Pine wood nematode, as an example of alien invasive species

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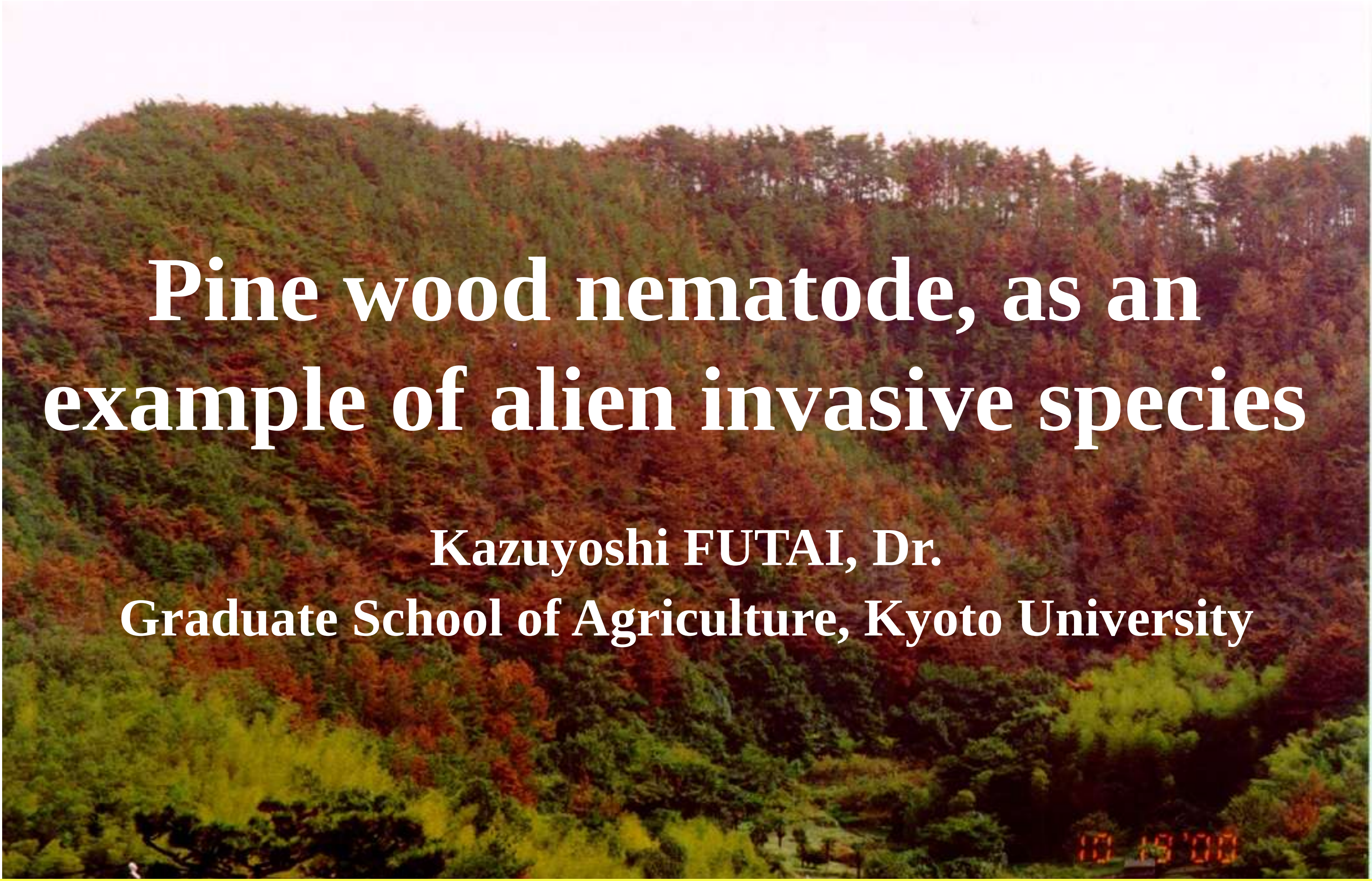
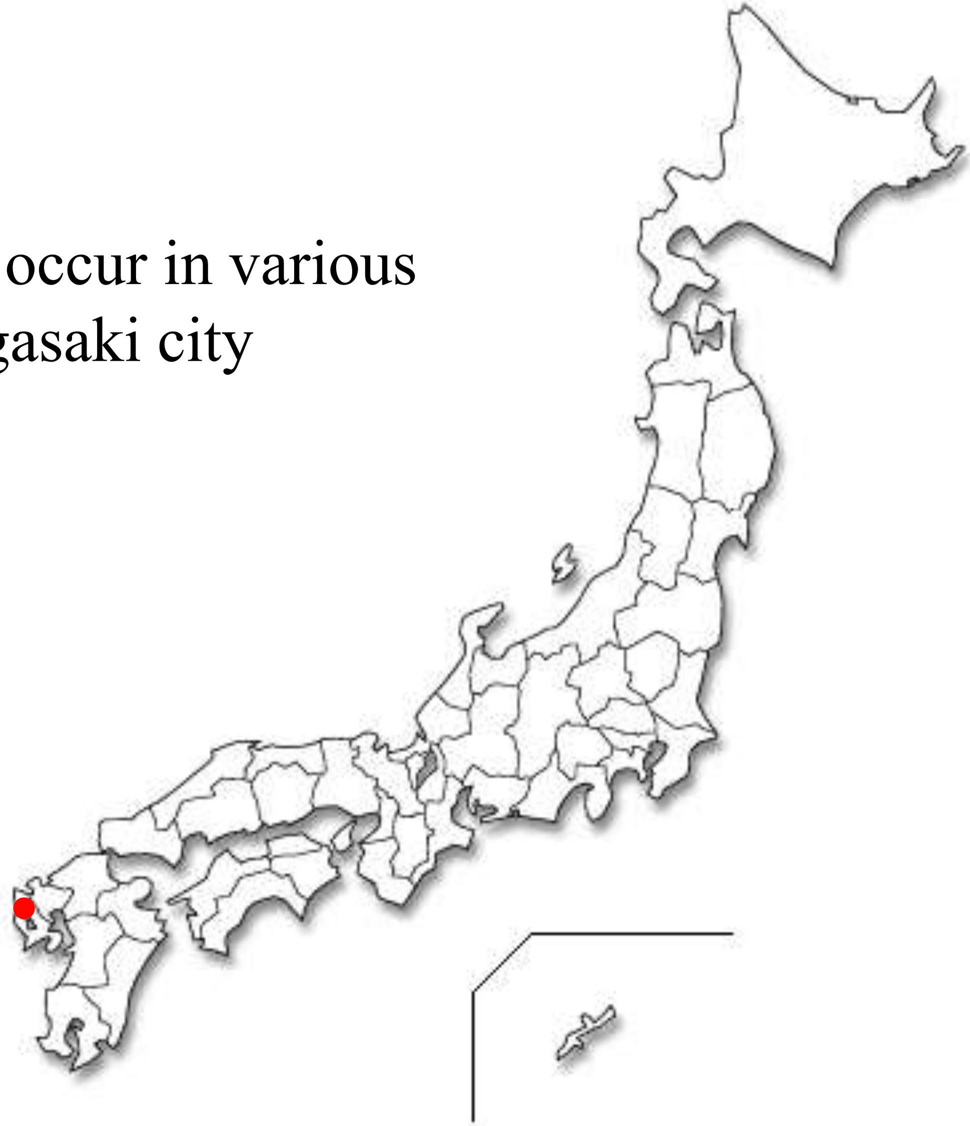


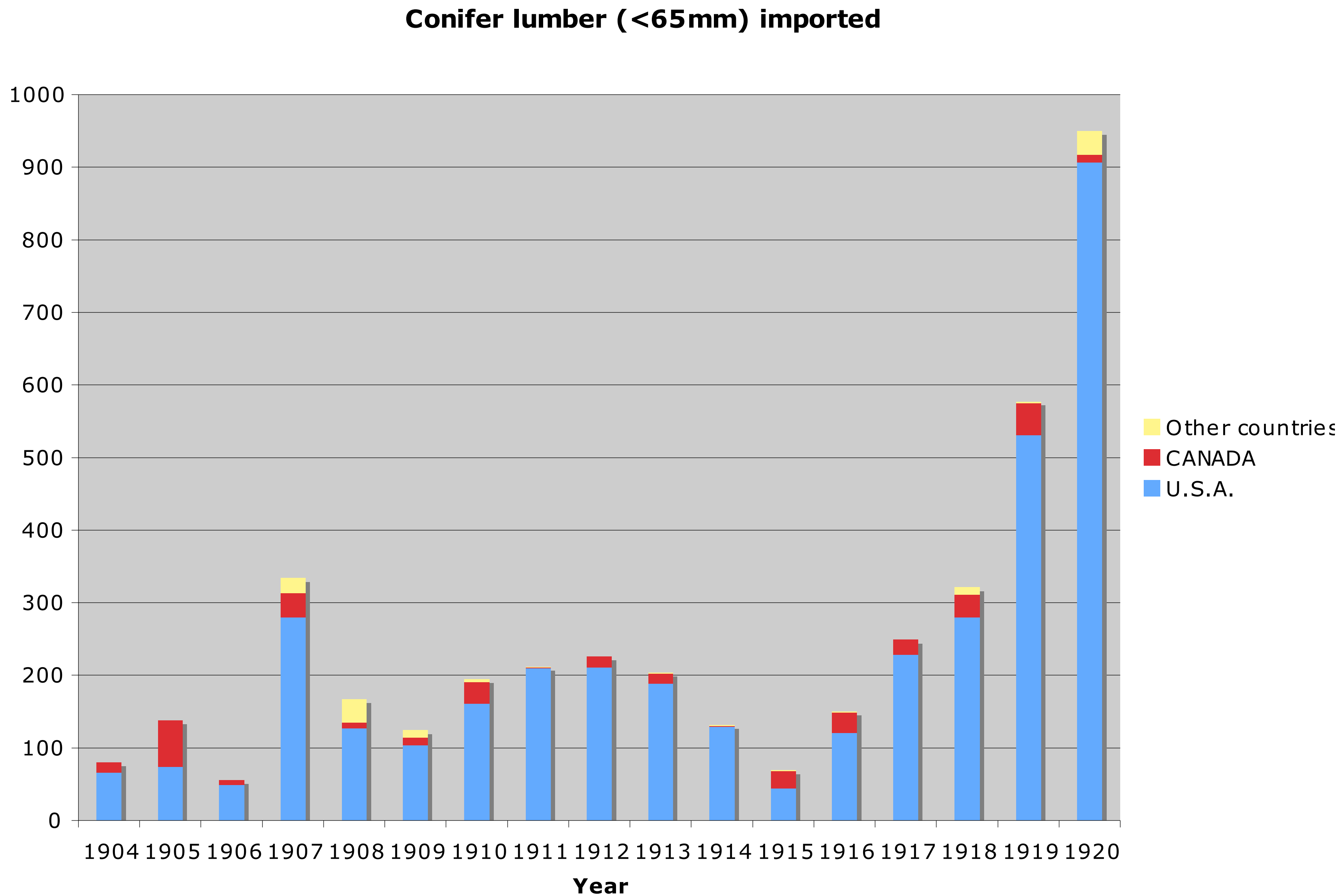
A mountain in Hiroshima, covered with Japanese red pines withering due to PWD. (Photo by Dr. Jikumaru)

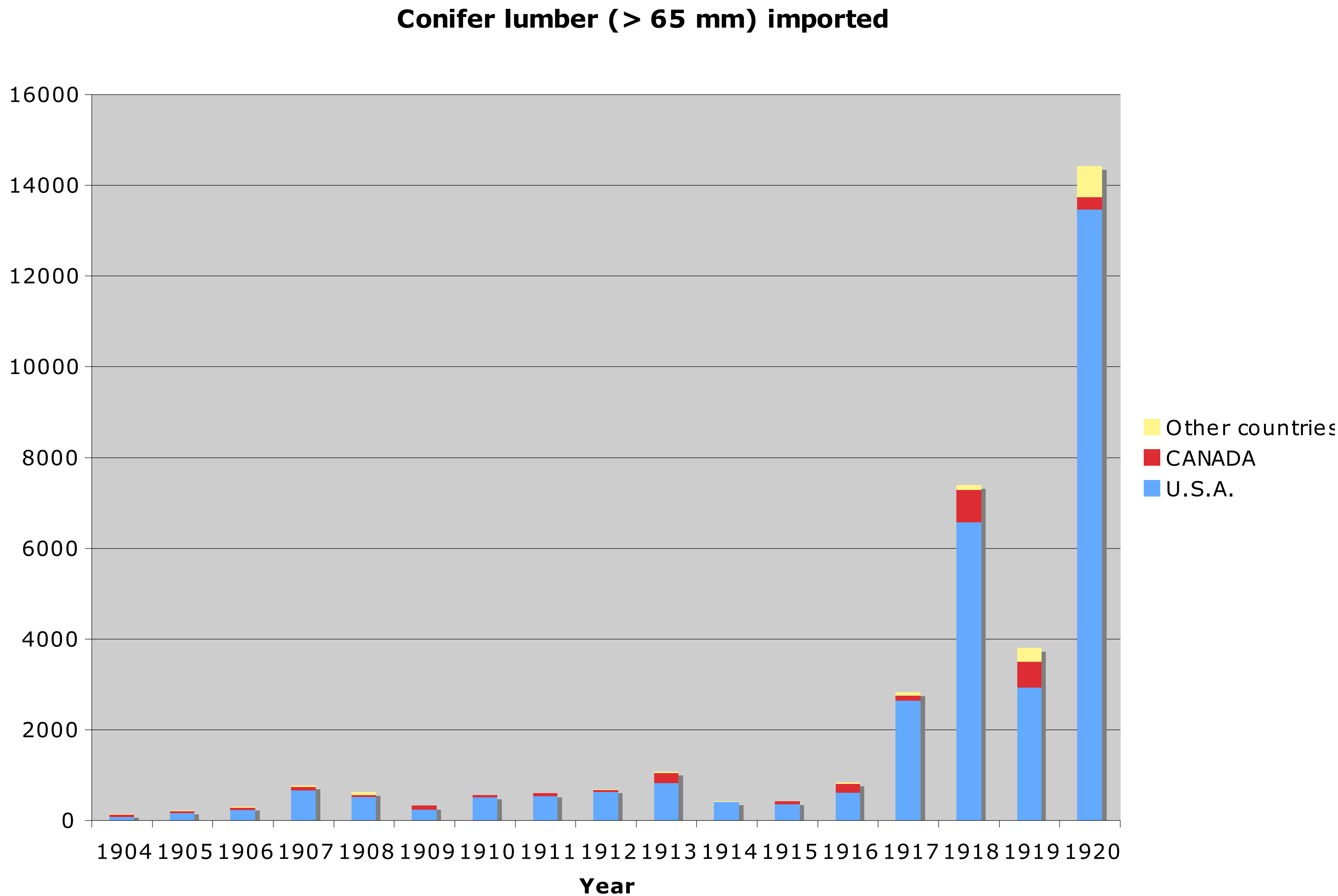
I. History of PWD expanding over Japan

1905

PWD started to occur in various locations at Nagasaki city

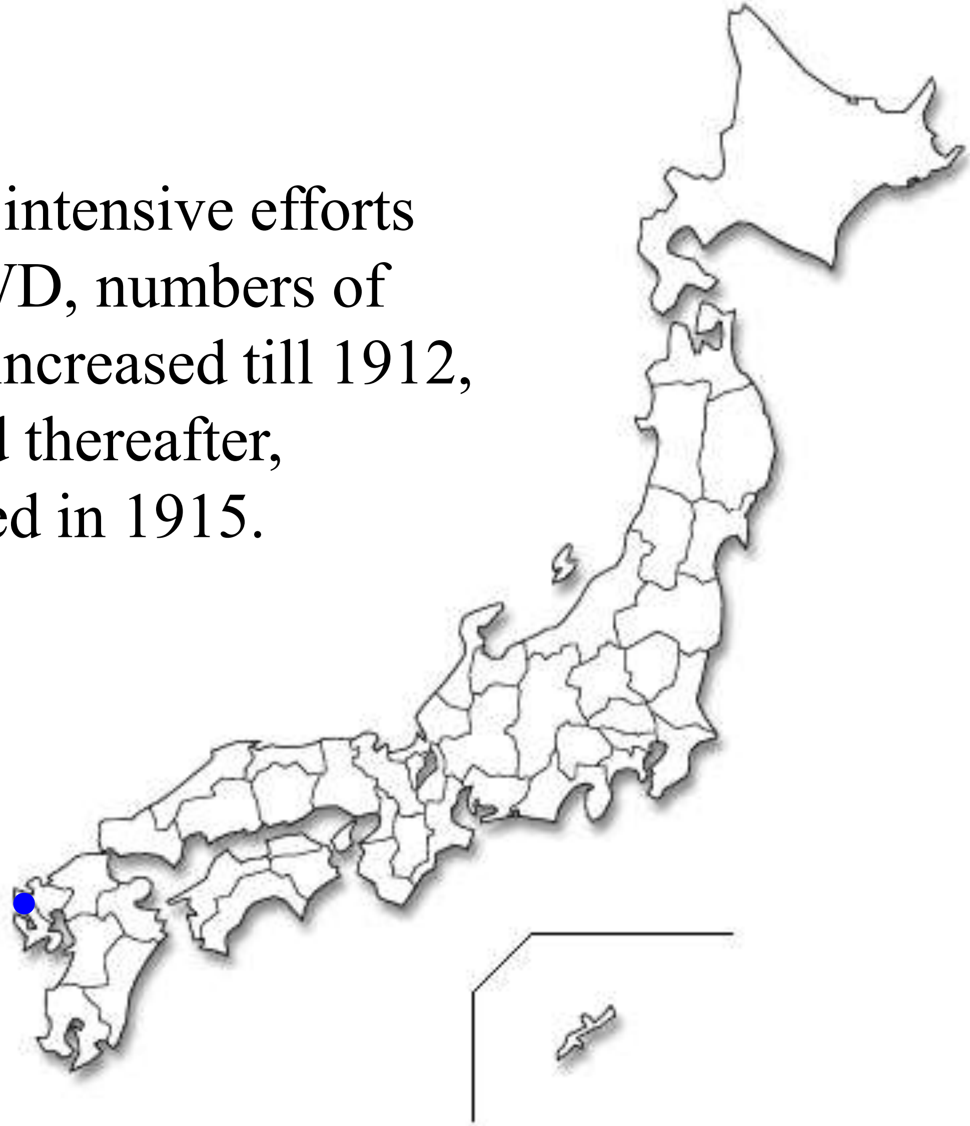



Conifer lumber (<65mm) imported
1000
900
800
700
600
500
400
300
200
100
0
1904 1905 1906 1907 1908 1909 1910 1911 1912 1913 1914 1915 1916 1917 1918 1919 1920
Year
Other countries
CANADA
U.S.A.


Conifer lumber (> 65 mm) imported
16000
14000
12000
10000
8000
6000
4000
2000
0
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
Year
Other countries
CANADA
U.S.A.

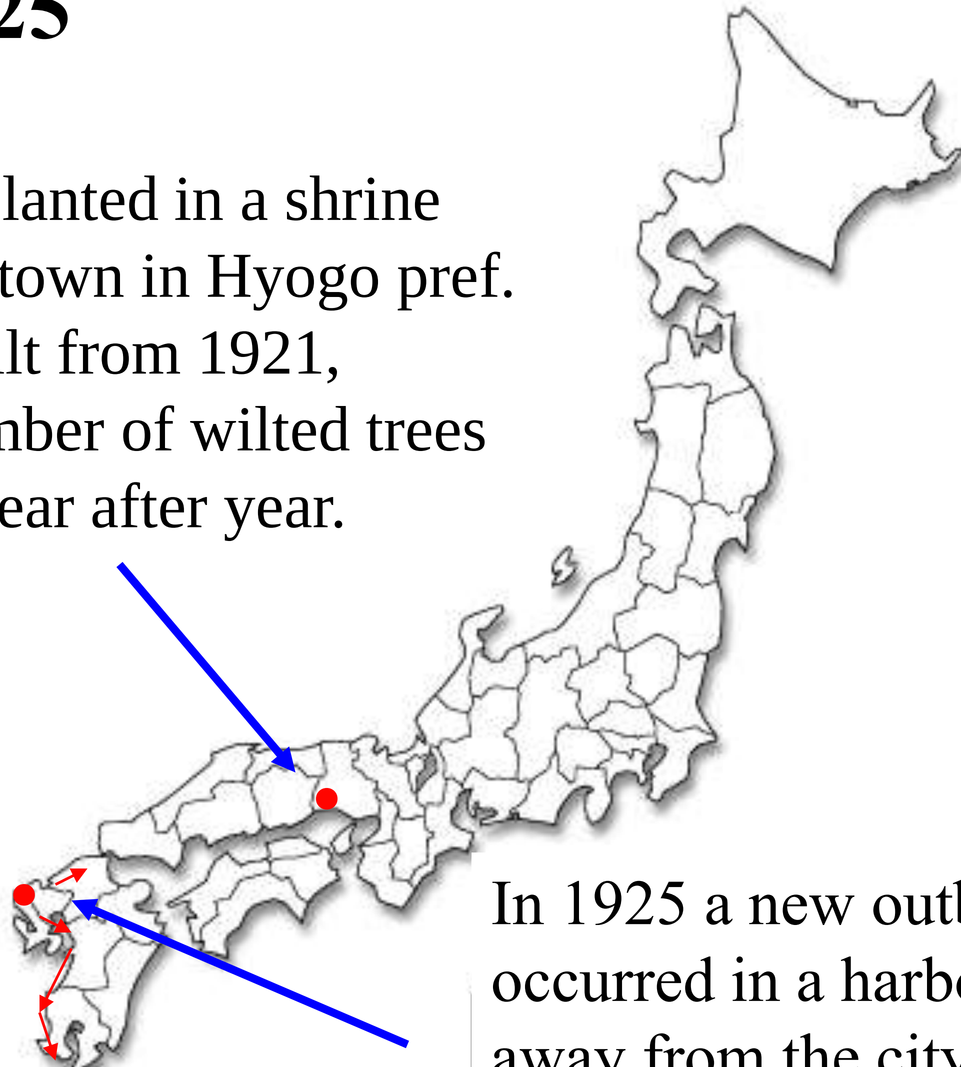
~ 1915

People made intensive efforts
to control PWD, numbers of
wilted pines increased till 1912,
but decreased thereafter,
and terminated in 1915.



1921～1925

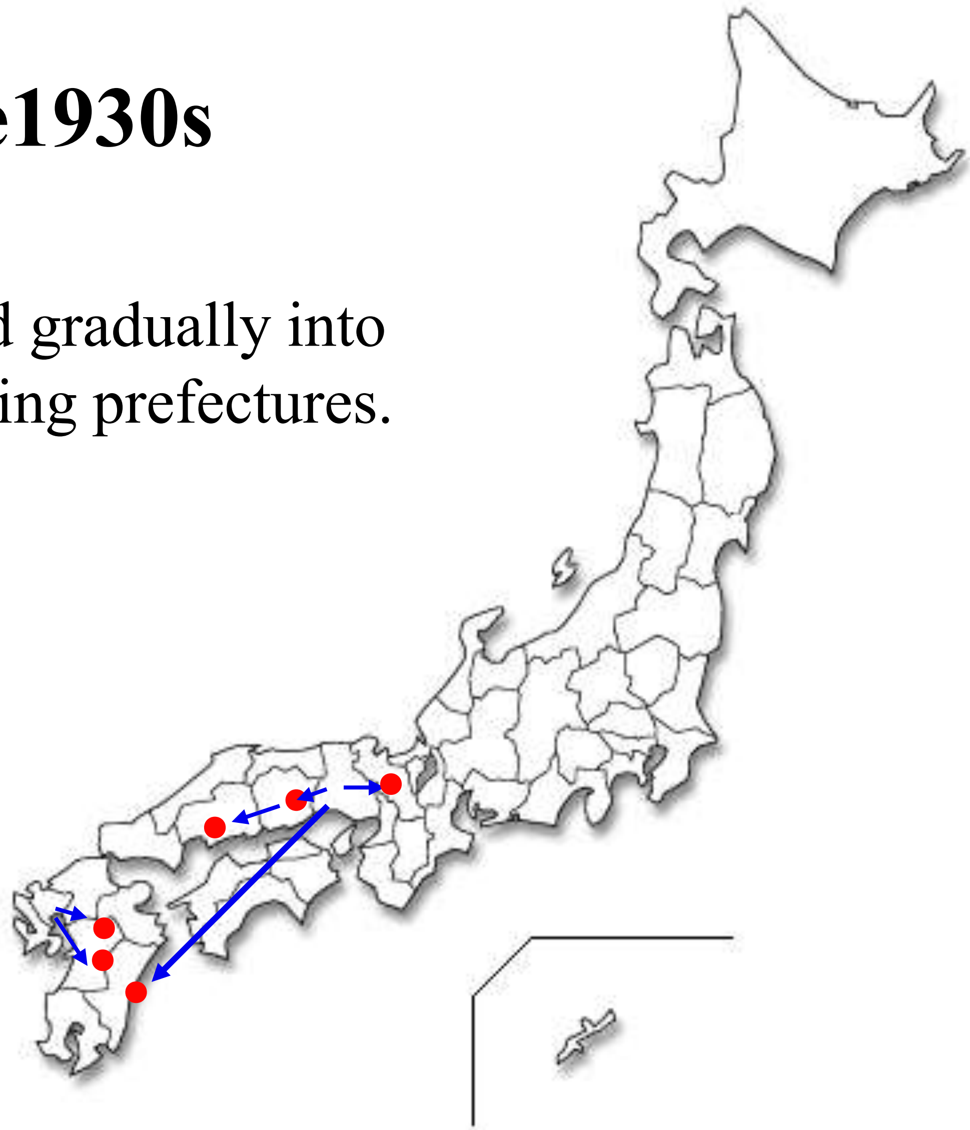
Old pines planted in a shrine
at a harbor town in Hyogo pref.
began to wilt from 1921,
and the number of wilted trees
increased year after year.



In 1925 a new outbreak of PWD
occurred in a harbor town 50 km
away from the city where the first
PWD was recorded, then gradually
spread into surrounding regions.

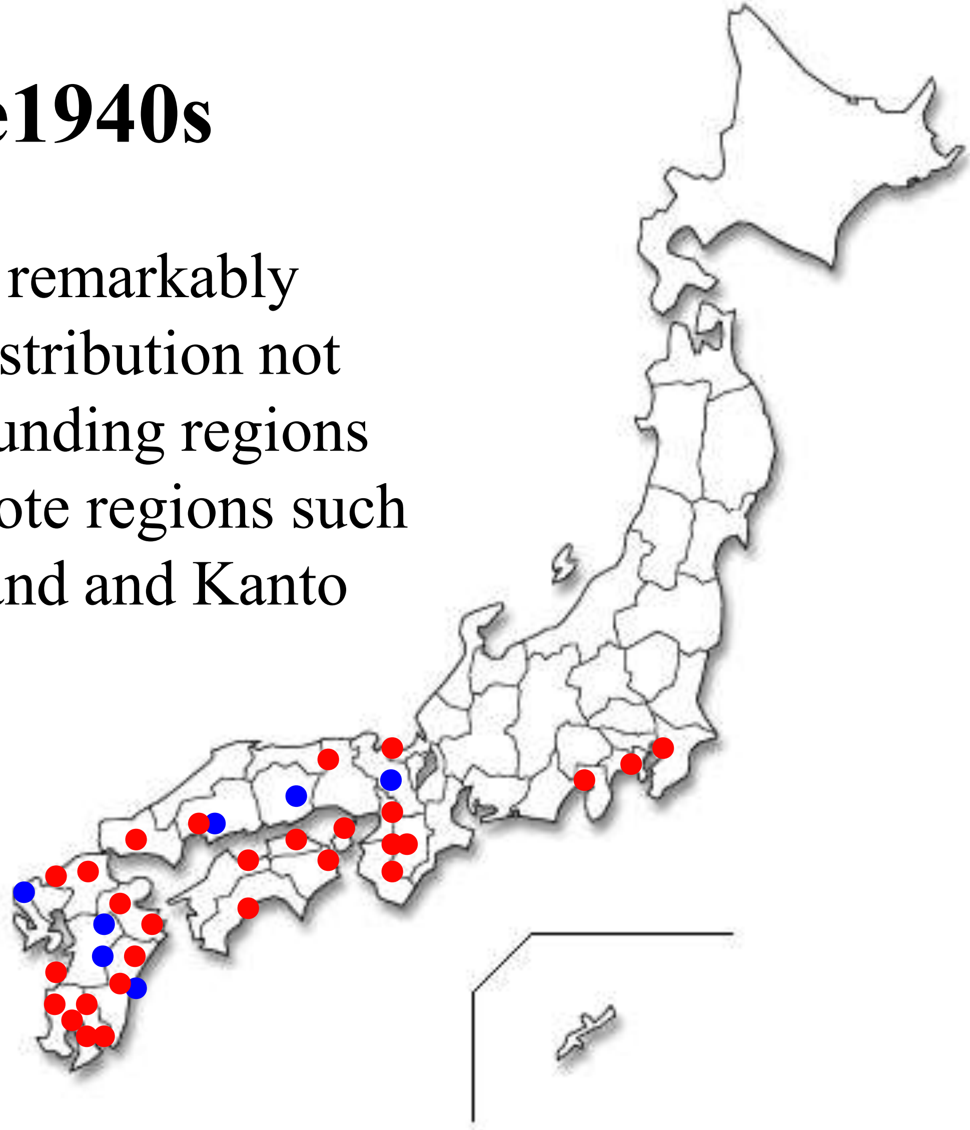
in the1930s

PWD spread gradually into neighboring prefectures.



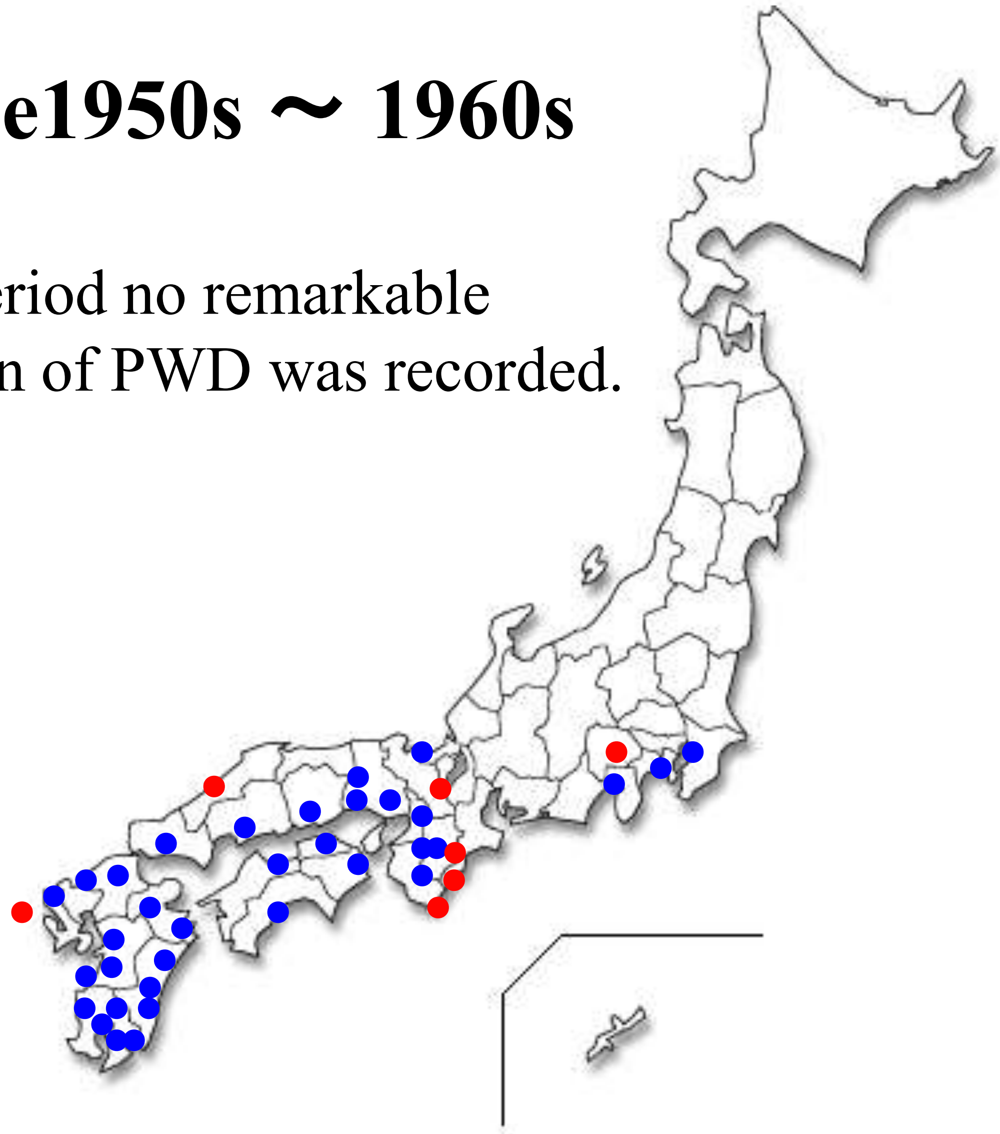
in the1940s

In 1940s PWD remarkably expanded its distribution not only into surrounding regions but also to remote regions such as Shikoku-island and Kanto districts.



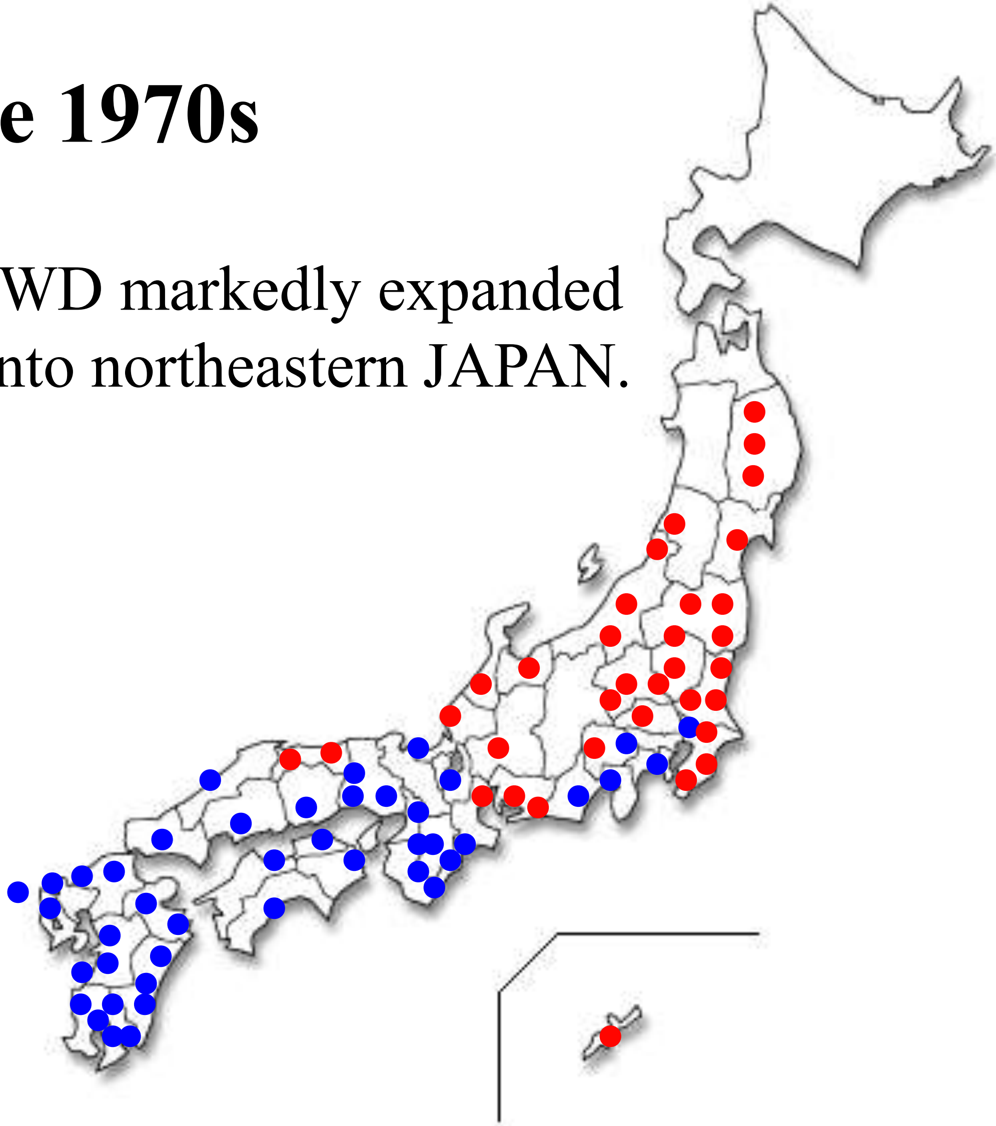
in the1950s ～ 1960s

In this period no remarkable expansion of PWD was recorded.



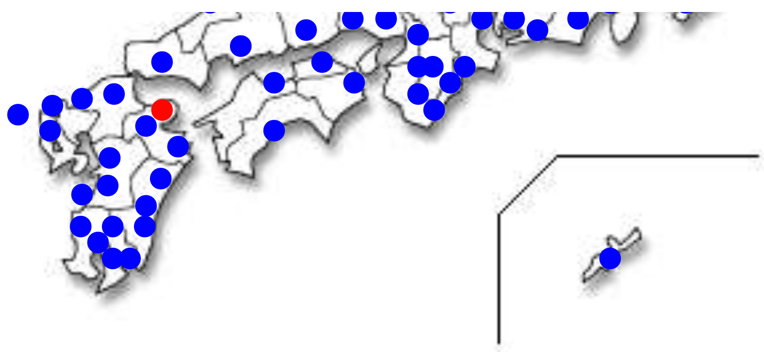
in the 1970s

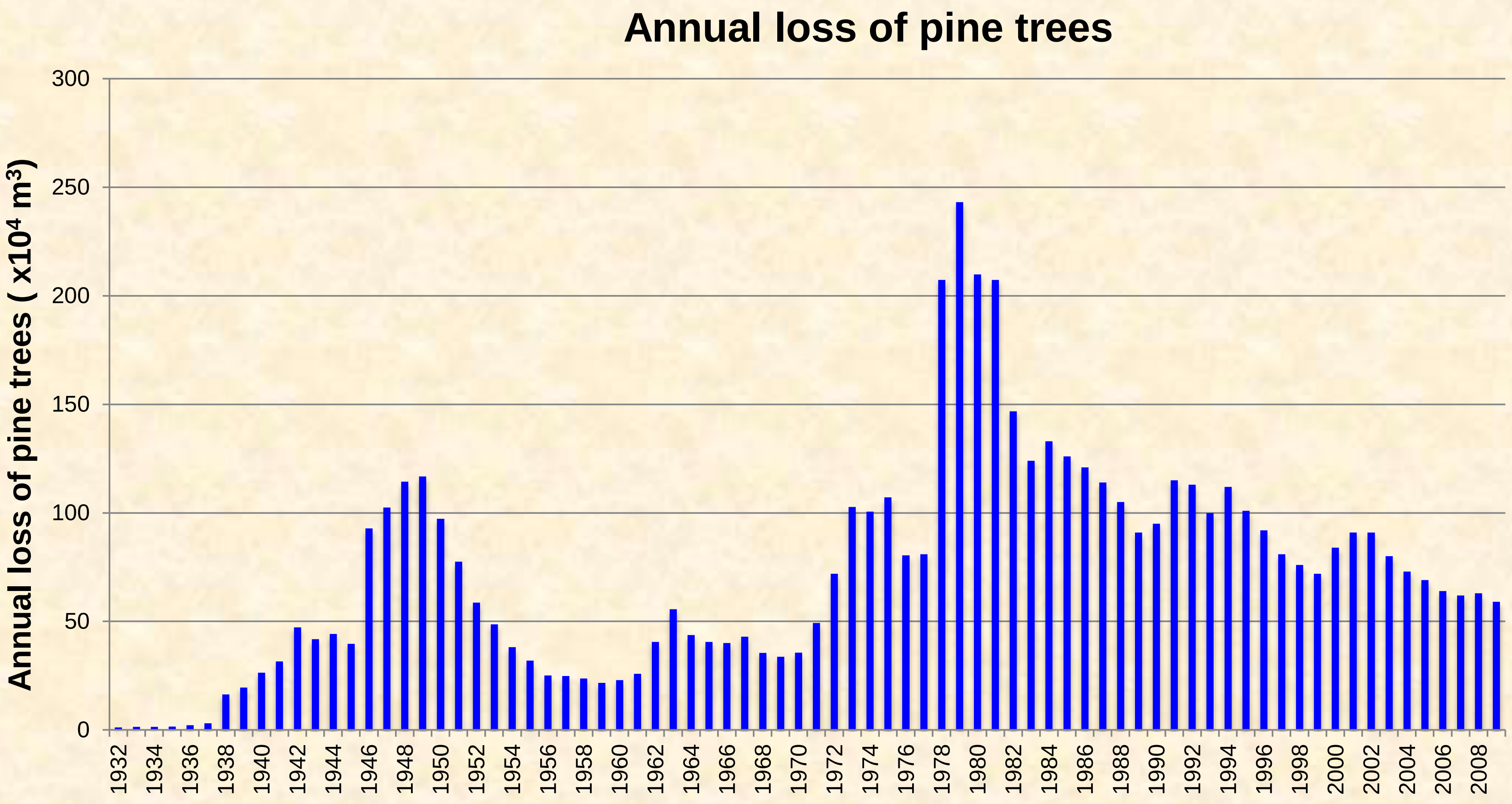
In this period, PWD markedly expanded its distribution into northeastern JAPAN.



in the1980s

Thus PWD became prevalent throughout Japan except for northernmost two prefectures, Aomori, and Hokkaido.





Annual loss of pine trees
Annual loss of pine trees (x10⁴ m³)
0
50
100
150
200
250
300
1932
1934
1936
1938
1940
1942
1944
1946
1948
1950
1952
1954
1956
1958
1960
1962
1964
1966
1968
1970
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1974
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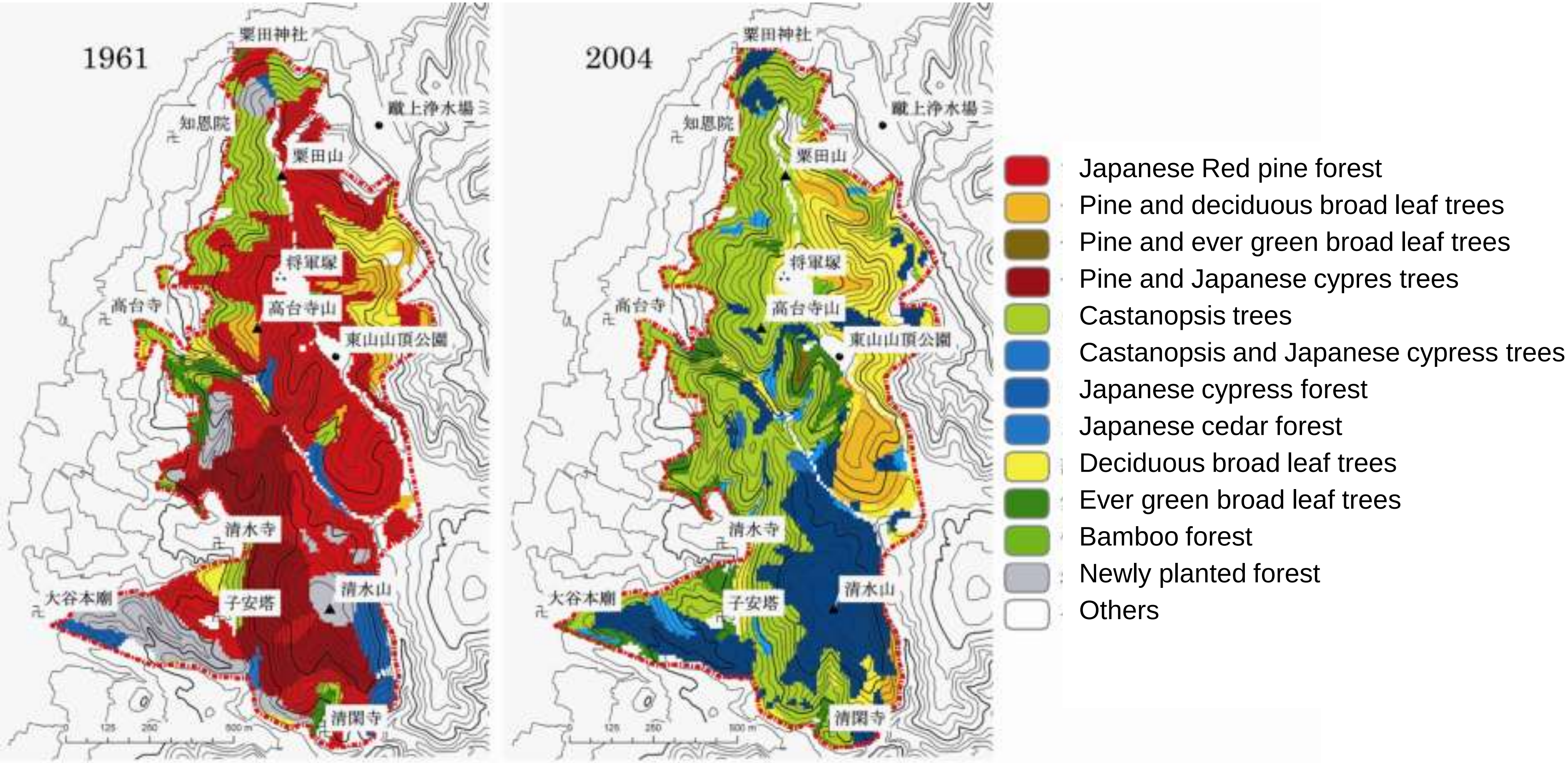
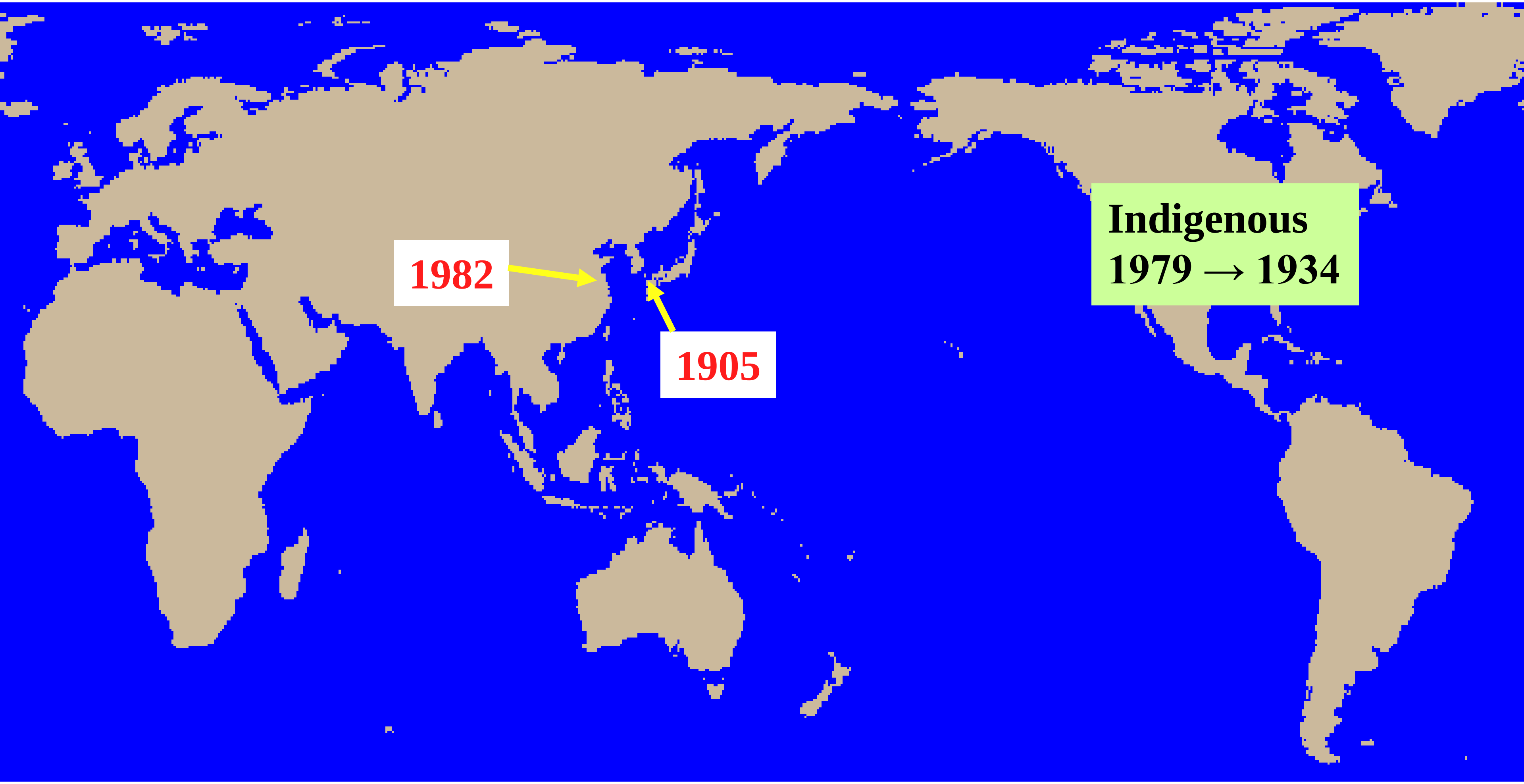
1998
2000
2002
2004
2006
2008



Vegetational changes in Higashiyama from 1961 to 2004
（Okuda and Takahara, Kyoto prefectural University, personal permission)

II. Global Expansion of PWD

Global Expansion of PWD（1）



Pine wilt in Nanjing, China

Pine Wilt in China

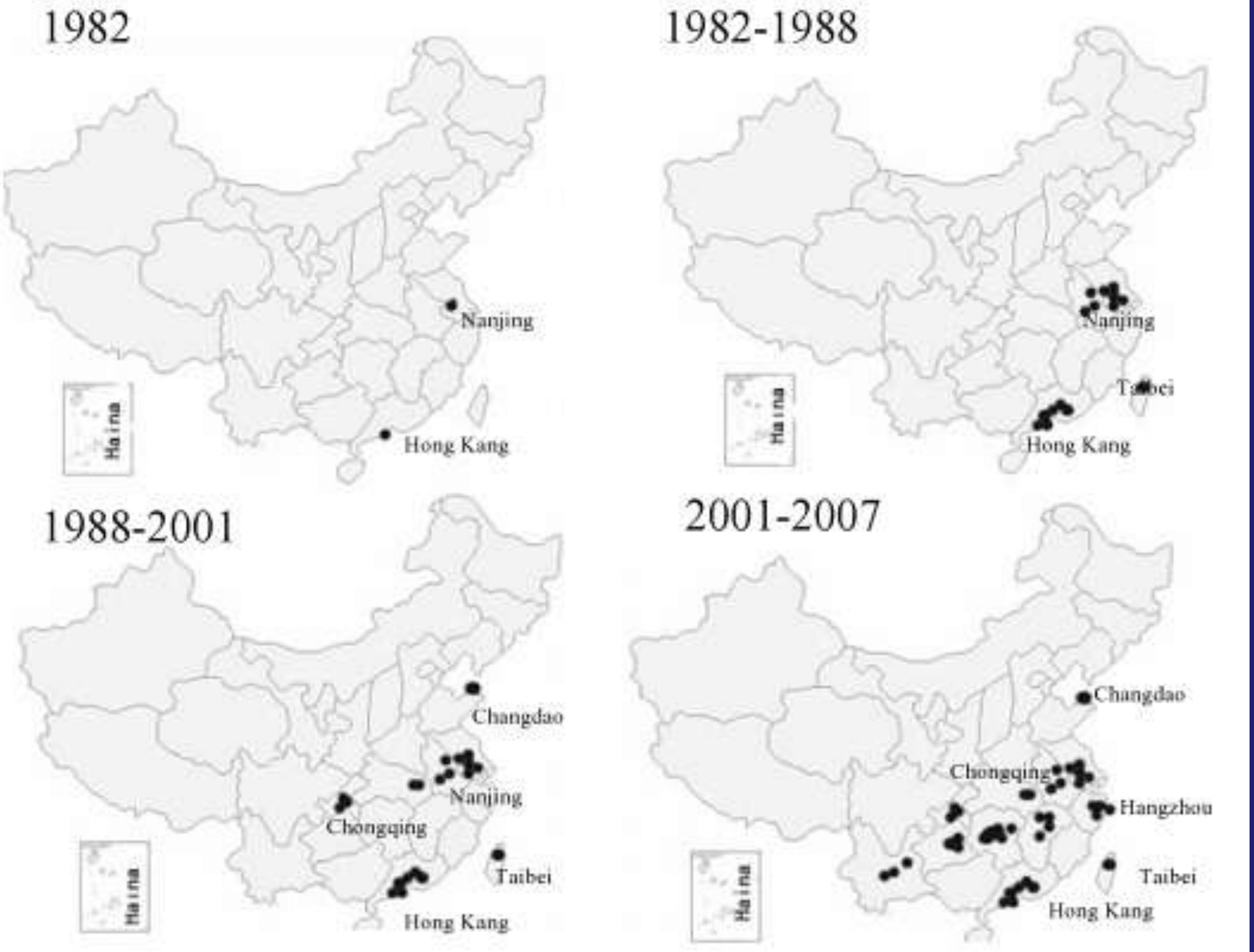
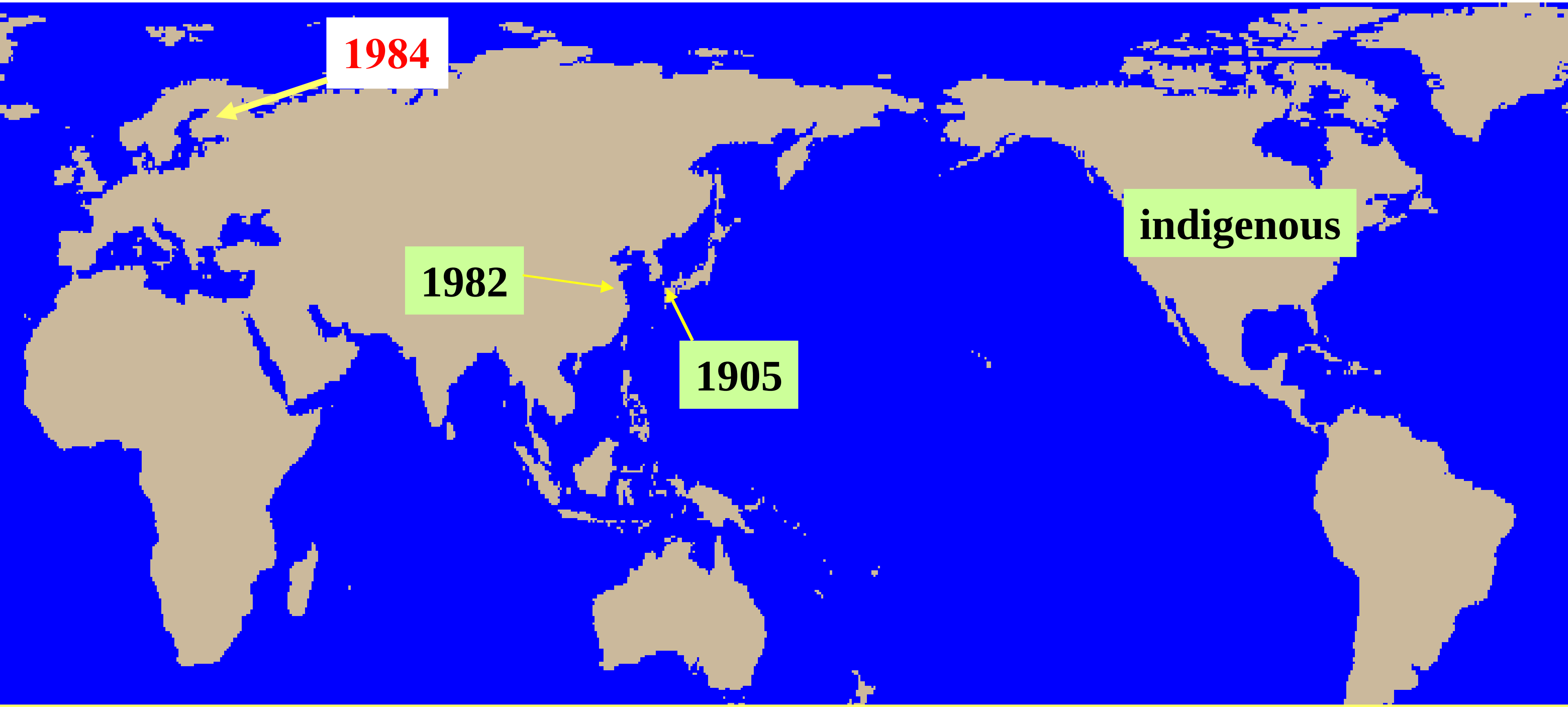


Fig. I.7 Distribution changes of pine wilt disease in China

Global Expansion of PWD（2）

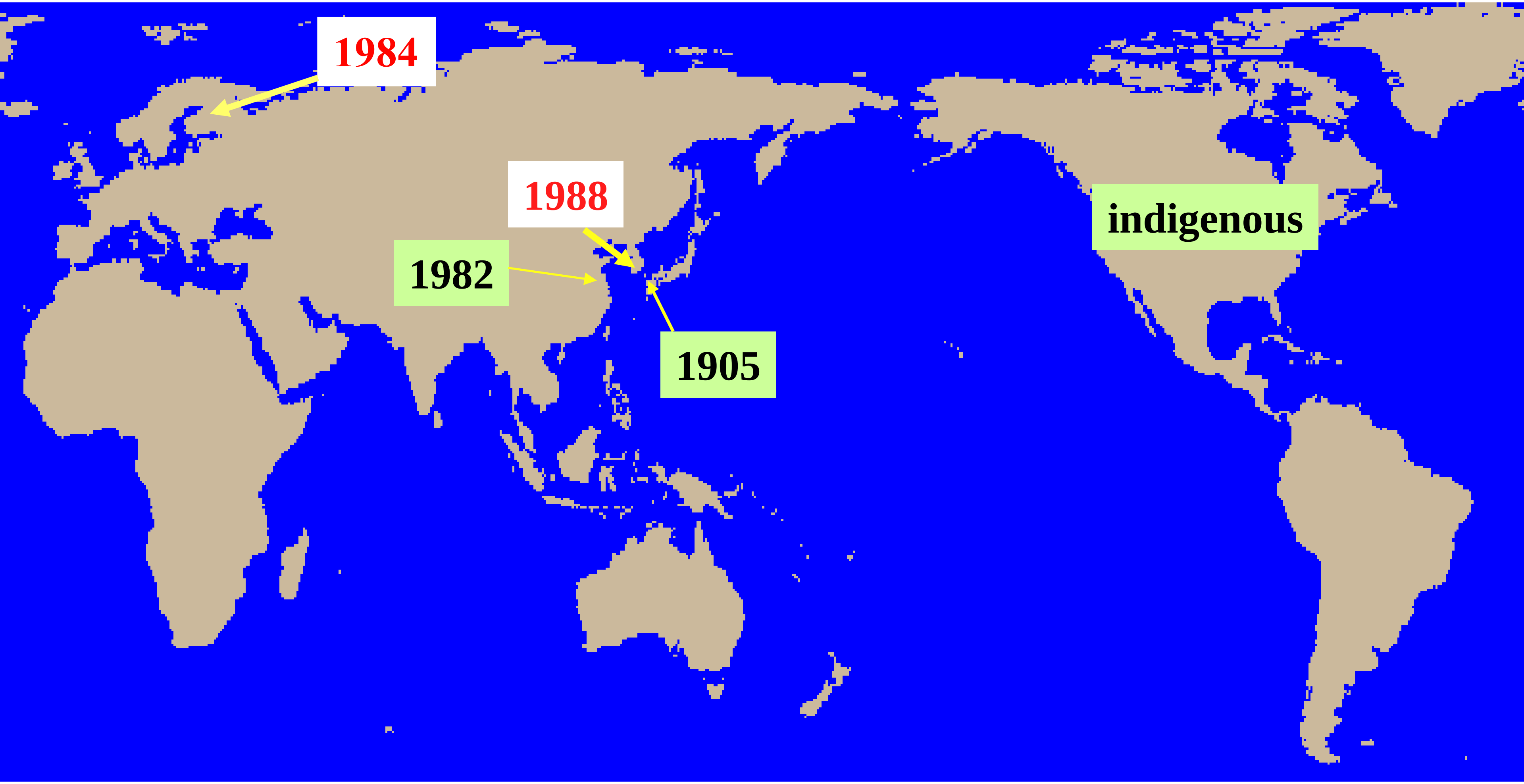


- Firstly Finland ● then Sweden and Norway placed a ban on importation
- In 1986 European Plant Protection Organization (EPPO) assigned PWD as A1-ranked pest.
- Due to this ban 13,000 people lost their jobs in USA, and suffered a loss of 60 million dollars.

The primary impact of the pinewood nematode in North America is on trade to European Union(EU) and other countries.

Slide shows pine chips being off loaded in Sweden. This was the last shipment of pine chips from North America to the EU.


Global Expansion of PWD（2）



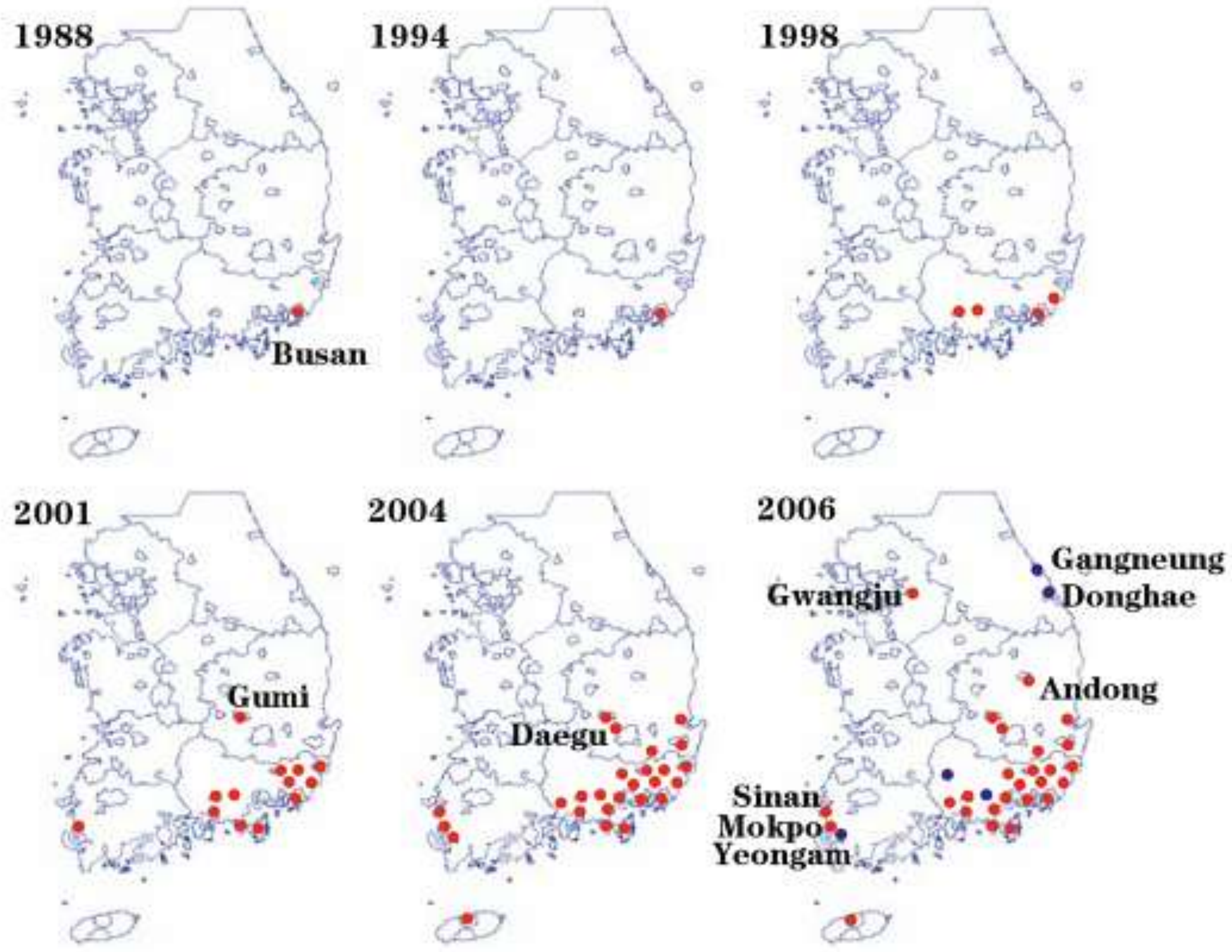
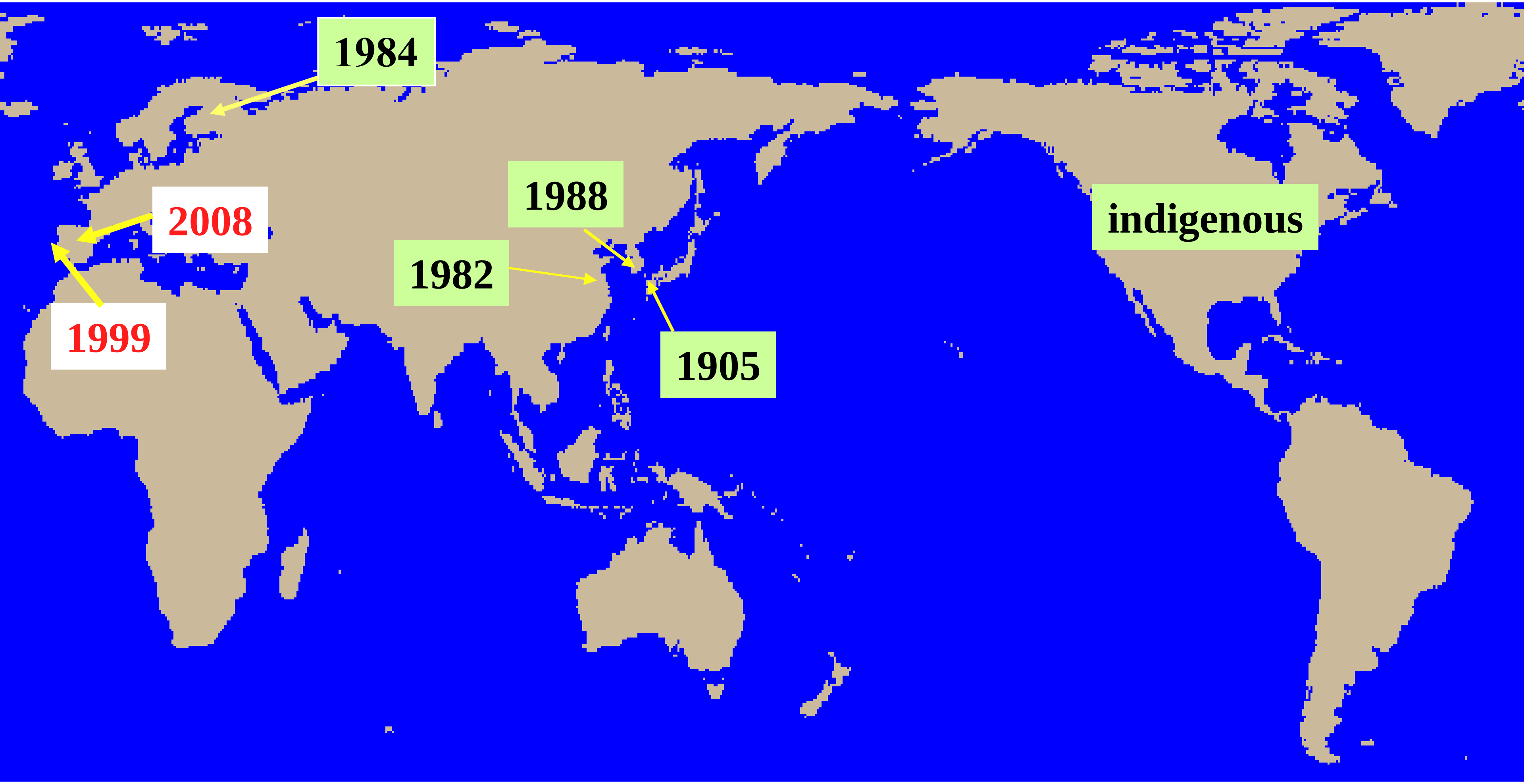


Fig. I.10 Spatial dispersal of pine wilt disease in Korea from 1988 to 2006. *Red dots* indicate the areas in which trees affected by pine wilt disease were observed and *blue dots* indicated the areas in which affected trees by pine wilt disease were found until 2005, but where no pine wilt disease has been observed since 2006 (see Color Plates)

韓国のマツ枯れ

“マツ枯れ”の世界への拡大（3）




Old-Modern Handicrafts

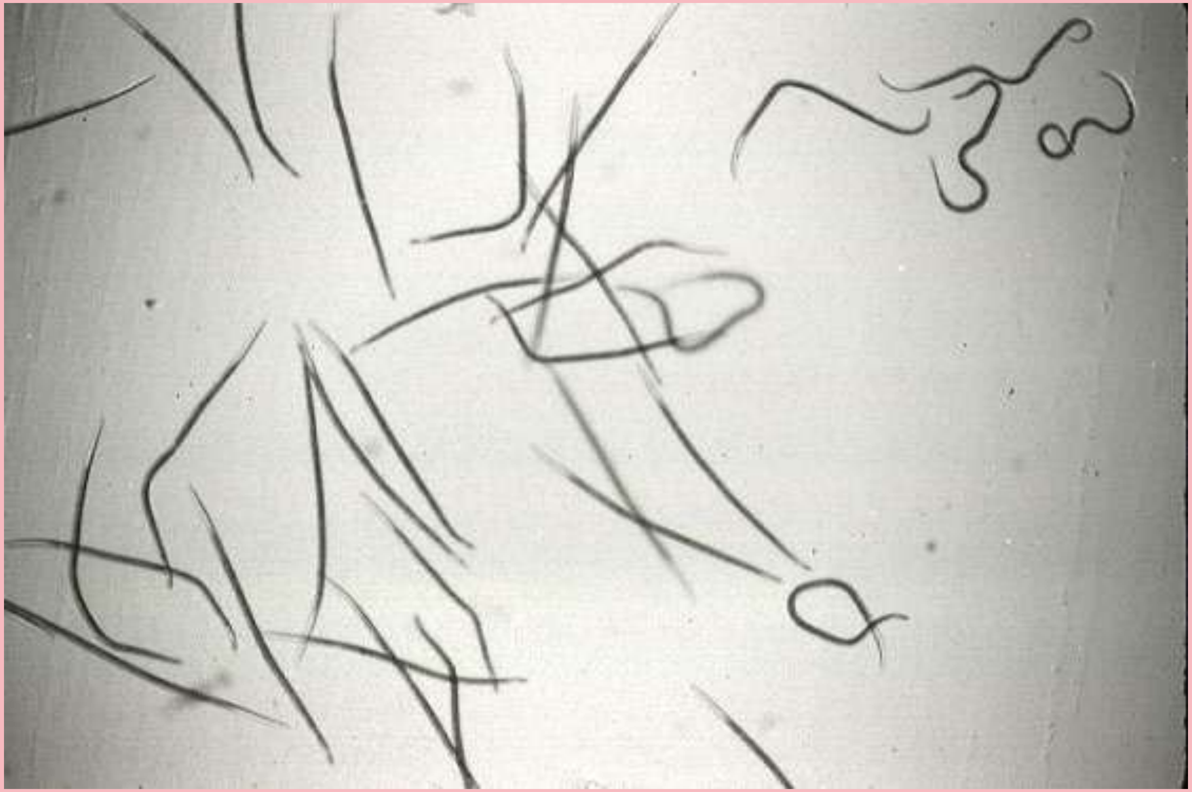
III. The Infection Cycle of PWD in East Asian Countries

Bark and wood borers related to dead pine trees

longicorn beetle

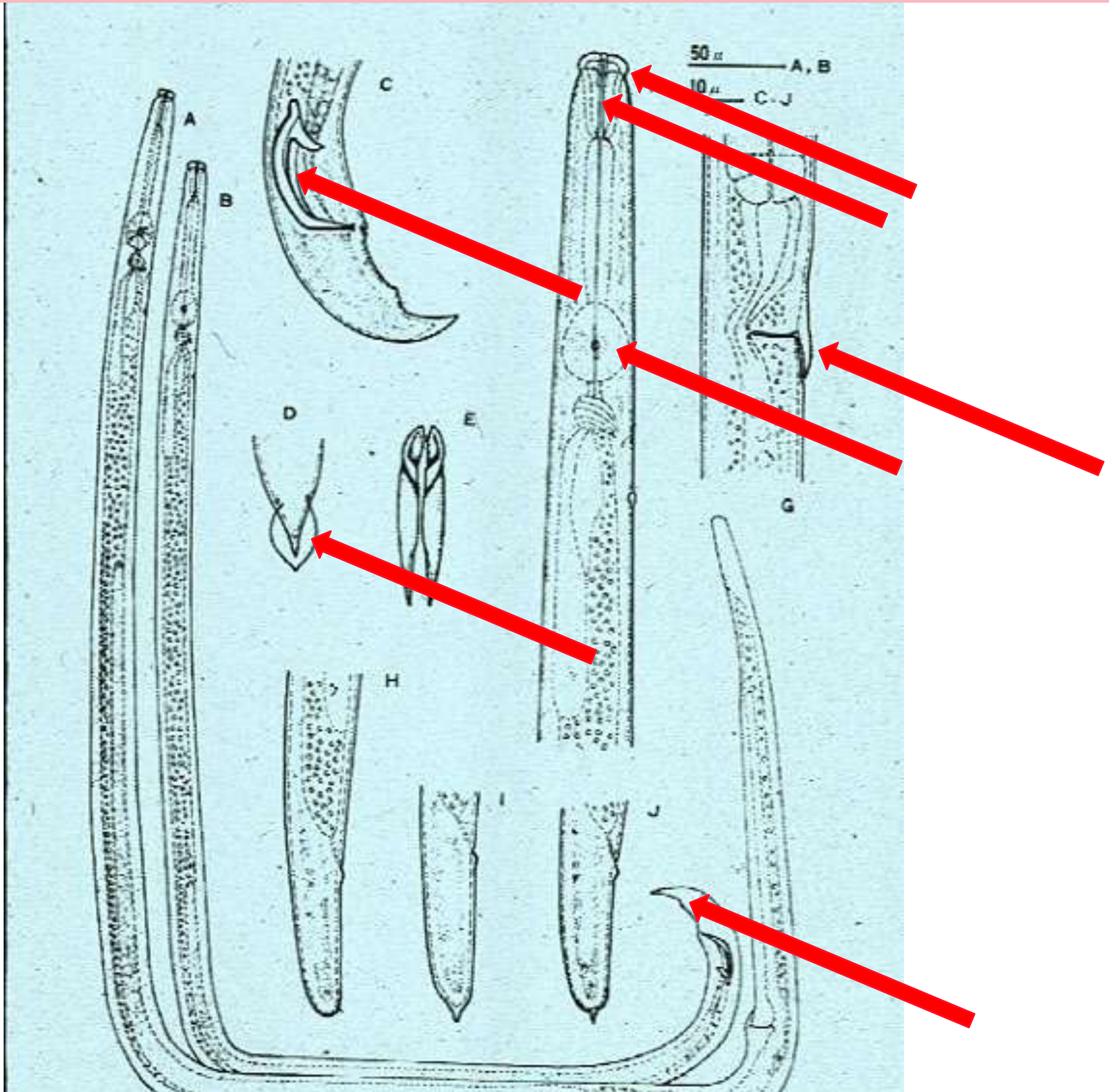
bark beetle

weevil



In the course of the survey, a tree pathologist, Dr. Tokushige, found numerous nematodes in his fungal cultures. He and his colleague, Dr. Kiyohara ventured to inoculate the nematodes onto healthy pine trees, though common sense suggested that plant parasitic nematode would never kill such big trees as pines.

Contrary to their presupposition, the pine trees inoculated with newly-found nematodes were killed in the same way as seen in the field.

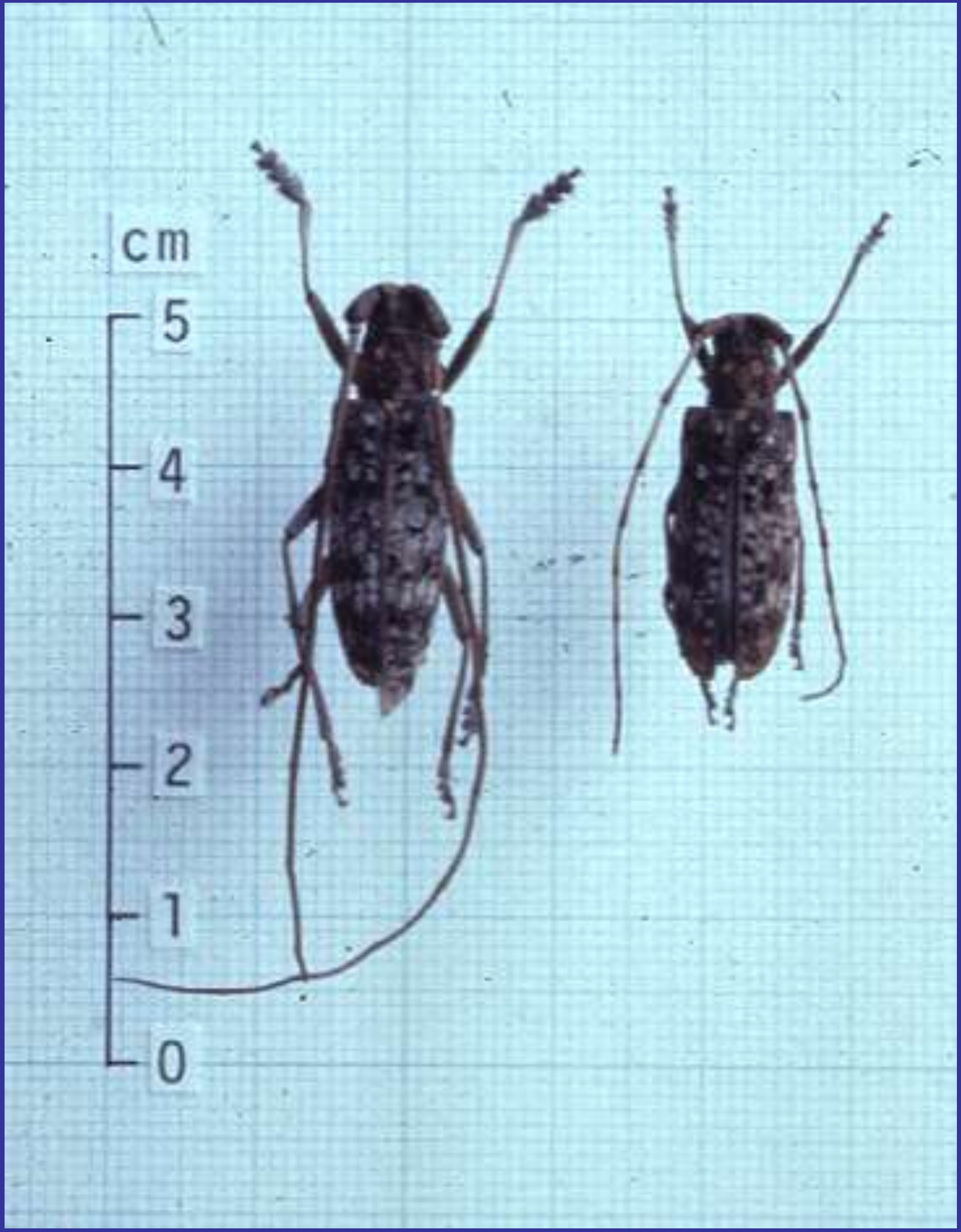


Pinewood nematode :

Bursaphelenchus xylophilus

This is the way of nematode inoculation to the sapling of Japanese black pine.

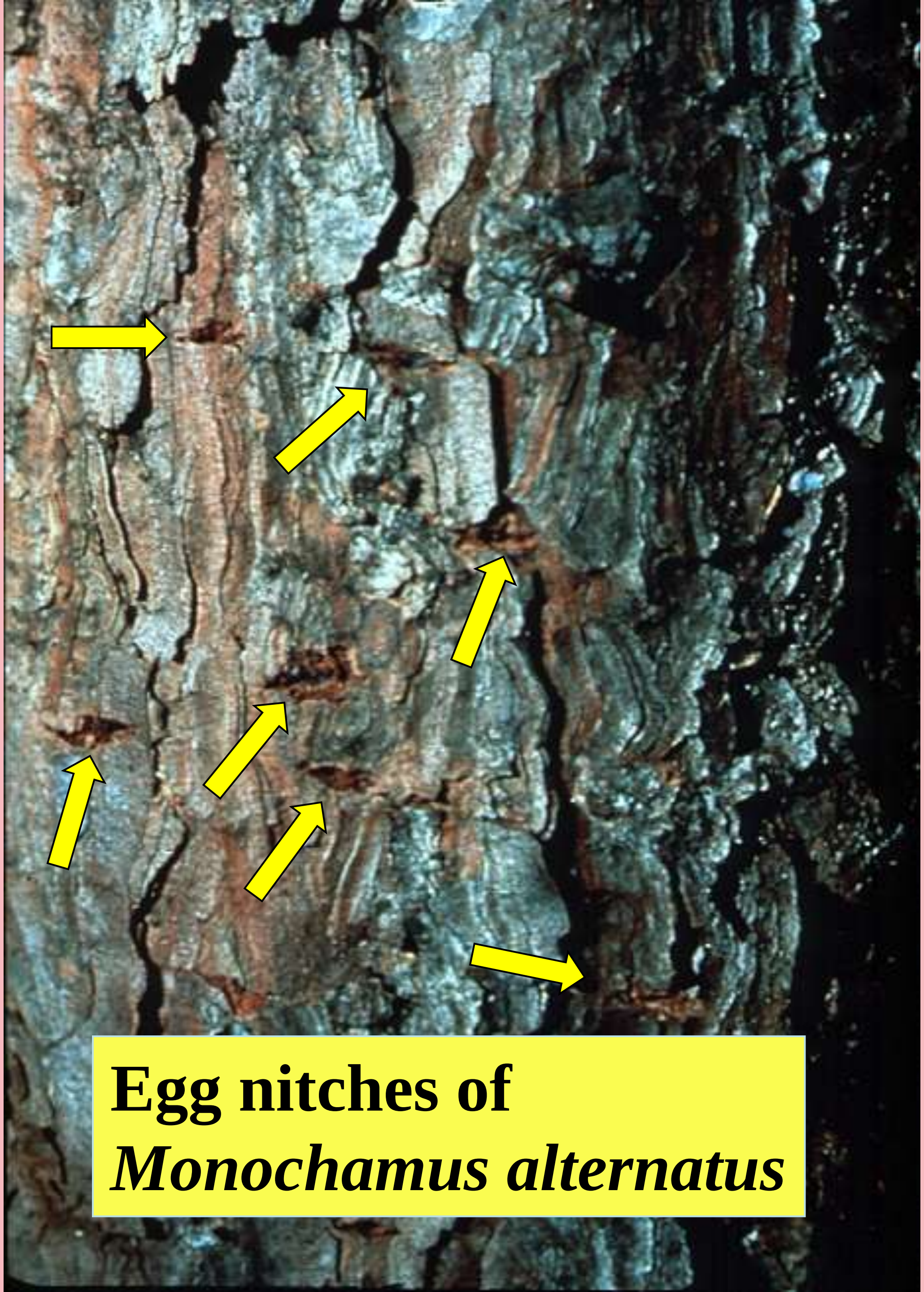
1. Firstly, we peel off a small part of bark,
2. Fix a cotton swab on the scar,
3. Pipett an aliquot of nematode suspension into the cotton swab,
4. Then, cover with parafilm not to be washed away by rain

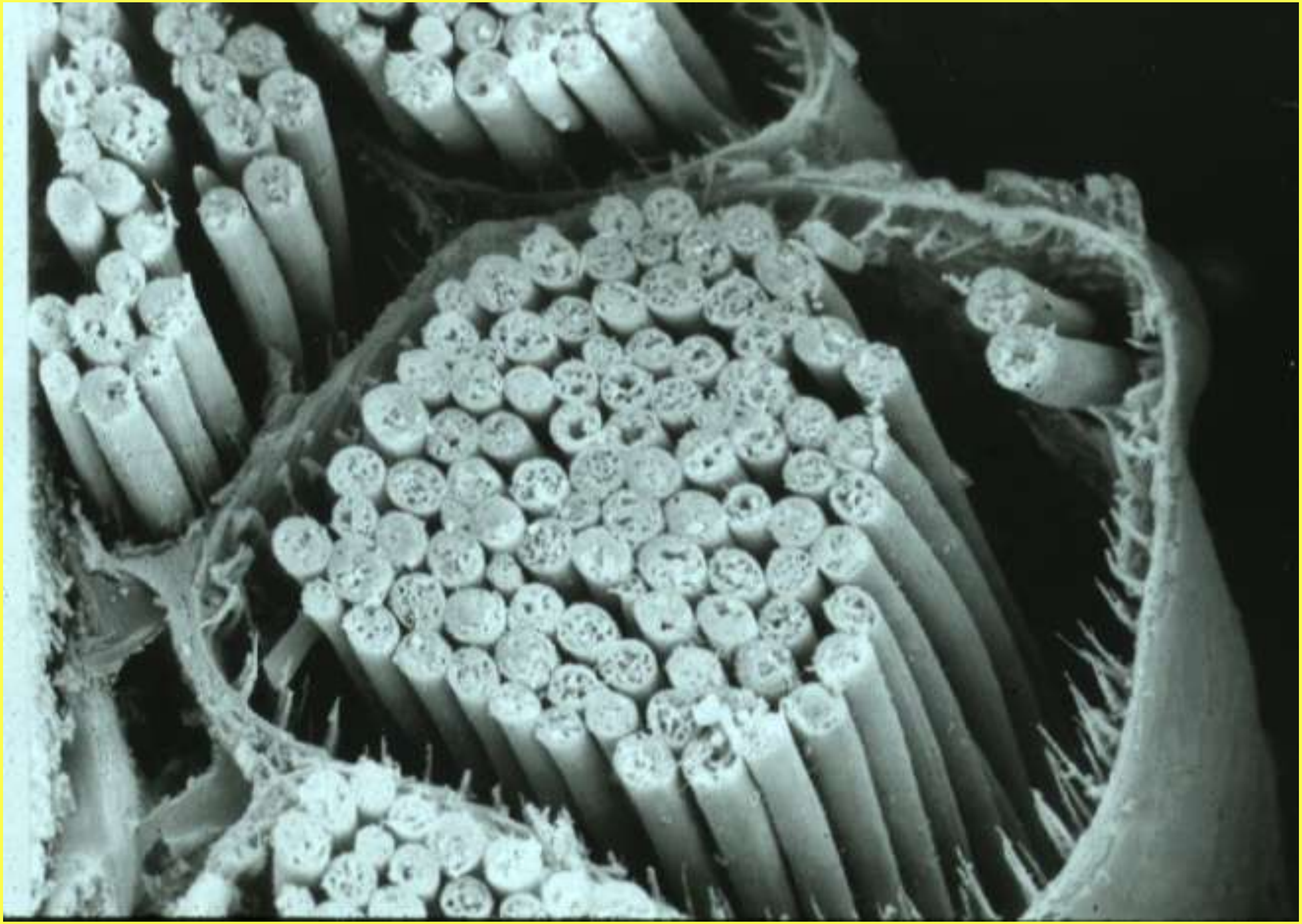

cm
5
4
3
2
1
0

When *Monochamus* beetles hatch, their sexual organs have not yet well-developed. So they have to keep on feeding to make their reproductive organs mature. For this purpose, they move from dead pine trees to healthy ones, and feed on young branches.

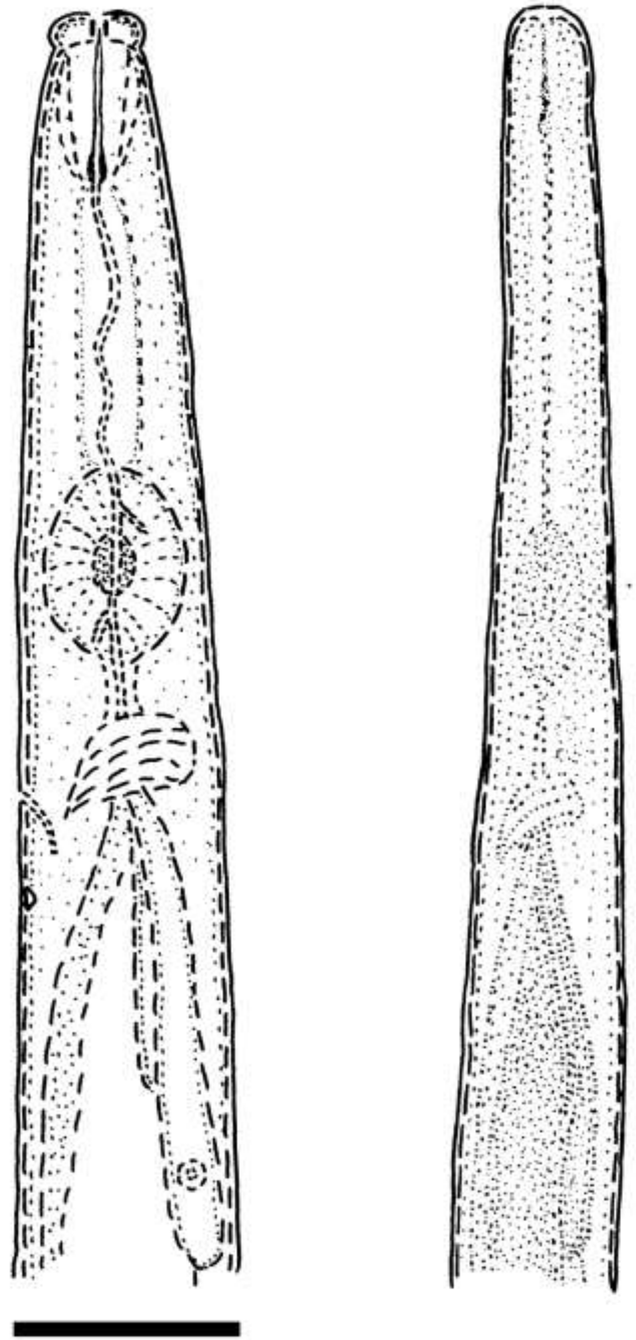
This is the feeding wound, through which nematodes invade the host tree.

Three to four weeks after nematode infection, pine trees cease their resin exudaion, and start to emit volatiles such as ethanol and monoterpenes. These volatiles attract matured *M. alternatus* to the diseased pine trees, where they mate and then lay their eggs making egg niches.



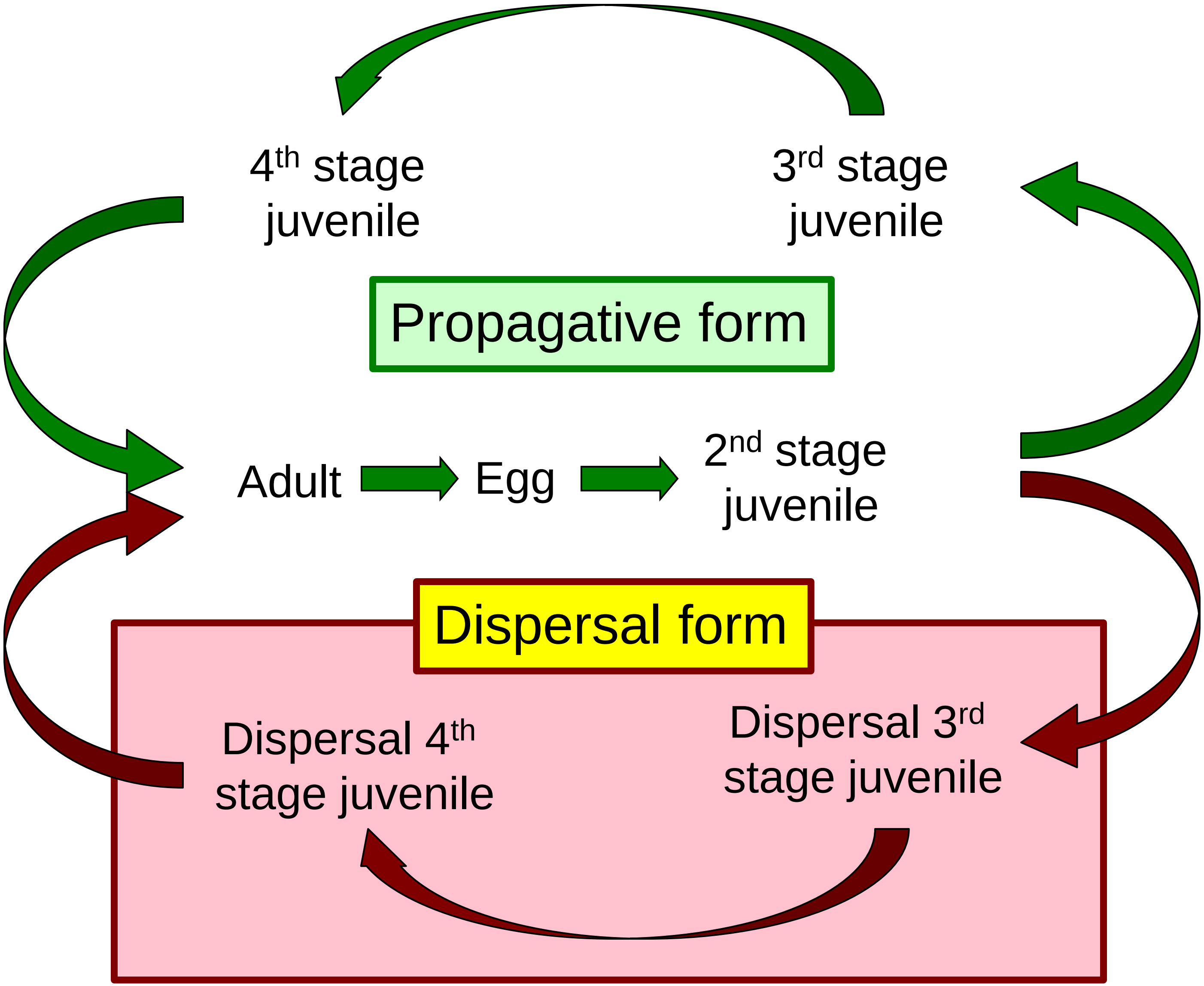


This is a sectional view of a tracheal tube filled with pinewood nematodes.



Life cycle of *Bursaphelenchus xylophilus*

Figure 1



***Monochamus* adult finishing its emergence hole**

From June to early July, *Monochamus* beetles emerge from dead pine trees through round holes, carrying numerous pinewood nematodes in their respiratory organs.

Common traits for invasive species

(1) Fast growth: → *Bx* grows slightly faster than *Bm*

(2) Rapid reproduction: → the reproduction velocity of *Bx* is higher than that of *Bm*

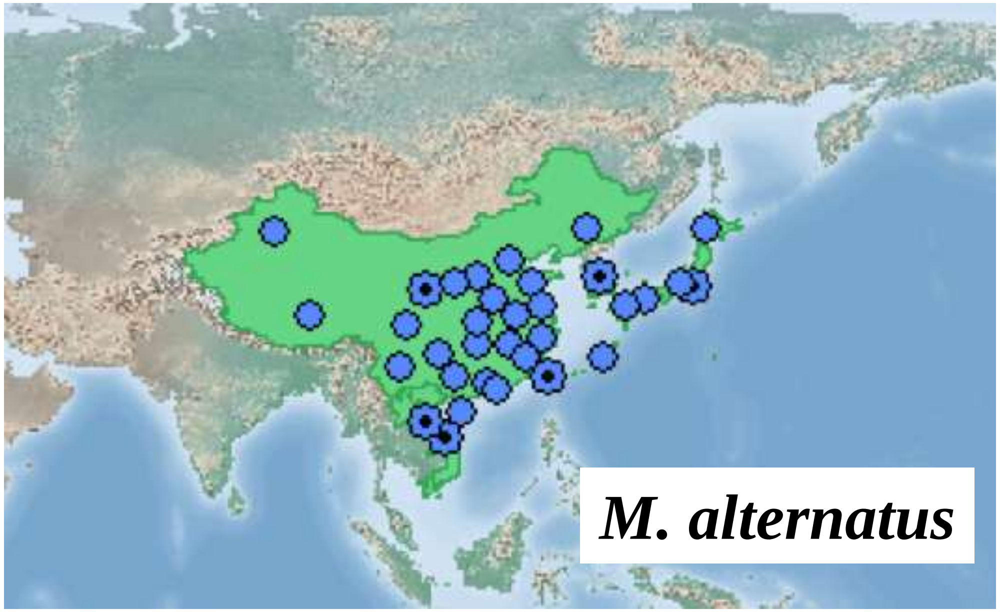
(3) Phenotypic plasticity (the ability to alter growth form to suit current conditions)

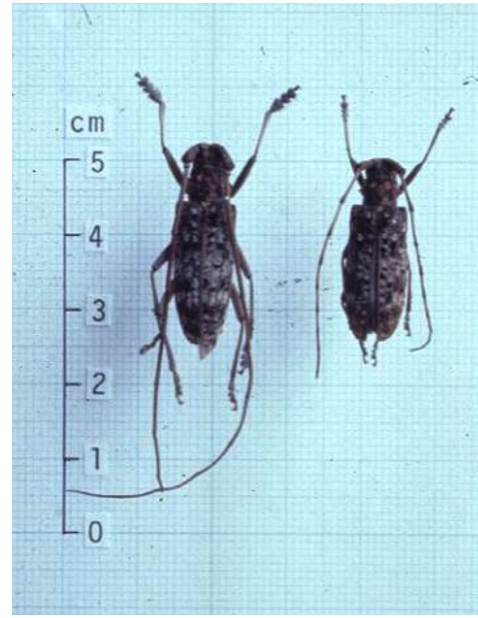
→ high adaptability to native *Monochamus* vectors

→ High dispersal ability

(4) Tolerance of a wide range of environmental conditions

→ *Bx* has a special stage (DL) adaptable to adverse conditions


M. alternatus


cm
5
4
3
2
1
0


M. saltuarius

Genus *Pinus*

Subgenus Pinus: ca.73 spp.
(two-needled pines)

Subgenus Strobus: ca 42 spp.
(five-needled pines)

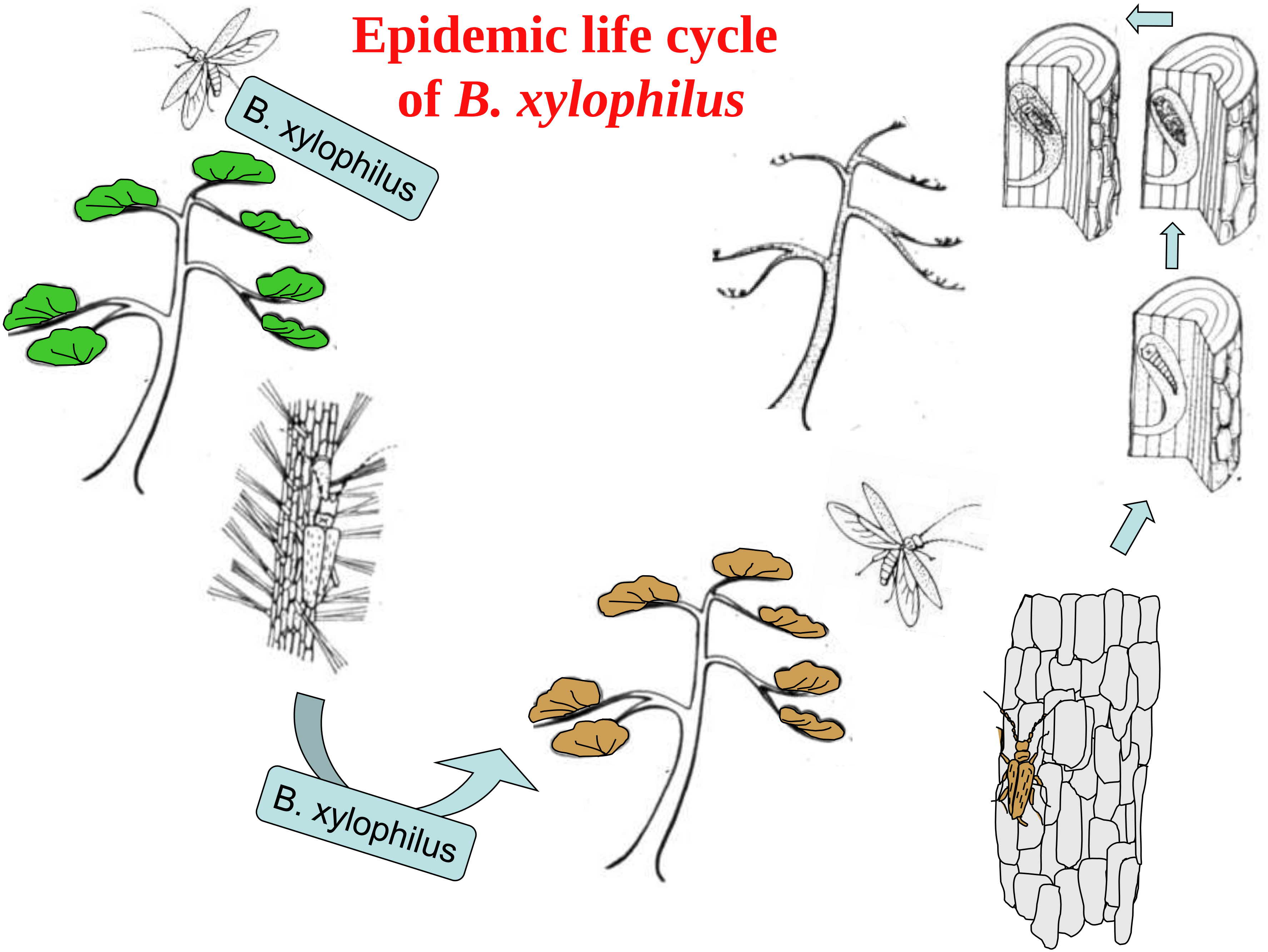
Japanese native two-needled pine are 3 species;

- ○ Japanese red pine: *P. densiflora*
- ○ Japanese black pine: *P. thunbergii*
- ○ Luchu pine：*P. luchuensis*

Japanese native five-needled pine are 4 species and one variety;

- ○ Japanese white pine：*Pinus parviflora*
- ○ Japanese five-needle pine：*P. pentaphylla*
- ○ Yakushima White Pine：*P. amamiana*
- ○ Korean pine：*P. koraiensis*
- ○ Siberian dwarf pine：*P. pumila*（？）

Epidemic life cycle of *B. xylophilus*



IV. The Infection Cycle of PWD in North American Countries

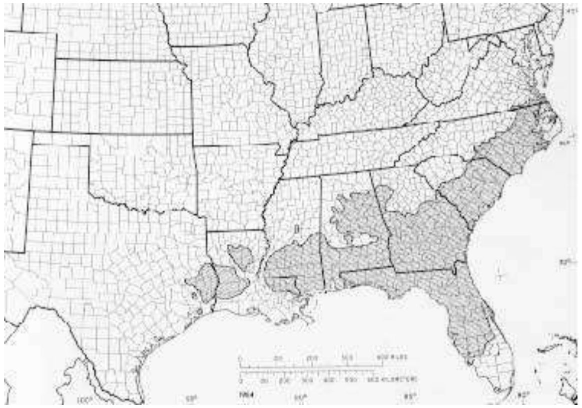
The history of pine wood nematode research

(Year)	(Event)	(Reference)
1929	*Aphelenchoides xylophilus* found in association with fungi in timer	Steiner and buhrer 1934
1969	*Bursaphelenchus* sp. found in wood of dead pine trees in Japan	Tokushige and Kiyohara 1969
1970	*Aphelenchoides xylophilus* transferred to *Bursaphelenchus*	Nickle 1970
1971	Pathogenicity of *Bursaphelenchus* sp. demonstrated by inoculating 25-year-old *Pinus densiflora*	Kiyohara and Tokushige 1971
1972	Pine wood nematode described as *B. lignicolus*	Mamiya and Kiyohara 1971
1972	Transmission of *B. lignicolus* by *Monochamus alternaturs* reported (maturation feeding)	Mamiya and Enda 1972
1979	*Bursaphelenchus mucronatus* is described	Mamiya and Enda 1979
1979	Pine wilt disease reportedly found in United States	Dropkin and foudin 1979
1981	*Bursaphelenchus lignicolus* placed as a synonym of *B. xylophilus*	Nickle and others 1981
1983	Transmission of *B. xylophilus* during oviposition of *Monochamus* vectors reported	Wingfield 1983
1983	*Bursaphelenchus xylophilus* was found in Canada	Knowles and others 1983
1984	*Bursaphelenchus xylophilus* intercepted in pine wood chips imported into Finland for the United States and Canada	Rautapaa 1986

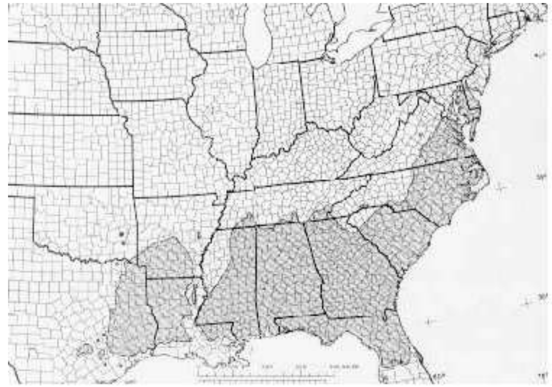
Initial Reaction to Rediscovery of Pine Wood Nematode in North America

When the pine wood nematode (as *B. lignicolus*) was found on dead Austrian (*Pinus nigra* Arnold) and Scotch (*P. sylvestris* L.) pines in Missouri (Dropkin and Foudin 1979) in 1979, this nematode was thought to have been introduced from Japan. The initial reaction by the scientific community, reflected in the literature of the time, was "We've been invaded!" (Holdeman 1980).

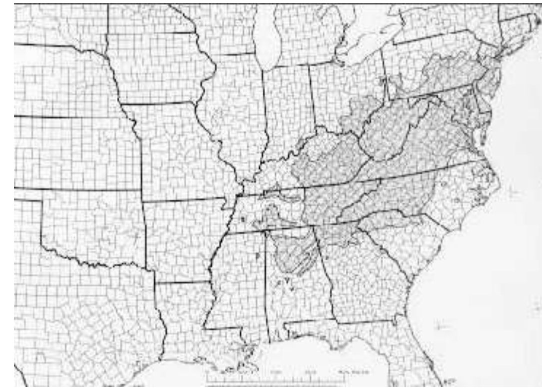
The Common North American Pines (1/3)



Pinus palustris (long leaf pine)

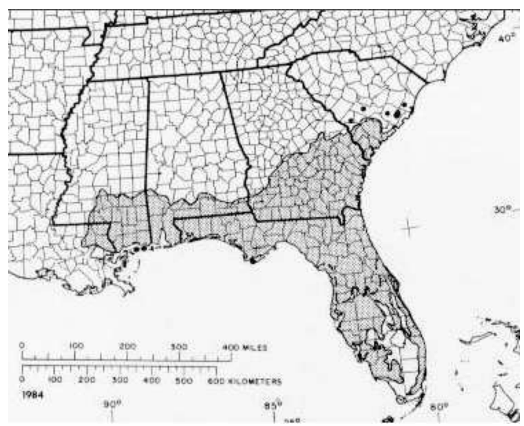


Pinus taeda (Loblolly pine)



Pinus virginiana (Virginia Pine)

Pinus echinata (short leaf pine)



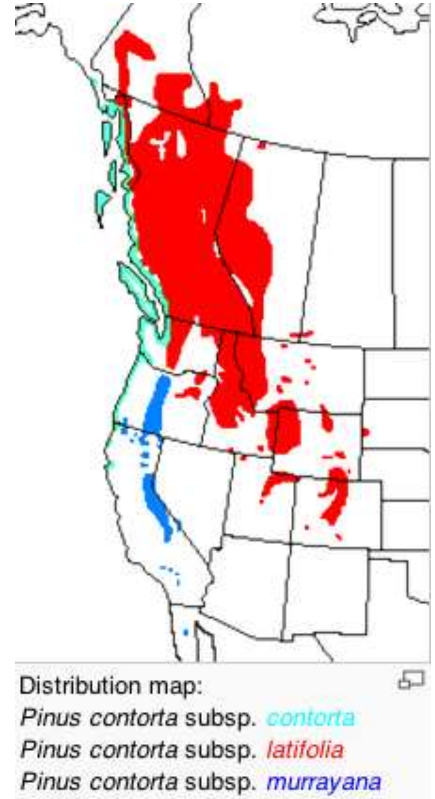
Pinus elliottii (slash pine)

pine)

The Common North American Pines (2/3)

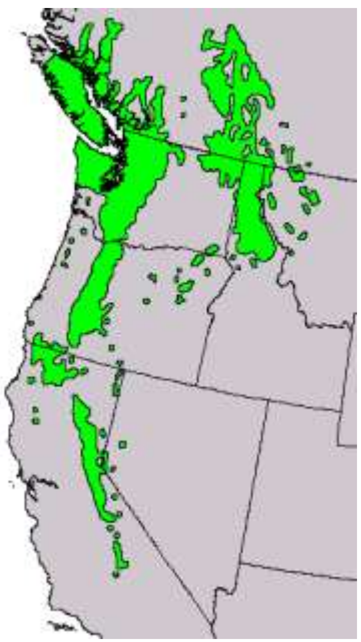
Pinus edulis **(Pinyon pine)**

Pinus jeffreyi **(Jeffrey pine)**



Pinus contorta **(Lodgepole pine)**

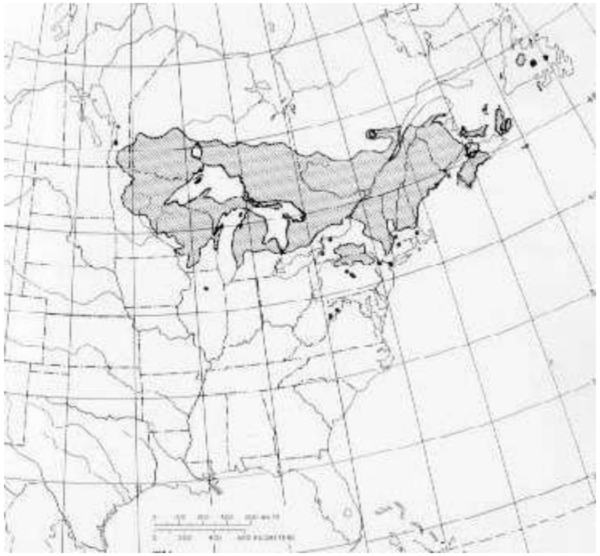
Pinus strobus **(Eastern white pine)**



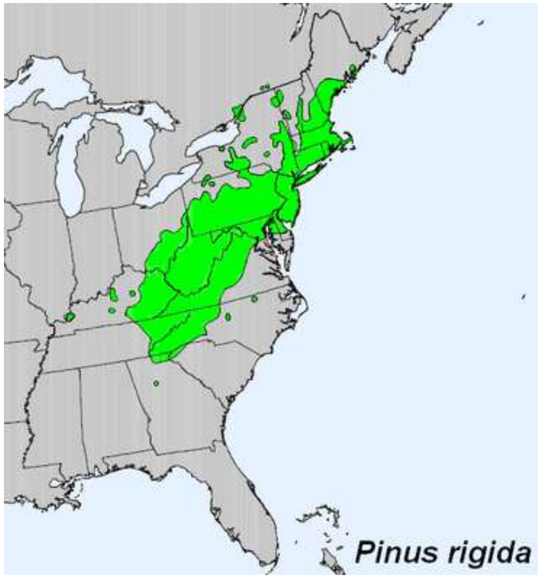
Pinus monticola **(Western white pine)**

The Common North American Pines (3/3)

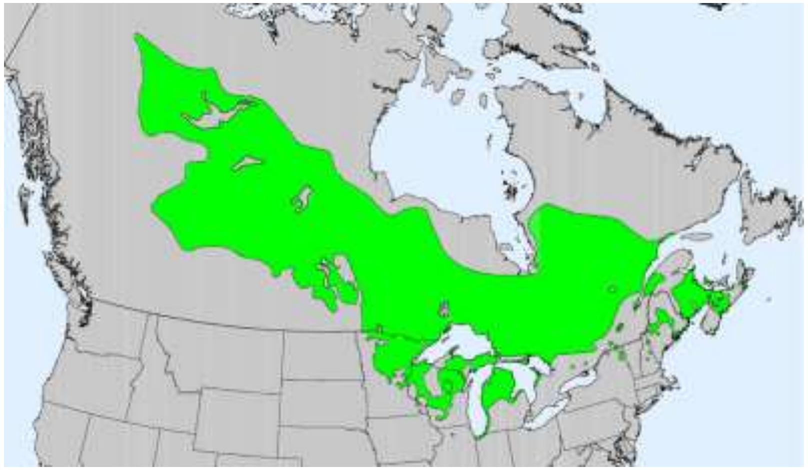
Pinus lambertiana (Sugar pine)



Pinus resinosa (red pine)



Pinus rigida (Pitch Pine)



Pinus banksiana (Jack pine)

In the United States,

dauerlarvae of *B. xylophilus* have been recovered from adult beetles of

***M. carolinensis*, *M. scutellatus*, *M. titillator*,**

***M. mutator*, and *M. notatus*.**

Monochamus carolinensis

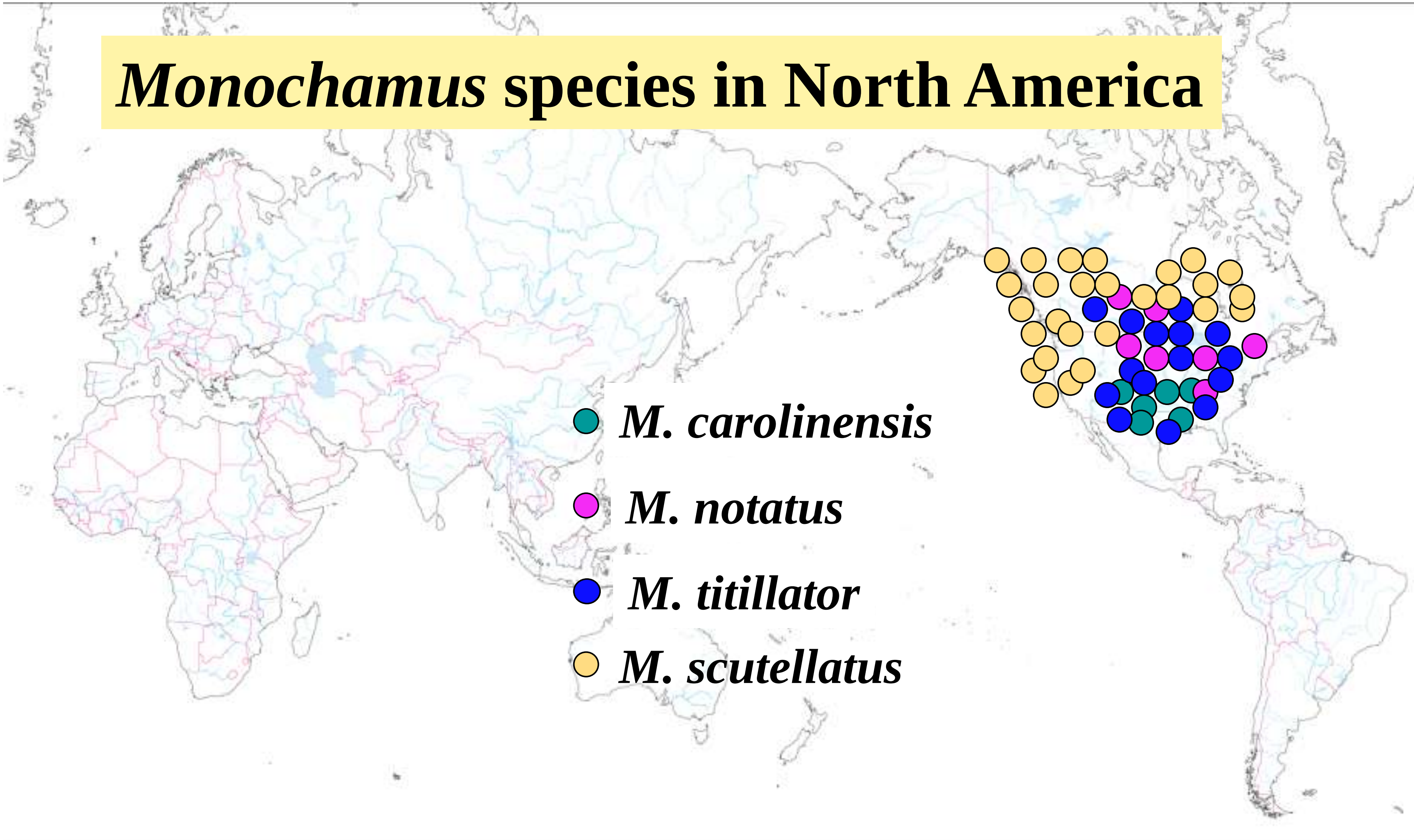
M. scutellatus

Monochamus sutor

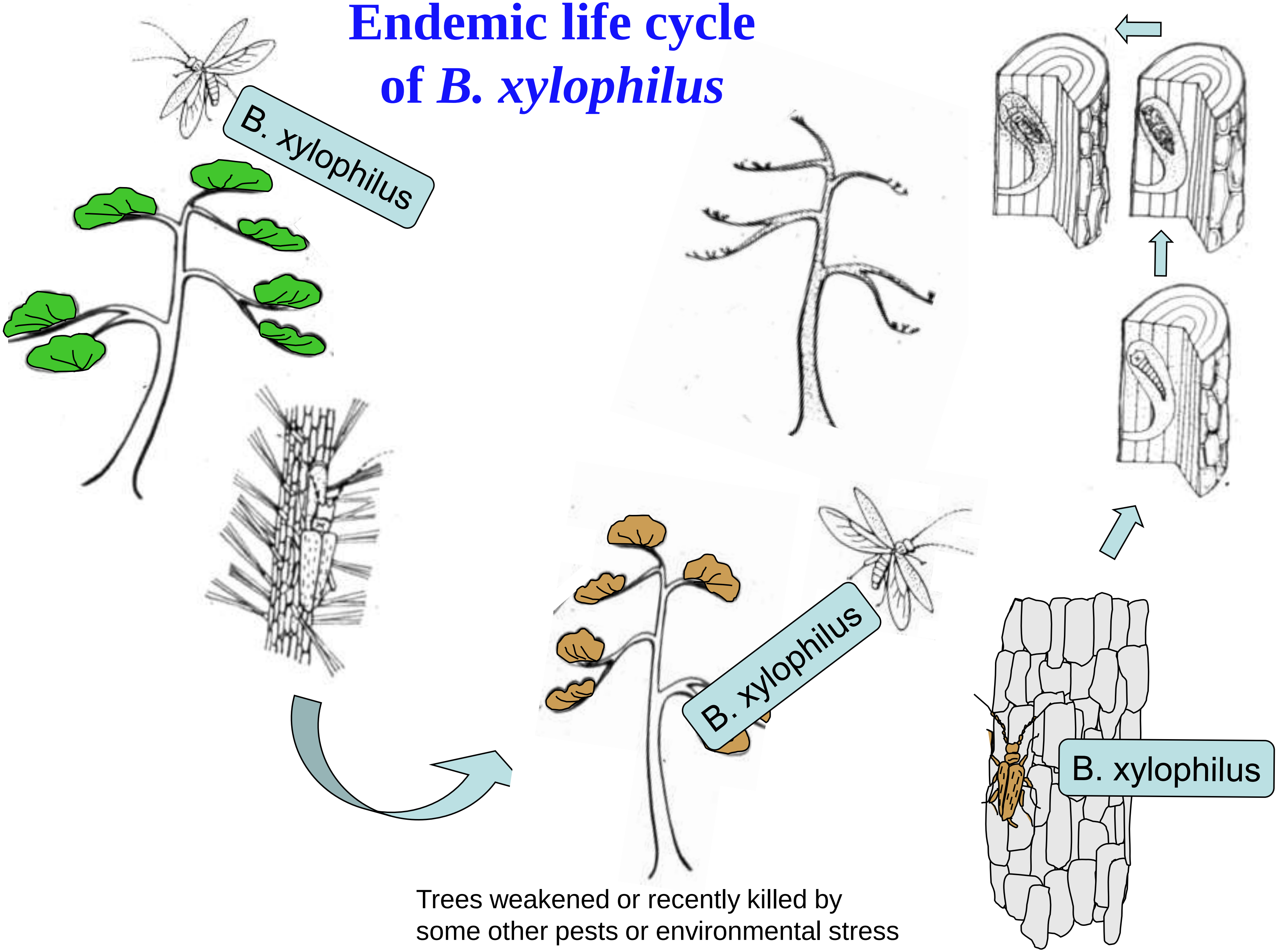
M. titillator

Monochamus notatus

Monochamus species in North America



Endemic life cycle of *B. xylophilus*



Trees weakened or recently killed by
some other pests or environmental stress

In North America,

(1) The pine wood nematode (*B. xylophilus*) is a secondary associate of native conifers

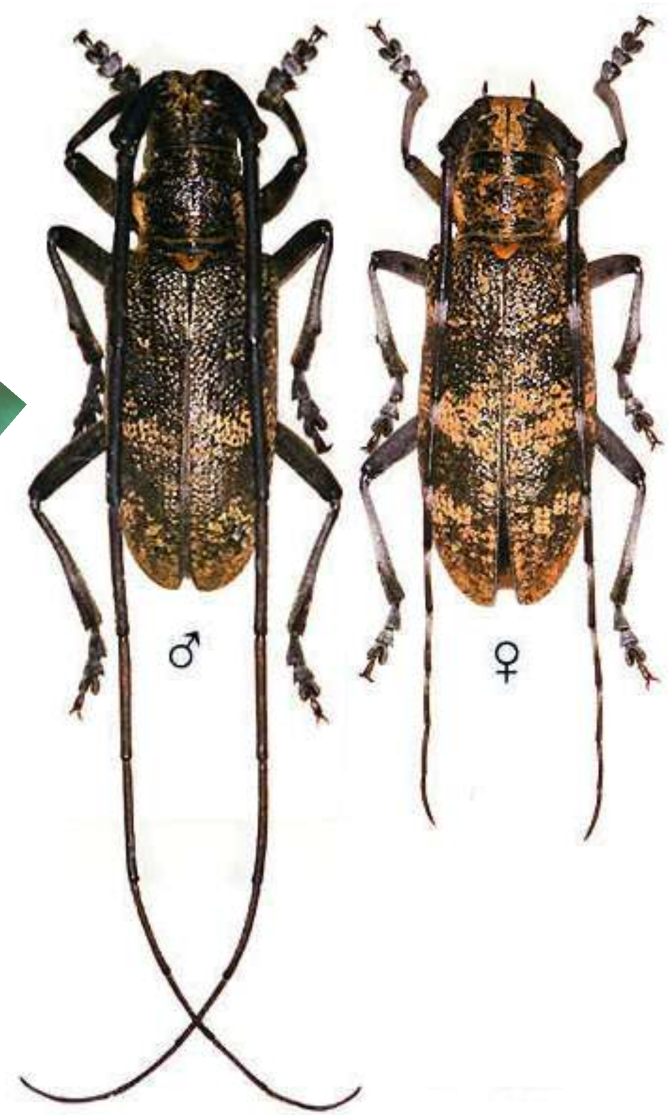
(2) The most common mode of transmission is during oviposition of the *Monochamus* vector.

(3) The pine wood nematode is a primary pathogen of exotic pines,

(4) Since the nematode is seldom a primary pathogen in North America, distribution there cannot be equated with the distribution of the pine wilt disease.

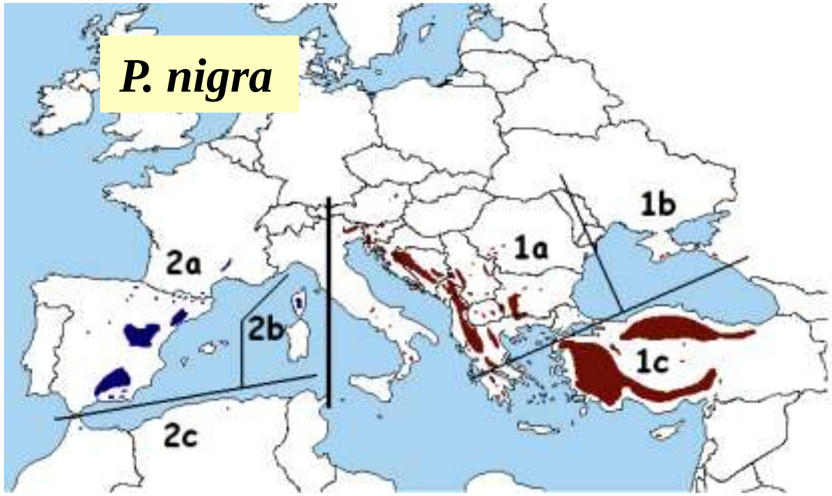
V. The Possibility of PWD spreading into European regions

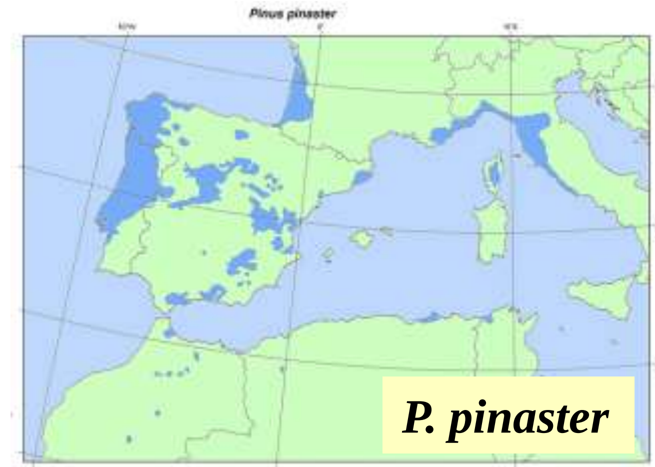
M. galloprovincialis

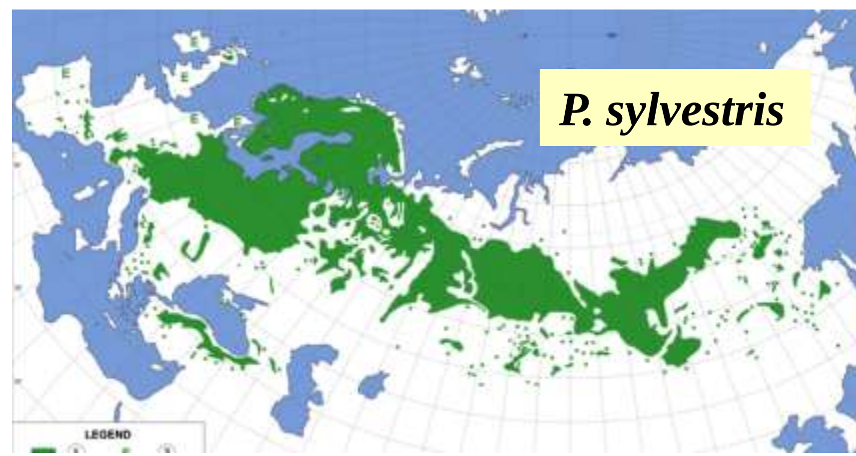


Monochamus galloprovincialis

European pine species susceptible to *B. xylophilus*







VI. Invasive species and native species

Comparison of several isolates of *Bursaphelenchus xylophilus* and *B. mucronatus*

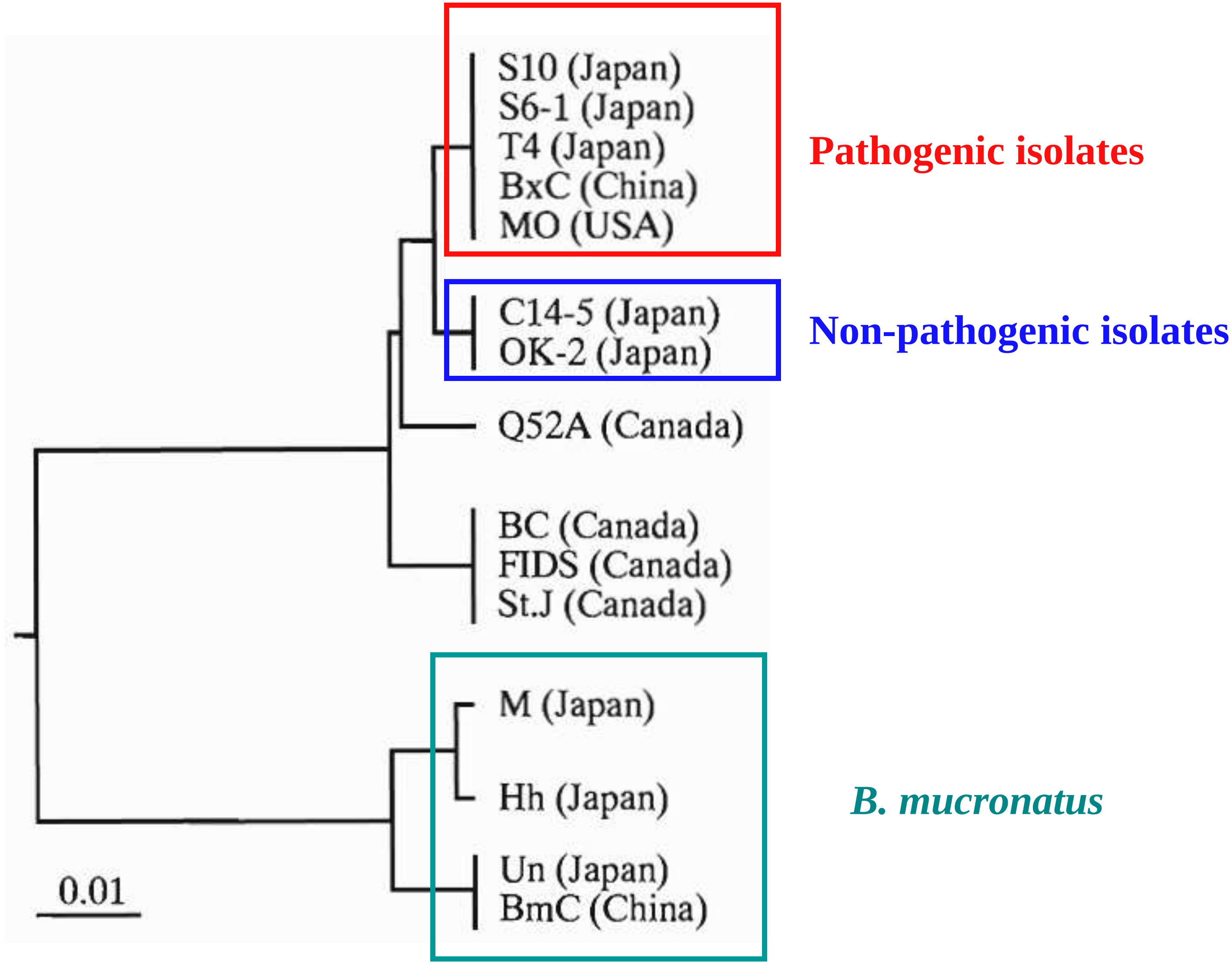
- **In 1998, we phylogenetically compared several isolaes of *B. xylophilus* and some isolates of *B. mucronatus* with PCR-RFLP analysis*.**

  * polymerase chain reaction – restriction fragment polymorphism analysis

- **The isolates of *B. xylophilus* examined were from Japan, the United States, China, and Canada and the *B. mucronatus* isolates from Japan, China, and France.**

Table 1. *Isolates and origins of nematodes used in this study.*

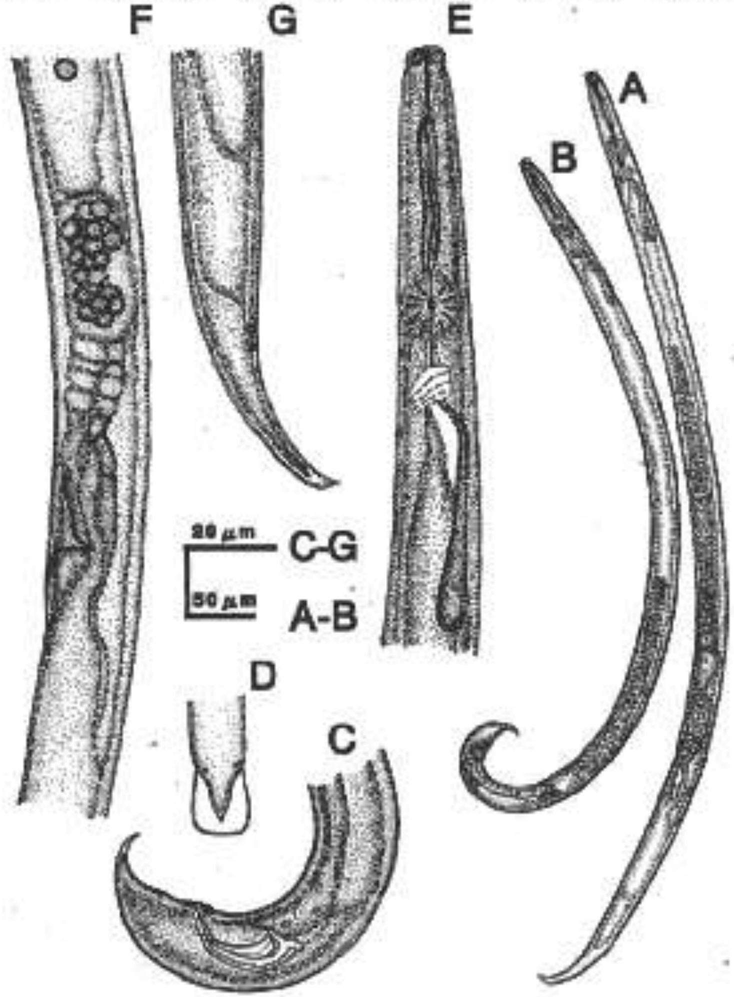
Species	Code	Isolate name	Origin	Source
Bursaphelenchus xylophilus	1	S10	Shimane, Japan	H. Iwahori
	2	S6-1	Ibaraki, Japan	H. lwahori
	3	T4	Iwate, Japan	T. Kiyohara
	4	C14-5	Chiba, Japan	H. Iwahori
	5	OK-2	Okinawa, Japan	T. Kiyohara
	6	BxC	Nanjing, China	B. Yang
	7	MO	Minnesota, United States	T. Kiyohara
	8	BC	British Columbia, Canada	T. Kiyohara
	9	FIDS	British Columbia, Canada	J. R. Sutherland
	10	St.J	New Brunswick, Canada	J. R. Sutherland
	11	Q52A	Quebec, Canada	J. R. Sutherland
Bursaphelenhcus mucronatus	1	M	Kyoto, Japan	H. lwahori
	2	Hh	Hiroshima, Japan	S. Jikumaru
	3	Un	Nagasaki, Japan	T. Kiyohara
	4	BmC	Sichuan, China	B. Yang
		F1	Saint Symphorien, France	G. de Guiran
Aphelenchus avenae	a		Iwate, Japan	H. Okada
Aphelenchoides besseyi	b		Shizuoka, Japan	T. Nishizawa
Aphelenchoides fragariae	f		Shizuoka, Japan	T. Nishizawa
Aphelenchoides ritzemabosi	r		Shizuoka, Japan	T. Nishizawa


S10 (Japan)
S6-1 (Japan)
T4 (Japan)
BxC (China)
MO (USA)
Pathogenic isolates
C14-5 (Japan)
OK-2 (Japan)
Non-pathogenic isolates
Q52A (Canada)
BC (Canada)
FIDS (Canada)
St.J (Canada)
M (Japan)
Hh (Japan)
Un (Japan)
BmC (China)
B. mucronatus
0.01

Some xylophilus group-*Bursaphelenchus* species, their vectors and host plants

Nematode spp.	*Bursaphelenchus xylophilus*	*B. mucronatus*	*B. conicaudatus*	*B. luxuriosae*	*B. doui*
Vector insects	*Monochamus alternatus* etc. 10-12 spp.	*M. alternatus*, *M. saltuarius*	*Psacothea hilaris*	*Acalolepta luxuriosa*	*Monochamus subfasciatus*
Host plants	Pinaceae	Pinaceae	Moraceae	Araliaceae	Broad leaved spp. (+ *Pinus* spp.)
Transmission	maturation feeding	Oviposition ?	oviposition	Oviposition ?	Oviposition ?
Pathogenicity of nematode	pathogenic	non-pathogenic	non-pathogenic	non-pathogenic	non-pathogenic
Which stage of nematodes, and where they are	Dispersal 4th stage nematodes are carried by vector insects being in their respiratory organ (tracheae).				
					

Bursaphelenchus conicaudatu



A: Female; B: Male; C: Male tail; D: Ventral view of male tail; E: Female ,anterior portion; F: Female, vulva; G: Female tail.


Psacothea hilaris
♀


Feeding marks made on mulberry leaves

	B. xylophilus	***B. conicaudatus***
Vector beetle	***Monochamus* spp.**	***Psacothea hilaris***
feeding preference	Fungal cells or plant parenchyma cells	Fungal cells or plant parenchyma cells
Average number carried by a vector	high to very high	rather low, up to some hundreds
the proportion of beetles infested with the nematodes	Different among stands, ranging from 0 to high proportion	High proportion of beetles are infested
Nematode release	**By mature feeding**	**By oviposition**
Speciation	None in Japan	**Subspecies appeared in accordance with vector's subspeciation**

Conclusion

Invasive *Bx* can use both living and dying/dead pines for their reproduction, while native *Bm* can use only dying/dead pines.

This must facilitate *Bx* to outcompete *Bm* in a common niche.

Thus, *Bx* is seemingly more adaptable to field conditions than *Bm*, however, the strategy of *Bx*, must be poorer than that of *Bm*, because their highly pathogenic traits apt to sweep away host trees from the field and thereby threaten their own survivability.

As is the case of most pathogenic bacteria, *Bx* must reduce its pathogenicity, and get more moderate association with host pine trees.

Thank you for your attention