Defense responses of oak sapwood in relation to wilt of oak trees in Japan

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Introduction

Mass mortality of oaks (mainly *Quercus serrata* and *Q. crispula*) has been appeared continually in Japan since 1980's (Figs. 1,2,3). Blockage of xylem sap ascent induced by the infection of a fungus *Raffaelea quercivora*, vectored by ambrosia beetle *Platypus quercivorus*, is considered to cause this mortality. We are investigating the defense responses of oak sapwood against the pathogen.

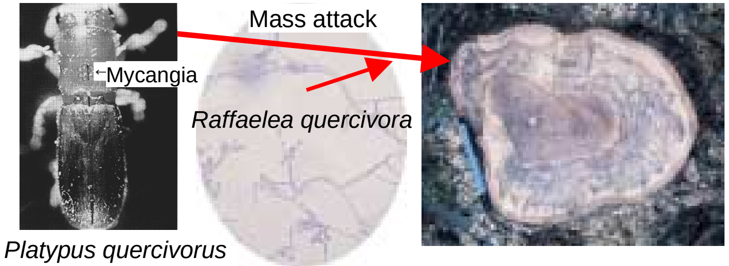


Fig.1 Attack of *P. quercivorus* and *R. quercivora*

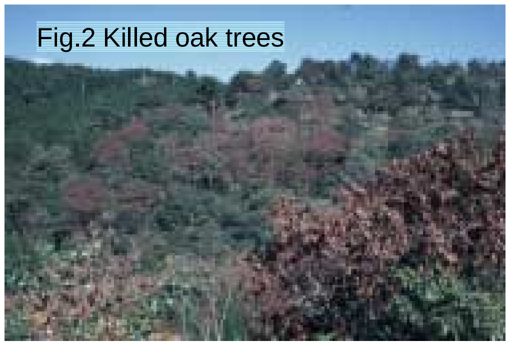


Fig.2 Killed oak trees

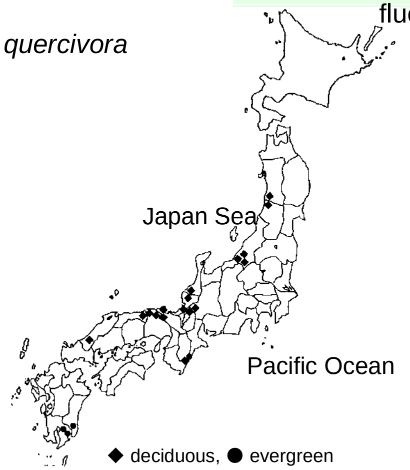


Fig.3 Distribution of the mortality

Materials

1. Sapwood of oak trees surviving natural attack by the beetles
2. Sapwood of wounded *Q. serrata* branches - to elucidate the time-course of defense events

Methods

1. Histochemical test for lignin, suberin, phenol, lipid & pectin.
2. Assay for soluble inhibitory substances

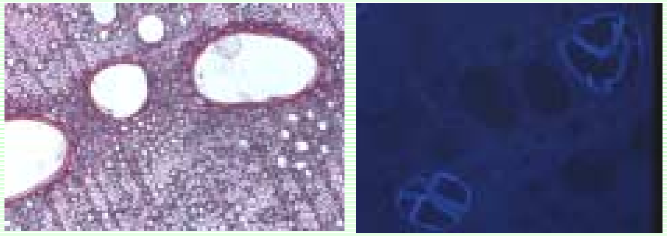


Fig. 4 Formation of tyloses (left) and their suberization (right- fluorescence)

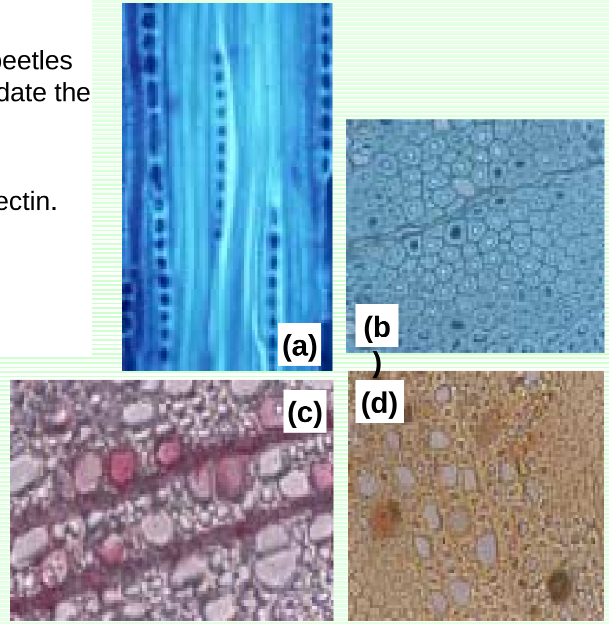


Fig.5 Insoluble deposits (a) fluoresced under UV illumination, (b) stained with Nile blue, (c) stained for pectin, (d) stained for phenolics

Results & Discussion

Naturally infected trees

Suberization of parenchyma cell wall was induced but not conspicuous in reaction zone barrier (RZB). Vessels in RZB were occluded with tyloses, and cell wall of tyloses was well suberized (Fig. 4).

Deposits were found to occlude fibre tracheids and small vessels in RZB. Most of deposits were insoluble to methanol or ethanol-benzene. Deposits in wood fibre region fluoresced under UV illumination and stained red with phloroglucinol-hydrochloric acid (Fig. 5a, 6), suggesting the presence lignin-like compounds. Additional staining tests showed that they consisted of pectin, phenolics, quinones, and lipids (Fig. 5). Major components of deposits appeared to differ between fibre region and tracheid / small vessel region.

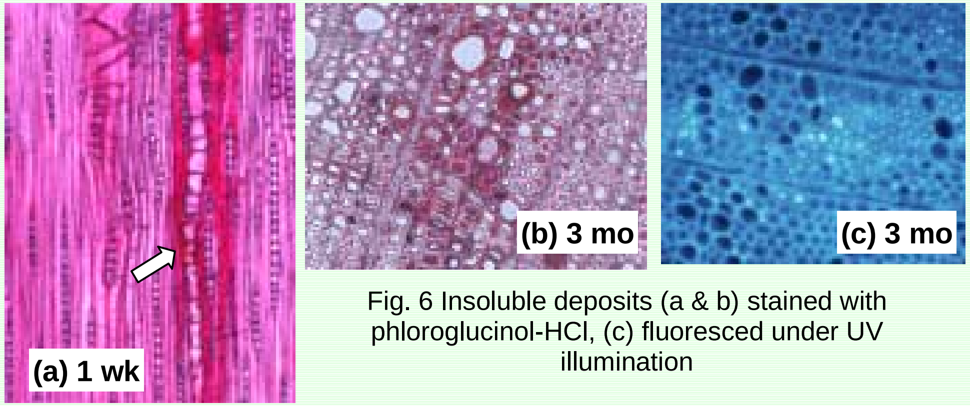


Fig. 6 Insoluble deposits (a & b) stained with phloroglucinol-HCl, (c) fluoresced under UV illumination

Time course of defensive events in wounded branches (Table)

Sapwood of wounded *Q. serrata* branches: Wood fibres were partially occluded with deposits 3 days after wounding. Then, occlusion of fibre tracheids became remarkable to form continuous barrier (Fig. 6,7). Tyloses and their suberization were in progress 3 to 7 days after wounding, and completed after 2 weeks. Accumulation of phenolic compounds was detected histochemically one week after wounding, and became remarkable after 2 weeks.

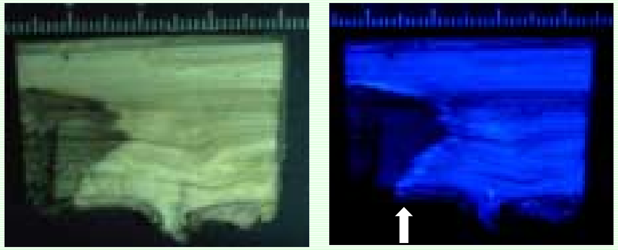


Fig. 7 RZB fluorescence (arrow) under UV illumination.
3 mo after wounding.

Barrier zone

Suberization, phenols accumulation and lignification were observed at barrier zone (Fig. 8).

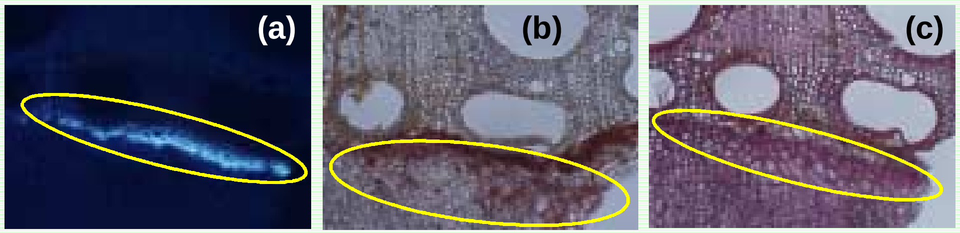


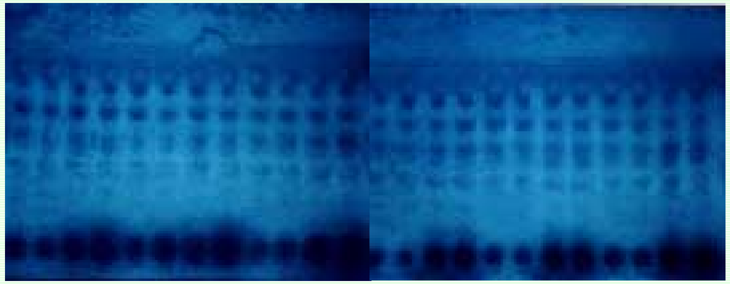
Fig. 8 Suberization (a), phenols accumulation (b) and lignifications (c) at barrier zone

Time course of defensive events after wounding

	3 d	1 wk	2 wk, 1, 3 mo after wounding
Deposits in fibers & tracheids	±	++	++
Tyloses	±(?)	±	+
Suberization of tyloses	−	±	+
Suberization of parenchyma	−	−	±
Phenols accumulation	−	+	++

Soluble inhibitory substances

Accumulation of extractives and soluble antifungal substances was not detected in RZB of naturally attacked trees nor wounded trees (Fig. 9).



C C W W C C W W C C W W C C W W C C W W C C W W
3d 1w 2w 1mo 2mo 3mo
after wounding

Fig. 9 TLC bioassay for soluble inhibitory substances with spore suspension of the pathogen. Inhibitory spots appeared as dark areas.
C, control; W, wounded

Conclusions

- Lack of soluble inhibitory substances accumulation
- Occlusion of xylem components with insoluble compounds - fastest & most conspicuous responses at RZB
- Suberization may be important at barrier zone, but probably not at RZB as an initial response

Future perspectives

- Chemical analysis of insoluble deposits
- Assessment of the role of insoluble deposits as defensive barriers