PREFECTURE OF IBARAKI

HORTICULTURAL EXPERIMENT STATION
AMI-MACHI INASHIKI-GUN
IBARAKI-KEN

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Mr. Fred Hebard
Virginia Polytechnic Institute
and State University
Blacksburg, Virginia 24061

Dear Mr. Hebard

Thank you very much for your **kind** letter and copy of translation of chestnut **Slight** bulletin, I **am** sorry for **the** deley in writing back. I have extended to write ever since then but I have been very busy.

I consent to your opinion on periderm and epiderms. In Japan, as follows.

外皮組織 tegumentary tissue
- 初生外皮組織 primary tegumentary tissue **(epidermis)**
- 次生外皮組織 secondary tegumentary tissue
  - 周皮 (periderm)
  - コルク層 (cork layer)

The wound periderm consists of two parts. The outer part is wound cork layer composed of several cork cells, and the inner part is the wound phellogen. The wound cork cells are always thin walled and folding **in** five or six rows, and they are larger in size than those of the underlying wound phellogen which actively produces the *cork* cells **upwards** as seen in the case of normal phellogen of bark.

Expressing my thanks for your opinion.

Enclosed are a few of my recent gapers on the chestnut disease.

Yours sincerely,

Kazuma Uchida

Kazuma Uchida

STUDIES ON ENDOTHIA CANKER OF JAPANESE CHESTNUT TREES CAUSED BY ENDOTHIA PARASITICA (MURRILL) P. J. ET H. W. ANDERSON

by

Kazuma Uchida

Forword

Cultivation of chestnut for nuts has been carried out in wide areas of Honshu, Shikoku and Kyushu but not Hokkaido. Its history is old, but during and after World War II, the chestnut gall wasp caused great damage, leaving chestnut orchards in ruin and endangering chestnut cultivation. Fortunately, the damage by gall wasp has decreased with a subsequent increase in chestnut cultivation.

Cultivation of chestnut is also worldwide; carried out in China and the Korean Peninsula in Asia and the United States and Europe. International exchange of nuts and seedlings is frequent.

At the beginning of this century, chestnut blight was discovered in the United States and spread rapidly. In Japan, the controversy as to the origin of the pathogen arose around 1915, when Hara (1915) first collected samples of blight. Later, Tsuji (1926) observed that blight was widespread in Honshu and Kyushu. Even, then, damage by chestnut blight appeared to be a problem in some chestnut growing areas. During the period of rapid industrial growth after World War II, advances in cultivation technology caused production of superior nuts, which increased the profitibility of the crop. At the same time, there was a move to control chestnut blight and other major diseases. Although many studies were done on chestnut blight, the majority of them dealt with the physiology and ecology of the pathogen, with little attention to control.

This is a report on studies started in 1961 at the Ibaraki-ken Horticultural Experiment Station (Ami-machi, Ihashiki-gun, Ibaraki-ken).

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The present Director of the Ibaraki Station, Dr. Sakae Iida, gave support essential to printing and publishing this bulletin. I would like to thank all these people.

Literature review

Chestnut blight is reported to have first been described by Merkel in 1904 in New York City, USA. The disease rapidly spread throughout North America, causing devastating damage. In Japan, incidence data were first collected by Hora in 1915, Nambu in 1918, and Tsugi in 1926. They found the disease to be widespread throughout the country (25).

The pathogen first was reported to be in the genus Diaporthe by Murrill in 1906. It later was transferred to the genus Endothia by Anderson and Anderson in 1912 and named Endothia parasitica (Murr.) P. J. et H. W. Anderson (1). In Japan, there was an international controversy regarding the origin and movement of the pathogen, since the pathogen of the blight in the United States was thought to have come from the Grient (25). Kitajima, in 1927 (30), found that the pathogens from the United States and Japan were the same species of fungus. Later, Kobayashi, in 1970 (33 and 34), reported results of taxonomic studies.

Anderson and Rankin (1), Shear, Stevens and Tiller (47), Stevens (48), Kitajima (30), Bazzigher (5) and Puhalla & Anagnostakis (42) have reported on the physiological characteristics and nutritional requirements of E. parasitica. The inhibitory effect of tannin on the pathogen was reported by Kitajima (30) and Aoyagi (3). Furthermore, Kitajima (30) indicated that the pathogen produced, in culture media, chemicals toxic to chestnut trees. Bazzigher (5) isolated that substance and demonstrated clearly its toxicity.

The ecology of the pathogen was studied by Rankin (43) and Heald & co-workers (18-22) who reported on the life history and modes of dissemination of the pathogen and effects of the environment in spread and incidence of the disease.

Aoyagi (2) and official reports of the Nara Prefecture Agricultural Experiment Station (38 and 39) reported on the relationship between environmental conditions and incidence of the disease in Tokushima and Nara Prefectives, respectively. Nishikado, et al. (41) conducted a comprehensive survey of disease incidence in the major chestnut-growing areas of Japan and reported on correlations between major environmental factors and incidence of chestnut blight.

Anderson and Rankin (1) reported tbat it was difficult to prevent the disease with protective fungicides. Rumbold (44 and 45) failed to relieve disease symptoms with theraputic injections of chemicals. Katsumata (28) found surgical removal of cankers and wound dressings to be ineffective, and Jaynes and Anagnostakis (27) were unsuccessful in causing permanent remission of symptoms by soil injection of fungicide in the root zone.

Disease control using blight resistant chestnut varieties is reported to have first been studied in 1906 in the United States. Interspecific hybridization of American and blight-resistant oriental chestnut species was begun around 1920. The favorable results prompted implementation of a full-scale breeding program (8, 13-15).

Bramble (11) and Biraghi (10) studied the histopathology of chestnut blight resistance. Nienstaedt (40) attributed resistance to the quality and quantity of tannins in the bark of chestnut trees, whereas Bazzigher (6 and 7) reported that enzymes produced by the fungus degraded tannins in the bark, calling into question their role as resistance factors.

II. Disease symptoms and morphology of the pathogenic fungus in nature

A. Symptoms

a. Materials and methods

Three hundred seedling grafts of cultivar 'Tsukuba' grown in Dejima and Chiyoda villages in Ibaraki Prefecture were collected at random in November, 1968.

Two-yr-old and 3-yr-old grafted scions of newly planted Tsukuba and Ginyose cultivars grown at this station were used in 1964 and 1965. The adult trees were 5- and 10-yr-old trees grown at this station. Three plots for the adult trees were established. These were:

1) A properly managed plot of 10-yr-old trees spaced 5 m x 5 m apart, giving 40 trees/10 a. Nitrogen was applied at a rate of 14 kg/10 a, phosphorus at 7 kg/10 a and potassium at 14 kg/10 a in a sod cultural orchard. (One a equals 100 m^2).

2) A plot of 10-yr-old trees spaced 5 m x 5 m apart with more trees in between, giving 50 trees/10 a. This was a densely populated orchard; the tree crowns crossed.

3) A plot of 5-yr-old trees spaced 2 m x 2 m apart, giving 250 trees/10 a, the weeds virtually abandoned, and no other management maintained.

b. Results

Incidence and symptoms are shown in Plate 1.

With the expansion of lesions, the branch and the leaves above the lesion brown and then wither. The brown leaves do not fall off when no new leaves form the following year, or when growing leaves die during spring and summer (Plate 1-A). Many of the trees which die of blight grow adventitious buds from the stock (Plate 1-B), for the stock stays alive even when the branches die. Lesions are generally brown or black-brown and concave (Plate 1-C) and form stromata 1-2 mm in height and 1-2 mm in diameter. Stromata are formed in the first third of May when the lesion forms after September of the previous year, and within one month of lesion formation when the lesion is formed from May to August or occasionally September. Stroma is orange-yellow (Plate 1-D). Pycnida formed inside the stromata are seen throughout the year and, in a wet climate after rain, they exude orange-ye1low, voluble, stem-1ike sporehorns (Flate 1-E). Formation of sporehorns occurred most often between June and mid-July, and between late September and mid-October.

Underneath the bark, a fan-shaped growth of yellow-white hyphae (mycelial fan) is observed (Plate 1-H).

In some trees, these symptoms occur with no outward signs of the disease (Plate 1-F). Sometimes brown spots occur inside (Plate 1-G). These lesions rarely expand.

Plate 2 shows lesions in seedling grafts. The graft union is where lesions most occur, especially when the union is abnormally swollen or imperfectly healed (Plate 2-A). On the other hand,,:lesions were not seen on smooth, healed graft unions (Plate 2-B). The lesions occurring in graft unions expand upward toward branches. In the stock, lesions start where the lateral and adventitious buds are clipped or there are bruises (Plate 2-C). In the case of branches, concave, brown lesions surround buds on them (Plate 2-D).

When diseased seedling grafts with no apparent symptoms are planted in the orchard, lesions generally appear from the first year. Plates 3 and 4 show symptoms of 2-yr-old grafts planted this year and 3-yr-old seedling grafts planted the year before. Normally, 2-yr-old grafts, when planted, are pruned (cutback) to the height of 1 meter. But, if the pruning stub is not treated, then the fungus colonizes the entire stub

(Plate 3-A). When the stub is above a side branch, the stub rarely becomes diseased (Plate 3-B). In either case, the stub becomes swollen from healing callus formation. Cankers are most prevalent on branch stubs; the graft unions are the sites where they are second most prevalent (Plate 3-C and D). Sometimes lesions form surrounding buds. Lesions start where branches rub against each other; at any rate, diseases start at wounds.

Three-yr-old grafts (trees), in their second year in the orchard tend to have disease on interior branches as well as on the main trunk. Most often, lesions are formed with a withered branch in the center. The brown flat lesions seen in seedlings and 2-yr-old grafts were less common in 3-yr-old grafts, but cracks in the bark near the base of branches or cracks caused by the expansion of the lesions were seen often (Plate 3-E, F and G). On the main stem, lesions were seen around the graft union (Plate 4-A) and at the base of the trunk, where there are browned or blackened concave spots probably caused by freezing (Plate 4-B). As in the case of seedlings and 2-yr-old grafts, 3-yr-old grafts showed lesion formation at branch stubs, at pruned stubs of sprouts from the stock and in exposed roots (Plate 4-C). Sometimes, at the rough-skinned union of side branches with the main stem, lesions form (Plate 4-D and E).

Plates 5, 6, and 7 show the symptoms of mature trees over 5 years old. In 5-yr-old trees (grafted), lesions are seen around the graft union; but in 10-yr-old trees, this does not occur often. Lesions swell and partially crack and shed diseased bark (Plate 5-A). Lesions on frostbit parts at the base of the tree are concave. The old lesions peel off (Plate 5-B). In the case of mature trees, lesions generally develop and expand from the branch into the main trunk (Plate 6-B). Lesions on side branches of 3-yr-old trees expand and on 5- and 10-yr-old mature trees, when the expansion of the lesion is rapid, reaches into the trunk of the tree, causing a crack in the bark (Plate 5-D and F). Lesions in the main trunk form from pruning stubs, and their expansion is very rapid when callus formation is absent (Plates 5-E and 6-C). Lesions do not occur when callus formation is good (Plate 6-D). Lesions on small and medium-sized branches start where the branches wither or are broken or cracked (Plate **6-i** and F; 7-C and D). They sometimes

expand from withered branches into the main branch and sometimes from there to the main trunk. In densely planted orchards, where interior branches wither, lesions occur not locally but spread rapidly through the branch with abundant fruiting and on initial restriction of the lesion (Plate 7-A and B).

Lesions normally start at mechanical wounds. They also start at insect wounds., Plant 8-A shows a wound made by a chestnut twig borer (Toxoscelus auriceps E. Saunders). The chestnut gall wasp (Dryocosmus kuriphilus Yasumatsu) also causes wounds in the small branches. White-striped longicorn beetles (Batocera lineolata Chevrolat) and larvae of Japanese swift bat moths (Phassus excrescens Butler) attack the trunk (Plate 8-B, D and F) and larvae of other lepidoptera, including Euzophera bigella Zeller, eat into branch crotches and cracks (Plate 8-C).

Furthermore, lesions occur as a secondary disease on already diseased (by another pathogen) parts (Plate 5-C). Lesions are categorized by their symptoms as follows: (1) rapid and extensive withering of bark, abundant fruiting occurring on all parts of the lesion - Plate 9-A; (2) lesions relatively flat, fast spreading - Plate 9-B; (3) callus forms surrounding the lesions, temporarily stopping spread of the lesion. But the lesion spreads again later so it is alternately concave then convex - Plate 9-C and D; (4) callus forms surrounding the lesion, stopping its spread, and the lesion exfoliates and heals - Plate 9-E and F.

B. Morphology of the pathogenic fungus

Two species of Endothia fungi parasitizing the chestnut tree are reported (39. Nara Prefecture Ag. Exp. Stn., 1938). Endothia parasitic? possesses strong pathogenicity of chestnut blight, while E. singularis has very weak pathogenicity.

a. Materials and methods

Cultivar Tsukuba 5-yr-old trees with natural occurrence of the disease. One hundred samples of lesions were collected in August and were observed for the morphology of stromata.

Microscopical observations were conducted on freehand sections mounted in glycerin:alcohol (5:5). Stromata were cultured for one month at 25°C. The compositions of the culture media were as follows: Potato-grape sugar-agar (grape sugar is dextrose): boil 200 g potatoes in

1 l distilled water. Skim off the top and add distilled water to make 1 l. Add 20 g grape sugar and 20 g agar. Chestnut fruit broth agar: 200 g chestnuts, 1 l distilled water, 20 g agar. Chestnut bark broth agar: 200 g live chestnut bark, finely cut, 1 l distilled water, 20 g agar. Oatmeal agar: add 1 l distilled water to 100 g oatmeal and cook it over hot water at 60°C for 1 hour. Let sit 1 hour. Skim off top and add distilled water to make 1 l. Add 20 g agar. Peptone agar: 5 g peptone, 5 g K_2HPO_4, 0.2 g $MgSO_4$, 50 g sucrose, 20 g agar 1 l distilled water. Richard's agar: 10 g KNO_3, 5 g $KHPO_4$, 25 g $MgSO_4$. 50 g sucrose, 200 g agar, 1 l distilled wster Richard's agar with yeast: 10 g yeast (Ebiosu powder) added to Richard's agar boiled for 20 min. Culture bases were autoclaved at 1.2 kg/cm^2 for 15 min.

Results

The pathogenic fungus forms stromata on the bark. Stromata grow diffused or in groups on flat lesions, or in a row on cracks in the bark. Strorna starts forming beneath the epidermis, swells up in a half-ball, then later pierces open the epidermis and protrudes its tip. Its surface is yellow-brown, but becomes yellow or orange-yellow when the pycnidium appears (Fig. 1-D). The size of strornata is 1-1.5 mm long, and approximately 1 mm in height. Stromata growing lined up in cracks in the bark are bigger - 1.5-2.5 mm long and 1.5-2.0 mm in height.

Pycnidia are formed within stromata, normally consisting of several irregular-shaped chambers. On the inner wall of the pycnidiam are numerous conidiophores with conidia on their tips (Plate 10-D).

A conidium is colorless but, in groups, conidia become orange-yellow or yellow. They are single-celled, short-rods, 3.0 to 5.0 µm long and 0.8 to 1.8 µm wide, averaging 4.1 x 1.3 µm (Plate 10-E). Conidia are mucilaginous and, when given moisture, spiral out of stroma and become orange-yellow (Plate 1-E). In dry season, they are a yellow or orange-yellow powder.

The conidiophore is colorless and grows straight or branches off. It is normally 3 times the length of conidium.

The peritheium is shaped like a iong-necked flask, growing in the bottom of stroma in irregular clusters (Plate 10-A). The diameter of the round part is 230 to 390 µm. The neck is long and black, opening

up at the tip of the surface of stroma. It is 390-1040 µm in length (Plate 10-B). Asci are club-shaped, some are curved (Plate 10-C). Their size is 37.5-50.0 x 5.0-7.5 µm, ordinarily 42.5-45.0 x 5.0-6.3 µm, averaging 44.3 x 5.8 µm.

In the ascus, 8 ascospores grow in two irregular rows. Ascospores are elliptical with rounded or pointed ends, colorless and have two cells (Plate 10-C). Their size is 6.3-9.3 x 2.5-3.8 µm. Ordinarily 7.5-8.8 x 3.00-3.8 µm, averaging 7.9 x 3.2 µm.

Underneath the bark of a stroma-forming lesion is a pale yellow mycelial fan (Plate 1-H).

The pathogenic fungus grew well on various culture meida but, depending on the type of medium, it differed in colony morphology and in formation of conidia. Table 1 shows the growth characteristics of the fungus in seven different culture media. On potato agar, it grew we11 and colonies were flat and orange-yellow. Clusters of pycnidia were small and grew densely on the whole surface. On chestnut fruit agar, growth was extremely good. Colonies were thick, velvety or yellow-white in color. Small or large clusters of pycnidia were mixed; small clusters grew densely in the central part; large clusters grew in the center and scattered a11 around. Large mucilaginons clusters were yellow. On chestnut bark agar medium, growth was fast but thin (like silk threads) and white. Pycnidial clusters were medium-sized, scattered in the center, but growing in a ring on the outer sphere. On the oatmeal agar medium, growth was inferior. Colonies were quite thin and white. Pycnidia clusters were extremely small, densely populated all over the surface with sparse growth in the shape of concentric circles. On the peptone agar medium, growth was very slow but colonies were thick and dense, like doubled flowers, and white. A few pycnidial clusters grew in the center, but they were very small. On the Richard's agar medium, colonies were thin, looking like silk threads and white. No conidial clusters were formed. On the yeast-amended Richard's agar medium, growth was very good. Colonies were thick and dense and velvety and pale-yellow. Conidia clusters were large and scattered all over the surface.

C. Discussion

Lesions were observed on chestnut trees of all ages from seedlings to mature trees. When lesions expand, encircling a branch or trunk, it withers and dies above the lesion. Normally, the root does not get diseased, although lesions form in the exposed part of the root. The tree is alive below ground when it is dead above, forming adventitious buds from the stump. The location of the lesion and symptoms differ, depending on the age of the tree and environment. But, in all cases, the lesion forms from a wound on the tree. It tends to expand more slowly when the tissues around the lesions swell up. There is no swelling of the tissues when lesion expansion is rapid. This may sound contradictory to the fact that callus formation on wounds swells up the bark, but the results of the dissection in Chapter II-B shows that callus formation around the wound suppresses lesion expansion. With the formation of callus, the lesion and surrounding tissues swell up. In graft unions, the disease does not occur when the callus is smooth and healthy, but there is a high incidience of the disease when the callus is scabrous and swollen like a tumor. Thus, it is understood that the rate of recovery of the graft union influences fungus invasion. The fungus invasion, the expansion of the lesion, and the callus formation of the tree are important and affect the symptoms. The following four types of lesions are, thus, conceived:

1) Acutely progressive: The whole tree or large parts of the tree rapidly wither and die. Then, later, the fruiting bodies of the fungus form simultaneously (Plate 9-A).
2) Progressive: Relatively rapid expansion of lesions; its surfaces remaining fairly smooth (Plate 9-B).
3) Chronic: Swelling around the lesion causes a temporary delay of expansion. The recurrent expansion results in an alternately concave, cnnvex surface (Plate 9-C and D).
4) Healing: Swelling of lesions, stopping expansion. Old diseased tissues peel off and heal (Plate 9-E and F).

The fungus forms stromata on the lesion. Within the strorna, pycnidia form with conidia inside, and perithecia with ascospores inside. The stromata are orange-yellow or yellow, scattered or in groups on the lesion. The surface of perithecial stroma is covered with the protruding necks of perithecia, and so is uneven. Normally, stromata are 1 to 2.5 mm long

and a little bigger in cracks in the bark. Several pycnidia, in the form of irregular small chambers, form within a stroma. Within the pycnidium are conidia which are colorless, single cell, short rods measuring 3-5 by 0.8-1.8 µm. Conidia appear after rain, forming orange-yellow clusters which are mucilagious and tendril-shaped. The perithecia are long-necked flasks, growing in the bottom of stromata in groups. Their diameter is 230-390 µm. Asci are club-shaped, 37-50 µm long by 5-7.5 µm wide, containing 8 ascospores in 2 irregular rows. Ascospores are colorless and 2-celled, measuring 6.3-9.3 by 2.5-3.8 µm with round or somewhat pointed ends. Underneath the surface of bark with stromata is a mycelial fan, a pale yellow-white in color.

III. Physiological characteristics of the pathogenic fungus

The influence of temperature, pH and the composition of nutrient media on fungus growth in culture has been reported by Anderson and Rankin **(1)**; Shear, Stevens and Tiller (48); Kitajima (30); and Bazzigher (5). Among them Kitajima (30) and Shear et al. (48) have compared several strains of the pathogenic fungus. Puhalla and Anagnostakis (42) reported on the influence of nutrients on spores and the hereditary influence of ultraviolet rays. Kitajima (30) and Aoyagi (3) reported on the relationship between tannic acid in the medium and fungus growth. Nienstaedt (40) stated that the amount of tannin in different genera of chestnut determined the resistance threshold of genera and species of chestnut for the disease. Kitajima (30) reported that the pathogenic fungus produced a substance in the medium which wilts the tree. Bazzigher (5, 6, 7) isolated the substance and analyzed its toxicity. He also reported on enzymes produced by the fungus.

A. Pathogenicity of strains

a. Materials and methods

Four strains from different locations (Table 2) were used with ten cultivars of 3-yr-old and 4-yr-old trees (Toyotama Wase, Ibuki, Yamato Wase, Chi-7, Tsukuba, Shichifuku, Ginyose, Rihei, Imakita, Akachu). On their branches were inoculated mycelial discs of the four strains grown for 10 days at 27°C in the potato-agar media. This was done in June of 1965 and 1966. In December, the number of diseased trees and the diameter of the lesion were determined. All the strains used after preliminary isolation had been inoculated into Ginyose cultivar trees and reisolated.

b. Results

Shown in Table 3. All 4 strains were pathogenic, but EP-7 was weaker with an incidence of 44% in 1965 and 8% in 1966. EP-1, EP-8 and EP-9 a11 had an incidence of 87-100%. There were no differences in pathogenicity among them.

B. Relationship of the growth of the fungus to incubation temperature and tannin concentration

1. Growth and temperature

a. Materials and methods

Pieces of mycelium were transferred into potato-agar in petri dishes 9 cm in diameter. These were placed in incubators at temperatures of 0-35°C, each having a 5°C difference. After 3 days of incubation, the diameter of colonies of mycelia was measured every day and the growth rate every 24 hrs. was calculated. The experiment was repeated twice with 5 petri dishes per temperature block. EP-1 strain was used.

b. Results

Shown in Table 4. No mycelial growth occurred at 0°C. At 35°C, swollen mycelial colonies formed around the transferred mycelial disc. Normal mycelial growth was observed at temperatures from 5 to 30°C. Maximum growth occurred at 25°C and was 7 mm in 24 hrs. The minimum temperature for growth was somewhere between 0°C and 5°C, the maximum between 30 and 35°C. Good growth was observed between 15-30°C. The optimum temperature for growth was 25-30°C.

2. Growth and tannin concentration

a. Materials and methods

The basal medium was Knop's solution (1 g $CaNO_2$, 0.25 g $MgSO_4$, 0.25 g KNO_3, 0.25 g K_2HPO_4, 3 drops 5% $FeCl_3$, 30 g sucrose, 1 l water). The tannic acid concentrations were 0.1, 0.2, 0.4, 0.6, 0.8 and 1%. Ten ml of 2% agar medium were placed in each 9 cm petri dish with 5 petri dishes for each tannin concentration. Hyphae were transplanted and incubated at 27°C. For 7 days, the diameter of mycelium was measured. The experiment was repeated once.

b. Results - Fig. 1

With 0.1% tannic acid added, mycelial growth was better than with no tannic acid. But above 0.2% tannic acid, growth was in inverse proportion to the tannic acid concentration.

3. The amount of tannin decomposition by the fungus

a. Materials and methods

Two hundred g shredded chestnut bark (raw) with 1 l distilled water added was boiled for 30 min. To this and Richard's solution (10 g KNO_3, 5 g K_2HPO_4, 2.5 g Mg SO_4, 50 g sucrose, 1 l distilled water) was added 0 and 1% tannic acid. One hundred ml of these solutions were poured into 200 ml flasks and autoclaved. A mycelium piece was transplanted and the flasks incubated at 27°C for a certain period. The mycelium was then strained, washed, dried, and weighed. The tannin in the filtrate was mezsured by the potassium permanganate method and the difference in tannic concentration of inoculated and uninoculated medium calculated. Three flasks were used for each treatment.

b. Results - Table 5

When the fungus was grown on a tannin-containing medium, the amount of tannin in the medium decreased as the fungus grew. The degree of decrease of tannin differed, depending on the kind of medium. In chestnut bark agar, 0.16-0.21 mg tannin decreased with the growth of 1 mg mycelium (dry weight) in 12 days; in Richard's medium, 0.23-0.31 mg tannin decreased with growth of 1 mg mycelium (dry weight) in 7 days.

C. Growth of fungus and nutrients

1. Nitrogen sources

a. Materials and methods

Richard's solution (10 g k NO_3, 5 g K PO_4, 2.5 g $MgSO_4$, 20 g grape (sugar (dextrose) 1 l distilled water) was the basal medium. KNO_3 was substituted by several nitrogen compounds (Fig. 2) to equal 1% KNO_3. One hundred ml of solution, each in three 200 ml flasks, were incubated at 28°C for 30 days. The mycelium was then strained, washed, dried at 60°C and weighed.

b. Results - Fig. 2

Many organic and inorganic nitrogen sources were used. No growth occurred in $(NH_4)HPO_4$, $(NH_4)_2C_2O_4 \cdot H_2O$ and urea. In the case of inorganic N compounds, growth was better in ammonia compounds than nitrate compounds. Growth was generally good on amino acid compounds, but a little slow on lycine or leucine and good on glutamic acid, tyrosine, asparagine and methionine. Best growth was observed on peptone.

Carbon sources

a. Materials and methods

An asparagine solution (2.5 g asparagine, 5 g K_2HPO_4, 0.2 g $MgSO_4$, 20 g grape sugar, 1 l distilled water) was the basal medium. Carbon sources were substituted to equal 2% glucose (Fig. 3). Seventy ml of this solution in each of three 100 ml flasks were incubated at 28°C for 10 days, and the mycelium weighed (see 1).

For the study of fungus growth on starches, 3% starch was substituted for 2% grape sugar in Richard's solution.

To study growth and sugar concentration, glucose, saccharose and water soluble starch were added to Richard's solution in concentrations of 0 to 5%.

The ratio of glucose and starch in Richard's solution was changed but the sum content was kept at 2%. In 70 ml of solution in 100 ml flasks (3), 1 ml of a conidial suspension was transplanted. The incubation temperature was 28°C. Growth was measured by dry weight of mycelium.

b. Results - Fig. 3

Monosaccharide, disaccharide and polysaccharide worked well as carbon sources for mycelium, but it did not grow on glycerin. The growth was inferior in lactose, mannite and inulin, but good on soluble starch and dextrin. Conidium formation was good on glucose, mannose, fructose, xylose, maltose, saccharose and inulin, but it was not observed in galactose, arabinose, lactose, soluble starch or dextrin.

Fig. 4 shows the relationship between fungus growth and the kind of starch used in the medium. Growth was good with soluble starch, corn starch and dextrin, but not as good with potato, sweet potato and wheat starches. No growth occurred on glucose.

No conidia were formed in any of the above media.

Fig. 5 shows the effect of carbohydrate concentration. Growth of the fungus is best on soluble starch, fair on saccharose and inferior on glucose. Conidia formation was observed only on saccharose. Up to a concentration of 2% soluble starch, 2% glucose and 3% saccharose, the growth increased in proportion to the concentration; but it decreased above these concentrations. Fig. 6 shows the effect of different ratios of glucose and starch. Growth of the fungus was poor on glucose alone but, with the addition of soluble starch to the glucose medium, it improved. The rate of growth changed little with the ratio of glucose and starch. Although conidia did not form on starch alone, they formed when glucose was added; and the ratio of glucose was over 70%. When the glucose ratio was below 80%, there was no conidia formation.

3. Vitamins

a. Materials and methods

In Richard's solution were added 1% each of grape sugar and soluble starch as carbon sources. Then one of the 10 vitamins in Table 6, or all the vitamins except one were added to the medium. Sixty ml of medium in each of three 100 ml flasks was sterilized for 30 min in a Koch sterilizer. One ml of a conidial suspension was transplanted. The incubation temperature was 27°C. Mycelium was washed, dryed, and weighed.

b. Results - Fig. 7

The fungus grew well on vitarnin-added media as well as the control. Growth was particularly good on biotin, nicotinic acid, cholin, and thiamin - vitamins in the B group.

D. Growth of the pathogenic fungus and composition of chestnut tree bark

1. Change in composition of the diseased tissue

a. Materials and methods

On 7/2/71, brown diseased bark with stomata formation and healthy bark from the same tree were collected from 6-yr-old individuals of the Tsukuba cultivar. The specimens were immediately heated at 95°C for 30 min, wind-dried at 60°C, powdered and kept in a dessicator. At the time of analysis, the powder was ground in a morter fine enough to pass a 100 mesh sieve.

To measure the content of cold water-, warm water-, acid- and alkaline-solubles, the powder form was used according to general timber analysis methods (51). The Somogyi method was used to measure total carbohydrates, and the method of Murayama, et al. (4) to measure types of carbohydrates.

b. Results

The results of the chemical analysis of the diseased bark is shown in Table 7. A reduction in chemical components is conspicuous in the diseased part compared to the healthy part. All solubles decreased with the exception of NaOH-solubles. The rate of reduction was 30% in cold water-solubles, 40% in warm water-solubles and 50% in HCl-solubles as compared to the elements of the healthy part. Carbohydrates decreased to 30% of the healthy part with a particularly drastic decrease in total sugars, soluble sugars, and hemicellulose (these to less than 20% of the healthy bark level). Sixty percent of crude starch remained in the diseased part.

2. Soluble substances in the bark and growth of the fungus

a. Materials and methods

Two-yr-old branches of 4-yr-old Tsukuba trees were used. The solution was a mixture of 20 g bark shreads and 100 ml water. For the cold water extract, the base solution was left to stand for 24 hr. For the hot water extract, the base solution was boiled for 15 min. The crude solutions then were filtered and cooked in a double boiler until the liquid evaporated. The solid remaining was dissolved in ethanol, and the soluble and unsoluble parts were separated to give the ethanol-soluble and insoluble solutions. They were mixed with 1% activated charcoal granules for 1 hr, then centrifuged at 3000 rpm for 12 min. Dilution of the supernatant to 100 ml gave the 1% charcoal filtered solution. The pellet was extracted with 75% ethanol to give 100 ml of solution. These solutions were added to Richard's solution to a concentration of 20%. One hundred ml of the media were poured into 200 ml flasks into which mycelial pieces were transplanted. These were incubated at 27°C, and the dry weight of mycelium was measured.

In addition to the cold water extract and hot water extract prepared in the above fashion, 1 N HCl, 1% NaOH, or nothing were added. The mixtures were boiled, then left at 25°C for 30 min. These neutralized mixtures and 5% activated charcoal granules were shaken for 15 min and centrifuged. Seventy ml supernatant was put into 100 ml where a mycelial plug was transplanted before incubation at 27°C. The pH of the cold water extract changed from 5.8 to 2.8 with the addition of HCl and 7-9.9 with the addition of NaOH. The pH of the boiled broth changed from 5.1 to 4.0 with HCl and to 8.0 with NaOH. The pHs were returned to the initial pH by neutralization.

b. Results

Table 8 shows the influence of ethanol and activated charcoal solubles on fungus growth. Twenty percent addition in both cases enhanced growth compared to the basal culture medium. Compared to the untreated crude solution, the ethanol-soluble solution showed only 30% growth, but the ethanol-insoluble solution showed as much or more growth as the untreated crude solution. The solution to which was added the activated charcoal-treated filtrate showed inferior growth, similar to the basal medium. The 75% ehtanol extract of activated charcoal-absorbed materials showed over 50% growth of the crude solution.

Table 9 shows the effect of acid, alkaline and activated charcoal treatments on growth. Growth on cold water extract was inferior to the growth on hot water extract. In other cold or hot water extracts, there was no difference in growth between HCl and NaOH or untreated solutions. Very little growth occurred on the activated charcoal-treated solution. Heat sterilization at 100°C for 30 min of the cold water extract had no effect on fungus growth.

E. Discussion

Studying the pathogenicity of four strains, we found that three were strongly pathogenic but the remaining one strain was weakly pathogenic. Kitajima (30) found that, of the 3 strains he studied, one was weak in pathogenicity and did not form mycelial fans in the lesion. All 4 strains we studied, however, formed fan-shaped mycelial colonies underneath the lesion.

The minimum temperatures for growth of the mycelium was between 0 and 5°C, the maximum temperature was 30-35°C, and the optimum temperature was 25-30°C (Table 4). Good growth occurred between 15 and 30°C. These results agree with previous findings by others (1, 5, 29, 48).

A 0.1% concentration of tannic acid enhanced growth, but more than 0.2% slowed down growth. This result agrees with Kitajima's (30), but not with Aoyagi's (3), which may be attributable to the different culture media used. The growing fungus decomposed tannin in tho medium (Table 5). This suggests that the fungus may possess a tannin-decomposing enzyme (7).

The fungus utilized all the organic or inorganic carbon compounds except a few inorganic compounds. Ammonium compounds or amino acids icnreased growth. Peptone caused excellent growth (Fig. 2). In the case of carbon compounds, many of the mono-, di- and pcly-saccharides were utilized as nutrients. Starch and dextrin gave particularly good growth (Fig. 3). The best concentrations of sugar for fungus growth was 2-3% (Fig. 5). In starch and dextrin, mycelia grew well, but pycnidia did not sporulate well. Many of the mono- and disaccharides enhanced the sporulation of pycnidia (Fig. 3). In the mixture of starch and glucose, pycnidiospores formed only when the glucose ratio was over 80% (Fig. 6). Pycnidial sporulation by addition of glucose was also confirmed by Puhalla, et al., (42).

The fungus grew well on vitamins, but they were not essential to fungus growth. This agrees with Puhalla et al.'s (42) finding.

The diseased bark contained reduced amounts of water-soluble and HCl-soluble compownds, carbohydrates, etc., compared to healthy bark (Table 7). On the other hand, the fungus grows well on water soluble compounds in the bark. These compounds are more soluble in warm water than cold water, are not deactivated by heat, acid or alkali, and get absorbed by activated charcoal (Tables 8 and 9). These characteristics are seen commonly in carbohydrates and some soluble nitrogen compounds, and correlated with the change in composition of diseased bark.

IV. Fungus invasion into the host and microscopic observation

Anderson and Rankin (1) found that the incidence was high throughout the year when a piece of diseased tissue was inoculated into the tree and that it was high from April through September when conidia, ascospores or mycelium were inoculated. In Japan, Aoyagi (2) reported a high incidence from April or May through December, and a low incidence from January to March.

Keefer (29) and Bramble, et al. (11) suggested that histological changes occur with the invasion of the pathogenic fungus.

The following experiments were conducted to study the time of invasion of the fungus, to observe microscopically lesions with different symptoms and to study the effects of environmental factors on callus formation in the tree.

A. Time of fungus invasion

1. Inoculation outdoors

a. Materials and methods

From November, 1961 to October, 1963, 4-yr-old and 5-yr-old trees of cultivar Ginyose were inoculated on the 15th day of the month. Inoculum was mycelial fans on agar circles 6 mm in diameter taken from the tip of a mycelial colony which had been incubated at 28°C for 5 days. Conidial cirri formed during a 20-day incubation were transferred to cotton balls 5 mm in diameter and also were inoculated. A corkborer (6 mm diameter) was used to pierce a hole in the bark of 2-yr-old branches down to the cambium. The inoculum was stuffed inside, and the hole was filled with the disk of bast removed previously. The hole then was covered with cotton impregnated with sterilized water and covered with polyethelene tape (Plate 11). After 1 month, the polyethelene tape and cotton were removed. Unless otherwise stated, this method was used throughout the experiment. Sometimes, callus formation occurs where the tree is bruised during inoculation. So, simultaneous with the inoculation experiment in 1963, the degree of callus formation at 10 holes (made by the above method) after 1 month was recorded as follows: the surface of the wound is completely covered with callus - index 2; half the surface is covered with callus - index 1; only around the peripheny of the wound is callus formed - index 0.5.

b. Results

Table 10 shows the results of outdoor inoculations during different seasons. During the 2 years of the experiment, incidence occurred from March to November when trees were inoculated with mycelium and from March to October when inoculated with conidia. The degree of incidence was higher with mycelial than conidial inoculum. Brown lesions appeared within 2 months of mycelial inoculations done in October, November and March, and within 1 month of inoculations done in April through September. When conidia were inoculated, brown lesions occurred in April-May of the next year when inoculated in October; within 2 months when inoculated in March, April and September; and within 1 month when inoculated in May through August. The time of highest incidence was August and September in 1962, July and August in 1963.

Figs. 8 and 9 show the average monthly temperature and precipitation. It was above 10°C from early April to mid- to late-November. Incidence occurred when the average temperature was above 10°C and after the mid-March inoculation. The range of incidence became higher during the high temperature season.

Table 11 shows the degree of callus formation and season. Callus was not formed on wounds inflicted from October to March of the next year until May. On wounds inflicted from April to September, callus was formed within one month of wound infliction, but the degree of callus formation within the one month varied with season. The highest degree occurred in wounds made in May and June, then July whose callus formation was 86% of the maximum degree of June at 100%. April and August were 44% of the maximum and September, 7%. Slight callus formation was sometimes observed on October wounds, but normally it did not occur.

Callus was often formed when the fungus was inoculated. Formation was vigorous when no incidence occurred.

2. Incidence and temperature

a. Materials and methods

The same 2-yr-old branches as 1. were used. They were cut into 15 cm pieces and kept in water. After inoculation, they were incubated at temperatures ranging from 5" to 35°C in 5°C intervals.

After incubation for a certain period of time, the incidence and diameter of the lesion were measured. Five to eight branches were used for each temperature setting. This experiment was conducted in March-April of 1963.

b. Results

Table 12 shows the results. Lesions formed within one month of inoculation of mycelium at 10°C and above, of conidia inoculated at 15°C and above. But in the case of conidia inoculation, no lesions were formed at 35°C.

All the hosts inoculated with mycelium got diseased, while only 2/7 and 1/7 of the branches inoculated with conidia did at 15°C and 20°C. But the incidence with conidia increased to 6/8 and 4/8 at 25°C and 30°C. Lesions also were formed when the conidia-inoculated branches were kept at 15°C after 27 days at 10°C. The time it took for mycelium to form lesions was 20 days at 10°C, 7 days at 15°C and above; that for conidia was 14 days at 15°C and above. The degree of lesion expansion was maximum at 25 and 30°C with mycelium, 30°C with conidia. Although lesion formation was observed at 35°C with mycelium, no expansion occurred thereafter.

Conidia formation, germination and temperature

a. Materials and methods

To study the relationship between conidia formation and temperature, naturally diseased branches with stroma were collected in February and May, 1963, put in water and sprayed with sterilired water. They were placed in glass jars and kept in the incubator at temperatures ranging from 5" to 35°C in 5°C intervals. The conidia formation was measured; three branches used for each temperature. To study the relationship between conidial germination and temperature, conidia grown on potato-agar at 25°C for 20 days were used. Conidia were added to a 20% solution of the broth of 200 g chestnut bark shreds and 1 l distilled water which had been boiled for 30 min. Using the hanging drop method with hollow glass slides, germination was measured at temperatures ranging from 5 to 40°C in 5°C intervals with three slide glasses for each temperature. One hundred buds on each slide were measured

and the germination percentage and average length of the germ tube were calculated.

b. Results

Table 13 shows the relationship between temperature and the amount of conidia formed. Conidia were formed at 15-35°C on the lesions collected in February. After 10 days at 25°C, many tendril-shaped conidial clusters were formed. After 20 days, they were formed at 15, 20, 25 and 30°C but not at 10°C. At 35°C, small amounts of conidia formation were observed after 10 days, but not after 20 days.

In the case of lesions collected in May, conidia formed at all temperatures from 5-30°C. Tendril-shaped clusters were formed at 15, 20 and 25°C and increased in size as the days passed, but no increase in formation was observed at 5 and 10°C. Table 14 shows the relationship between germination and temperature. Germination occurred at 10 to 35°C and was optimal at 25 and 30°C; 96-97% of conidia germinated within 24 hrs. The temperature range for germination was between 5 and 10°C as lowest and between 35 and 40°C as highest.

B. Dissection of the diseased part

a. Materials and methods

Lesions with various symptoms were collected from the orchard in April, 1974. Lesion pieces were excised horizontally or vertically, immediately immersed in a glycelin-alcohol solution **(I:1)** for several minutes, hand sectioned, mounted in glycerin and microscopically observed. At the same time, for the observation of callus, 2-yr-old branches were cut and put in water. The section was covered with wet cotton, wrapped in a plastic bag, and kept in an incubator at 25°C.

Four types of lesions were studied according to Experiment 11-A (Plate 10).

b. Results

1. Macroscopical observations of diseased tissue

Lesion type I is an acute, progressive, withering type, browning and withering the whole of a trunk or branch suddenly. Histological changes of the lesion are shown in Plates 12-A, B, C, D and Fig. 10. The lesions are widespread and the surface is smooth.

The bast was all brown and dried and xylem also withered. Both tissues lost moisture and dried up, but no histological changes were seen.

Lesion type II is progressive, expands relatively fast, is smooth and somewhat concave compared to healthy parts. Histological changes of the lesion are shown in Plates 13-A, B, C, D and Fig. 11. The lesion is smooth. The browned part is gradually concave with no sudden drop. Browning is almost completely limited to the bast layer, with few cases of xylem browning. As is seen from the cross section, the color change of this lesion is similar to that of type I at the center, but in the surrounding area of the bast layer, the color change is complex and occurred in the inner layer when the outer surface appeared healthy. The border between the browned part and the healthy part is unclear, and sometimes the color gets lighter in gradation. No histological change occurred due to swelling in the bast and xylem layers.

Lesion type III is a chronic type of lesion with swelling in the surrounding area. The expansion is intermittent with repeated cessation and expansion of the lesion which results in an alternately convex then concave surface. Plate 14-A, B, C, D and Fig. 12 show the lesion and histological changes in sections. The lesion became layered unevenly and, in many cases, dried out in the center. As can be seen in cross sections, the xylem discolored, its growth was suppressed and the annual ring thinned in some cases. In the bast, discoloration was remarkable, and there was a brown zone at the lesion boundary. In the cambium, callus increased pushing up the bast, which peeled off the xylem in some cases. There was same discoloration in the xylem, but no clear band like in the bast. The formation of annual rings was suppressed in the diseased xylem, in contrast to the annual rings in healthy xylem.

In Lesion type IV, the area surrounding the lesion swelled, and the lesion ceased expanding. Furthermore, the lesion sloughed off and the canker was cured. The change in the lesion is shown in Fig. 13 and Plate 15-A, B, C and D. Sometimes, the area

surrounding the lesion swelled and the bast peeled off from the xylem, cracked and fell off, and the xylem became exposed. But in small lesions, the wound was sealed by formation of callus tissue. In the bast, the boundary of the lesion formed a clear band, and the old diseased tissue sloughed off. In the xylem, the boundary of discoloration was not clear. In the swollen area surrounding the lesion, callus formed in the cambium and then differentiated into xylem and bast where the expansion of the lesion stopped.

2. Microscopical observations of diseased tissue

Plate 16 shows brown, dried, diseased tissue of Lesion type I. Diseased tissue in the bast showed an overall browning with no brown band or gradient of color. The cells were plasmolysed and many of them were sloughed off, creating internal spaces (holes) in the bast (Plate 16-A and B). In the tissues, hyphae often were observed, especially as mycelium (aggregates of hyphae) in the internal spaces (holes) (Plate 16-C and D).

Plate 17 shows the boundary of a discolored lesion and a lesion with an unclear brown band, as seen in Lesion type II. The boundary between the brown bast and the healthy part was unclear, with discolored and healthy cells intermixed. Even in the discolored area, bast fiber bundles did not discolor, although the parenchymatous tissue and rays were discolored (Plate 17-A and B). Even in the tissues which formed an unclear brown band, the discolored area surrounding the band had healthy-appearing cells intermingled with obviously diseased cells (Flate 17-C and D).

Plate 18 shows the tissue that form a brown band between diseased and healthy tissue as in Lesion type III. The boundary between the brown and healthy areas was clear, especially in areas surrounding the bast fiber bundles (Plate 18-A and B). In the areas surrounding the boundary, the disposition of cells was more uniform and they were closely packed together, the cells being suberized and forming a wound periderm (Plate 18-C, D and E). Furthermore, due to the development of the phellogen, the diseased and healthy areas were separated (Plate 19-B). Periderm often formed next to the fiber bundles (Plate 19-C and D).

Plate 19 shows the sloughing off of the diseased bast area seen in Lesion type IV. Cork cambium developed between the diseased and healthy area forming phellem; and the outside lesion tissue peeled off. The surface of the inside, healthy area was protected by a new outer periderm (protective outer skin).

Plate 20 shows the tissue formed between xylem and bast at the boundary between healthy and diseased tissue as seen in Lesion types III and IV. In the early stages, several parallel layers of cork tissue form on the surface with the formation of parenchyma cells on the inside, many of which became clumped (Plate 20-A and B). By the formation of this scab tissue (callus), the lesion sloughed off. On the inside, this tissue differentiated into xylem, forming vessels and medullary rays. On the outside, the scab tissue differentiated into the phloem, expanding into the bast and sloughed off the lesion (Plate 20-C and D). In some cases, the expanding callus grew over the diseased tissues. Also, many tyloses were observed in previously formed vessels.

3. Callus formed under high humidity conditions

Plate 21 shows a callus which formed rapidly at 25°C after applying damp cotton on the bruised part. Several days after injury, callus began to form from the cambium; at 10 days, it swelled into a cloud shape (Plates 21-A and B). Right after the damp cotton was removed, the surface was white, but it turned yellow gradually. In the section at 3 days, the outer layer consisted of parallel, suberised cells. The inner layer was filled with parenchyma,;, which were similar to the callus tissue formed in the cambium of Lesion types 111 and Ⅳ (Plate 21-C). Newly formed callus with a white surface had an irregular disposition of non-uniform cells, but with suberized phellem underneath (Plate 21-D).

C. Relationship between callus formation and growing conditions

1. In relation to plant density

a. Materials and methods

On 12/25/63, three 2-yr-old branches from five 4-yr-old trees (variety Ginyose grown in the orchard on brown volcanic ash soil) were collected. Each of the 15 branches, 15 cm long, was put in water in a room with high humidity at 27°C. On 1/18/64,

callus formation on the sections was measured according to the following criteria:

(1) Callus formation on the whole surface (index 2);
(2) callus formation on over half the surface (index 1);
(3) partial formation (0.5); (4) no callus (0).

$$\text{callus formation ratio} = \frac{2n_1 + n_2 + 0.5n_3}{2N} \times 100$$

n_1, n_2, n_3 - callus formation (1), (2), (3), respectively
N - number of samples

Tree density was 5 x 5 m in a sparsely populated block, 2.5 x 2.5 m in a densely populated block. The fertilized block received 10 kg N & K each, 5 kg P per 10 a.

b. Results

Shown in Table 15. Callus formation ratio was highest at 73.0 in the fertilized, sparsely populated block, 15.0 in the fertilized, densely populated block. One branch out of 15 in the unfertilized, sparsely populated block and zero in the unfertilized, densely populated block. Therefore, under the same fertilization condition, the denser tree planting gives less callus formation. When the distance between trees is equal, callus formation is inferior when trees are unfertilized.

2. Defoliation and root shearing

a. Materials and methods

Used 3-yr-old trees of the Tsukuba cultivar. The developing leaves were all removed on 6/15/72 and 8/1/72. Also on these dates, I dug a hole 60 cm in diameter and 60 cm in depth around the trunk, lifted the roots and cut them back. On the three trees of each treatment, bark was removed with cork borer at three places which were then covered with wet cotton and covered with vinyl. Two months later, callus formation was measured according to the standard shown in (1).

b. Results

In Table 16. Although callus did not form as much in the treated blocks compared with the untreated block, there was seasonal variation. In the June block, the callus formation ratio was 61 in the untreated, 50 in the cut-root trees and zero in the plucked-leaf trees. In August, in the untreated trees, the callus formation ratio was 100 (all wounds covered by callus), 86 in the plucked-leaf trees and zero in the cut root trees. As for the condition of the trees, new leaves started a month after plucking in June; they started to grow several days after plucking when they were plucked in August. In the blocks where roots were cut, in some trees, leaves withered temporarily, but the degree of withering was light in the June block and new branches grew after July. In the August block, withering was severe and some leaves fell off.

3. In relation to fertilizer

a. Materials and methods

One-year seedlings of variety Tsukuba were grown in concrete troughs (1 m^2, 1 m deep) and grown in the orchard (9 trees per 20 m^2 plot with 20 g each of N, P_2O_5, K_2O per tree of fertilization, 9 kg per 10 a). In the concrete troughs, 3 trees per trough were grown in 5 g each of the three nutrients per tree (15 kg/10 a) for the standard fertilization treatment. Two-thirds of the urea (N) and potassium (K) and all of the superphosphate (P) were applied before planting and the rest applied early in July. Fertilization was applied in three 'areas of each trough. On July 8, two 8 mm diameter bark disks (including functional phloem) were removed with a cork borer. Callus was measured on 10/23. The standards were: (1) callus formed on the whole surface (index 2); (2) more than half the surface (index 1.5); (3) about half (index 1); (4) partial (0.5); (5) no callus (0).

$$\text{callus formation ratio} = \frac{2n_1 + 1.55\ n_1 + n_3 + 0.5\ n_4}{N} \times 100$$

$n_1 \ldots n_4$ is the number of (1)...(4)

N is the number of samples

b. Results

Shown in Table 17. The rate of callus formation was the least in a non-fertilized area whether it was in the orchard or in concrete troughs (44.0 in field, 68.0 in concrete enclosure). The rate was also low in the no-nitrogen block (56.5 and 72.0). In blocks fertilized with all 3 essential chemicals, callus formation was better. There was no difference in callus formation depending on the amount of fertilization. The relationship between the callus formation rate and the rate of lesion spread (Tables 26 & 27) showed a correlation coeffient of $r = 0.912^{**}$ in the field and $r = 0.729^{**}$ in an inverse relationship (Figs. 14 and 15).

4. Moisture in soil

a. Materials and methods

One-yr-old trees of the Tsukuba cultivar were grown in a concrete trough 1 m deep, 1 m in diameter. Three trees were planted in each trough on 3/26/75. Between 7/1 and 9/27, the soil conditions were adjusted to dry, normal and moist. In the dry block, a lid was put on the trough to prevent rainwater from going in, and trees were watered when the leaves became so dry as to curl inward. The normal and moist blocks were left with no lid and watered when appropriate. Soil moisture was measured by the water column method (using a DIK tensiometer). Before planting, 100 g of chemical fertilizer (3 nutrients, all 15 kg/10 a) was applied to each trough.

b. Results

Shown in Table 18. Callus formation was inferior in the dry block at 75.0 as compared with 92.0 in the normal block and 94.5 in the moist block. Fig. 16 shows the inverse relationship ($r = 0.823^{**}$) between callus formation and the length of lesion.

D. Discussion

In indoor inoculation, fungus invasion into the tree was observed between mid-March and mid-November in case of hyphae and from mid-March to mid-October in case of conidia (Table 10). The results more or less agreed with Anderson's (1). During this period, when the average temperature was 10°C, conidia did not form lesions within one month, but did if the temperature rose subsequently (Table 12). This agrees with results obtained concerning the relationship between incidence and temperature in the field. When inoculation took place in mid-November, hyphae caused disease but conidia did not. This is probably due to the minimum temperature for germination of conidia being between 10 and 5°C while that for the growth of hyphae is between 5 and 0°C.

The formation of conidia and release from pycnidia, considered to play a very important role as a source of infection, was good at 15-30°C (Tables 4, 13). The conidia already in the pycnidium came to the surface even at below 15°C if moisture was obtained (Table 13). Therefore, it appears that conidia are present throughout the year and the possibility of infection exists all year round. Outdoors, an average temperature of 10°C and above will constitute the season for possible infection.

Incidence and spread of lesions is vigorous at high temperatures - August and September outdoors; but it was less vigorous in June and July, which suggests the temperature is not the sole contributor (Tables 10, 22). When comparing the rate of callus formation in different seasons, on the other hand, it was highest from May through July, but declined after August (Table 11). Because the fungus invades the wound to cause blight, swift recovery from wounding by way of callus formation prevents invasion. Also, as was seen in the histopathological study, development of fungi that are already in the tissue is obstructed by callus formation; and when callus formation is vigorous, it was observed the diseased tissue peels off from the healthy tissue, resulting in healing (Plates 15, 19, 20; Fig. 13).

The role of callus formation varies, with season, age of the tree and growth conditions, being most vigorous in May-July when trees grow fast and form new branches. It was least vigorous in the non-fertilized, no-nitrogen block and when the soil was kept dry in summer. It varied with the period it took the trees to recover from abnormalities caused by removing leaves and cutting roots. Trees with inferior callus formation

showed expanding lesions, whereas trees with good callus formation showed slower lesion spread, with a highly inverse relationship between the two.

The resistance reaction of chestnut to the invasion of the blight fungus has been studied on American chestnut (C. dentata) by Keefer (29) and Bramble (11) and on European chestnut (C. sativa) by Biraghi (10). They all report the resistance is a temporary one as was found in this study with C. crenata as progressive lesion. In Japanses chestnut, further resistance leading to healing was observed, which was strongly related to the growth of tree and callus formation, which is influenced by environmental conditions. This means that the growing conditions of the tree are important to prevention of chestnut blight.

v. Incidence and environment

With respect to the relationship between incidence and damage and growing conditions, Aoyagi (2), the Nara Agriculture Station (38, 33) and Nishikado (41) suggest that weather, particularly low temperature and precipitation, soil richness, moisture, fertilization, age of tree and fruiting all influence the incidence of chestnut blight. But the interrelationships have not been understood clearly. Experiments were conducted on the incidence and growing environment.

A. Age of tree, environment, incidence and symptoms

a. Materials and methods

Three hundred Tsukuba seedling grafts were picked at random from plants grown in the towns of Dejima and Chiyoda in Ibaraki Pref. in November, 1968. One hundred-fifty each of 2-yr-old and 3-yr-old seedling grafts of the Tsukuba and Ginyose varieties grown on volcanic ashes were used in 1964 and 1965 as the 2- and 3-yr-old trees. For adult trees, 5-yr-old and 10-yr-old trees grown on volcanic ashes were used in 1971. These adult trees were grown in three types of orchards. Growing conditions in the properly managed orchards were 5 x 5 m spacing - 40 trees per 10 a, 14 kg nitrogen, 7 kg phosphorus, 14 kg potassium per 10 a, grassy land, and the tree crowns barely touching each other (10-yr-old). Densely populated orchards had auxiliary trees planted later between an original 5 x 5 m spacing. There was little sunlight on the ground and insufficient undergrowth (10-yr-old).

Abandoned orchards had 2 x 2 m spacing, giving 250 five-year-old trees per 10 a, with the weeds little managed. At the time of planting, trees with no outward lesions were selected.

b. Results

Table 19 shows the results according to the part of the seedling grafts. Fifty-nine of three hundred (20%)already showed distinct symptoms before removal from the nursery in November. A few plants died at the nursery, but they were not counted in the experiment. The incidence and the number of diseased trees do not agree because some trees had 2 or more lesions. Grafts had 31 incidences (10%), about half the number of diseased trees, the highest number. Then 22 trees were diseased in the scarred area of the trunk (7%). Then 14 trees in branches including those whose disease started at the grafting area and scarred parts (5%). Then 3 trees in the buds (1%). Lesions were formed from the places where the grafting wound has not been healed completely, where side branches were sawed off from the trunk, where scars were created during management of trees, and where buds had witherea.

Even with healthy seedling grafts planted in blight-free areas, many developed lesions in their infancy after planting. Table 20 shows the incidence of 3-yr-old trees (planted same year) and 3-yr-old trees (planted previous year). At the time of planting, branches of 2-yr-old trees were pruned at about 1 m to streamline their appearance. Pruned branches frequently withered and then became blighted. The incidence was 56% (84 trees out of 150). In most cases, lesion formation stopped at the topmost branch below the pruning stub, where callus formed. Almost all of them healed after the peeling off of lesions. Except for the above mentioned example, 2-yr-old trees showed traits similar to seedlings. Twenty-seven of one-hundred fifty trees developed the diseases (18%), twenty-one in the grafted area (14%), four in the withered buds (aver 3%), one in the injured part caused during planting, and one in the torn bark of a branch.

In the 3-yr-old trees, added to incidence on the main trunk, lesions occurred in new branches. Sites of lesions increased dramatically, but they appeared on the same tree. The number of trees

diseased was 33 out of 150 (22%) but with 107 lesions. Lesions developed most often in the withered buds, composing 68 of 107 total lesions (64%). Ten lesions were from a grafted area, frozen part, and concave part, 8 from torn limbs, and 13 from torn bark caused by branching.

Table 21 shows the incidence in 5-yr-old adult trees. The incidence was 19% in properly managed orchards, 39% in densely populated orchards, and 38% in abandoned orchards. As for the number of lesions, 31 occurred on the 29 diseased trees in the properly managed orchard, 44 on 43 diseased trees in the abandoned orchard, but in the densely populated orchard, 186 occurred on 74 diseased trees. In the latter, many branches intertwined, and inside branches tended to weaken and die. Lesions developed in these accounting for 54% of the incidence. Otherwise, lesions developed where branches had been cut or torn, accounting for 81% of incidence in the properly managed orchard, 23% in the densely populated orchard (43 lesions) and 43% in the abandoned orchard. Next, the site of injury (wouna) promoted lesion formation. In the abandoned area, 21% occurred in the grafted area, 16% at primary branches from the trunk, 16% right at the ground, and 9% in insect-eaten areas. Those lesions occurring mainly in the trunk were serious.

B. Symptoms and environment

1. Years after planting or shift in number of diseased trees

a. Materials and methods

Variety Tsukuba seedlings with no apparent lesions were planted in an established chestnut orchard (Chiyoda village, 1961), a newly-tilled area (Ami town, 1967), and an already tilled area (Ami, 1967). The number of trees planted (at 2.5 x 2.5 m spacing) were 65, 148 and 137, respectively. Every December thereafter, the number of trees dead from chestnut blight was counted.

b. Results

Table 22 shows the shift in the death caused by chestnut blight under different environmental conditions. There was a high percentage of death (25%) during the first year of planting. The death rate decreased as years passed.

This tendency was most striking in a new orchard with no chestnut orchards nearby. In the established chestnut orchard, the death rate showed a resurgence after 3 years of planting.

2. Seasonal shift in expansion of lesions

a. Materials and methods

In June and November of 1963, the extent of expansion on naturally occurring lesions was determined on pruned branches of 2-yr-old trees of variety Tsukuba planted in March, 1963. Additionally, lesions were induced by inoculation on main branches of 3-yr-old Tsukuba trees. From April, 1962 to April, 1963, the lengths of these lesions were measured every month. The rate of lesion expansion was calculated by subtracting the previous month's diameter (length) from the current measurement.

b. Results

Fig. 17. Two-year-old trees planted this year showed expansion of lesions from the cut branch-tip, where lesions originated, down to the branching point (Fig. 18). In all trees, lesions spread to the topmost branching point by November of the same year. When the branch was pruned right above a healthy side branch, no lesion was observed, as in Fig. 17-B and number 1 in Fig. 18 or else, if disease occurred, the lesion did not spread below the side-branch. Those which spread below the side-branch, as in numbers 2 and 4, hardly spread further.

When cut in places other than where side branches were, lesions spread to side branches by the time of observation in November, regardless of disease occurrence in June. The development between June and November was especially swift. Furthermore, in April of the next year, lesions were similar to those observed in November of the previous year. They showed little spread after November.

Monthly spread of the lesions which grew during the previous month is shown in Table 23. Lesions grew slowly between December and March and spread fast between April and November; especially fast were the 15 mm for August to September and the 11 mm for

September to October. The average temperature during the vigorous spread between mid-May and mid-October was above 15°C. The average temperature was similar in May and October, June, July, and September (Fig. 8, 9), but lesion spread was more vigorous in September and October than in June, July and May.

3. Lesion spread and temperature

a. Materials and methods

To discover the effect of temperature on lesion spread and hyphal development, 2-yr-old branches of Tsukuba which had been diseased by inoculation the previous August were collected in the Winter of 1963. The branches were cut 20 cm long with the lesion in the center and put in water. They were maintained for 1 month in 10, 15, 20, 25, 30 and 35°C incubators. The diameter of the lesion was measured and compared with that prior to treatment. Six lesions per treatment were provided.

b. Results

Table 24 shows the results. At all temperatures, lesion spread, but the rate was 1 mm in 25 days at 10°C. No mycelial fan formed underneath. Lesion spread was 7 mm at 15°C, 13 mm at 20°C, 23 mm at 25°C and 24 mm at 30°C. Between 15-30°C, concurrent with or slightly prior to lesion spread, newly grown, white, Pan-shaped hyphal colonies were observed. At 35°C, 8 mm of lesion spread occurred. The hyphal colony was yellow-white and smaller than the lesion with no white expanding area as seen at 15-30°C. Between 15 and 30°C, conidia were formed.

C. Incidence and soil moisture

When stromata get moist from rain, spores are released from pycnidia and perithecia (20). It is said (17) that incidence decreases in the summer when precipitation is low and spores are not being released as much; but in Japan, incidence tends to increase with decreased precipitation. Incidence increases dramatically with summer drought (38, 39, 41). The following experiment was conducted to study the reiationship between soil moisture, deeply related to precipitation, and incidence and tree growth.

a. Materials and methods

Nine trees each of 1-yr-old Tsukuba trees grown in troughs 1 m in diameter and 1 m deep were used. In each trough was placed brown volcanic ash field soil and 100 g of chemical fertilizer ($N15 \cdot P_2O_5 15 \cdot K_2O 15$) on 3/26/75. Three trees were planted in each trough. On 6/2, cultured hyphal segments were inoculated using a cork borer. From July to September, moisture was maintained at dry, normal and moist levels. In the dry block, a lid was placed on the trough to prevent rain from draining in. When leaf edges began to curl in a little, the trees were watered. The normal and moist blocks were left naturally and watered when needed. Change in soil moisture was measured by one DIK tensiometer placed 20 cm underground. Also, in order to find out the loss of nitrogen by irrigation, soil collected prior to planting, after fertilization, just prior to water treatment, one month after water treatment, and on October 15 was analysed by the phenol-SO_4 method for NO_3-N (see D, a). Incidence was observed on October 22. Growth of trees was measured at 6/13 and 10/23. The maximum possible water content of the soil was 124.5%. Its chemical composition is shown in Table A. The change in water tension at 20 cm underground is shown in Fig. 19.

b. Results

Table 25 shows the results. In the dry block, all nine trees were diseased with a mean lesion length of 34 mm; in the standard block, 3 of 8 were diseased with a mean lesion length of 10 mm; in the high moisture block, 6 of 9 with a mean lesion length of 16 mm. The growth in diameter of trees was 3.1 mm in the dry, 5.3 mm in the standard and 4.8 mm in the moist block. Based on these measurements, the ratio of tree growth (in diameter). and lesion length was an inverse one: $r = 0.823^{**}$ as shown in Fig. 20. Table 26 shows the change in NO_3-N due to irrigation. On April 15, there were 34-36 mg of N from 0 to 20 cm below ground and 19-21 mg from 20 to 40 cm. On June 25, 10-11 mg of N were at 0 to 20 cm and 12-13 mg from 20 to 40 cm.

Prior to watering, all blocks showed similar amounts of N in soil. On 7/25, after wateri'ng, the dry soil had 3.7 mg at 0-20 cm and 6.4 mg at 20-40 cm, which was higher than in the moist soil. On 10/15, it was 2.3 mg and 14.6 mg in the dry soil, 0.9 mg and 2.5 mg in the regular soil, and 0.6 mg and 0.5 mg in the moist soil. The electrical conductivity was highest at 0.87 mU in the surface layer on 4/15 (after fertilization).

D. Fertilization and incidence

It is reported that the incidence rate is high in lean soil (38, 39, 41); high in orchards with lean soil fertilized with chemical fertilizer (41), especially if heavily fertilized at the time of planting, but deficient later (39, 41); high when fertilized to proinote growth of tree in the autumn (2); and high in a year after excessive fruiting (39). It is also reported that the use of potassium fertilizer inhibits incidence (3).

a. Materials and methods

One-yr-old trees of the Tsukuba variety were grown in concrete troughs 1 m square and 1 m deep, and in 20 m^2 plots. In the latter were planted 3 rows of 3 trees each. Twenty g per tree of fertilizer (9 kg per 10 a) N, P_2O_5, and K_2O were applied. In the concrete troughs were planted 3 rows of 1 tree each with 5 g each (per tree) of fertilizer (15 kg per 10 a) applied on 3/26, prior to planting. Two-thirds of the urea and P_2O_5 and all of the K_2O were applied mixed in all the surface soil down to 20 cm'deep in the concrete troughs. In the field plots, the fertilizer was mixed with the soil used to fill the 40 cm diameter, 30 cm deep planting holes. The rest of the fertilizer was applied on 7/9-10 by mixing on the surface soil around the tree. Salt concentrations in different layers of soil were measured on 4/15 and 7/25, and NO_3-N right after fertilization and on 10/15. Electrical conductivity was determined with an electrode (Toa Model-CM-2A) after adding water (1:5 ratio) and shaking 30 min. NO_3-N was measured with the phenol-SO_4 method using a Hitachi 200-10 double-beam, prism spectrophotometer. Hyphae from cultures were inoculated using a cork borer on June 2-4 and the number of diseased trees and lesion length were measured on 10/21-22. The difference in lesion length between

6/13 and 10/13 was used as the degree of growth. Soil conditions prior to fertilization, analysed on 3/25, are shown in Tables B and C.

b. Results

Tables 27 and 28. In the orchard (Table 27), the incidence rate was highest in the non-fertilized block, with all trees diseased and a mean lesion length of 96 mm. The no-nitrogen block had 95% incidence with a 60 mm mean lesion length. Fertilization with N or all 3 elements lowered incidence; incidence was 60%with a mean lesion length of 43 mm in the standard 3-element fertilized block, 66.7% with 48 mm in the block fertilized with a double dose of N. The incidence rate was even lower in the blocks with a triple dose of N (33.3%, 26 mm), the block fertilfzed with a double dose of all 3 elements (43.9%, 33 mm), and the block fertilized with a triple dose of all 3 elements (45%, 29 mm).

In the concrete troughs also (Table 28), all trees were diseased with 33 mm and 36 mm mean lesion length in the non-fertilized block and the no-nitrogen block, respectively. Incidence was lower in the fertilized block; 2 out of 8 trees were diseased, with a mean lesion length of 10 mm in the standard block; 3 out of 8 with 11 mm in the blocks double dose N; 4 out of 7 with 19 mm in the triple dose of N; 4 out of 7 with 15 mm in the double dose of all 3 elements; and 3 out of 8 with 11 mm in the triple dose of all elements. Little difference occurred due to the amount of fertilization, whether N or all 3 elements, but trees without fertilization were more diseased. As for tree growth, in the cultivated field, the growth was least, being 1.5 mm in the non-fertilized block, 2.5 mm in the no-nitrogen block, 2.6 mm in the standard fertilized block, 3.4 mm in the double-nitrogen block, and 3.8 mm in the triple-nitrogen block and the double- and triple-fertilized blocks. In the concrete trough, the growth was 2.3 mm in the non-fertilized block, 3.8 mm in the non-N blocks, and 5.2-7.6 mm in blocks fertilized with all 3 elements.

As shown in Figs. 21 and 22, the relationship between tree growth and lesion length was r = 0.818* in the cultivated field and r = 0.778* in the concrete trough.

Table 29 shows the change in NO_3-N during the experiment period of March to October, 1975. In the orchard, the maximum figures obtained on July 25 were 18.2 mg in the soil 0-20 cm in depth and 20.9 mg in the soil 20-40 cm in the standard fertilization block; 5.1 mg and 5.9 mg in the non-fertilized block; 5.7 mg and 4.9 mg in the no-nitrogen block; 41.8 mg and 33.4 mg in the double-nitrogen block; 35.7 mg and 29.6 mg in the double-fertilization block; 53.4 mg and 35.4 mg in the triple-nitrogen block; and 54.9 mg and 35.1 mg in the triple-fertilization block. On October 15, the non-fertilized and no-nitrogen blocks showed extremely low values of 0.5-0.7 mg in all soil depths. The standard fertilization block showed 1.2 mg in the soil depth of 0-20 cm and 4.2 mg in 20-40 cm; the double-nitrogen block, 3.4 mg and 8.9 mg; and the triple-nitrogen block 6.1 mg and 16.0 mg. In the concrete trough experiment, the highest values were obtained on April 15 and July 25 (after fertilization); 31.5-33.4 mg in 0-20 cm in the triple fertilized block in April and 30.1-35.5 mg in July; 20.0-25.2 mg in April and 17.4-17.7 mg in July in the double-fertilized block; 17.5 mg in April and 12.7 mg in July in the standard fertilized block; 2.9 mg in April and 2.3 mg in July in the non-fertilized block; and 1.5 mg in April and 1.8 mg in July in the no-nitrogen block. On October 15, all blocks in the soil depth of 0-20 cm had less than 3 mg with little variation. But in the 20-40 cm soil depth, the standard fertilization block had 11.4 mg; the non-fertilized block, 5.5 mg; the no-nitrogen block, 4.4 mg; the double-nitrogen block, 13.7 mg; the double-fertilization block, 14.8 mg; the triple-nitrogen block, 19.9 mg; and the triple-fertilization block, 17.8 mg.

The change in conductivity (souble salts) is shown in Tables 31 and 32. The highest value was 1.02 mU in the 20-40 cm soil profile of the double N block in the cultivated field and 1.14 mU in the 0-20 cm profile of the triple fertilized block (both on 4/15). The rest were all below 1 mU.

E. Growing methods and incidence

The growth of chestnut trees is vigorous and, in normal orchards, the plot becomes densely vegetated several years after planting. Incidence also is influenced by the growing conditions.

1. Tree density

a. Materials and methods

In 1960, 120 trees of the variety Ginyose were planted at 2.5 x 2.5 m spacing. Two years later, in 1962, one half of the field was left at that density, while in the other half trees were eliminated to give 5 x 5 m spacing. Each block was further divided into two; one side was fertilized with 10 kg nitrogen 5 kg phosphate, 10 kg potassium per 10 a; the other half was left unfertilized.

After planting in October, 1960, trees which had died from chestnut blight were cut and 5 of them were placed in each block as a source of inoculum. In November, 1963, incidence was measured. After that, 10 healthy trees were chosen from each block and were inoculated with cultured mycelium. Trees and lesion tissues were measured in November, 1964. Ten trees were measured for growth and two trees for root distribution. In November, 1964, the tree growth and root distribution of each block were studied. Using 10 trees each for branches and 2 trees each for roots, the root distribution at the 30 cm and 150 cm circumferences was measured.

b. Results

Table 33 shows density and incidence. Incidence in non-inoculated trees was 34% in the dense, non-fertilized block, 22% in the dense, fertilized block, and none in the sparsely populated blocks. For the inoculated trees in the dense, non-fertilized block, eight out of ten trees got disease with one tree dead. Five of ten trees were diseased in the fertilized block. In the sparsely populated block, 2 trees were diseased in the fertilized block, and none in the non-fertilized block. In the dense block, many branches withered and died where the disease occurred, especially in the non-fertilized block.

Growth is shown in Figures 23 and 24. In the dense block, the tree height was 3.3 m, fertilized, and 2.9 m, non-fertilized, and the limb diameter was 5 m and 4 m; in the sparse block, height was 3.5 m, 3.3 m and diameter 5 m and 4.6 m. In the dense block, branches touched and mixed, with branches on the inside dying often.

Root distribution is shown in Figures 25 and 26. At the section 30 cm south of the trees, tree roots spread to 80 cm deep in all blocks, but no deeper. Horizontal distribution was similar to branch distribution. Roots were touched by those of neighboring trees at 1.2 m in dense block and 2 m in sparse block.

Many fine roots were dead where they touched each other. At 150 cm to the south from the trunk, roots spread to 2.3-2.5 m on one side in the sparsely populated block, and there was a little touching of roots in the fertilized block; but in the densely populated block, roots spread to about 1 m on one side and roots touched on all sides. Above and udnerground, callus formation was good in the fertilized block and not good in the non-fertilized block.

2. Cultivation management

a. Materials and methods

To study the relationship between incidence and tree density, sod culture and clean culture, 120 trees of varieties Tsukuba and Tanzama were planted alternately with 2.5 x 2.5 m spacing in March, 1967, in an orchard where grapes had been grown (Ami, Ibaraki). Three years later, in 1969, the plot was divided into two; one to sod culture and the other to clean culture. In November of the same year, the two plots were further divided into two; in one, trees were removed to give a spacing of 5 x 5 m and in the other, the tree density was maintained the sáme as at the time of planting. In November, 1971, the incidence of blight was measured.

To study tree variety, sod culture and clean culture in the plot where chestnuts had been grown in Chiyoda, Ibaraki, the varieties Nakaotamba, Ginyose (10-12 year old) and Tsukuba (4 year old) were planted in clean culture. In 1955, 3 plots were established: clean culture, fertilized clean culture and fertilized clean culture with Timothy and Rajin clover. Incidence was measured in October, 1968.

b. Results

Table 34 shows the results. On the grassy field, 3 of 15 trees in the sparse block became diseased and 11 of 30 9j the dense block (20% and 37% incidence). In the clean field, 2 of 14 (15%) trees were

diseased in the sparse block, and 12 of 30 (40%) in the dense block. Table 35 shows the cultivar and incidence. In the grassy, fertilized field, 2 of 25 Nakaotanba (8%) trees were diseased and 3 of 33 Ginyose trees (9%). None of Tsukuba were diseased. In the clear, fertilized field, 16% of the Nakaotauba, 19% Ginyose and 15% Tsukuba became diseased. In the clear, non-fertilized block, incidence increased: 22% of the Nakaotanba, 30% of the Ginyose, 60% of the Tsukuba trees became diseased. Tsukuba showed the most difference in incidence according to treatment.

F. Grafting, cold damage and the disease

a. Materials and methods

Branches of Tsukuba were grafted on wild chestnuts at 0, 5, 25, 50, and 75 cm above ground level. The next March, in 1966, 50 trees for each grafting height were planted in the orchard. Cold damage and incidence were studied for 4 years until the fall of 1969. Cold damage was determined by discoloration of the bark.

b. Results - Figure 27.

Frost burn appeared near the grafts. The bark turned black-brown and a little concave. Frost burn occurred more on lower grafts; 70% of the grafts at 0 cm had frost burn, 66% at 5 cm, 36% at 25 cm, 6% at 50 cm and less at 75 cm.

Lesions occurred often where frost burn killed the limb. The incidence was 50% at 0 cm, 26% at 5 cm, less than 10% above 25 cm and 0% at 75 cm. Frost burn and incidence were roughly proportional

G. Root cutting, light and incidence

a. Materials and methods

Five trees each of 3-yr-old Tsukuba were inoculated with mycelium on 7/3/64 and measured for the number and size of lesions in October.

Roots: The limb length was 120 cm, so we assumed the roots were the same length and cut them to half that length, so that the root balls were cut to 60 cm in diameter and 60 cm in depth.

Light: Limbs were covered with black cloth for 3 months until the end of September. At noon on a sunny day, the rate of sunshine beneath the cloth was about 50%.

Roots and light: Both treatments were done on the same tree.

b. Results - Table 36.

In the untreated block, 2/5 trees became diseased with a mean lesion length of 14 mm (small). In the trees whose roots were cut, all 5 got diseased and the mean lesion length was 48 mm; one tree died. In the shaded trees, four were diseased with a mean lesion length of 32 mm. In the block with both root and light treatment, all 5 trees were diseased with 44 mm as the mean lesion length; three trees died.

H Discussion

Sites of lesion formation vary with the age of tree and growing conditions. In the seedling grafts, lesions occurred most often on grafts, especially when the grafting area was not smooth, but had swollen tissues and a rough surface.

Even if seedling grafts with no apparent lesions were planted in a clean place, lesions sometimes formed. This was because lesions had formed underneath the skin (Plates 1-F, G). Lesions often occurred at sites of injury.

The number of diseased trees tended to increase with the years after planting (Tables 20 and 21), but the death rate decreased (Table 22). In young seedlings, the disease occurred most often on the trunk, which often cuases the seedling to die. As the trees grew older, the disease occurred localized on the branches.

Lesion expansion was vigorous in summer and fall, and slow in winter and spring (Table 23). From mid-May to mid-October, when it was vigorous, the average temperature was above 15°C (Figures 8 and 9). This agreed with the results of growth of the fungus (Table 4) and lesion expansion (Table 24). The results with American chestnut by Anderson (1) and those with European chestnut by Bazzigher (5) that expansion was most vigorous in July and August were in agreement with our results. But, in the case of C. crenata, there was a difference in expansion at the same temperature from June to August compared to that from September to October. It is attributable to the difference in callus formation, which was vigorous in May-July and slow after September (Table 11). Callus formation and lesion expansion were in inverse proportion (Figures 14, 15 and 16). It is reported that growth of the fungus on site is prevented by the formation of cork layer (11, 29). Our results (IV-B) agree with this.

Incidence increased with drying of soil in summer, when tree growth was inhibited in the dry block and the trunk expansion slowed (Table 25). This agrees with the findings that incidence of blight increased with summer drought (38, 41) and that the growth of chestnut is influenced by a lack of soil moisture (36). Of course erosion, especially leaching of NO_3-N, must be taken into account, but it had little effect on tree growth in the months of June and July (Table 26) which are crucial to tree growth (24).

Fertilization: Lesion expansion increased in non-fertilized and no-nitrogen blocks, and was inhibited by increased application of all three elements and nitrogen (27, 28). This agrees with the results that the lack of fertilization promoted disease incidence (38, 39, 41). The growth of the trunk and the length of lesions were in inverse proportions (Figs. 21 and 22), and the growth of the tree was correlated with incidence. The lesion expansion rate did not differ much with the amount of fertilization except with non-fertilization and no-nitrogen fertilization. But, since the chestnut has a wide range of fertilization, especially nitrogen (24), all amounts used in the experiment were suitable and promoted tree growth and inhibited disease. Judging also from chloride concentrations (Tables 31, 32) growth was not inhibited by over-fertilization.

Incidence increased with density of trees (Table 33). With crossing and touching of branches (Figs. 23, 24, 25 and 26), branches died, which became an opening for fungus invasion,. Root shearing and light blocking prevented absorption of nutrients, water and photosynthesis, respectively. These were physiological stresses on the trees (31) and also resulted in lesion spread (Table 36), suggesting physiological stress as a cause of lesion occurrence and spread.

Because the fungus invades injury sites, the injury caused by frost burn becomes an infection site. Frost burn occurred near the graft in young seedlings, especially on grafts made 25 cm above the soil line. The disease occurred often there (Fig. 27).

Summary

The Endothia canker has been well known as one of the most important diseases of chestnut trees.

Up to the present time, studies on the physiology and ecology of the causal fungus, Endothia parasitica (Murrill) P. J. et H. W. Anderson, have been reported by many pathologists, but very few studies on the ecological control of the disease by experimental methods have been reported.

This study was conducted: on the ecology of the Endothia canker (E. parasitica (Murrill) P. J. et H. W. Anderson) of chestnut trees, Castanea crenata Sieb. et Zucc., under different cultural conditions; on the resistance reaction of chestnut trees to invasion by Endothia parasitica; and on the morphological and physiological characteristics of the causal fungus. The results are summarrized below.

Symptoms: The symptom of Endothia canker due to attack of the fungus was observed on chestnut trees of every age level, but there were cases where the latent infection of the fungus'was observed on seemingly normal barks. The fungus attacked trunks, branches and exposed roots of the chestnut trees. The initial invasion of the fungus occurred on the injured tissue of the chestnut trees.

Hypertrophied or swollen symptoms were often considered to be signs of a resistance to Endothia canker. The symptoms of Endothia canker were classified into four types: acutely progressive type, progressive type, chronic type and recovering type, according ot the degree of development of the lesions.

Morphological characteristics of the causal fungus: Stroma were scattered or gregarious on smooth bark and often confluent. Perithecia were embedded in the lower part of stroma. Pycnidia were formed under the periderm, pycnospores exuded out from stroma as sticky ye1lowish orange-colored tendrils under moist condition. The fungus produced fan-like mats of mycelium within the cambium of chestnut trees.

Physiological characteristics of the causal fungus: The minimum temperature for mycelial growth was 0-5°C. The sporulation of pycnidiospores was observed within the range of temperatures between 15 and 30°C. The minimum temperature range for the germination of pycnidiospores was 5-10°C. Optimum temperature range for the germination of pycnidiospores was 25-30°C.

The growth of mycelium was hastened by adding 0.1% tannic acid to the media; however, its growth was inhibited by adding a higher percentageof tannic acid (0.2% and above). The amount of tannic acid in media decreased with growth of mycel ium.

The most suitable nitrogen source for mycelial growth of the fungus was peptone, although many of the nitrogen sources tested were suitable for the fungus. Among the carbon sources tested, soluble starch and dextrin were favorable for mycelial growth. For the sporulation of pycnidiospores, the suitable carbon sources were monosaccharide and disaccharide. The best concentration of sugar source for sporulation of pycnidiospores was 2-3%.

The fungus did not require vitamins as indispensable factors for mycelial growth, but the growth of mycelium was accelerated by adding vitamins to the media.

From the results of chemical analysis of chestnut bark, it was found that, of the barks affected by the causal fungus, the substances which were soluble in water or 3% HCl solution, and soluble sugars were considerably less than those of healthy barks.

The mycelial growth of the causal fungus was accelerated by adding chestnut bark extract to synthetic media. The growth substances were extracted from chestnut barks with water, hot water and 75% ethyl alchhol and they were absorbed by activated carbon granules and stable under acid or alkaline reaction and in boiling water. The substances were suggested to be carbohydrates and amino acids.

Infection period of the causal fungus: Endothia canker infection upon the periderm or its wounds of the Japanese chestnut was possible from mid-March to mid-October with the optimum season from August to September.

The invasion of the causal fungus was influenced by temperature and rate of callus formation on the chestnut trees.

Reaction of chestnut trees to invasion by the causal fungus: The results of anatomical observation on the canker are summarized as follows: Under favorable conditions for the invasion of the fungus, the mycelia affected the tissues so rapidly that the formation of defensive tissues could not take place. When the growth of host trees was active the defensive tissues surrounding the affected area developed rapidly.

Microscopical observations of the resistant chestnut trees showed that the fungus infection was restricted to the layers of bark and that a brown zone had been formed surrounding the infected areas. At the same time, a wounded cork layer appeared in the tissues inside the brown zone, and separated the infected areas from adjacent uninfected tissues. In the next stage of the healing process, callus formation took place at the cambium, and complete exclusion of the infected tissues appeared to require the formation of periderm.

A negative correlation was obtained between the development of Endothia canker and the amount of the callus formation over the wounds of chestnut trees. The callus formation was possible only during the growing season of chestnut trees under natural conditions, and its formation was retarded in unsuitable conditions for the vegetative growth of chestnut trees.

Ecology of the disease: Many young trees, especially 1- to 2-yr-old ones, were rapidly withered by the attack of the fungus. When mature trees were planted under suitable conditions for growth, the development of Endothia canker was rather slow.

The development of the lesions occurred from early spring to late autumn, with the best season for the development from August to September, and was related to temperature and the rate of callus formation on the chestnut trees.

The development of Endothia canker was accelerated by drought of soil moisture in summer and nitrogen deficiency. The vegetative growth and callus formation of chestnut trees were prevented by the above-mentioned conditions, and a negative correlation was obtained between the development of Endothia canker and the growth rate of trunk diameter of chestnut trees.

The damage by Endothia canker was increased in densely-planted chestnut orchards in poor soil with little fertilization.

The damage by Endothia canker in the sod cultural orchards was less than that in clean cultural orchards.

More damage by Endothia canker was observed on young trees of low grafting compared with those of high grafting. The attack of the fungus was connected with freezing injury on the grafting point of chestnut trees, and the freezing injury of chestnut trees was increased on grafting points lower than 25 cm in height.

The development of the lesions was accelerated by such abnormal treatment as root pruning and shearing.

Conclusion: From these results, it can be said that, in addition to surgical and fungicidal treatment, improvements in growth of chestnut trees by cultural measures are very important for the control of Endothia canker of Japanese chestnut trees.

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FIGURE CAPTIONS

FIG. 1 Relationship between fungus growth and tannic acid concentration
abscissa - tannic acid concentration (%)
ordinate - diameter of mycelial colony (cm)
O - O: EP-1, △...△ : EP-6, □...□; EP-7, x-·-·-x: EP-9
7 days at 27°C. 5 petri plates each

FIG. 2. Source of nitrogen in culture medium and fungal growth
abscissa - fungus dry weight (mg)
ordinate - source of N
Basic culture medium - Richard's. Cultures grown 30 days at 28°C.

FIG. 3. Source of carbonydrate in culture medium and fungus growth
abscissa - fungus dry weight (mg)
ordinate - source of carbon
Basic culture medium: asparagine medium. Cultures grown 10 days at 28°C.
Average value of 3 weights each. P: conidia formed; +: growth negligible.

FIG. 4 Relationship between type of starch and fungus growth
abscissa - fungus dry weight (mg)
ordinate - type of carbon
Basic medium: Richard's. Cultures grown 20 days at 28°C. Average
value of 3 flasks each.

FIG. 5 Relationship between concentrations of starch and sugar and fungus growth
abscissa - fungus dry weight (mg)
ordinate - concentration (%)
Bssic medium: Richard's. Cultures grown 15 days at 28°C. Average
value of 3 flasks. P: conidia formed; +: growth negligible

FIG. 6 Relationship of ratio of starch and grape sugar (dextrose) to fungus growth
abscissa - fungus dry weight (mg)
ordinate - ratio of S and G
P: conidia formed; S: starch; G: grape sugar
Cultures grown 20 days at 28°C. Average value of 3 flasks.

FIG. 7 Relationship between type of vitamin and fungus growth
abscissa - fungus dry weight (mg): left - 1 vitamin added; right - 1 vitamin omitted
ordinate - type of vitamin
Basal medium: Richard's salts with glucose and starch added
Cultures grown 15 days at 27°C. Average value of 3 flasks.

FIG. 8 Average temperature and precipitation at time of inoculation from 1961 to 1962 **(1).**

FIG. 9 Average temperature and precipitation at time of inoculation from 1962 to 1963 **(2).**
abscissa - month
ordinate - temperature (°C) (left), precipitation (mm) (right)

FIG. 10 Cross section of lesion type I
B: bast, browned & dead; X: xylem

FIG. 11 Cross section of lesion type II
A. Cross section. B. Magnification of A
B: bast; X: xylem; Br: browned, diseased area

FIG. 12 Cross section of lesion type III
A. Cross section B. Magnification of A
B: bast; X: xylem; C: callus formed between bast and xylem
BZ: brown zone formed between browned, diseased area and healthy area;
Br: browned, diseased area

FIG. 13 Cross section of lesion type IV
A. Cross section B. Magnification of A
B: bast; X: xylem; DB: dead bast; Br: brown, diseased area;
BZ: brown zone formed between brown diseased area and healthy area.

FIG. 14 Relationship between callus formation and lesion growth (orchard).

FIG. 15 Relationship between callus formation and lesion growth (trough grown)

FIG. 16 Relationship between callus formation and lesion growth (soil moisture controlled)
abscissa - callus formation (%)
ordinate - lesion length (mm)

FIG. 17 Incidence and pruning point. A: When pruning occurs beyond a healthy branch, a lesion starts at the pruning point and extends to the branch.
B: This does not occur with pruning at a healthy branch.

FIG. 18 Diagram of area of incidence on juvenile trees
abscissa - tree number
ordinate - height of bud above grafting point (cm)
-o: topmost, healthy branch : extent of canker in June
x: diseased on top in June : extent of canker in November

FIG. 19 Daily change in water column from July to September
abscissa - day of the month
ordinate - water column (mm)
●——● soil maintained dry; ● — — — — ● soil maintained moist;
●—·—● soil maintained wet

FIG. 20 Relationship between trunk diameter and lesion growth (soil moisture controlled)
abscissa - net trunk growth (mm)
ordinate - lesion growth (mm)

FIG. 21 Relationship between trunk growth and lesion growth (orchard)

FIG. 22 Relationship between trunk growth and lesion growth (concrete trough)
abscissa - net trunk growth (mm)
ordinate - lesion growth (mm)

FIG. 23 Diagram of tree density and crown and root growth (1, 5 x 5 m, sparse planting density)
abscissa - distance between tree parts (m)
ordinate - tree height (m, top) and root depth (m, bottom).
top diagram - fertilized block
bottom diagram - unfertilized block

FIG. 24 Diagram of tree density and crown and root growth (2, 2.5 x 2.5 m, dense planting density)

FIG. 25 Root distribution of trees of different density (1, 5 x 5 m sparse planting density)
abscissa - distance between tree parts (m)
ordinate - depth below grown (m)
top diagram - non-fertilized, 30 cm from trunk
second diagram - fertilized, 150 cm from trunk
third diagram - fertilized, 30 cm from trnnk
bottom diagram - non-fertilized, 150 cm from trunk
x: planting position ◎ : roots larger than 10 mm diameter
o: roots larger than 15 mm diameter fine roots
roots overlapping with roots of next tree

FIG. 26 Root distribution of trees of different density (2, 2.5 x 2.5 m dense planting density)
top diagram - fertilized, 30 cm from trunk
second diagram - fertilized, 150 cm from trunk
third diagram - non-fertilized, 30 cm from trunk
bottom diagram - non-fertilized, 150 cm from trunk

FIG. 27 Position of frost death and incidence
abscissa - height of graft (cm)
ordinate - death rate from frost (%, left) and blight incidence (%, right)
50 each height of wild chestnut stock grafted with Tsukuba scions. Frost death and incidence measured from 1966-1969 (4 years).

Plate 1

A. Blight incidence in an orchard. When a tree dies with the leaves still on it, they often do not abscise.

B. When the trunk withers, adventitious buds often sprout below the cankers.

C. On the canker, the bark turns a lusterless black-brown and becomes sunken.

D. On the canker are formed many pycnidia 1-2 mm in diameter.

E. In wet weather, curly, orange-yellow tendrils of conidia exude from the pycnidia.

F. A canker which appears healthy outwardly. Such cankers normally do not expand.

G. Scraping away the surface of a canker, such as in F, you can see the brown tissue inside.

H. Underneath the surface of a canker with pycnidia and a black-brown discoloration, you can see colonies of fan-shaped mycelia.

Plate 2 - Characteristics of seedlings

A. A high rate of blight incidence is observed on abnormally shaped grafts whose wound periderm is rough.

B. Low incidence is observed on normal grafts which are smooth and show good healing.

C. Canker formed at the site of a cut side branch.

D. Canker formed at a bud.

Plate 3 - Characteristics of 2-yr-old and 3-yr-old trees (1)

A. Canker formed on the pruning wound of a seedling pruned at the time of planting. Canker normally heads in the pruning stub.

B. Cut surface right above a healthy secondary branch. Good wound periderm development and no canker.

C. Canker formed on graft.

D. Canker formed on graft.

E. Canker formed on the bud.

F. Canker started from the bud splitting the surface of the branch.

G. Canker started from the bud splitting the bark horizontally and vertically.

Plate 4 - Symptoms of 3-yr-old trees (2)

A. A canker on the trunk, which appeared to have started from the graft, illustrating the swollen, rough surface and splitting.
B. Canker which appeared to have started at a site of frost burn. The canker is sunken more than usual. These often occur at ground level.
C. Canker formed on a root exposed above ground.
D. Canker formed from the split on a bud near a branching point.
E. Canker formed in a bark crack occurring at a branching point, after the crack becomes rough; cankers occur mainly on the upperside.

Plate 5 - Symptoms of adult tree trunk

A. Canker on the trunk. Canker is swollen and split, the old parts of the canker often slough off.
B. Canker which appears to have started from frost damage.
C. Canker occurring as secondary disease on bark diseased by another pathogen. The canker is sunken with traces of secretion of black juice from small slits in the bark surface.
D. Canker started from a pruned branch, spread to the trunk.
E. Canker developed from inside a pruned branch stub.
F. Canker spread from the secondary branch to the tronk and the main branch.

Plate 6 - Symptoms on the branches of an adult tree (1)

A. Canker on the big branch which spread from the small branch.
B. Canker on a branch; the canker originated, at the branching point.
C. Canker formed in a pruning wound.
D. Wound periderm on a pruning wound, there is no canker.
E. Canker formed at a broken-off branch. It is often swollen and split.
F. Canker formed from the dead branch.

Flate 7 - Symptoms of the branch of an adult tree

A and B. Canker on withered branches as often occurs in densely planted orchards. Canker covers the whole branch forming fruit bodies.

C. Canker in the middle branch. Canker formed at a branch fork and an injury site. It is swollen and split.

D. Canker spread from the small branch to the middle branch, small branches are dead.

Flate 8 - Cankers on insect-damaged plants

A. Canker formed at the burrowing site of a chestnut twig borer larvae (Kuritamamushi). Occurs in small branches and juveni1e trees.

B and D. Canker at burrowing site of a bat moth larvae (Komoriga) occurs mostly on the bark and on branches.

C. Canker at burrowing site of a larvae of a moth. Occurs mainly at branching points and cracks in the trunk.

E. Blight occurs on almost all trees damaged by bark beetles and other related insects.

F. Canker at feeding site of a longicorn beetle. Cankers also develop at oviposition sites and sites of larval burrowing. Occurs most often on the main trunk.

Plate 9 - Canker types depending on rate of spread

A. Very rapid: Discoloring wide areas quickly. Stromata appear later.

B. Rapid: Fairly smooth with little ridging. Relatively fast spread.

C and D: Chronic: Swollen around the canker, temporary stoppage of spread, then more spread causing ridging. Sometimes the bark is split.

E. Healing: Callus develops around the canker, stopping its spread. The old canker peels off.

F. Healing: Callus forms on the canker formed from the wound. The old canker tissue peels off.

Plate 10 - Histology of the blight fungus

A. ascostroma

B. peri thecium

C. asci and ascospores

D. pycniostroma

E. conidia

Plate 11 - Inoculation method

A. Cut holes

B. Inoculate

C and D. Replace bast

E. Cover with wet cotton

F. Cover with vinyl

Plate 12 - Lesion type I

A. Outward appearance

B. Cross section

C. Longitudial section

D. Magnified cross section

Plate 13 - Lesion type II

A-D. As in Plate 12

Plate 14 - Lesion type III

A-D. As in plate 12.

BZ: brown belt, C: callus, X: new xylem

Plate 15 - Lesion type IV

A-D. As in plate 12.

X: new xylem, DB: dead bast, BZ: brown belt

Plate 16 - Microscopical appearance of lesion type I

A. Cross section of diseased bark, showing internal spaces.

B. Magnification of A.

C and D. Hyphae (H) in the lesion

Plate 17 - Microscopical appearance of lesion type II

A. Browning occurs in the bast parenchyma (BP) .but not the bast fibers (BF).

B. Magnified view of A

C. The lesion is surrounded by a brown zone (BZ)

D. Magnified view of C

Plate 18 - Microscopical appearance of lesion type III (1)

A. A brown zone (BZ) occurs around the lesion, often at the border between the bast parendeyma (BP) and bast fibers (BF). No discoloration has occurred in the bast fibers except in rays traversing them (RT).

B. Cork layer (CO)

C and D. Magnified view of cark layer (CO)

Plate 19 - Microscopical appearance of lesion type III (2)

A. Cork layer (CO)

B. Separation (S) of bast parenchyma (BP) at the brown zone (BZ)

C. Separation (S) of bast parenchyma (BP) and bast fiber (BF) at brown zone (BZ)

D. Magnified view of C

Plate 20 - Microscopical appearance of tissues near the cambium in lesion types III and IV

A. Callus (C) formed between wood (W) and bast (B)

B. Cork (CO) in exterior layers of callus

C. Xylem (X) and bast (B) formed in callus (C). Diseased bast (DS) is enclosed and tyloses (T) have formed.

Plate 21 - Callus formed under humid conditions

A. Callus formation: Callus (C) and wood (W)

B. Magnified view of A

C. Callus exposed to air for several days forms a cork tissue (CO) on Its exterior.

D. New callus just starting to form cork (CO).

Table 1. Morphology of the fungus on solid culture media

Type of medium	Colonies of mycelial fan growth[a]	appearance[b]	Conidia formation[c]
Potato-agar	68m	Flat, orange-yellow	Small, dense all over
Chestnut juice agar	77	Thick velvety, yellow-white	Large & small, dense in center
Chestnut skin agar	60	Thin, silk thread-like, white	Medium, around the rim in circles
Oatmeal agar	37	Very thin, white	Very small, dense in concentric circle
Peptone agar	13	Thick, doubled, white	Very small, extremely sparse in center
Richard's agar	52	Very thin, silk thread-like, white	None
Yeast-amended Richard's	73	Thick velvety, light . yellow	Large, sporadic all over

4 petri plate each were used; a) diameter of colony after 7 days at 25°C; b), c) after one month at 25°C; c) orange-yellow, sticky

Table 2. Pedigree of fungus isolates

Isolate	Location	Variety of tree	Date	Type
EP-1	Koganei, Tokyo	Okano wase	September, 1953	Ascospore
EP-6	Kamazaki, Hyogo Prep	Unknown	February, 1963	Lesion
EP-7	Kikuchi, Kunamoto	Rihei	May, 1953	Lesion
EP-9	Ami, Ibaraki	Tsukuba	April, 1963	Lesion

EP-1 was given by Ministry of Agriculture, Forest Research Station, Protection Department.

Table 3. Comparison of pathogenicity of fungus isolates

Isolate	1965				1966			
	No. trees	No. diseased trees	Incidence (%)	Length of lesion (mm)	No. trees	No. diseased trees	Incidence (%)	Length of lesion (mm)
EP-1	18	18	100	37	38	34	90	47
EP-6	21	20	95	30	38	33	87	42
EP-7	16	7	44	35	38	3	8	4
EP-9	23	23	100	47	37	36	97	116

The branches of 3-yr-old and 4-yr-old trees were inoculated in early June. The number of lesions and lesion length were measured in early December.

Table 4. Relationship between fungus growth and temperature

Temperature	Growth rate of mycelial colony[a]	Morphology of mycelial colony after 8 days
0° C	0 mm	———
5	0.4	Thin mycelium
10	1.7	Fairly thin mycelium
15	4.0	Medium mycelium, conidia formation sparse
20	5.5	Medium mycelium, conidia formation medium
25	7.0	Medium mycelium, conidia formation dense
30	6.5	Medium mycelium, conidia formation dense
35	0	Thick mycelium, growth arrested

a) Potato agar culture, rate of growth at 24 hrs. 5 petri plates each. Strain EP-1 was used.

Table 5. Tannin ccnsumption by the fungus

Isolate	Fungus dry weight (mg)	Tannin consumption (mg)	Tannin consumption per mg/fungus
Chestnut bark medium (12 days at 27°C)			
EP-1	476	101	0.21
EP-6	634	105	0.16
EP-7	589	105	0.18
EP-9	497	83	0.17
Richard's medium with 0.1% tannic acid (7 days at 27°C			
EP-1	124	36	0.29
EP-6	121	34	0.28
EP-7	154	35	0.23
EP-9	147	41	0.31

Average value of 31 flasks each

Table 6. Added vitamins and concentrations

Vitamin	Concentration µg/ml
Thiamin	0.1
Riboflavin	0.5
Pyridoxin	1.0
Calcium pantothenate	1.0
Biotin	0.005
Nicotinic acid	1.0
Cholin	1.0
p-Aminobenzoic acid	0.5
i-Inositol	1.0
Folic acid	0.005

Table 7. Composition of helathy bark and bark diseased by blight

Component	Conpcsition (dry wt %) Healthy bark	Diseased bark
Water soluble		
Cold water	12.4	8.0
Hot water	28.4	17.9
1%NaOH	43.6	42.8
3%HCl	41.5	22.0
Carbohydrates		
Total carbohydrates	16.0	5.0
Total sugars	6.1	0.9
Soluble sugars	5.5	0.9
Starch	5.0	3.3
Hemicullulose carbohydrate	4.9	0.8

Six-yr-old Tsukuba samples collected on 7/2/71 from healthy bark and the browned bark of stromata.

Table 8. Influence of ethanol and activated charcoal treatment of water soluble components of bark on the browth of the blight fungus

Treatment	Fungus dry weight	
	Hot water extract (rng)	Cold water extract (mg)
Crude solution	329	300
100% ethanol soluble	103	100
100% ethanol insoluble	328	375
1% activated charcoal filtered solution	71	63
75% ethanol extraction of activated charcoal-absorbed components	224	150
Basal culture medium	57	35

The mean value of fungus dry weight in 3 flasks of Richard's medium, amended with 20% of bark fractions, after incubation at 27°C for 15 days.

The fungus was obtained from 4-yr-old Tsukuba on May 20, 1970.

Table 9. Influence of acid, alkali and activated charcoal treatment of water solubles or chestnut bark on the growth of blight fungus

Treatment	Fungus dry weight	
	Hot water extract (mg)	Cold water extract (mg)
Crude solution[a)]	--	13
Heated crude solution	56	18
1N HCl treated	56	14
1N NaOH treated	56	12
5% activated charcoal treated	2	2

a) Still extraction for 24 hrs., then sterilized and filtered. 4-yr-old Tsukuba collected 6/20/70. Cultured 20 days at 27°C. Average of 3 flasks.

Table 10. Relationship between time of inoculation and incidence

Time of inoculation	1961-62		1962-63	
	Mycelium	Conidia	Mycelium	Conidia
November	1/5	0/9	5/5	0/5
December	0/	0/	0/	0/
January	0/	0/11	0/	0/
February	0/	0/	0/	0/
March	4/	3/13	3/	1/
Apri1	2/	2/12	2/	2/
May	0/	5/13	2/	0/
June	5/	2/	4/	2/
July	4/	3/	5/	5/
August	5/	6/	5/	3/
September	5/	6/	4/	1/
October	5/	1/5	3/	0/

Fractions are: incidence/number of inoculations

Table 11. Time of injury and callus formation

Month injured[1].	Callus formation		
	Month of	Amount[a]	Ratio to amount in June[b].
1	5		
2	5		
3	5	-	
4	5	43	44
5	6	95	98
6	7	97	100
7	8	83	86
8	9	43	44
9	10	7	7
10	5		
11	5		
12	5		

1. Injured on 15th of the month; measured for callus on the 15th of next month.

a. Amount of callus formation: excellent (n_1) ... index 2
good (n_2) ... index 1
a little (n_3) ... index 0.5

$$\text{amount} = (2\ n_1 + n_2 + 0.5\ n_3)/N \text{ (number of samples)}$$

b. Maximum of 100 in June.

Table 12. Relationship between incidence rate and temperature

Temperature	Mycelial inoculum			Conidial inoculum		
	14 days after inoculation	25 days after inoculation		11 days after inoculation	27 days after inoculation	
	Fraction diseased	Fraction diseased	Mean lesion length	Fraction diseased	Fraction diseased	Mean lesion length
5 C	0/5	0/5	- mm	0/7	0/7	- mm
10	0/	5/	2.3	0/	0/[b]	-
15	4/	5/	5.3	0/	2/	5.5
20	5/	5/	6.7	0/	1/	4.0
25	5/	5/	44.0	0/8	6/8	6.7
30	5/	5/	37.3	0/6	4/6	29.0
35	5/	branch withered	(5.0)[a]	0/7	0/7	

[a] Measured 14 days after inocolation

[b] Diseased after 1 month, heated. Fractions are: incidence/number of inoculations.

Table 13. Relationship between conidia formed in pycnidia and temperature

Temperature	Winter[a]		Summer[b]		
	10 days later	20 days later	3 days later	Conidia washed off	3 days later	10 days later
5 C			+		+	+
10	-	-	+		+	+
15	+	++h	+h		+h	++h
20	+	++h	++		++h	++h
25	++h	++h	++		+h	++h
30	+	+h	+		-	+
35	+	-				

- none formed, + formed, ++ many formed, h conidial cirrus; a) early February; b) late May

Table 14. Relationship between conidial germination and temperature

Temperature	24 hours		48 hours		72 hours		96 hours	
	Percent germination	Germ tube length	Percent germination	Germ tube length	Percent germination	Germ tube length	Percent germination	Germ tube length
5 C	0	- µm	0	- µm	0	- µm	0	- µm
10	0	-	11	3	43	9	98	57
15	16	7	89	41	97	55	-	-
20	75	13	91	38	100	63	-	-
25	96	25	100	52	-	-	-	-
30	97	38	100	63	-	-	-	-
35	34	14	82	40	91	59	-	-
40	0	-	0	-	0	-	0	-

Experiment conducted January, 1964. 100 conidia measured on each of 3 slides.

Table 15. Callus formation and planting density of trees

Density and fertilization	Number of samples	Callus formation index
Sparse, fertilized	15	73.0
Sparse, not fertilized	15	3.3
Dense, fertilized	15	15.0
Dense, not fertilized	15	0

Sparsly planted is a 5 x 5 m spacing. Densely planted is a 2.5 x 2.5 m spacing. Two-yr-old branches of 4-yr-old Ginyose variety trees cut in 12/25, maintained in wet room at 25C. Measured for callus formation on 1/16.

Table 16. Callus formation and time of removing leaves and roots

Treatment	Month of treatment	Number of samples	Callus formation index
Removed leaves	June	9	0
Removed leaves	August	9	86.0
Removed roots	June	9	50.0
Removed roots	August	9	0
No treatment	June	9	61.0
No treatment	August	9	100.0

Used 3-yr-old Giuyose variety trees. Nine wounds were made on 3 trees for each block. Wounds made on 6/15 and 8/1, right after treatment. Callus formation measured 2 months later.

Table 17. Callus formation and fertilization

Fertilization	Callus formation	
	Orchard	Trough
No fertilization	44.0	68.0
Fertilized; no N	56.5	72.0
All 3 nutrients; standard fertilization	72.5	79.5
Standard fertilization; double dose N	66.5	83.5
Standard fertilization; triple dose N	83.5	82.0
Double fertilization	77.5	91.0
Triple fertilization	78.0	89.0

One-yr-old Tsukuba, callus formation of holes of the trunk 7/8/75 holes punched;

10/23 measured callus formation rate = $\frac{2n_1 + 1.5n_2 + n_3 + 0.5n_4}{2\,N} \times 100$

$n_1 \ldots n_4$ number per degree of formation, N ... number of measurements

Table 18. Callus formation and soil moisture

Moisture	Callus formation
Dry	75.0
Normal	92.0
Moist	94.5

One-yr-old Tsukuba variety trees were used. The experiment ran from 7/1 to 9/27/75. The water tension of soil 20 cm underground (water column mm) ranged from: dry 10-600; normal 10-457; moist 10-253.

Table 19. Place of occurrence of lesions on seedling grafts

Location	Number of seedling grafts	Percent diseased
Total number	59	20
Trunk	22	7
Graft union	31	10
Bud	3	1
Branch	14	5

Incidence measured in November, 1968 on 300 Tsukuba variety seedlings.

Table 20. Place of occurrence of lesions on juvenile trees

2-yr-old trees		
Location	Number of trees	Percent incidence
Total number (without pruning site)	27	18
Graft union	21	14
Dead bud	4	3
Injury site	2	1
Pruning site	84	56

Experiment done 1964. 150 two-yr-old trees of Tsukuba & Ginyose varieties

3-yr-old trees		
Location	Number of trees	Percent incidence
Injured trunk	10	9
Trunk branch pruning wound	8	8
Other branching point	13	12
Injured branch	3	3
Dead branch	68	64
Ground level adventitious bud	4	4
Exposed root	1	1
TOTAL	107	100

Experiment done 1964. 150 three-yr-old trees of the Tsukuba & Ginyose variety were used. Thirty-three trees were diseased for an incidence of 22%.

Table 21. Place of occurrence of lesions on mature trees

	Properly managed[a]		Dense plantings[b]		Abandoned orchard[c]	
Number of trees tested	150		191		114	
Number of diseased trees	29		74		43	
Percent	19		39		38	
Location	Number of lesions	Percent	Number of lesions	Percent	Number of lesions	Percent
Pruning wound	25	81	43	23	19	43
Broken branch	2	7	9	5	3	7
Injured bark	1	3	7	4	1	2
Dead small branch	1	3	7	4	2	5
Dead secondary branch	0		101	54	0	
Branch tumor	2	7	8	4	0	
insect wound	0		5	3	4	9
Branching point	3	10	4	2	7	16
Graft union	1	3	2	1	9	21
Ground level	1	3	0	-	7	16
TOTAL	31	100	186	100	44	100

[a], [b] Ten-yr-old trees

[c] Five-yr-old trees

A mixed orchard of Tsukuba, Tanzana and Ibuki cultivars, measured May, 1971.

Table 22. Incidence of tree death on juvenile trees

Year	Established orchard			Newly tilled, newly planted			Old tilled, newly planted		
	Number trees planted	Number dead	Percent	Number trees planted	Number dead	Percent	Number trees planted	Number dead	Percent
1	65	15	23	148	35	24	137	35	26
2		8	12		26	18		30	22
3		11	17		20	14		14	10
4		6	9		14	9		0	0

Trees of Tsukuba cultivar; dead trees assessed in December.

Table 23. Lesion growth during the season

Date	Number of lesions which grew	Mean net growth of growing season
5/16	64	2.5 mm
6/19	59	6.8
7/14	29	5.8
8/13	35	7.2
9/14	38	15.0
10/16	35	11.9
11/17	22	4.2
12/13	36	1.8
1/16	44	1.7
2/16	49	1.6
3/15	44	1.5
4/15	30	3.2

The trees measured were 3- to 4-yr-old Tsukuba cultivar.

Table 24. Lesion expansion on excised branches and temperature

Temperature	Mean net growth[a)]	Remarks
10	1 mm	No expanded mycelial fan colonies
15	7	Expanded mycelial fans - conidium formation
20	13	Expanded mycelial fans - conidium formation
25	23	Expanded mycelial fans - conidium formation
30	24	Expanded mycelial fans - conidium formation
35	8	Staid yellow mycelial colonies

a) Lesion 25 days after temperature treatment. , Two-yr-old branches of Tsukuba cultivar trees. Numbers are means of 6 lesions. Experiment done March, 1963.

Table 25. Incidence, tree growth and soil moisture

Moisture status	Number of trees	Number diseased	Percent	Mean lesion length	Mean net increase up trunk diameter
Dry	9	9	100	34 mm	3.1 mm
Normal	8	3	38	10	5.3
Moist	9	6	16	16	4.8

Trees were 1-yr-old Tsukuba cultivar. Water treatment extended from July to September, 1975. Diameter growth was between June and October.

Table 26. Changes in electrical conductance (EC) and NO_3-N in soils of different moisture.

Moisture status	Soil depth (cm)	April 15 EC (mʊ)	April 15 NO_3-N (mg)	June 25 NO_3-N (mg)	July 25 EC (mʊ)	July 25 NO_3-N (mg)	October 25 NO_3-N (mg)
Dry	0-20	0.87	33.9	11.2	0.45	3.7	2.3
	20-40	0.44	19.9	13.1	0.35	6.4	14.6
Normal	0-20	0.85	34.0	10.2	0.27	2.8	0.9
	20-40	0.43	20.7	12.9	0.36	3.9	2.5
Moist	0-20	0.81	35.7	10.9	0.33	2.8	0.6
	20-40	0.44	19.2	11.9	0.39	5.6	0.5

The soil was a brown volcanic ash in concrete troughs 1 m in diameter. Moisture was controlled from July to September, 1975.

Table 27. Incidence, tree growth and fertilization in the orchard

Fertilizer regime	Number of trees	Number diseased trees	Percent	Mean lesion length (mm)	Mean net increase in trunk diameter (mm)
No fertilizer	19	19	100	96	1.5
No nitrogen	22	21	95	60	2.5
Standard	25	15	60	43	2.6
Double nitrogen	18	12	67	48	3.8
Triple nitrogen	21	7	33	26	3.8
Double standard	21	9	44	33	3.8
Triple standard	20	9	45	29	3.8

One-yr-old Tsukuba variety trees were inoculated on 6/4/75. Incidence was measured on 10/22. Tree diameter growth was between June and October. Standard fertilization: 20 g each of N (urea), P_2O_5, and K_2O per tree on 3/26 and 7/10.

Table 28. Incidence, tree growth and fertilization in concrete troughs

Fertilizer regime	Number of trees	Number diseased trees	Percent	Mean lesion length (mm)	Mean net increase in trunk diameter (mm)
No fertilizer	9	9	100	33	2.3
No nitrogen	9	9	100	36	3.8
Standard	8	2	25	10	6.0
Double nitrogen	8	3	38	11	7.6
Triple nitrogen	7	4	57	19	5.8
Double standard	7	4	57	15	5.2
Triple standard	8	3	38	11	7.4

The troughs were 1 m square, 1 m deep. One-yr-old Tsukuba variety trees were inoculated on 6/4/75. Incidence was measured on 10/21/75. Tree diameter growth was between June and October. Standard fertilization was 5 g each of N(urea), P_2O_5, and K_2O per tree on 3/26 and 7/9.

Table 29. Seasonal change in NO_3-N in soils with different fertilization regime in the orchard

Fertilization regime	Soil depth (cm)	NO_3-N (mg) 4/15	6/26	7/25	10/15
Non-fertilized	0-20	1.1	0.9	5.1	0.7
	20-40	1.6	0.8	5.9	0.7
	40-60	3.4	-	-	-
No nitrogen	0-20	1.1	1.2	5.7	0.7
	20-40	2.3	1.5	4.9	0.5
	40-60	3.7	-	-	-
Standard	0-20	5.4	1.8	18.2	1.2
	20-40	6.4	15.4	20.9	4.2
	40-60	4.1	-	-	-
Standard plus	0-20	18.4	16.2	41.8	3.4
Double nitrogen	20-40	11.2	18.1	33.4	8.9
	40-60	5.6	-	-	-
Standard plus	0-20	18.5	21.2	53.4	6.1
Triple nitrogen	20-40	13.1	19.2	35.0	16.0
	40-60	5.4	-	-	-
Double standard	0-20	16.0	17.9	36.7	5.4
	20-40	10.7	21.1	29.6	10.2
	40-60	4.0	-	-	-
Triple standard	0-20	19.0	23.6	54.9	4.4
	20-40	11.3	24.5	35.1	23.1
	40-60	4.7	-	-	-

The soil was a brown volcanic ash. Standard fertilization was 9 kg each of N(urea), P_2O_5 and K_2O. Two-thirds of N and K and all of P were applied 3/26 and one-third of N and K on 7/10.

Table 30. Seasonal change in NO_3-N in soils with different fertilization regimes in concrete troughs

Fertilization regime	Soil depth (cm)	NO_3-N (mg)				
		3/25	4/15	6/26	7/25	10/15
No fertilization	0-20	1.2	2.9	0.7	2.3	2.3
	20-40	2.3	3.6	1.4	2.4	5.5
No nitrogen	0-20	2.1	1.5	0.7	1.8	1.7
	20-40	1.7	1.8	1.1	2.6	4.4
Standard	0-20	1.5	17.5	6.9	12.7	2.5
	20-40	2.1	10.6	8.9	11.2	11.4
Standard plus	0-20	1.5	20.0	12.5	12.4	2.8
Double nitrogen	20-40	1.4	10.3	12.8	16.3	13.7
Standard plus	0-20	1.0	33.4	17.3	35.5	3.1
Triple nitrogen	20-40	2.3	13.8	19.3	22.4	19.9
Double standard	0-20	1.0	25.2	11.8	17.7	2.7
	10-40	2.2	13.8	14.1	18.3	14.8
Triple standard	0-20	0.9	31.5	17.7	30.1	2.4
	20-40	1.4	13.2	17.8	26.7	17.8

Troughs were 1 m square and 1 m deep. The soil used was a brown volcanic ash. 15 g each of of n, P_2O_5 and K_2O were applied per trough. Two-thirds of N & K and all of P were applied on 3/26, one-third of N & K on 7/9.

Table 31. Change in moisture comtent and conductance of soils in the orchard under different fertilization regimes

Fertilization regine	Soil depth (cm)	April 15 moisture (%)	April 15 EC (mʊ)	June 25 moisture (%)	June 25 EC (mʊ)
No fertilizer	0-20	35.4	0.12	40.0	0.22
	20-40	37.1	0.12	42.1	0.22
	40-60	45.9	0.14	-	-
Fertilized but no nitrogen	0-20	35.6	0.26	40.8	0.33
	20-40	37.7	0.30	40.5	0.43
	40-60	45.7	0.17	-	-
Standard	0-20	36.8	0.15	40.8	0.52
	20-40	39.1	0.22	43.1	0.45
	40-60	45.3	0.16	-	-
Standard plus double nitrogen	0-20	36.7	0.81	40.5	0.80
	20-40	37.5	1.02	41.3	0.45
	40-60	43.6	0.24		
Standard plus triple nitrogen	0-20	37.1	0.81	40.8	0.81
	20-40	37.9	0.48	42.0	0.63
	40-60	45.2	0.21		
Double standard	0-20	37.3	0.23	40.8	0.63
	20-40	37.3	0.68	42.0	0.53
	40-60	46.1	0.19		
Triple standard	0-20	37.0	0.68	39.8	0.73
	20-40	37.7	0.48	40.5	0.69
	40-60	46.9	0.20		

The soil was a brown volcanic ash. Standard fertilization was 3 **kg** each of N (urea), P_2O_5, and K_2O. Two-thirds of the N & K and all the P were applied on 3/26, one-third of the N & K on 7/9.

Table 32. Change in moisture content and electrical conductance of a soil in concrete troughs under different fertilization regimes

Fertilizer	Soil depth (cm)	3/25 moisture (%)	3/25 EC (m℧)	4/15 moisture (%)	4/15 EC (m℧)	7/25 moisture (%)	7/25 EC (m℧)
No fertilizer	0-20	47.1	0.08	46.2	0.14	49.2	0.24
	20-40	49.2	0.09	48.2	0.13	49.5	0.28
No nitrogen	0-20	49.1	0.06	45.7	0.20	49.9	0.27
	20-40	49.5	0.11	48.8	0.13	49.6	0.28
Standard	0-20	47.8	0.07	48.8	0.78	50.3	0.36
	20-40	49.6	0.12	48.6	0.26	51.6	0.32
Standard plus double nitrogen	0-20	48.6	0.08	46.6	0.65	49.2	0.38
	20-40	47.7	0.08	49.6	0.26	49.9	0.34
Standard plus triple nitrogen	0-20	49.9	0.06	46.3	0.77	49.3	0.45
	20-40	49.5	0.09	49.2	0.26	50.9	0.41
Double standard	0-20	47.8	0.06	46.6	0.76	49.6	0.41
	20-40	49.8	0.08	48.8	0.37	50.9	0.40
Triple standard	0-20	47.0	0.06	46.6	1.14	49.0	0.49
	20-40	48.3	0.09	49.4	0.34	50.8	0.38

Each trough was 1 m square and 1 m deep. The soil was a brown volcanic ash. Standard fertilization was 15 g each per tree of N (urea), K_2O_5 and P_2O. Two-thirds of the N & K and all the P were applied on 3/26. One-third of the N & K was applied on 7/9.

Table **33.** Relationship of tree density and incidence

Density	Fertilization regime	Non-inoculated			Inoculated		
		Number of trees	Number diseased trees	Percent	Number of trees	Number diseased trees	Percent
Dense	Fertilized	30	7	23	10	5	50
	Not fertilized	30	10	33	10	8	80
Sparse	Fertilized	18	0	0	10	2	20
	Not fertilized	18	0	0	10	0	0

In 1960, Ginyose variety trees were planted. Incidence was measured on the 5-yr-old trees in November, 1964. The densely planted block was spaced at 2.5 x 2.5 m; the sparse block at 5 x 5 m. Fertilizer was applied at a 10 a rate of 10 kg N (urea), 5 kg P and 10 kg K.

Table 34. Relationship between management and incidence

Management	Population	Number of trees	Number of trees diseased	Incidence (%)
Sod culture	Sparse	15	3	20
	Dense	30	11	37
Clean culture	Sparse	14	2	18
	Dense	30	12	40

Tsukuba and Tanzawa seedling grafts were planted alternately in 1967. No. of diseased trees counted November, 1971. Grassy fields: weeds were left growing since 1969. Densely populated - 2.5 x 2.5 m, sparsely populated - 5 x 5 m.

Table 35. Management and incidence

Management	Variety	Number of trees	Number of diseased trees	Incidence (%)
Sod culture, fertilized	Nakao-Tamba	25	2	8
	Ginyose	33	3	9
	Tsukuba	30	0	0
Clean culture, fertilized	Nakao-Tamba	25	4	16
	Ginyose	32	6	19
	Tsukuba	33	5	15
Clean culture, non-fertilized	Nakao-Tamba	18	4	22
	Ginyose	23	7	30
	Tsukuba	20	12	60

Treated in 1965. Incidence measured in October, 1968.

Table 36. Relationship between incidence and root cutting and light blocking

Treatment	Number of trees	Number of diseased trees	Dead trees	Lesion length (mm)
Root cut	5	5	1	48
Shading	5	4	0	33
Root cut & shading	5	5	3	45
No treatment	5	2	0	14

Three-yr-old Tsukuba. Treated 7/3/64. Shading for 3 months until September. Incidence measured October, 1964.

Table A. Soil composition

Moisture status	Depth	Percent moisture	pH N·KCl	pH H_2O	Ec (ma)	NO_3-N (ppm)
Dry	0-20	49.3	5.1	5.9	0.10	1.3
	20-40	50.1	5.1	5.9	0.09	1.2
Normal	0-20	48.6	5.0	5.8	0.10	0.9
	20-40	50.1	5.0	5.8	0.13	0.9
Moist	0-20	49.2	5.1	5.9	0.13	1.4
	20-40	50.2	5.2	5.9	0.11	1.8

The soil was a brown volcanic ash; measurements made 3/25/75.

Table B. Composition of orchard soil

Depth	Soil characteristic	Color	Maximum water capacity	pH N·KCl	pH H_2O	EC mʊ	NO_3-N mg/100 g
0-20	L	7.5YR3/4	115	4.9	5.5	0.10	1.2
20-40	L	7.5YR4/4	121	5.1	5.6	0.12	2.9
40-60	L	7.5YR4/6	125	5.3	5.8	0.14	2.4

Brown volcanic ash soil on 3/25/75

Table C. Composition of trough soil

Depth	Soil characteristic	Color	Maximum water capacity	pH N·KCl	pH H_2O	EC mʊ	NO_3-N mg/100 g
0-20	L	7.5YR4/4	121	4.8	5.5	0.07	1.3
20-40	L	7.5YR4/4	121	4.8	5.5	0.09	1.9

Brown volcanic ash soil on 3/25/75.

Castanea species and cultivars in Fruit Tree Research Station

2nd Lab. of Fruit Breeding

1. Wild chestnut	3 species	11	clones
(1) Japan			
C. crenata		5	clones
C. crenata var. sakyacephala		1	clone
C. crenata pendula Sandoguri		2	clones
		3	clones
(2) China			
C. seguinii			
C. henryi			
(3) America			
C. pumila			
(4) Hybrid			
crenata x seguinii		10	clones
mollissima x seguinii		1	clone
pumila x crenata		2	clones
2. Cultivar	191 cultivars		
(1) Japan	106 cultivars		
(2) Korea	14 cultivars		
(3) China	33 cultivars		
(4) Europe	6 cultivars		
(5) Hybrid			
sativa x crenata	5 cultivars		
crenata x mollissima and its reciprocals	27 cultivars		

for dwarfing has also been started.

Among the gall wasp resistant varieties obtained through the above-mentioned progrews, the representative ones are as follows.

1) Tanzawa: The average weight of the fruit is around 20 gr. The color is slightly lighter but the fruit is of good quality. Early maturing.

2) Ibuki: The average fruit weight is around 20 gr. The appearance and quality of the fruit are both superior, but it is susceptible to damage by the Peach pyralid moth (Dichocrocis punctiferalis GURNEE). Early maruting.

3) Tsukuga: Strong tree vigor and highyielding. The average fruit weight is 20-25 gr. Medium-maturing variety of good appearace and quality. With desirable storage quality it is the most popular variety at present.

4) Ishizuchi: The average fruit weight is over 20 *gr*. Outstanding variety amontg the late-maturing ones. The tree is not so vigorous, and intensive orchard management is required.