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SUMMARY OF DOCTORAL THESIS

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Studies on roles of ethylene, jasmonic acid, and salicylic acid in defense reactions to traumatic stimuli in stems of woody species

樹幹の傷害刺激に対する防御反応におけるエチレン、ジャスモン酸、およびサリチル酸の役割

Ethylene, jasmonates, and salicylic acid have been considered the essential components involved in the establishment of disease resistance in a wide variety of plant species. In this study, effects of ethylene, methyl jasmonate and salicylic acid on the induction of physiological defenses against injury and pathogen infection were elucidated in the stems of broad-leaved and coniferous trees. Roles of the potent hormone-induced resistant mechanism in *Quercus* species that were widely attacked by the Japanese oak wilt caused by bark beetle (*Platypus quercivorus*) infection by the vectored fungi *Raffaelea quercivora* was studied in *Quercus serrata* trees. Ethrel (Et), an ethylene releasing compound, methyl jasmonate (MJ), methyl salicylate (MS), sodium salicylate (NS), and mixed combinations of these chemicals were horizontally injected into stems to induce defense responses in *Quercus serrata*. Discoloration of sapwood as a part of physiological defense reactions differed in individual and combination applications of these compounds. Four months after wounding with the application of those chemicals, the extent of sapwood discoloration was observed in tangential and axial directions. The combination of MJ and Et (MJ+Et) induced the greatest discoloration among all treatments. Sodium salicylate (NS) or methyl salicylate (MS) alone increased the discolored area to a lesser degree than did MJ, but defensive responses were obviously more accelerated when the former were added to the latter in the combination treatments. In particular, induced discoloration was noticeably achieved following MJ or Et combined with NS rather than as individual treatments. In contrast, neither salicylate appeared to promote discoloration when combined with the MJ+Et treatment. Wounds challenged with an inoculation by a bark beetle vectored fungus, *Raffaelea quercivora,* developed significantly greater sapwood discoloration than did non-pathogen inoculation, in all directions.

We continued to study the direct evidence of ethylene production in susceptible oak trees damaged by ambrosia beetles and their symbiotic fungi. We found that ethylene production in living and dying stems (survived and killed, respectively) of *Quercus serrata* trees attacked by ambrosia beetles, *Platypus quercivorus*, was compared to production in non-attacked stems. Attack by these beetles is associated with infection by the symbiotic fungus *Raffaelea quercivora.* High levels of ethylene production occurred in stem segments of a survived tree compared with those in non-attacked or killed trees. Attacks were generally concentrated at the stem bases of both survived and killed trees. Earlywood vessels occluded with tyloses significantly increased in the survived tree compared with the non-attacked tree. Current-year wood located close to the cambial region had high ethylene production ability, unlike the inner sapwood or intermediate wood in the non-attacked tree. Such ethylene production was significantly enhanced in the survived tree, which had many fungal hyphae in current-year vessels. The increase in ethylene in current-year wood may be related to the mechanism of induced resistance to pathogen infection in oak trees. Many earlywood vessels occluded with many tylose were observed in the stem of the survived tree, of which ethylene production was vigorous. These results suggest that ethylene production from xylem may be related to tylose formation in vessels in ambrosia beetle-attacked and *R. quercivora* infected *Q. serrata* trees.

In coniferous species, Et, MJ, NS, and combinations of these compounds were applied to unwounded or wounded stems of *Thujopsis dolabrata* cuttings and *Metasequoia glyptostroboides* seedlings to clarify the mechanism of traumatic resin duct formation. Wounding treatment significantly increased the stimulation of resin duct formation in the bark of *T. dolabrata* cuttings over that in non-wounded cuttings of all treatments. The application of MJ, in addition to wounding, clearly induced the formation of abundant traumatic resin ducts. In both unwounded and wounded stems of *M. glyptostroboides*, Et+MJ strongly induced massive tangential resin ducts in the xylem, whereas applications of lanolin (control) and NS showed little effect. The NS application did not promote the formation of resin ducts in either species. Additionally, the application of NS with Et decreased resin duct formation in *M. glyptostroboides*, compared with single Et application. A surprising observation in *M. glyptostroboides* seedlings was the tangential row of plugging cells along the thick boundary of lignified fibres and swelling parenchyma cells in the phloem that appeared after combination applications of Et and MJ, but not single applications. Thus, the Et+MJ application is a strong elicitor that responds to the severity of an attack, leading to an increased magnitude of defence induction in *M. glyptostroboides*. This implies that a complex compartmentalization process, consisting of the activation of large secondary metabolite accumulation in phloem cells and formation of TRDs in xylem tissues, can be concurrently induced to provide an effective barrier to inhibit aggressive invaders. In addition, the combination of Et+MJ+NS resulted in accumulated resin exudation in tracheid cells under the ring of resin ducts of *M. glyptostroboides*, a feature not observed in single Et or MJ applications or combined Et and MJ (Et+MJ) treatment. Salicylic acid may be a subsequent agent that increases the magnitude of or is otherwise involved in the remote defence response following biotic or abiotic attacks on woody species. However, this suggestion remains speculative.

Induction of discolored wood with exogenous applications of potent defense inducers, including Et, MJ, NS and combination of these compounds was investigated to elucidate a formation of agarwood in *Aquilaria crassna*. Single applications of Et, MJ slightly increased the discolored wood on a stems of *A. crassna*. In contrast, Et together with MJ in combination treatments (Et+MJ) acted as a strong elicitors to activate the discolored wood in both *A. crassna* trees and seedlings. Particularly, the discoloration was substantially induced when NS was combined together with Et and MJ (Et+MJ+NS). It is probable that ethylene, methyl jasmonate and salicylic acid may synergistically stimulate the formation of agarwood within the wood of *A. crassna*. This study showed the similar evidence as occurred in the experiment in *Quercus serrata* that the sapwood discoloration in *A. crassna* extends farther in the axial (discolored length) than in the radial or tangential (discolored width) direction after induced wounding and chemical applications. These phenomena can be described by a compartmentalization of decay in trees (CODIT) model. In CODIT model, the vascular system has considered as the weakness component that provide microorganisms or elicitors spreading farthest resulted in the elongated discolored wood in the vertical direction. In contrast, the radial and tangential direction, which are composed of ray cells is strongest compared with the vertical direction. We have also found that the discolored width in *A. crassna* slightly differs among all treatments compared to the discolored lengths. This has been attributed to the relative strength of ray cells that effectively inhibit the movement or spread of invading elicitors. No changes of cell compositions were observed in the wood tissues in *A. crassna* following the treatments. The wood tissues of discolored wood were embedded with deposits that consist of phenol, lipids and fatty acids, indicating by positive tests with ferric chloride, Sudan black B, and Sudan III, respectively.

In overall, an ability to form the sapwood discoloration or TRDs is differed among tree species and by variation of potent inducers that are utilized by host trees to establish the defense mechanisms. Generally, the Et+MJ treatments showed to be the strong elicitor to induce the defense responses in this study.