Biological Control of Fusarium Wilt of Carnation by Application of Nonpathogenic *Fusarium oxysporum*

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Nonpathogenic *Fusarium* species isolated from the root tissue of carnation were screened for biocontrol activity to fusarium wilt of carnation caused by *Fusarium oxysporum* f. sp. *dianthi.* Some isolates showed suppression of fusarium wilt in carnations. The most effective isolate, No 108, was identified as *Fusarium oxysporum,* and is nonpathogenic to major crops such as tomato, radish, eggplant, and cucumber. No 108 isolate showed a 70% reduction in disease severity 16 weeks after transplanting in field trials. Pre-inoculation with No 108 isolate is considered to be a practical biocontrol agent of fusarium wilt of carnations because it is effective in field trials and nonpathogenic to major crops. Moreover, it showed protective effect when it was inoculated not only just before transplanting, but also at the cutting rooting stage.

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* is one of the most serious diseases worldwide in carnation culture (Garibaldi, 1979). Steam disinfection and chemical fumigants, such as chlorpicrin, are commonly used to protect against this disease. But these methods are somewhat unreliable, eliminate the antagonistic microflora that normally inhibit this pathogen, and excess application of chemical fumigants causes soil and air pollution. The application of antagonistic microorganisms was examined to develop a new control system in harmony with the natural environment.

MATERIALS AND METHODS

Isolation of *Fusarium* Species and Screening in Pot Tests. Isolates of *Fusarium* species, isolated from carnation roots using Komada medium (Komada and Ogawa, 1980), were screened for biocontrol activity to fusarium wilt of carnation, by dipping the roots of rooted cuttings (cv. Lena) into the bud-cell suspension (10^7 bud-cells ml^{-1}) of each isolate just before transplanting. Rooted cuttings pre-inoculated with tested *Fusarium* species were transplanted into 18-cm-diameter pots containing soil infested with the pathogen (10^4 spores per g dried soil). Five plants were transplanted into each pot and grown in a glasshouse at 28C. Control plants were dipped in distilled water. Twenty plants were examined for each treatment. The plants were examined for symptoms of fusarium wilt every 7 days after transplanting.

The index of symptoms was recorded as follows: 0, no external symptoms; l, chlorotic leaves or crooked neck shoots; 2, widespread light wilting or partially severe wilting; 3, widespread severe wilting; and 4, complete wilting (death).

$$\text{Disease severity} = \frac{\text{Sum of index value (0-4)}}{\text{Number of tested plants} \times 4} \times 100$$

$$\text{Protective value} = \frac{\text{(Disease severity in untreated control) - (Disease severity in pre-inoculated with tested isolates)}}{\text{(Disease severity in untreated control)}} \times 100$$

Field Trial. A field trial was conducted in the soil bed of a glasshouse with the same No 108 isolate utilized in the pot test. The same method and inoculation concentration were used as in the pot test. The carnation cultivar and pathogen infestation level were also the same as in the pot test. The trial was carried out over 6 months from May to November.

Effect of Inoculation at Cutting Sticking Stage. The bud-cell suspension of No. 108 isolate was sprayed on cuttings prior to rooting. They were rooted, stored at cool conditions (0 to 1C) for 2 weeks, and then transplanted into pots containing pathogen-infested soil. Plants were cultivated in a glasshouse at 28C.

RESULTS AND DISCUSSION

Selection of Effective Isolate to Fusarium Wilt of Carnation. The protective use of*Fusarium* species against fusarium wilt has been widely reported (Garibaldi, 1987; 0gawa 1984; Tezuka, 1991). In this experiment, more than 100 isolates of *Fusarium* species were isolated and tested for their biocontrol activity on fusarium wilt of carnations. Some isolates showed biocontrol activity. The patterns of protective effect in tested isolates were classified into three groups:

1) The isolates in which the effect was maintained for a long time;
2) The isolates in which the effect was shown for only a short time;
3) The isolates in which no protective effect was shown (Fig. l).

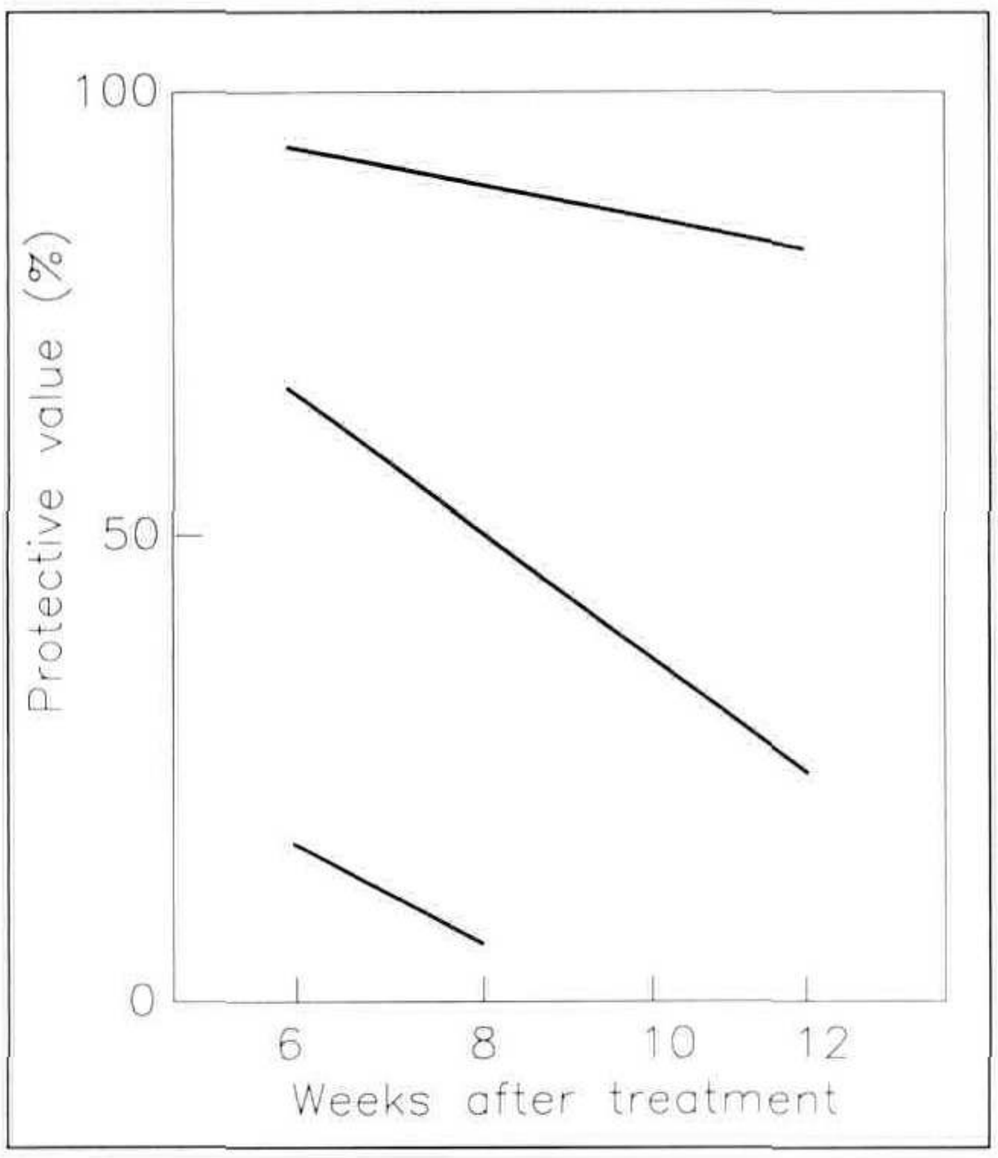

Figure 1. Three patterns of protective effects with tested isolates.

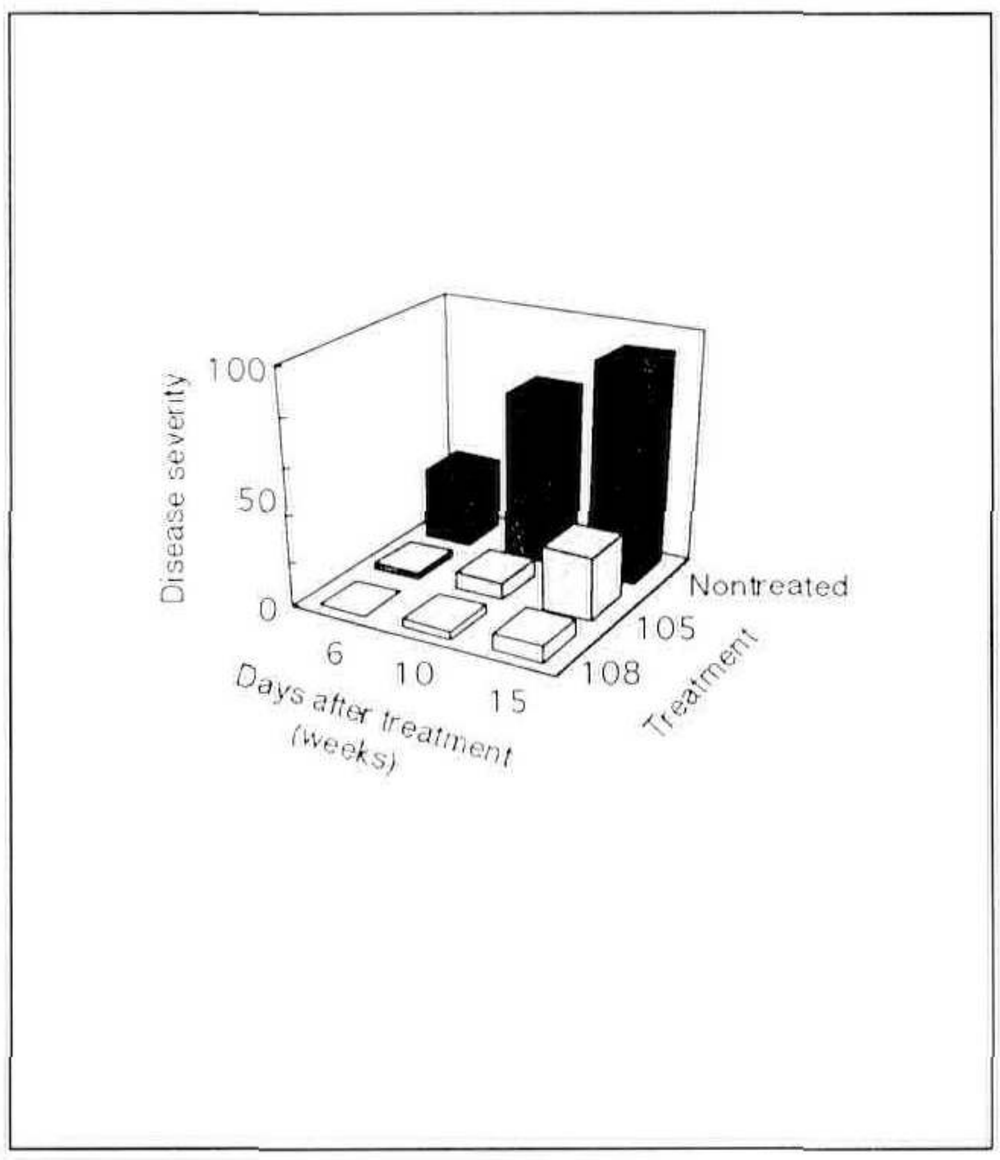

Figure 2. The effects of selected isolates in pot tests.

The most effective isolate, No. 108, showed 80% protection even 15 weeks after transplanting in a pot test (Fig. 2). No. 108 isolate was identified as *F. oxysporum* by microscopic test, and confirmed by inoculation tests to be nonpathogenic to major crops (such as tomato, radish, eggplant, and cucumber).

Field Trial. The effect of the No.108 isolate was examined in a glasshouse under near normal growing conditions. In control plants, 100% of the tested plants showed disease symptoms, and disease severity reached 60 at 10 weeks after transplanting. In contrast, in pre-inoculated plants with No.108 isolate, only 20% of tested plants showed disease symptoms and disease severity was low. The protective value of No.108 isolate was maintained for a long time and registered 70% at 16 weeks after transplanting (Figs. 3 and4). Therefore, it may be concluded that No. 108 isolate is able to be applied in practical conditions as a biocontrol agent.

Effect of Inoculation at Cutting Rooting Stage. No. 108 isolate showed a protective effect when it was inoculated not only just before transplanting, but also at cutting stage (Table 1). In the future, rooted carnation cuttings resistant to fusarium wilt may be pre-inoculated with No. 108 isolate at cutting sticking stage. An examination of the effect of application at cutting rooting stage in field trials will be needed for practical use.

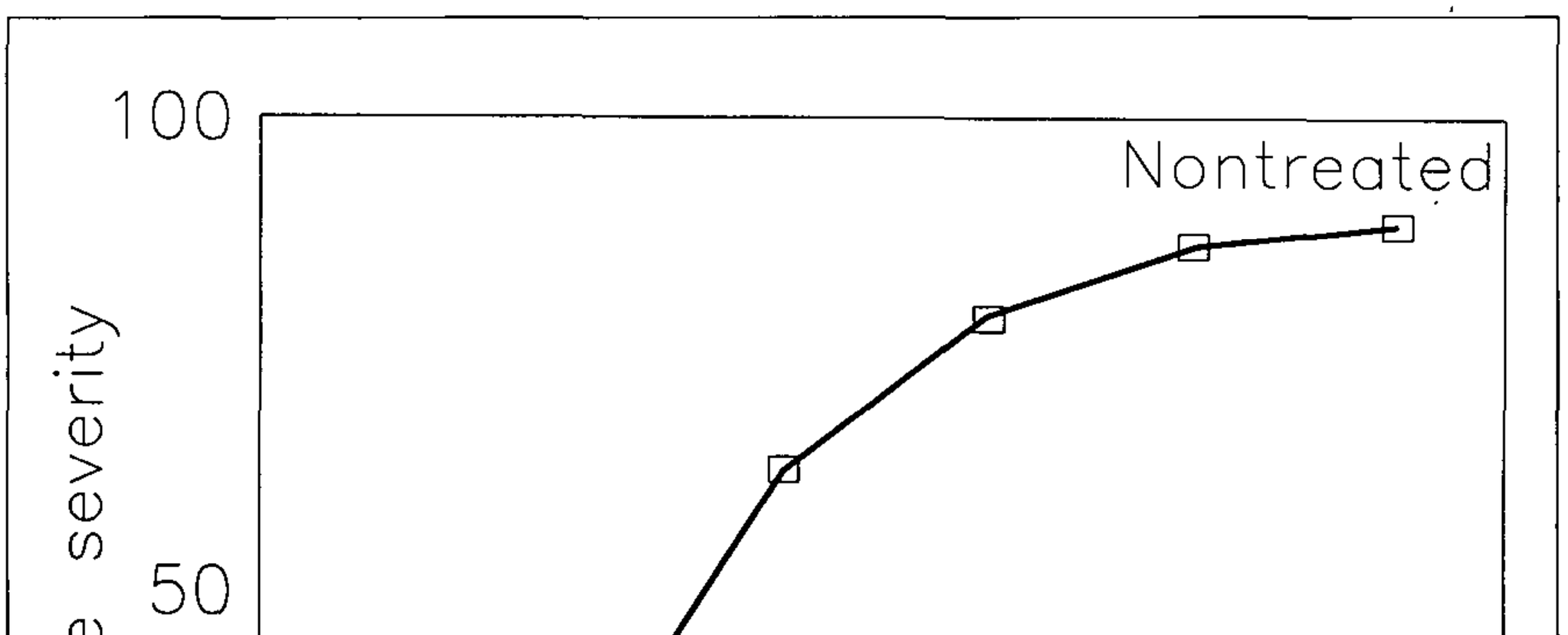

Figure 3. The effect of No. 108 isolate in a field trial.

Figure 4. The effect of pre-inoculation with No. 108 isolate in a field trial 10 weeks after transplanting (left: pre-inoculation with No. 108; right: control).

Table 1. The effect of isolate No. 108 on disease severity of carnation cuttings inoculated at pre and post rooting stages.

Time of treatment	No. of tested plants	No. of diseased plants	Diseased plants (%)[z]	Disease severity	Protective value (%)
Cutting sticking[x]	15	4	26.7	10.0	88.5
Cutting rooted[y]	12	2	16.7	10.4	88.0
Non-treated	15	14	93.3	86.7	NIL

[x] 2 liters of bud cell suspension (10^6 bud cells ml^{-1}) was sprayed on 100 rooted cuttings.
[y] The roots of the rooted cuttings were dipped in the bud cell suspension (10^6 bud cells ml^{-1}) for 30 min.
[z] Values represent the results 12 weeks after transplanting.

LITERATURE CITED

Garibaldi, A. 1979. Fungal and bacterial diseases of carnation and gerbera. Proc. Eucarpia Meeting, Alassio. p. 69-88.

Garibaldi, A. and **M.L. Gullino.** 1987. Fusarium wilt of carnation : present situation, problems, and prospects. Acta Horticulturae 216:45-54.

Komada, H. and **K. Ogawa.** 1980. Estimation of *Fusarium* species. In *Fusarium* Disease of Cultivated Plants (Matsuo, T et al.). Zenkoku Nosan Kyoiku Kyokai Publishing Co Ltd. , Tokyo. p. 201-228.

Ogawa, K. and **H. Komada.** 1984. Biological control of Fusarium wilt of sweet potato by non-pathogenic *Fusarium oxysporum.* Ann. Phytopath. Soc. Japan 50:1-9.

Tezuka, N. and **T. Makino.** 1991. Biological control of Fusarium wilt of strawberry by nonpathogenic *Fusarium oxysporum* isolated from strawberry. Ann. Phytopath. Soc. Japan 57:506-511.