Pine Wilt Disease and the Decline of Pine Forests

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A Global Issue

By

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PROLOGUE

FAMILIAR ENVIRONMENT NO LONGER: DISAPPEARANCE OF PINE FORESTS

Disappearance of pine forests: A case report

Kyoto the old capital of Japan for 1000 years until 1868, now home to nearly 1.5 million, is situated in a basin surrounded by gently sloping mountains. The Kamigamo Experimental Station of the Kyoto University Forest, where my research began is situated in the northern part of Kyoto. The area of the experimental station is covered with 60% natural secondary forest and has an area of about 50 hectares. About 60 years ago, Japanese red pine (red pine hereafter), *Pinus densiflora*, was the main tree species of the forest, along with cypress and hardwood. Since the mid-1960s, pine wilt disease (PWD) has killed many trees and devastated the forest (Fig. 1).

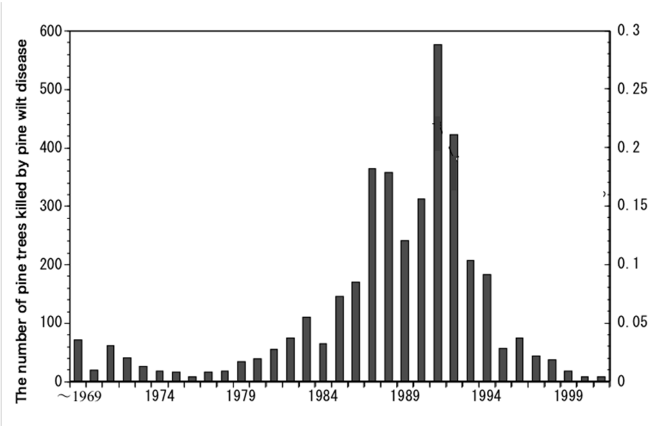

Fig. 1 Annual pine loss at the Kamigamo Experimental Station of the Kyoto University Forest.

University forest officers since 1969 made enormous efforts to control pine wilt disease by cutting down dead pine trees and removing them from the forests each year. However, PWD could not be managed, and the disease spread so extensively that the red pine trees could no longer be found within the experimental station. When I started my studies on PWD in 1977, the forest damage due to this disease was already serious, though many red pines remained in the station. In the 1970s, I enjoyed the relaxing experience of walking and driving through the pine trees. The red pine trees disappeared from the forest and it became almost impossible to find a pine tree even for experiments. How did the red pine forests that were everywhere totally disappear from the experimental station? To answer this question, I studied how PWD had spread in the station. After looking for a stand where the red pine trees were still growing, I finally found one stand.

I counted all the surviving red pine trees at this stand and plotted their locations on a map in 1995. I found 178 red pine trees in a forest area of 1.8 ha. Then I overlaid the distribution of the pine trees killed over the preceding ten years in the area. The study showed that 810 red pine trees were distributed in 1985, suggesting that almost 78% of the trees had been killed by PWD thus far (Fig. 2). All of the university forest rangers were surprised. Even those engaged in the control of PWD every year could not remember the forest being densely covered with red pine trees 10 years earlier. It was hard to understand the progress of PWD without accurate statistics of the live and dead pine trees at the Experimental Station. So I collected the data and made a figure (Fig. 3) showing the reduction in red pine trees and also the change in mortality ratio.

Fig. 3 shows that the mortality rate remained low in the early stage of PWD, then increased and reached a peak (25%) resulting in serious damage. Finally, the rate decreased along with the decrease in the number of surviving trees. The mortality rate of 25% means that one-quarter of the pine trees that were surviving in early summer were dead by late autumn. This severe damage continued in the pine forest from 1992 to 1994 and the numbers of surviving trees dropped to 20% in ten years (Futai, 1999).

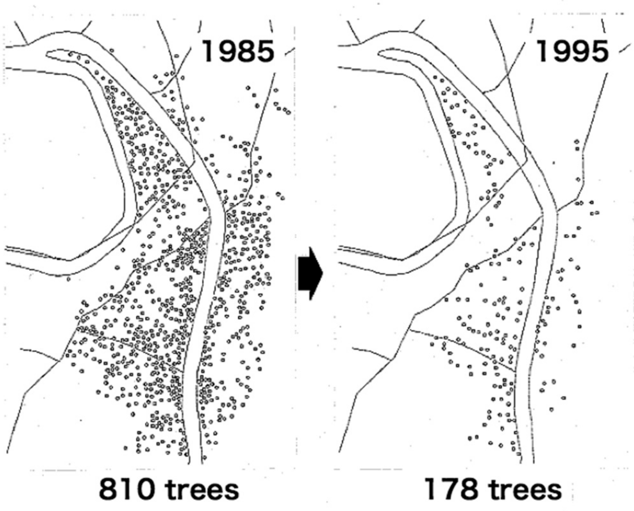

Fig. 2 Almost 78% of the Japanese red pine trees at Kamigamo Experimental Station were killed in 10 years.

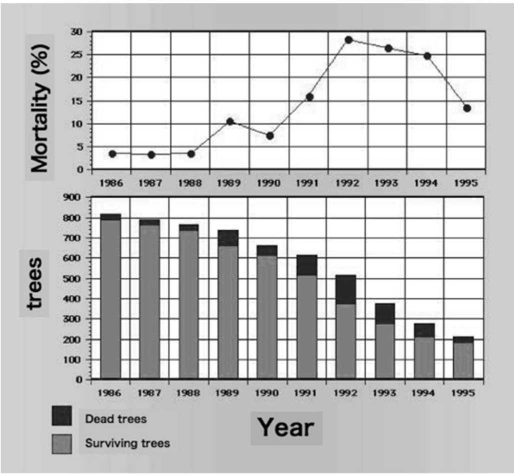

Fig. 3 Changes in the number of surviving and dead pine trees (bottom), and mortality ratio (top) at Kamigamo Experimental Station.

Loss of pine forests

Most of the low mountains surrounding the Kyoto basin were covered with beautiful red pine forests before the serious devastation caused by PWD. Local residents enjoyed visiting nearby mountains, harvesting miscellaneous trees (bush) for their rituals, and collecting edible mushrooms such as the precious pine mushrooms, matsutake, in autumn. However, the red pine forests of the mountains have almost disappeared. Being one of the world's leading forest countries, with forests covering about two-thirds of the total country area, 251,000 square km, and 10% i.e., 21,000 square km are said to be woods composed of the red pine or black pine, *Pinus thunbergii*. However, more than a quarter of the trees have been affected by PWD. Therefore, it is difficult to estimate how many healthy pine forests remain. A survey of the production of matsutake by a forest officer over a wide area of red pine forests in Kyoto Prefecture revealed that there were more than 1000 square km of pine tree-dominated forests in Kyoto Prefecture. However, most of such pine forests were devastated by PWD and replaced with various species of broad-leaved trees. The surviving JRP trees appear to be buried in the broad-leaved forest. Primary pine forests dominated by red pine and black pine decreased sharply to less than 50 square km. In other words, it had decreased to one-twentieth of the area in 30 years. However, this dramatic decrease in pine forests was not recorded in the annual governmental report probably due to the government's inadequate knowledge about the decline of pine forests.

For example, according to the governmental report, the area of man-made pine forests in Kyoto Prefecture in 1990 was about 100 square km, which was similar to that in 1980. However, the area with pine trees killed by PWD during those ten years was at least 400,000 cubic meters (equivalent to about 1,200,000 pine trees). This discrepancy may have been due to the way the area was recorded: thee forest with at least one surviving pine tree was recorded as a pine forest irrespective of the number of remaining pine trees. Therefore, the real damage and loss of JRP could not be grasped.

When the number of dead pine trees first appearing as brown spots on the green mountainside, and the discolored pines started covering the whole mountainside (Fig. 4), people finally realized something undesirable was happening and became concerned. In Japan, the mild climate conditions prevailing from spring to summer and high precipitation during the rainy season accelerate the replacement of dead pines with other tree species. Within a few years, only the skeleton of the dead pines remained leaving stems and branches on the forest floor, and soon even the bleached trees were swallowed in the sea of green trees. Before long, people forgot that many of their nearby mountains used to be covered with red pine trees or black pine trees.

Fig. 4 A number of dead pine trees appeared as discolored spots on a mountainside. (photo courtesy of Dr. Furuno, T.)

Pine forests spread with human activity

Until PWD became an epidemic, pine trees had been growing on many nearby mountains and were considered a part of the natural scene. I wondered if it was also so in ancient times. It is possible to determine the species of plants growing in the area and their dominance by identifying the species and the abundance of the pollen buried in the stratum.

This field of study is called Palynology. A palynologist reviewed the results of pollen analyses reported from various districts in Japan and presumed that the red pine and the black pine began to propagate roughly 1,500 years ago, and finally became dominant in the Japanese vegetation 500 years ago. When paddy rice cultivation began 3,000 years ago in Japan, the population in those days was sustained with sufficient food obtained from naturally available paddy fields. A stable food supply achieved through agriculture brought about a boom in the population and then urged the expansion of paddy fields by reclamation of the surrounding forests.

The inhabitants cleared the forests covering mountain slopes by cutting and burning trees for 1,500 years. Then they started intensive slash-and-burn agriculture by cultivating buckwheat, millets (foxtail millet, sorghum), beans, and tubers. After several years of slash-and-burn cultivation, soil nutrients were exploited. The agricultural land with poor soil was abandoned, and this accelerated the invasion of red pine trees. The red pine trees have symbiotic relationships with mycorrhizal fungi on their fine roots and thereby can grow on land with poor soil (the Mycorrhizal symbiosis is described later). The accumulation of fallen leaves makes the soil nutritious. As a result, saprophytic fungi begin to spread. Therefore, the symbiotic mycorrhizal fungi colonizing the roots of pine trees are suppressed by the active saprophytes. When the mycorrhizal symbiosis is compromised, water and nutrient availability is reduced and the host pine tree is replaced by the surrounding competing tree. Without human intervention, the red pine forest eventually becomes a climax forest such as an evergreen broadleaf forest.

Red pine trees flourish on mountainsides where the surface is exposed after deforestation and in abandoned wastelands surrounding newly-developed farms. Pollen of the red pine trees exceeded 50% of all pollen examined from about 500 years ago. This was the time when the techniques of agriculture such as the two-crop system, use of fertilizers, and equipment in irrigation facilities developed rapidly.

The introduction of the monetary-based system promoted the cultivation of commercial crops. Fallen leaves were collected for use as compost for the fields, which accelerated the exploitation of the forest. This made the site more comfortable for the red pine, which resulted in its dominance.

The Edo period or period of Tokugawa Shogun continued for 265 years from 1603 until the transition of power to the Meiji emperor, in 1868 when the capital was moved from Kyoto to Tokyo. The Tokugawa government promoted the development of new paddy fields, and the total area of paddy fields in Japan nearly doubled by the early 18th century. This reclamation of land for agriculture undoubtedly led to the destruction of the forests. On the other hand, various projects were carried out to protect coastal regions from erosion including the planting of Japanese black pine trees in different regions in the 17th century. Thus, man-made black pine forests gradually took over Japan's coastal lines.

The first record of Pine Wilt Disease

PWD devastated the flourishing red pine and black pine forests. The first record of the PWD in Japan dates back to 1905. In 1913, an entomologist, Munemoto Yano, reported the occurrence of withered pine trees in the vicinity of Nagasaki city (①: see Fig. 5). The aspects of the pine disease, such as symptoms, seasonal symptom development, and spreading manner of the disease, he reported are totally in accordance with what we currently refer to as the pine wilt disease.

At almost the same time, another record of high mass mortality involving pine trees was reported in Fukuoka Prefecture (②) in Kyushu. A few years later, a large number of black pine trees that were planted along Fukiagehama Beach in Kagoshima Prefecture (③) to safeguard against salty wind died suddenly for unknown reasons. In 1915, many old pine trees were killed at a shrine in Ako City (④), Hyogo Prefecture, and massive pine extinction spread to the mainland. In the 1920s, the pine forest damage gradually spread into surrounding prefectures in southwestern Japan.

During World War II, Japan's forests became seriously devastated and became a hotbed for the sudden increase in pine tree mortality after the war. There are several possible explanations for this sudden increase. The access to the pine forests surrounding naval ports and other military installations was severely limited. Moreover, the dead pine trees were left in the woods without any treatment and might have become an infection source. However, the major reason was the abandonment of steady efforts to keep their own mountain clean due to the lack of labor and harsh living conditions during the war.

Fig. 5 Early pine wilt occurrence sites in southwestern Japan.

PWD-damaged pine logs were moved from place to place with the movement of a large number of pine logs during and after the war. As a result, PWD spread north to over 27 prefectures from Kyushu to the Kanto area. The annual loss of pine trees caused by PWD reached 720,000 cubic meters (equivalent to about 2,000,000 trees). The General Headquarters of the occupation forces (GHQ) became concerned about the severity of PWD and were worried that the devastating scene of the pine trees in the mountains might further upset the Japanese people, already depressed in defeat. Therefore, the GHQ summoned an American entomologist, Dr. R.L. Furniss (USDA) to investigate the damaged forests and submit a proposal on the control of the disease. The method ultimately suggested was simple: cut down the dead pine trees, peel off their bark, and burn the bark. The forest agency of Japan carried out this powerful PWD control under the authority of GHQ. The result was remarkable, and the spread of PWD stopped after 1950. This simple method achieved remarkable success because of social situations and abundant availability of labor. Each local government thoroughly implemented the method under the absolute authority of GHQ, and the dead pine logs sold as firewood, like hotcakes.

How pine forests became seriously devastated

Japan was in a post-war reconstruction period from 1945 to 1955. Later, Japan celebrated a period of rapid economic growth, with a significant change in lifestyle, which rapidly promoted the fuel and fertilizer revolution.

People used to go to the red pine forests in the nearby mountains and scrape the floor to collect fallen needles, which were useful as fuel or fertilizer. The forest floor of the red pine forests was generally without litter sedimentation and thus poor in soil nutrients. Thereby, ecological succession became stagnant and maintained the pioneer red pine forests at the subclimax condition. As new fuel and fertilizer became available, fallen pine needles accumulated on the forest floor, resulting in the eutrophication of red pine forests.

The decline of the precious Matsutake production could be ascribed to this eutrophication of the red pine forests. On the other hand, the red pine and black pine trees were used as raw materials for pulp production. Large-scale pine afforestation was carried out, and the area of pine forests increased rapidly from the end of the 1920s to the 1930s.

However, broad-leaved trees began to be used as a natural material for pulp production in place of pines, and the stronger yen currency stimulated the import of much cheaper material (wood). The economic value of the pine forest was thus reduced and the forests were left unmanaged. The increase in the abandoned and/or eutrophicated pine forests that appeared in this period were unsuitable for the growth of the red pine trees, which are adapted to poor soil conditions. The red pine trees were constantly challenged with physiological stresses because of competition with broad-leaved trees for water and nutrients. These stresses reduced the resistance of pine trees against pests and pathogens, and damage of the pine wilt became widespread and severe.

Before the epidemic of pine wilt, the pine accounting for total timber production was almost the same as those of Japanese cedar and cypress. However, the timber production of pines decreased with the severity of pine wilt damage. This is clearly shown by the comparison of the proportion of the pine trees cut for total timber production in the areas where pine wilt damage is severe compared with that where the damage is still small. For example, the damage caused by pine wilt during the 10 years from 1986 to 1995 was 410,000 m^3 in Hyogo Prefecture in western Japan, but it was 97,000 m^3 in Iwate Prefecture in northeastern Japan where the damage was still small. In this case, the proportion of pine trees in total timber production in Hyogo dropped to less than half, but there was almost no change in the production in Iwate Prefecture (Fig. 6).

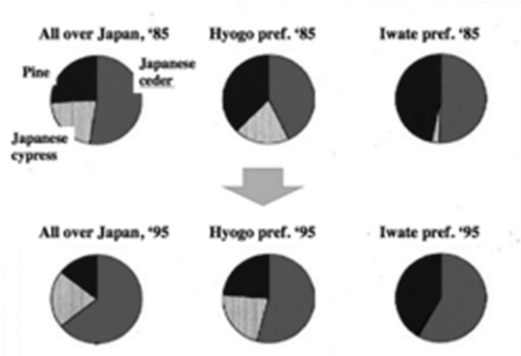

Fig. 6 Changes in the proportion of three major tree species in total timber production from 1985 to 1995.

I. EXPLORING THE TRUE CAUSE OF MASSIVE PINE DEATH

1. So-called "Pine-eating beetles" – Relationship between wood-boring beetles and trees

How "Pine-eating beetles" received this name

When the dead pine trees are cut, the bark falls off and exposes a large number of insect larvae and various tunnels they made (Fig. 7).

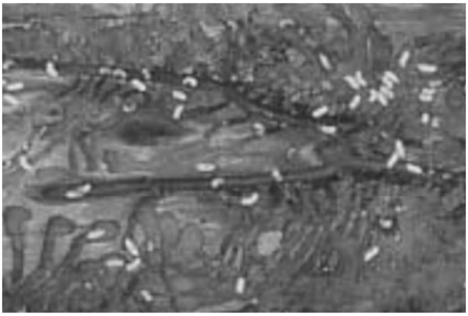

Fig. 7 Many larvae and tunnels of beetles under the bark of a tree.

Most of the insects called wood-boring beetles belong to several species and families. The most common three families are longhorn beetles, bark beetles, and weevils (Fig. 8). These insects bore holes, make tunnels under the bark, and then move into the wood. They then feed on wood tissues and/or fungi growing on them.

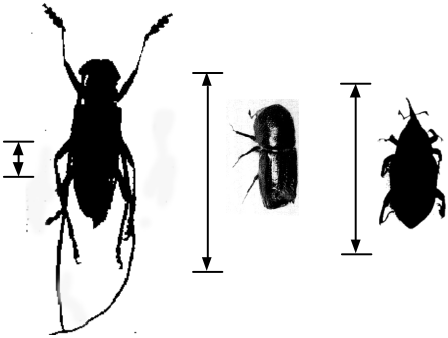

Fig. 8 The most common wood boring beetles (from left to right, a longhorn beetle, a bark beetle, and a weevil). Arrows mean 1 cm in length.

Since most of the dead pine logs harbor numerous wood-boring beetles, they were considered as a causative organism of the massive death of pines. Scientists ascribed pine death to these insects and targeted them for disease control before and after World War II.

Even 40 years after the nematode was identified as the true causal agent of the disease, the Japanese media still blame the "matsukuimushi" (Japanese word for pine eating beetle) as the cause of pine wilt disease damage. However, "matsukuimushi" is neither a scientific Japanese name nor a scientific entity, but merely a common name for the insects infesting dead pine logs. Incidentally, this word was first used by a newspaper reporter, who attended a meeting on pest control of pines held in Hyogo Prefecture, in 1941. Why did so many researchers consider "matsukuimushi" as the causal agent of the massive pine death? For a long time, whether or not the beetles damaged the living trees remained a controversial issue.

Primary forest pests and secondary forest pests.

A tree pest beetle known as the sugi* bark borer (Fig. 9 left) causes serious damage to Japanese cedar (*Cryptomeria japonica*) and Japanese cypress (*Chamaecyparis obtusa*) trees (Ito and Kobayashi, 1991). Female beetles insert their ovipositor through cracks in the bark of healthy cedar or cypress trees and lay their eggs. The larvae hatch after 8 to 18 days and stay for a while in the outer bark, then go through the inner bark and feed on the cambium tissues. When attacked, the healthy host trees exude resin to cover the wounds made by the beetles. The larvae might be hampered by the sticky resin and thereby might be killed. The beetles that can break through active host resistance and damage healthy trees are called primary pests, while those that can attack only trees or logs with reduced resistance such as withering or felled trees are called secondary pests. Wood-boring beetles can be classified into these two categories.

Fig. 9 Tree pest beetles. Left: Sugi* bark borer (*Semanotus japonicus*), Right: Spruce bark beetle (*Ips typographus*)

* Sugi is the Japanese name for *Cryptomeria japonica*

However, when the roots are cut off due to strong typhoon winds or their leaves are consumed by an abnormally high number of herbivores, many trees become weakened or die. Then the numbers of secondary pests increase abnormally and begin to attack the surrounding healthy trees. This phenomenon is called “a phase transition from secondary to primary pest”. Most of the wood-boring beetles are secondary pests, but such phase transitions have been reported for several species of beetles. Spruce bark beetle (*Ips typographus,* Fig.9 right), a famous pest insect of Yezo spruce (*Picea jezoensis*) and Sakhalin fir (*Abies sachalinensis*) in Hokkaido island, is principally a secondary pest. However, this beetle becomes a primary pest when a large number of trees are felled by strong wind or when the tree logs are left at a felling site (Furuta, 1989). Then, what is the cause of the difference between the primary and the secondary pests?

Tree wood is composed of three polymers; cellulose, hemicellulose, and lignin. All of these polymers, mainly made of carbon, oxygen, and hydrogen, are indigestible and persistent. However, wood contains little nitrogen, which is essential for organisms to produce protein. The ratio of carbon to nitrogen called the “C/N ratio” is an indicator of the quality as a food resource, production efficiency, and decomposition degree.

Generally, the quality as food is in parallel with the nitrogen ratio, a lower C/N ratio means higher food quality. For instance, fallen leaves have a low C/N ratio ranging from 40 to 170 depending on the tree species. Tree wood has a higher C/N ratio, ranging from 350 to 1,250 (Cowling 1970). Generally, wood contains lots of carbon, but hardly any nitrogen. Therefore, tree wood is not a useful food resource. Even the wood-boring insects cannot live on wood alone as a dietary source and have overcome this challenge in several ways.

A strategy of feeding on living tissue
– In the case of bark beetles –

Bark beetles, a group of wood-boring beetles eat the inner bark, that is, living tissue, including the cambium. These living tissues contain cytoplasmic matrices and nutrients. However, the host trees do not expose their important tissues unnecessarily to pest attack; they have accumulated repellant or toxic substances such as monoterpenes and/or polyphenols to protect themselves from beetle attacks.

Resin secretion is the most effective defense mechanism that coniferous trees have. The larvae of Sugi bark borer feeding on inner bark tissues are captured by the sticky resin, and thereby, most of them are eliminated. To overcome this host resistance, some primary bark beetles have developed a strategy called “mass attack,” in which a large number of beetles attack a host trunk together (Fig. 10 top right). The aggregation pheromone, which is a signaling substance, makes the mass attack possible (Clark et al. 2012).

A few beetles stay under the bark of the host tree and release the aggregation pheromone. Due to the release of the pheromone, numerous beetles of the same species are attracted to the tree resulting in a mass attack. In a mass attack, the amount of resin each beetle is exposed to is reduced thereby resulting in reduced resistance. Thus, it becomes possible for bark beetles to feed on nutritious inner bark tissues without being exposed to the dangerous host resin. The mountain pine beetle (Fig. 10 top left) in western North America and the southern pine beetle in the southern USA are known to have adopted this strategy.

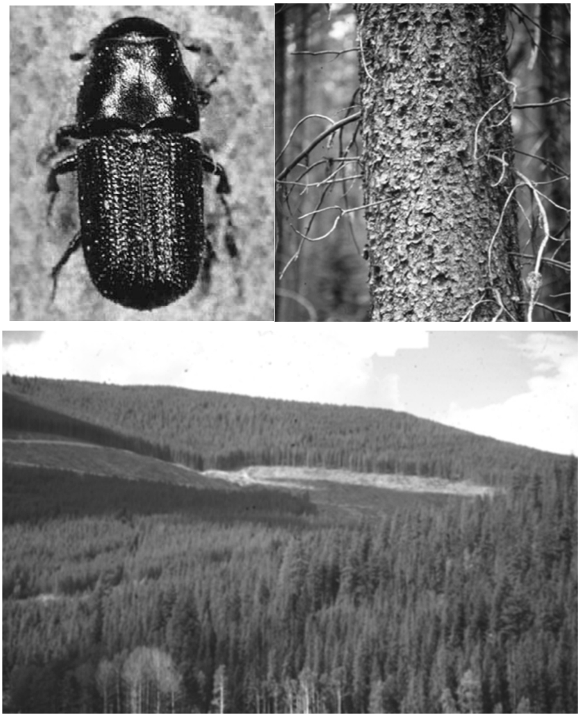

Fig. 10 The mountain pine beetle (MPB). Top left: adult beetle, top right: the trunk of a lodgepole pine with mass attack by MPB. The bottom figure: wide areas of Canadian lodgepole pine forest damaged by MPB. *See centerfold for this image in color.*

This group of beetles also adopted a distinct strategy of cooperating with pathogenic fungi to suppress the resistance of the host tree (Safranyik et al. 1973). Blue-stain fungi belong to a group of Ascomycetes that attacks sapwood at a relatively early stage after the tree dies and discolors the sapwood blue and black. The mountain pine beetle, for example, keeps blue-stain fungi such as *Grosmannia clavigerum* or *Ophiostoma montium* in sac-like mycangia on its maxillary and transports the fungi to new host trees (Whitney and Farris, 1970). The fungus that is carried depends on the species of the beetle.

Fungi moving into the host trunk by the beetle, attack the surrounding living tissues, spread over the sapwood in one month, and inhibit water conduction within the host stem. Thus, blue-stain fungi suppress the host resistance and provide the beetle a suitable breeding ground, while the beetles ensure consistent transport and continuity of breeding for the fungi (Yamaoka, et al. 1995).

There are “three major forest diseases in the world” that appear in textbooks on forest pathology. They are Dutch elm disease, white pine blister rust, and chestnut blight, and these three forest diseases have caused severe damage to indigenous trees in newly introduced locations.

Dutch Elm Disease

Generally, indigenous trees have never encountered such diseases, and consequently have no resistance to them. Among the three major diseases, *Ophiostoma ulmi,* the pathogen of Dutch elm disease, was transported to the new host tree by some bark beetle species (3 domestic species in Europe, an invasive European, and a domestic bark beetle in North America), and resulted in an epidemic (Fig. 11).

Fig. 11 Dutch elm disease. Top: An elm tree damaged by Dutch elm disease, Bottom lefr: European elm bark beetle (*Scolytus multistriatus*), Bottom right: spores (conidia) of *Ophiostoma ulmi*

Therefore, the relationship between the blue-stain fungus (*O. ulmi*) and certain bark beetles is similar to that between the blue stain fungus and North American pine beetles, such as mountain pine beetle and southern pine beetle. However, the main tactic of attacking healthy trees by these beetles is the behavior of the beetle itself, “mass attack”, while Dutch elm disease, the blue stain fungus with strong pathogenicity kills the trees, and ensures the propagation of the next generation of the vector beetles.

Bark beetles in Dutch elm disease were found to rely more on the accompanying blue-stain fungus than these beetles. Thus, the spread of Dutch elm disease is strongly influenced by the pathogenicity of the accompanying fungus. *O. novo-ulmi*, which is more pathogenic than *O. ulmi*, emerged during the disease spread and accelerated the expansion of damage in Europe. Meanwhile, in North America, the pathogenic fungus was brought in with elm logs, in the 1930s. Later, a new American strain of blue-stain fungus appeared and intensified the damage.

The American strain of blue-stain fungus enhanced the pathogenicity re-landed from North America to Europe and killed numerous elm trees by overwhelming the host resistance that had been produced by long breeding efforts.

Bark beetles that did not adopt the tactic of “mass attack”

Bark beetles that lacked the habit of mass attack are considered a secondary pest. The number of bark beetles increased abnormally and attacked the surrounding healthy trees due to large amounts of withering trees because of strong winds or an outbreak of herbivorous insects. This is mentioned above as “a phase transition from secondary to primary pest”. Spruce bark beetles (*Ips typographus*) and larch bark beetles (*Ips cembrae*) are secondary pests, but *Ips* beetles have stronger primary pest traits than other secondary pests. These bark beetles also are deeply related with the blue-stain fungus. Still, they do not have the special organ, “mycangia”, to carry their partner fungus, suggesting that they do not have the intimate relationship that the primary bark beetles have.

Beetles that use mushrooms as partners for nutrient absorption.

Most animals that have no wood-degrading enzymes cannot utilize wood as a carbon resource. On the other hand, many wood-rotting fungi can use persistent substances of wood. White-rot fungi can degrade cellulose, hemicellulose, and lignin to the same degree, while brown-rot fungi can degrade only cellulose and hemicellulose. Scarlet bracket-fungus, purple pore bracket, artist’s bracket, and the like belong to the former group, while train wrecker and rusty gilled polypore to the latter group.

Fungi inhabiting the wood compensate for nitrogen deficiency by incorporating nitrogen from the outside of the wood by expanding their mycelia, or some fungi, like the Oyster mushroom, capture nematodes using their specialized mycelial traps and use them as nitrogen resources (Thorn and Barron 1984). Some bacteria with nitrogen-fixing ability also inhabit the wood by taking advantage of their capabilities (Griffiths et al. 1993: Perry 1994: Crawford et al. 1997). Some insects obtain carbon from wood material degraded by fungi, and nitrogen from fungal mycelia or bacteria.

By using their mycelial network, fungi inhabiting wood accumulate nitrogen and phosphorus that are present in extremely small amounts in the wood and concentrate them in their cells. Insects feeding on decaying materials absorb nutrients concentrated in these microorganisms at the same time. For instance, the larvae of giant stag beetles (*Dorcus hopei*), which is a popular pet for children, feed on the used mushroom cultivation logs (Araya 1993) (Fig. 12). Other beetle larvae that feed on rotting wood such as longicorn beetles and weevils also make use of the wood with these microorganisms.

Fig. 12 In the rotten wood, the larvae of giant stag beetles (*Dorcus hopei*) (left) feed on the mycelia of white-rot fungi such as turkey tail (*Trametes versicolor*) (right).

Avoiding the scramble for food – In the case of ambrosia beetles

Many wood-boring beetles called bark beetles utilize living tissues under the bark which is exceptionally rich in nutrients. However, the life under the bark is very competitive for common nutritive tissues among various species of insects. The part under the bark is close to the surface and easily exposed to parasitic insects' attack from the outside. It is safer deep in the wood than right under the bark, but nitrogen deficiency is severe.

The insects that utilize rotting wood overcome nitrogen deficiency by eating nitrogen-rich microorganisms along with the wood. However, they must fight with competitors and natural enemies present in that environment. Fresh wood of the declining tree, on the other hand, is a safer environment with far fewer competitors and natural enemies. The "Ambrosia beetle" has adopted a more advanced way of utilizing a safer space deep in the wood of the declining tree. These beetles bring bait fungi deep into the wood and cultivate them as food and overcome the problem of nitrogen deficiency.

Wood-boring beetles include about 7,000 species of Family Scolytidae and about 1,500 species of Family Platipodidae. Among them, adults of all species of Platipodidae and 10 genera of Scolitydae have storage organs (mycangia) on specific parts of their bodies and keep spores of bait fungi. These beetles dig tunnels deep into the wood of the withering tree, inoculate the spores on the tunnel wall, and cultivate them for their progeny (Endoh, R. et al. 2011). The fungi that the next generation larvae feed on are called ambrosia fungi, and these wood-boring beetles are called "ambrosia beetles" (Fig. 13).

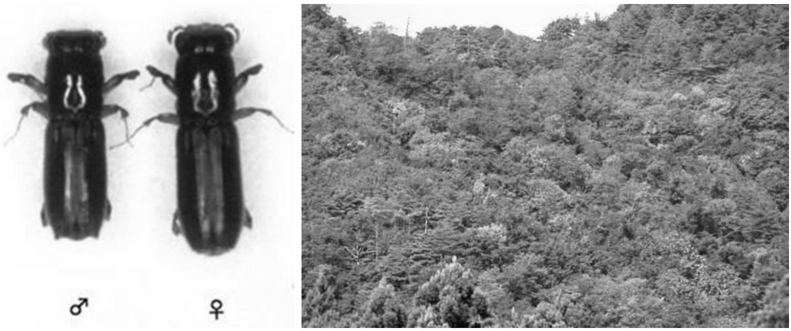

Fig. 13 The oak ambrosia beetle (*Platypus quercivorus*) and the dieback of oak trees by the infection of a pathogenic fungus, *Raffaelea quercivora*, vectored by the beetle. *See centerfold for this image in color.*

The word "ambrosia" coming from a Greek myth, means "food of God," for immortality.

Besides the ambrosia beetles, several groups of insects, such as wood wasps (horntail), leafcutter ants, and fungus-related termites, have also evolved their fungus-cultivating systems.

2. New developments in clarifying the cause of PWD

The beetle falsely accused of causing PWD

My story has departed considerably from the main subject. The conflict among researchers on the relationship between the beetles and fungi lies in the beetle's ability to kill trees. Researchers were suspicious, but could not ignore the general opinion that wood-boring beetles are responsible for killing pine trees, and have been conducting extensive studies. The above-mentioned examples suggested that wood-boring insects might weaken the pine trees to the point of death. Thus, the suspects were considered to be the insects living in the pine trees just starting to show wilting symptoms. Based on this, eight wood-boring beetles were indeed listed as the candidates for possible causal agents of massive pine death in 1942. They are four Scolytidae beetles (bark beetles), three Curculionidae beetles (weevils), and a Cerambicidae beetle (Japanese pine sawyer). Since then, there were conflicting opinions over the causal agent of the massive pine death between two opposing points of view. Some scientists regarded the pine-wood boring beetles as being primary insect pests, while others regarded them as secondary pests that laid their eggs only on such pines that had been weakened due to other causes.

Several experiments have been carried out to answer this question. Three species of insects whose eggs and/or larvae were frequently found under the bark of the dead pine trees in most of the devastating pine forests were studied. For instance, a species of pine bark beetle (*Cryphalus fulvus*), a species of pine bark weevil (*Shirahoshizo* sp.), and the Japanese pine sawyer (*Monochamus alternatus*) were forced to lay their eggs on the healthy pine stems, or their eggs were artificially inoculated under the bark of a healthy pine stem, to examine their harmful effects.

The results of the experiments, strongly suggested that these pine bark beetles are not the primary pests, but typical secondary pests and cannot kill healthy pines. That is, “The beetle was falsely accused.” The studies on the causal agent of pine wilt disease had to return to the starting point of basic research.

New Clues – What field research revealed

In 1964, researchers at the Japanese Forest Experiment Station established a 2.4 hectares survey area in a national forest within the Chiba forestry office’s jurisdiction. More than 1800 pine trees were planted in this survey area for researchers. After observation of the seasonal changes, disease symptoms of the trees, and the feeding marks of wood-boring beetles, 300 trees were cut down in the following July, September, and October of 1965. The amount of resin exudation and the degree of damage by wood-boring beetles in each tree were examined. Focusing on the exudation amount of resin, many trees seemed healthy, but their resin exudation on the cut surface of their stumps was markedly reduced.

Moreover, the trunks of more than half of the trees were not infested with wood-boring beetles. Many such trees with the decline of resin exudation, were ill before the attack of wood-boring beetles. Thus, the hypotheses on the massive pine deaths to the wood-boring beetles had to be fundamentally reviewed. A research project team started work in 1968 to reveal the actual causal agent of the enormous pine death. Not only entomologists but also tree pathologists, tree physiologists, and pedologists, were involved in this team.

Launch of research project

The research of this team were summarized in the interim report in the “Forest Pests” vol. 9 (1970). According to the report, the following studies were carried out to elucidate the causal relationship between the attack of wood-boring beetles and the physiological disorder of pine trees: (1) Surveillance on the amount of damage and the change in the pest insect fauna in the specific research forest, (2) survey on the roots of pines, (3) survey on the infesting microorganisms (4) study on the influence of the blue-stain fungus that infest pine stems, and (5) studies on the influence of chemical and physical traits of soil, and that of meteorological factors on the occurrence of pine wilt.

To determine which conditioned pine trees are vulnerable to the attacks from wood-boring beetles, this research project team established a simple method for examination of tree health by utilizing the amount of resin exudation from artificially-made wounds and studied the changes in the physiological functions and the chemical tree constituents after withering.

Also, this team studied a pre-treatment method for victim pines against the attack of wood-boring beetles and a regeneration method after massive pine death. The scientists switched the research targets from wood-boring beetles to other unknown factors and widened their research net for both biotic and abiotic factors. This brought a big transformation from the fixed regime of research teams composed of entomologists to a large project team involving scientists from various fields.

Many tree pathologists who joined this research project assumed that microorganisms must be the real causal agent of the massive pine death. They especially concentrated their efforts on examination of micro-organisms isolated from withering pines in which resin exudation just began to decrease or had ceased, and thereby attracted wood-boring beetles for egg-laying on their stems.

Koch's postulates

Sample materials were obtained from various parts of the tree for investigation. Small pieces were cut, placed on the nutrient medium, and various fungi and bacteria grew out of the small pieces of wood after incubation. Then, such microorganisms detected from withering pines were selected, and each of them was transferred aseptically to another medium one by one (known as "**isolation**"). By observing the microorganisms thus isolated under a microscope, and examining the color and morphology of the colonies formed on the medium, or investigating the nutritive requirements based on growth on various media, microbiologists can determine the species of the organisms ("**identification**"). When a microorganism is reported to be a pathogenic microorganism or closely related species, then its pathogenicity is examined by inoculating it onto pine trees. This series of operations is based on the rules to prove that the discovered microorganism is a pathogen. This method initially developed by the German microbiologist H. Robert Koch the founder of modern bacteriology who identified the causal agents of tuberculosis, anthrax, and so on, is called **Koch’s postulate**

As the next step in Koch’s postulate, researchers had to examine the pathogenicity of a microorganism by inoculating it to the host organism and confirm the disease development (“**confirmation of pathogenicity**”). Then they tried to re-isolate the same species of the microorganism from the diseased host (“**re-isolation**”). (The last postulate was added by US plant bacteriologist, E. F. Smith). Japanese researchers participating in the project were vigorously exploring pathogenic microorganisms according to the old well-known postulates.

In the course of exploring the real causal agent of the massive pine death, tree pathologists discovered several candidates of pathogens in its aboveground parts, such as leaves and branches. Still, these were not involved in the withering of pine trees observed in the field. From roots, pathogenic fungi such as *Rhizina undulata* (pine fire fungus), *Cylindrocarpon* sp., *Armillaria mellea*, and so on were detected. These fungi were found less frequently in the forests devastated by the pine wilt, so they were not considered to be true pathogens of the pine wilt.

Rhizina undulata (Fig. 14), for example, is resistant to high temperatures and propagates rigorously in the soil after a bonfire or fire in the pine forest, causing the successive death of pine trees. However, the expansion of pine death is limited within the area of fungal concentric development. It is also different from the peculiar spreading pattern of the pine death due to the pine wilt disease where the withering disease spreads out was from tree to tree and from forest to forest.