Seed Transmission of Fusarium Wilt of Bottle Gourd, *Lagenaria siceraria,* used as Rootstock of Watermelon

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Grafting watermelon on the rootstock, bottle gourd, has hitherto been practiced commonly in Japan for many years in commercial production of watermelon (Plate 1). It is a very useful method for the control of Fusarium wilt in watermelon caused by *Fusarium oxysporum* f. sp. *niveum* Snyder et Hansen, because the rootstock is immune to the causal fungus. Matuo and Yamamoto (1967), however, reported that the rootstock plant, bottle gourd, was attacked by another *Fusarium*, *Fusarium oxysporum* f. sp. *lagenariae* Matuo et Yamamoto. The incidence of this Fusarium wilt attacking the rootstock has increased remarkably in recent years, causing great damage, thus giving rise to a new difficult problem to the watermelon production. The source of primary infection of this disease was supposed to be the seeds infected with the pathogen. Therefore, the following experiments were conducted to clarify the details of the seed transmission.

Plate 1. Seedling of watermelon grafted on bottle gourd

Detection of the pathogen in seeds and secondary dissemination of the pathogen by grafting operation

The commercial seed samples tested were found to be infected with the pathogen at the rate of 2 to 5%. It was proved that some of the seeds carried the pathogen in their internal tissues. The seeds, collected from the fruit borne on infected plants and allowed their fruit mesocarp to decay following the routine method practiced by seed collecters, were found to be infected with the pathogen at the rate of 2.0 to 16.8%. The rate of seed infection varied greatly with samples from different mother fruit.

It was demonstrated that the disease was transmitted at the time of grafting by grafting instruments contaminated with the sap of diseased plants at a considerably high fre-

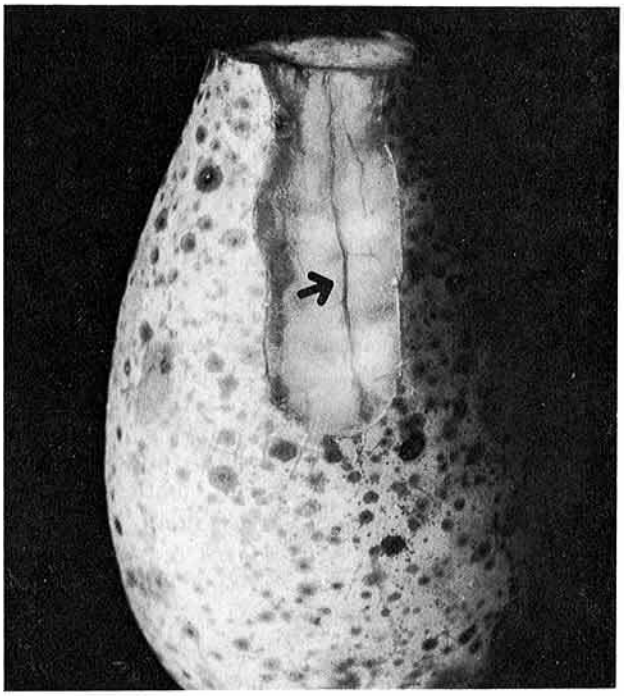

Plate 2. Infected fruit of bottle gourd showing brownish discoloration of its principal vascular bundles in epicarp (arrow)

quency; at least, one to four seedlings were infected from one diseased seedling. The infection occurs readily by the seedlings showing so slight symptom that it is very difficult to distinguish them from healthy ones.

Migration of the pathogen from infected stem to fruit and seed

The causal fungus was isolated consistently from discolored xylem tissues of roots, stems and peduncles in the infected plants. It shows the migration of the causal fungus to the apex through vascular bundles from roots and stems. In unripe fruit, borne on diseased plants, the invasion of the causal fungus was restricted to the base of peduncle and no fungus was detected in any other parts of the young fruit. In mature fruits borne on the diseased plants, however, brownish discoloration was found in the principal vascular bundles in epicarp (Plate 2) and the causal fungus was readily isolated from those parts. Following two courses of seed infection was observed:

1) Seed infection occurred in the course of decomposing mesocarp of the fruit

With advance of maturity of diseased fruit, the causal fungus latent mostly in the discolored principal vascular bundles proliferated saprophytically within the decaying mesocarp and then penetrated into the tissues of seed coats. Some of them penetrated into cotyledons and even into embryos. High rate of seed infection i.e., 16.8 to 46.7%, resulted from decomposition of mesocarps. The causal fungus were deep-seated in the seed coats from its sub-epidermal layer to parenchyma tissues in the forms of mycelia and chlamydospores (Plate 3).

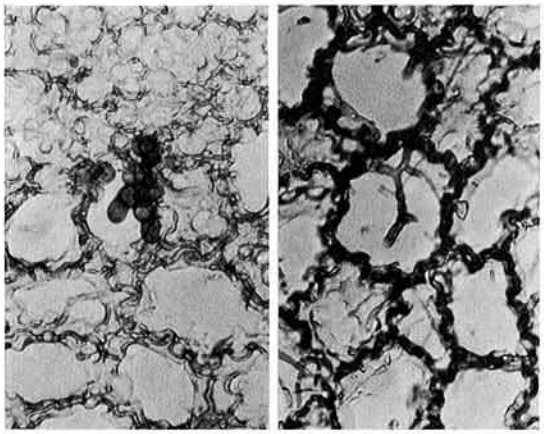

Plate 4. Chlamydospores observed in vas-pathogenic fungus invaded in tissues of seed coat

2) Direct seed infection through vascular bundles

The infected fruit borne on diseased stems and still free from the decay of fruit tissues, were used as materials for observation. As one of the anatomical features of the seeds developed from anatropous ovules such as seeds of bottle gourd, a distinct vascular bundle extending from hilum through rahpe was observed in the seed coats. The distribution of the causal fungus in the vascular bundles of loculus and placenta as well as of seed coats of infected fruit was examined with the purpose of tracing the direct invasion through the vascular bundles to the seeds. Presence of the causal fungus in loculus and placenta was proved by putting the surface sterilized tissue sections on a solid medium and observing the causal fungus development. Out of about 1000 seeds harvested from the infected fruit mentioned above, 23 seeds were proved to be infected with the causal fungus. In 6 seeds among the infected seeds, chlamydospores assumed to be of the causal fungus (Plate 4) were observed in vessels and in parenchyma of vascular bundles of the seed coats.

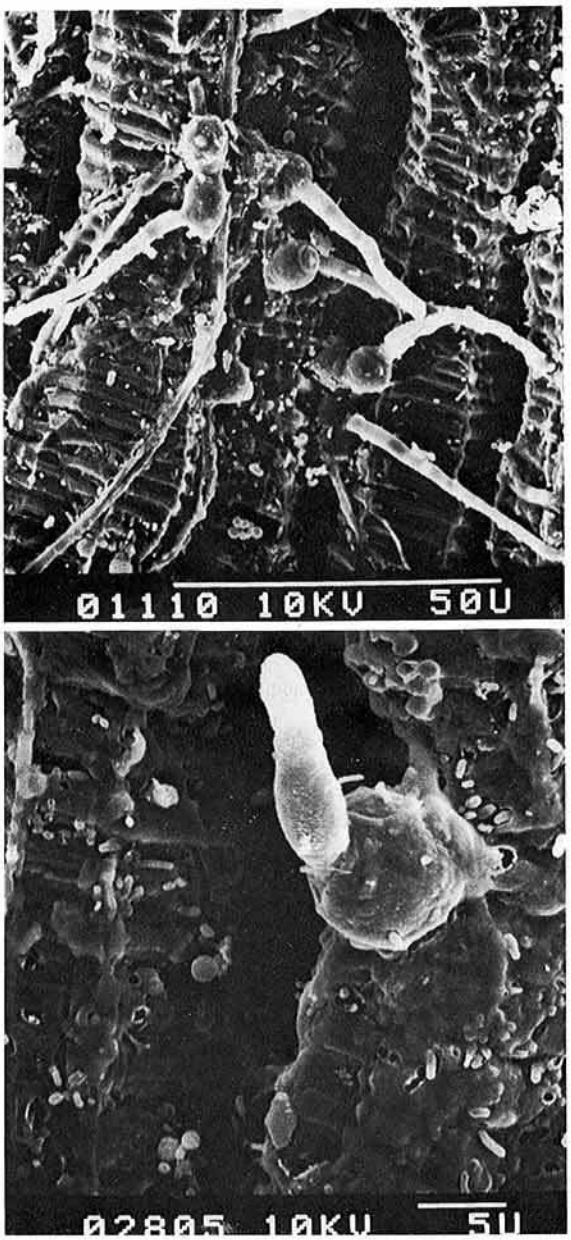

Plate 4. Chlamydospores observed in vascular bundles of the seed coat.
(The spores were germinating as the seeds were incubated on the medium at 25°C for 24 hrs before fixing)

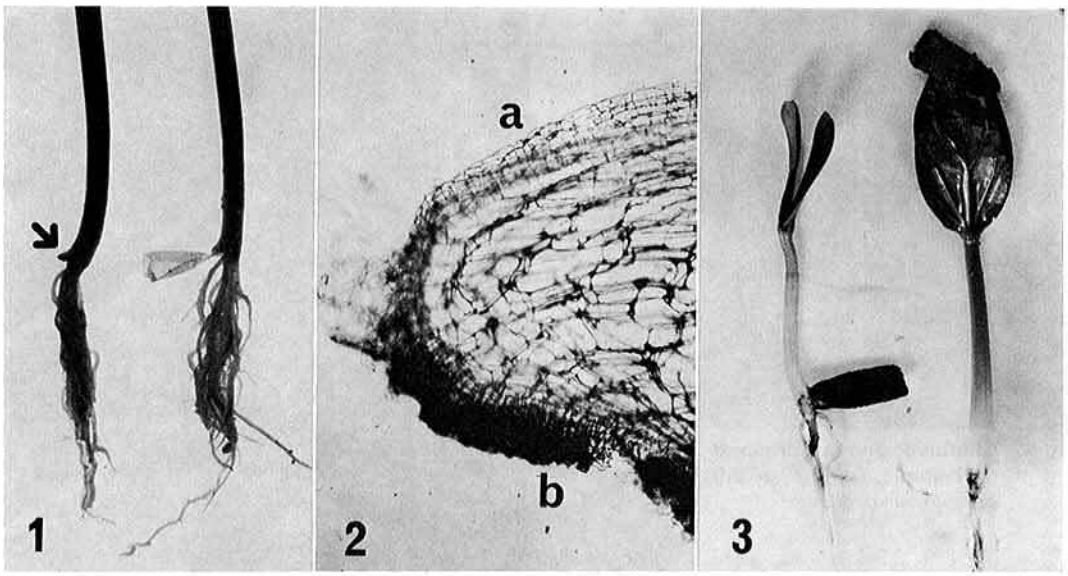

Plate 5. 1: Peg of seedling of bottle gourd (arrow) and degenerate albumen attached to peg
2: Longitudinal section of peg, a; upper side of peg, b; lower side of peg
3: Two types of germination, left; the seed coat attached to peg, right; the seed coat attached to cotyledon

Course of seedling infection by the seed borne pathogen

Seed costs of bottle gourd remained, in most cases, attached to the pegs of seedlings after germination (Plate 5) and the causal fungus present in the seed coats proliferated to serve as the primary inoculum to seedings. Infection rate of seedlings with pegs accompanied by seed coat ranged from 14 to 18%, while that of seedlings having their seed coats attached to cotyledons was 2 to 3%.

Among various parts of seedlings, the density of Fusarium propagules was highest in pegs followed by roots. When roots or pegs of healthy seedlings were inoculated, the rate of disease outbreak was as high as 90 to 100% irrespective of inoculation methods. When hypocotyls or cotyledons were wound-inoculated, seedlings were infected, but practically no disease occurred when these parts were inoculated without wounding. Thus the pegs and roots were the major sites for the penetration of the causal fungus. Histological observation indicated that the causal fungus penetrates from lower side epidermis of the pegs and extends its mycelia throughout the whole tissues of the pegs.

The results suggest that in the process of germination of seeds, the causal fungus latent in the infected seed multiplies in the tissues to build up the inoculum potential to a level sufficient for causing infection and that the penetration of the causal fungus to seedlings takes place mostly from the lower side epidermis of pegs.

Survival of the causal fungus in stored seeds

Almost all of the causal fungus adhering to the surface of the seed coats in forms of conidium or mycelium died out during seed storage for 4 months. On the contrary, the causal fungus deep-seated in the seed coats in the form of chlamydospore showed high tolerance against a long term storage of the seeds.

Isolation percentage of *Fusarium oxy-*

sporum in two seed samples stored for 12 months did not changed so much from the value at the beginning of the storage showing 100% and 65% respectively. By sowing the seeds in sterilized soil infected plants appeared at 18.5 and 5.0%. respectively. After 16 months' storage, however, the isolation percentage reduced significantly to 32% and 16% and infected plants appeared at the rate of 5% and 3% respectively by sowing the stored seeds. *Fusarium oxysporum* was still isolated from the seeds stored for 2.5 years at the rate of 5% and 3%, and infected plants appeared at about 2% from each seed smaple by sowing them in the soil.

From the results, the causal fungus was proved to survive for considerably long term in the form of chlamydospore and seed storage for 2.5 years was not sufficient to free seeds from the pathogen.

Seed disinfection

1) Dry-heating as a practical means of seed disinfection

The fusaria in seeds were killed almost completely by heating the seeds at 75°C for 7 days. Predrying of the seeds at 40°C for 24 hrs was recommended in order to prevent the loss of seed viability by reducing the moisture content of seeds to around 5%.

When the seeds were subjected to heating at 75°C in an electric oven with the forced-air circulation, their moisture content was rapidly reduced from around 9% to 2% or lower within about 24 hrs. Thereafter, the moisture content reached an equilibrium at the level of about 1.5%. No damage in germinability occurred.

Influence of moisture content of seeds on germination and disinfection was examined by heating the seeds with various moisture contents, enclosed in sealed conical flasks at 75°C for 24 hrs. When the moisture content was 5.3 to 5.6%, the fusaria were killed completely, and seed germination was also reduced to only 6 to 10%. When the moisture content was less than 2.4%, the viability of both fusaria and seeds was not affected by the heat treatment. From these results, the difference in the heat resistance was hardly found between fusaria and seeds in relation to the moisture content in seeds.

When the moisture content was less than 2.5%, both the seeds and the fungi could withstand the heat 75°C for considerably long duration. The fungi, however, were found to be killed completely in 6 or 7 days. On the contrary the seeds could withstand the heat for the longer duration. It is considered that the practical effectiveness of dry heating in disinfecting seeds without reducing seed germination could be caused by the quantitative difference in heat resistance between the seeds and the fungi.

2) Seed disinfection with fungicides

Benlate T (a complex of methyl 1-(butylcarbamoyl)-2-benzidazole carbamate and Thiram) and Homai (a complex of dimethyl 4,4-o-phenylene *bis* (3-thioallophanate) and Thiram) were proved to be effective by dressing dry seeds at the dosage of 0.4 and 0.5% of dry seed weight respectively. Damage to seed was not observed by the treatments.

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