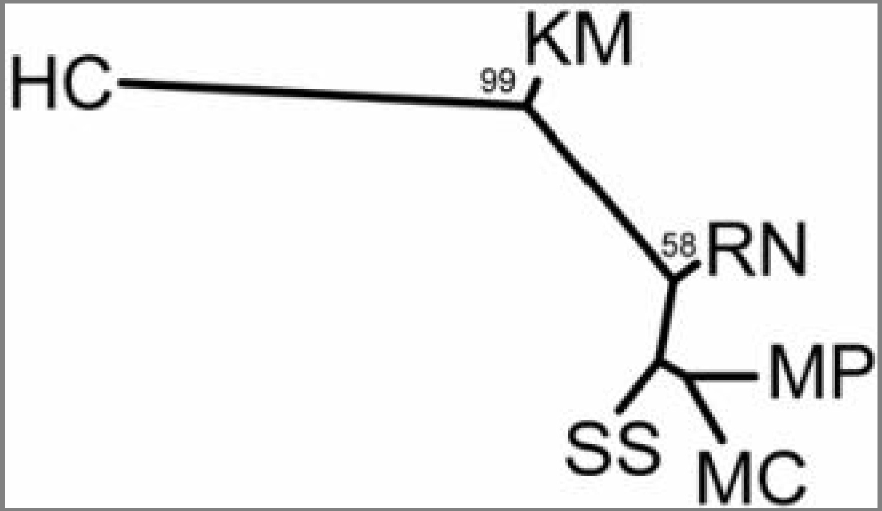
1) Effects of host resistance on C. ribicola

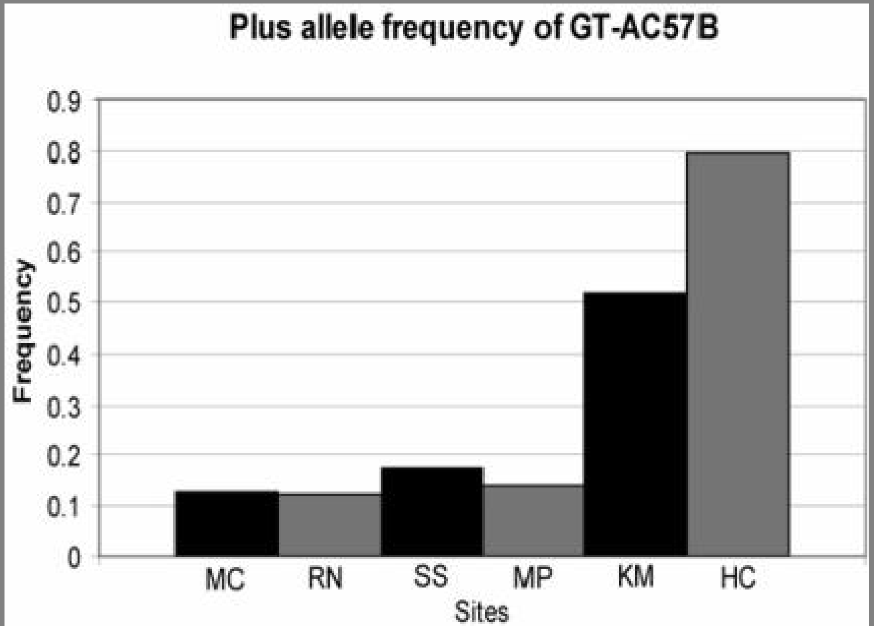
Materials + Methods



- 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
- Fst= 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.

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, site 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

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

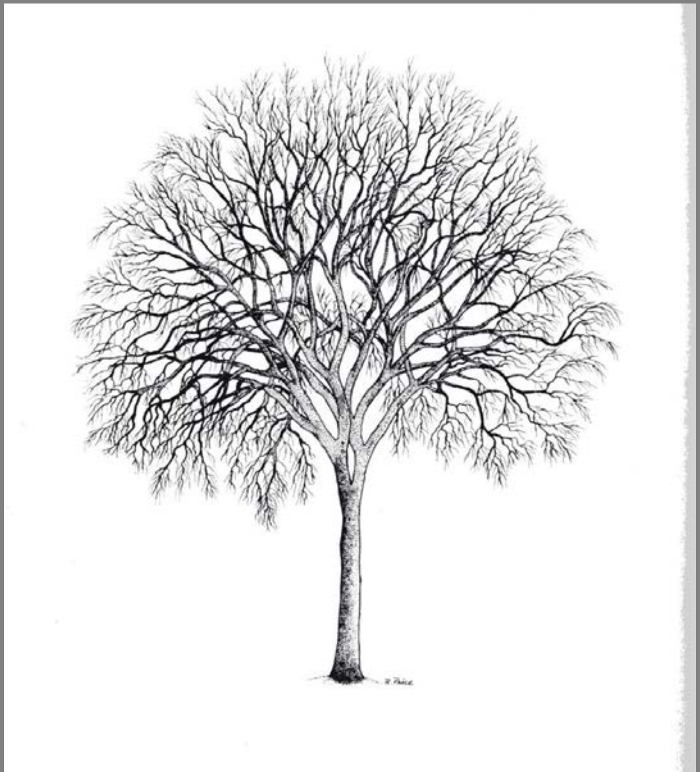
Fig. 25. Street scene of arching American elms in Evanston, Illinois (1976). (Courtesy E. B. Himelick)

- #1 urban tree in U.S east of the Rockies, and in large parts of Europe and Asia (Heybroek, 1993)

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* ssp) and spreads through the vascular system
-
- Caused by ascomycete fungi (genus *Ophiostoma)*
- Vectored by beetles (family *Scolytidae*) and root graft

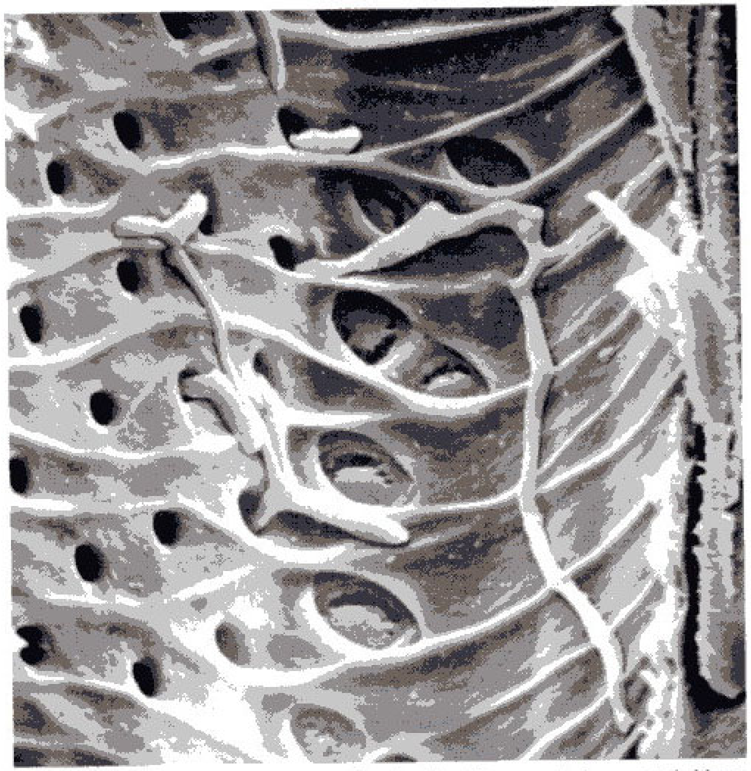
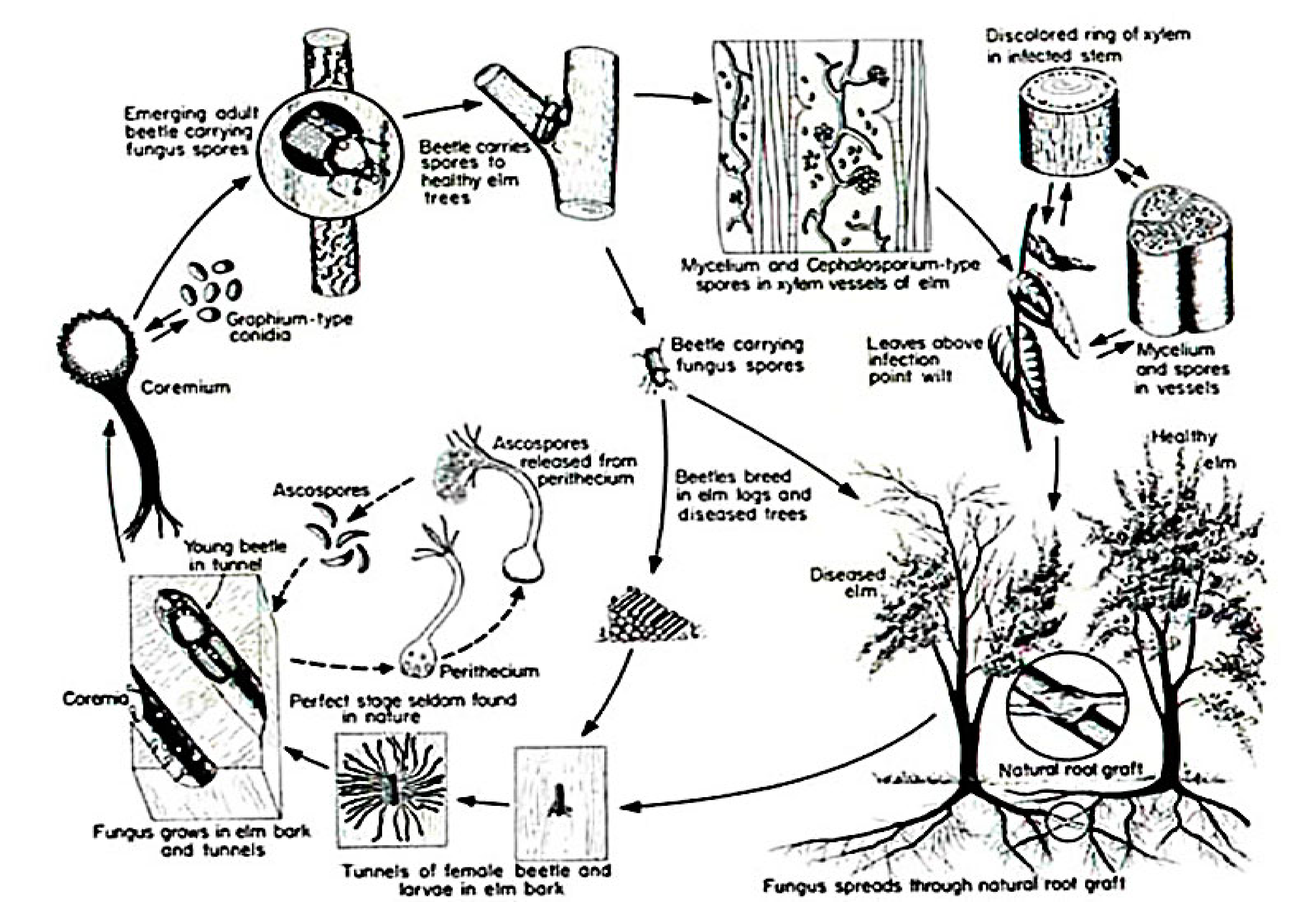


Fig. 22. Hyphae of *Ceratocystis ulmi* in diseased elm wood. Note passage of fungus through pit openings between vessels. (Courtesy D. M. Elgersma)

Life Cycle of *Ophiostoma ulmi*



Vectors of disease

- <u>Insects</u>: 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

- <u>Root grafts</u>: when elms are within 50 feet of one another, their roots can grow together and disease passes easily along. Important in urban settings

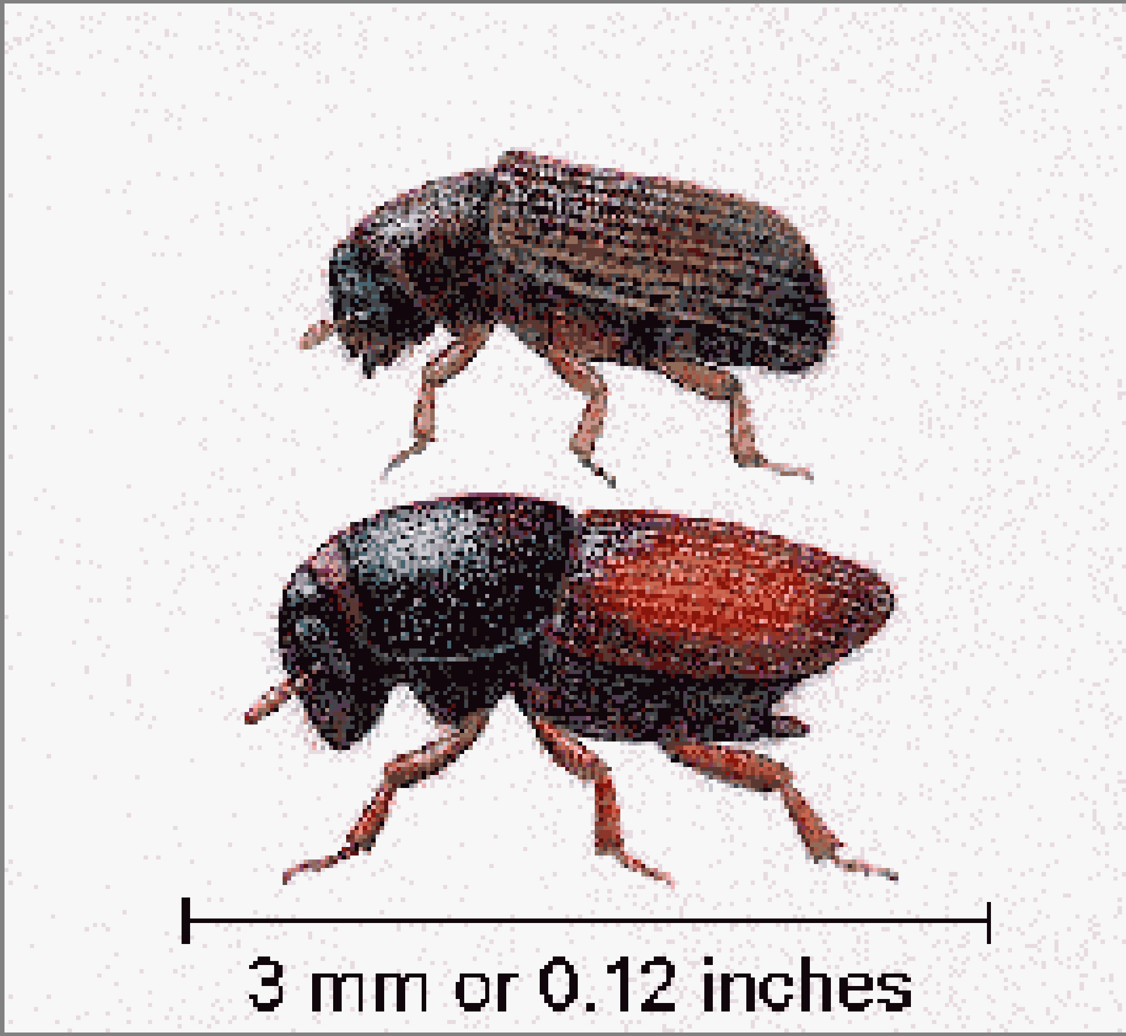
- <u>Infected logs</u>: Often transferred long distances

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.

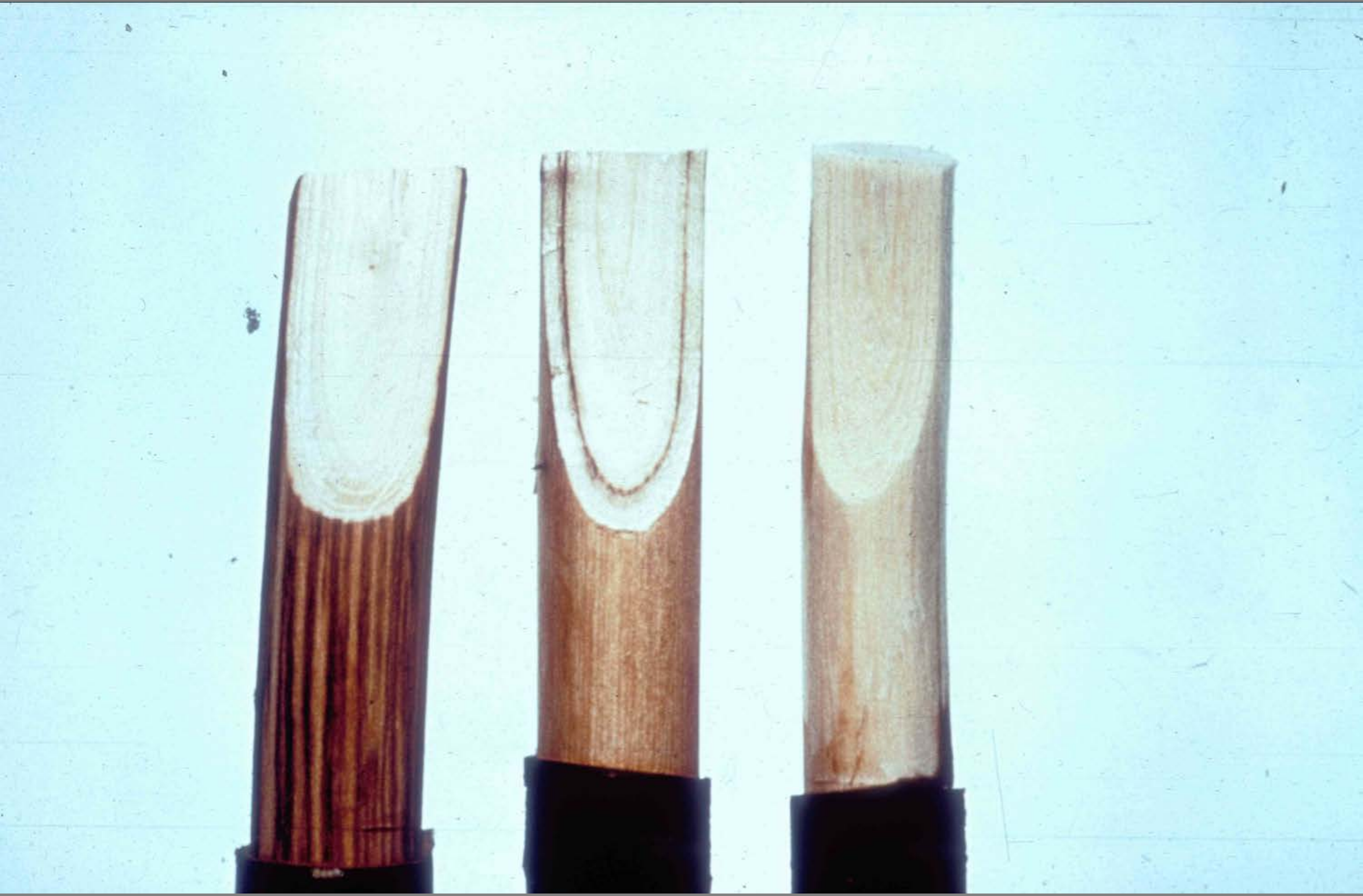
However, temperatures below -6F kill the larvae.

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 efffective.
- 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.