Biological Control of Fusarium Wilt of Sweet Potato with Cross-Protection by Prior Inoculation with Nonpathogenic *Fusarium oxysporum*

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Introduction

Cross-protection is considered to be a useful measure for biological control of soilborne diseases by which a biocontrol agent induces resistance in a host rather than through the mechanism of direct antagonism against the pathogen. Twenty or more examples of cross-protection against soilborne pathogens have been published, especially more works have been carried out on the vessel diseases caused by *Fusarium oxysporum*[1,3,4,5,6,7,9,10,14,16] and *Verticillium dahliae*.[2,11,15] However, only a few have been applicable to commercial production, presumably because in most cases protection was induced by an avirulent strain of a pathogen or by an organism pathogenic to other species of crop.

This study was attemped to apply the cross-protection phenomenon for controlling Fusarium wilt of sweet potato, caused by *Fusarium oxysporum* f. sp. *batatas*, by prior inoculation with nonpathogenic *Fusarium oxysporum* isolates which were often found in the vessels of healthy sweet potato plant and natural soils. The isolates obtained from the healthy plants were nonpathogenic not only to sweet potato but also to several other species of major vegetable crop.

Material and methods

1) *Isolation of nonpathogenic F. oxysporum*

Nonpathogenic *F. oxysporum* isolates were obtained from the vascular bundles of stems of healthy sweet potato sprouts, and sometimes from those of tubers, using Komada's *Fusarium*-selective medium.[8] To examine each isolate for pathogenicity to sweet potato and to several other species of major vegetable crop, the isolates were grown in shake cultures for 7 days, then the bud cells were used to infest potting soils. Cut sprouts of sweet potato or seeds of other species of crop were planted in the infested soil.

2) *Prior inoculation of nonpathogenic F. oxysporum*

Each nonpathogenic *F. oxysporum* isolate was tested for cross-protection ability against Fusarium wilt by dipping fresh cut ends of sweet potato sprout in a diluted suspension of bud cells of each isolate produced in 7-day-old shake cultures. Sometimes, instead, fresh cut ends were smeared with a condensed bud cell suspension paste.

3) *Planting of sweet potatoes*

Sweet potato plants were always planted by cuttings. Normally, cut ends of the sprout were diagonally inserted into the test soils. In a certain case, the sprouts were bent, and the middle portions of the stem were buried in the soil with the cut ends remaining out of the soil.

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Preliminary reports of these results were published.[12,13]

4) Soils and infestation with the pathogen

Volcanic ash soil was usually used. Soils of the experimental or commercial field were naturally infested with the pathogen. In the greenhouse experiments, the potting soils were often artificially infested by drenching with a bud cell suspension of the pathogen or by mixing with a small amount of a soil-wheat bran culture of the pathogen.

Results

Many *F. oxysporum* isolates were obtained from healthy sweet potato plants, and most of them were not pathogenic to all the crops tested, such as sweet potato, tomato, cucumber, bottle gourd, melon, radish and cabbage. Furthermore, some of the nonpathogenic isolates showed cross-protection against Fusarium wilt of sweet potato when sweet potato plants were inoculated with them previous to planting.

1) Greenhouse experiments

When sweet potato sprouts were previously inoculated with a nonpathogenic *F. oxysporum* isolate, and then planted in naturally infested soil, or in natural or autoclaved soils artificially infested with the pathogen, a high degree of cross-protection was observed against the wilt, as shown in Plate 1. However, drenching soil

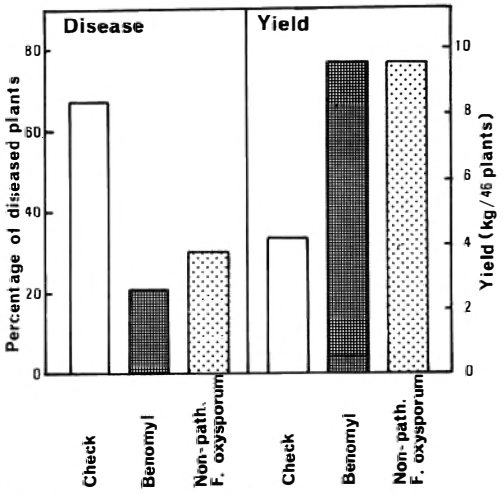

Fig. 1. Effect of prior inoculation with nonpathogenic *F. oxysporum* on disease incidence of Fusarium wilt and yield of sweet potato in comparison with dipping the sprouts in benomyl suspension (500 times of 50% w.p.) in naturally infested field

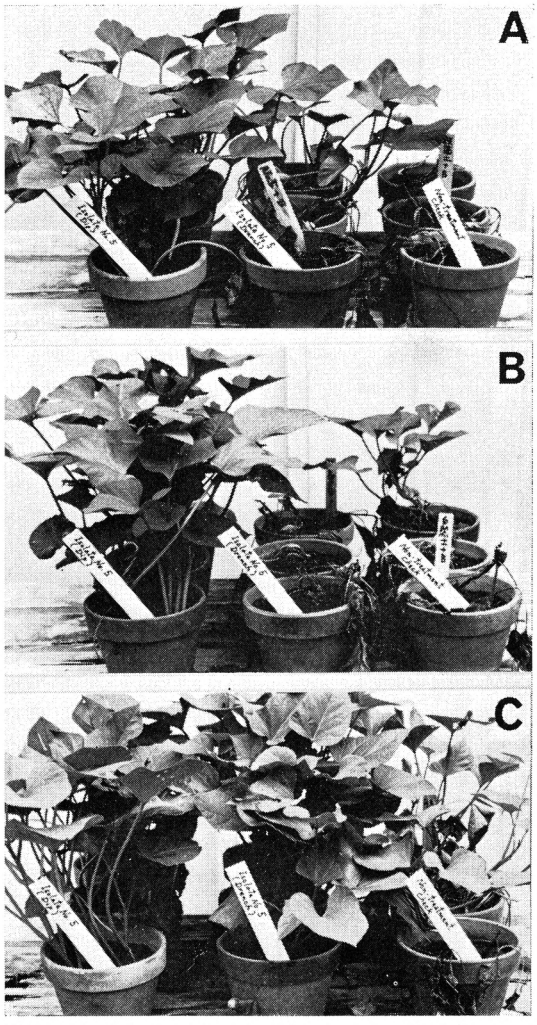

Plate 1. Cross-protection of Fusarium wilt of sweet potato by prior inoculation with a nonpathogenic isolate of *Fusarium oxysporum* in a pot test using aritificially infested autoclaved soil (A), aritificially infested natural soil (B) and naturally infested soil (C)

Left row: Previously inoculated by dipping cut ends of sprouts in a bud cell suspension of the isolate.
Center row: Previously inoculated by drenching soil with a bud cell suspension of the isolate.
Right row: Non-inoculated control.

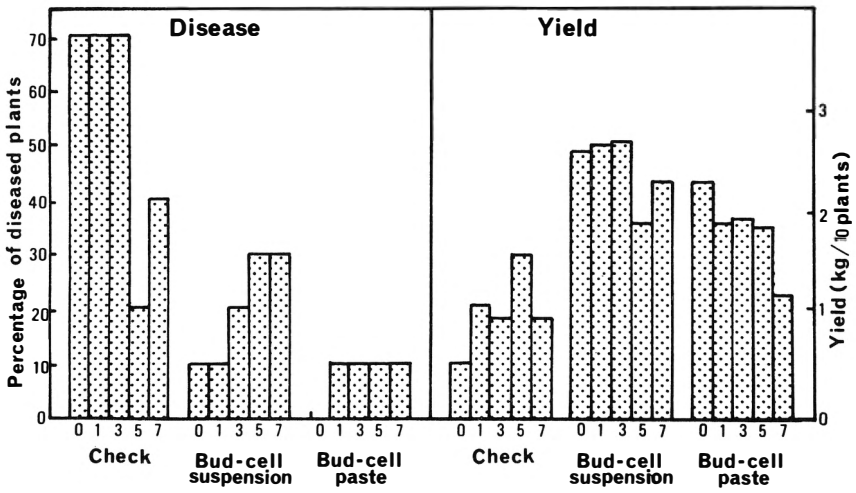

Fig. 2. Biological control of Fusarium wilt of sweet potato by prior inoculation with nonpathogenic *F. oxysporum* using a bud cell suspension or a condenced bud cell suspension of the fungus in the naturally infested field
0, 1, 3, 5 and 7 represent interval in days from prior inoculation, carried out immediately after cutting, to planting, respectively.

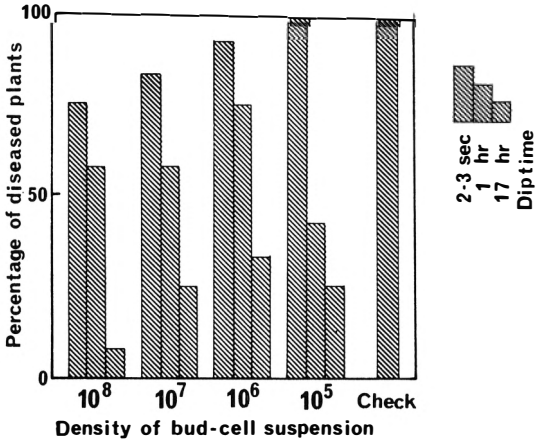

Fig. 3. Influence of dilution of bud cell suspension of a nonpathogenic isolate of *F. oxysporum* and of time of dipping sprouts in the cell suspension on cross-protection of Fusarium wilt of sweet potato

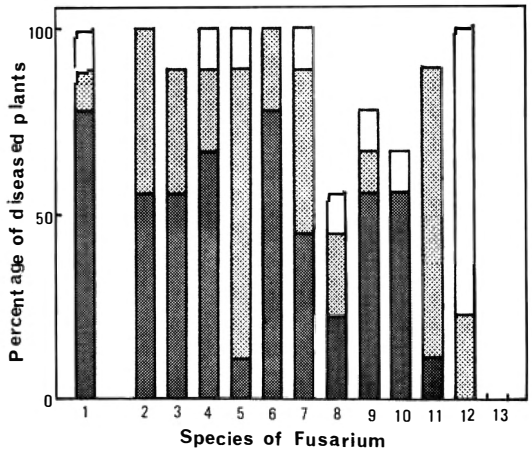

Fig. 4. Control of Fusarium wilt of sweet potato by prior inoculation with several species of *Fusarium*
1: Not previously inoculated control.
2—13: Previously inoculated with *F. episphaeria* (2), with *F. tricinctum* (3), with *F. rigidiuscula* (4), with *F. moniliforme* (5), with *F. roseum* 'Graminearum' (6), *F. roseum* 'Semitectum' (7), with *F. roseum* 'Avenaceum' (8), with *F. roseum* 'Culmorum' (9), with *F. roseum* 'Equiseti' (10), with *F. solani* f. sp. *Mori* (11), with *F. oxysporum* (12) and with nonpathogenic *F. oxysporum* isolate No. 101—2 (13).
■ : Percentage of diseased plants at 8 days after planting, ▨ : Percentage of lethal plants at 24 days after planting, □ : Percentage of lethal plants at 41 days after planting.

with a bud cell suspension of the fungus was effective only in naturally infested soil in which the inoculum density of the pathogen was assumed to be lowest among the three infestation methods.

2) Field experiments

In the naturally infested experimental or commercial fields, cross-protection by prior inocula-

tion with nonpathogenic isolates of *F. oxysporum* has always brought about a remakable decrease in wilt incidence and a remarkable increase in yield of sweet potato. The effects were equivalent to those obtained in chemical treatment in which cut ends of the sprouts were dipped into a benomyl suspension (500 times of 50% w.p.) for 30 min, as shown in Plate 2 and Fig. 1.

Smearing cut ends of sprouts with a condensed bud cell suspension of the fungus, or dipping cut ends of the sprouts in a diluted bud cell suspension, brought about a remarkable decrease in wilt incidence and a remarkable increase in yield. And more yield and less wilt incidence were evident when sprouts were planted as soon as possible after the prior inoculation, as shown in Fig. 2.

Prior inoculation was effective not only for the disease caused by the pathogen transmitted from the infested soils but also for that transmitted from the infested tubers.

The denser the bud cell suspension, and the longer the duration of dipping the sprouts in the bud cell suspension, the greater was the cross protective effect, as shown in Fig. 3.

3) Mechanism of cross-protection

Among 12 pathogenic and nonpathogenic Fusaria belonging to 7 species, only the non-

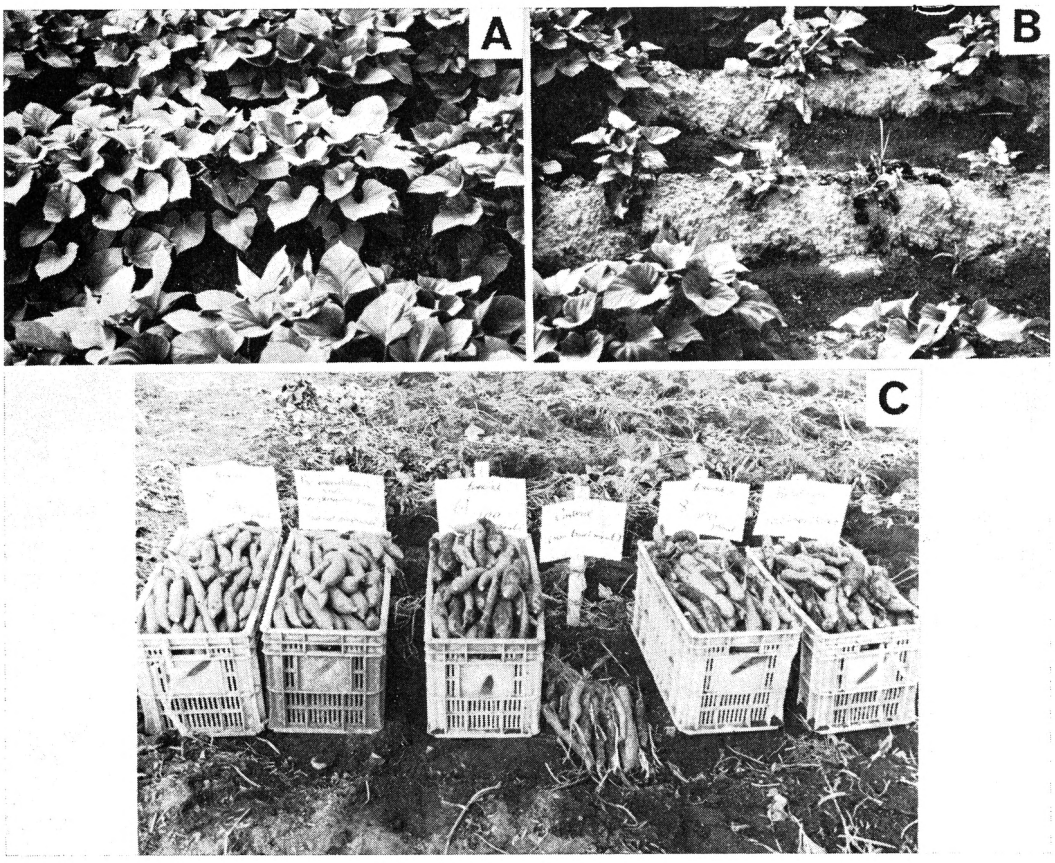

Plate 2. Field test for cross-protection of Fusarium wilt of sweet potato with nonpathogenic *F. oxysporum*

Sweet potatoes grown from cuttings that had been dipped in a bud cell suspension of a nonpathogenic *F. oxysporum* (A), or that received no treatment (B). Comparison of yield (C) from the cross-protected plot (two containers on the left), with those from benomyl-treated plot (do on the right) or non-treated plot (center).

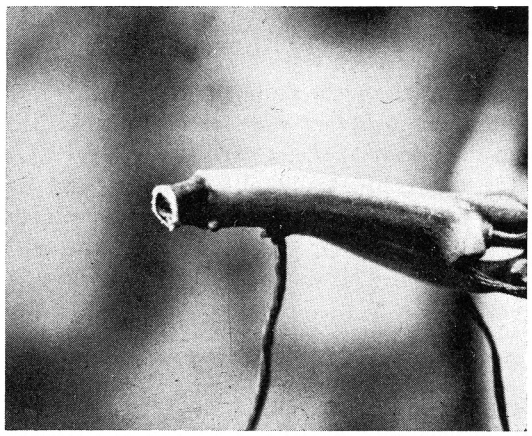

Plate 3. Local lesion at a cut end of sprout caused by a non-pathogenic isolate of *F. oxysporum* which induces cross-protection of Fusarium wilt of sweet potato

pathogenic isolates of *F. oxysporum* showed cross-protection, as shown in Fig. 4.

No antagonism was observed between the cross-protective isolates and the pathogen in confronting plate cultures.

When the cut ends of sprout were dipped in liquid paraffin, no protection was observed against the disease. This suggested that the cross-protection was not due to a mechanical plugging of wounds with the bud cells.

Only living bud cells of the isolates distinctly caused cross-protection against the disease. Heat-killed bud cells did not cause any cross-protection. Bud cell germination exudate caused slight cross-protection. But the effect of culture filtrate was indefinite, presumably due to disturbance by unexpected impurities.

Even if the previously inoculated sprouts were bent and the bent portions were planted, cross-protection was satisfactorily obtained. Cross-protection was also observed when the previously inoculated sprouts were planted in water, and then inoculated with the pathogen by injecting a bud cell suspension into the stems about 10 cm upper from the cut ends. These results suggested that the cross-protection was revealed systemically on the plant. If the cut ends were excised 2 days after prior inoculation, cross-protection was shown, but no cross-protection was observed if the cut ends were excised immediately after prior inoculation. The cross-protective isolates were not pathogenic to sweet potato, as mentioned above, however, colonization of the cut ends was followed by a remarkable development of local lesions there, as shown in Plate 3. These results suggested that the systemic cross-protection was induced mainly by fungal colonization of the wounded cut ends.

Reaction of sweet potato plants against phytotoxic substance(s) produced by the pathogen, resulting in wilting and yellowing of stems and leaves, was nullified by prior inoculation with cross-protective isolates.

Discussion

Cross-protection by prior inoculation with *F. oxysporum* isolates which are nonpathogenic not only to sweet potato but also to several other species of major vegetable crop shows promise for biological control of Fusarium wilt of sweet potato, commercially. It is effective not only for the disease caused by soilborne inoculum but also for that transmitted from infested tubers, and does not require the introduction of any complicated operation because sweet potatoes are usually planted by cuttings in Japan. Therefore, it will be one of a few examples of the biological control using cross-protection in commercial practice.

Although the research is continuing to elucidate mechanism of the cross-protection phenomenon, it is assumed that the cross-protection is due to the resistance which is induced by the nonpathogenic *F. oxysporum* colonizing and bringing about local but severe infection at the cut ends of sprouts. It is also assumed that the plants react to infection by producing resistance product(s) such as sort(s) of phytoalexin which translocate systemically from the basal to the upper stem of the plants.

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