Mycopathologia **117**: 65–71, 1992.
© 1992 *Kluwer Academic Publishers. Printed in the Netherlands.*

Interactions of *Fusarium moniliforme*, its metabolites and bacteria with corn

Charles W. Bacon & Joseph W. Williamson
Toxicology and Mycotoxin Research Unit, Richard B. Russell Agricultural Research Center, USDA/ARS, P.O. Box 5677, Athens, GA 30613, USA

Received and accepted 25 September 1991

Key words: Review, fumonisins, *Fusarium moniliforme* Sheldon, *Zea mays* L., mycotoxins, phytotoxins

Abstract

Fusarium moniliforme Sheldon is an economically important pathogen of corn (*Zea mays* L.) which causes stalk, root and ear rot. Several mycotoxins have also been isolated, identified and implicated in both animal and human toxicoses. The fungus can be disseminated in symptomless corn seed and can also survive in crop residues in the soil. Asymptomatic infection may be related to different corn cultivars, fungal strains, and environmental factors. Symptomatic expression of pathogenicity may vary, but usually the result of such infections is death of the plant. The greatest concern is the asymptomatic infection, since it is in this form that fungal toxins may surreptitiously enter animal and human food chains. *F. moniliforme* produces both fusaric acid, which is phytotoxic to corn and interferes with seed germination, and plant growth regulators that may affect pathogenicity of the fungus or be associated with the production of mycotoxins. Other metabolites, including fusarin C, moniliformin, and the fumonisins, may or may not be phytotoxic, but are associated with animal and human toxicoses. The control of *F. moniliforme* in corn is therefore quite important. One potential means to accomplish this reduction is biocontrol by the application of antagonistic rhizobacteria to corn kernels at planting. To be effective the bacteria must be able to colonize the corn root system and be able to prevent root infection by successful competing with *F. moniliforme* which may be accomplished by siderophore and or antibiotic activity.

Introduction

Fusarium moniliforme Sheldon, the anamorphic state of *Gibberella fujikuroi* (Sawada) Wr., is a biotrophic pathogen that can also grow saprophytically [1]. The fungus has a wide geographic distribution, but is restricted to the warm temperate, the humid tropical and subtropical regions of the world. Nevertheless, the fungus is cosmopolitan enough to cause stalk, root and ear rot of corn [2], resulting in severe economic losses to corn and other cereals and food crops throughout the world [3].

In addition to causing losses in corn, *F. moniliforme* is implicated in animal and human toxicoses [4]. Although several mycotoxins have been isolated and identified from *F. moniliforme*, the total number of mycotoxins produced is un-

known. The toxicological reports on *F. moniliforme* indicate that a wide range of animal species is affected and that there is a world-wide occurrence of toxic isolates.

This paper is a review of the physiology of *F. moniliforme* pertaining to its host-parasite associations and the interactions of fungal metabolites produced in corn, which must be considered relative to animal toxicity and eventual control of the fungus. Because the fungus may be seed-borne or survives in crop residues in the soil, its control should be designed to prevent fungus growth and inoculum buildup within the soil. One possible control strategy is biocontrol, utilizing soil bacteria and their metabolites to suppress *F. moniliforme*. Thus, biocontrol will be reviewed to stimulate interests in using bacteria to control this fungus since the effects of bacteria and their metabolites on *F. moniliforme* have not been studied in detail.

Host-fungus relationships

Corn-fungus associations

Fusarium moniliforme can be a seed-borne pathogen. Numerous surveys indicate that the occurrence of the fungus on corn kernels ranges from 1 to 100% [2, 3]. This range in infection probably reflects differences in corn cultivars, fungal strains and environmental conditions. Studies indicate that several high-lysine and supersweet hybrids which contain the opaque-2 and shrunken-2 endosperm mutations, respectively, are more susceptible to kernel infection by *F. moniliforme* [5, 6]. However, the open pollinated field and sweet corn cultivars are also susceptible but show extreme variation [2]. Lines of corn with the brown midrib are also susceptible [2]. Both inbreds and hybrids show variation in susceptibility from location to location, suggesting an environmental influence. The mechanism for corn resistance to *F. moniliforme* has not been identified, although progress in breeding for resistance has met with some success.

The fungus is distributed over the pericarp of kernels and since it is cultured from surfaced disinfected kernels, it is also located within the kernel. Studies utilizing scanning electron microscopy have determined that the fungus is located in sound-appearing kernels at the pedicel or tip cap end of a kernel well beyond the vascular bundles (Figs. 1–5). Fungal hyphae were not found within the embryo or endosperm of sound-appearing kernels, although in kernels associated with toxicity the embryo and endosperm are invaded. The finding of this fungus within the pedicel of sound-appearing corn indicates that the seed serve as an effective dispersal unit from which plant infection may take place. Further, it implies that external seed treatments using fungicides might not be an effective control.

F. moniliforme is also considered an endophyte since hyphae occur systemically in leaves, stems, roots, and cobs [7]. Isolates of *F. moniliforme* can be divided into symptom-inducing and non-symptom-inducing endophytes. These terms are intended to define specific fungal strains, although similar terminology was used earlier to describe cultivars of infected corn [5]. Symptom-inducing strains of *F. moniliforme* produce disease signs on kernels and plant parts while symptomless strains do not. Kernels from symptom-inducing strains should never be used for food. Current information indicates that *F. moniliforme* is not confined to the initial infection site; it is instead uniformly distributed but the plant shows little or no response [8].

The symptomless expression on corn produces the greatest concern, since it is possible that mycotoxins can enter the food chain undetected when symptomless but infected corn is consumed. Symptomless infection of corn by *F. moniliforme* may be viewed as a latent infection, although this type of infection is probably not metabolically inactive. For example, this symptomless infection might be similar to infection caused by endophytic fungi of grasses, where a variety of toxins are produced by the endophytic fungus without host symptoms occurring [9]. Because *F. moniliforme* is endophytic, plant residues also play an

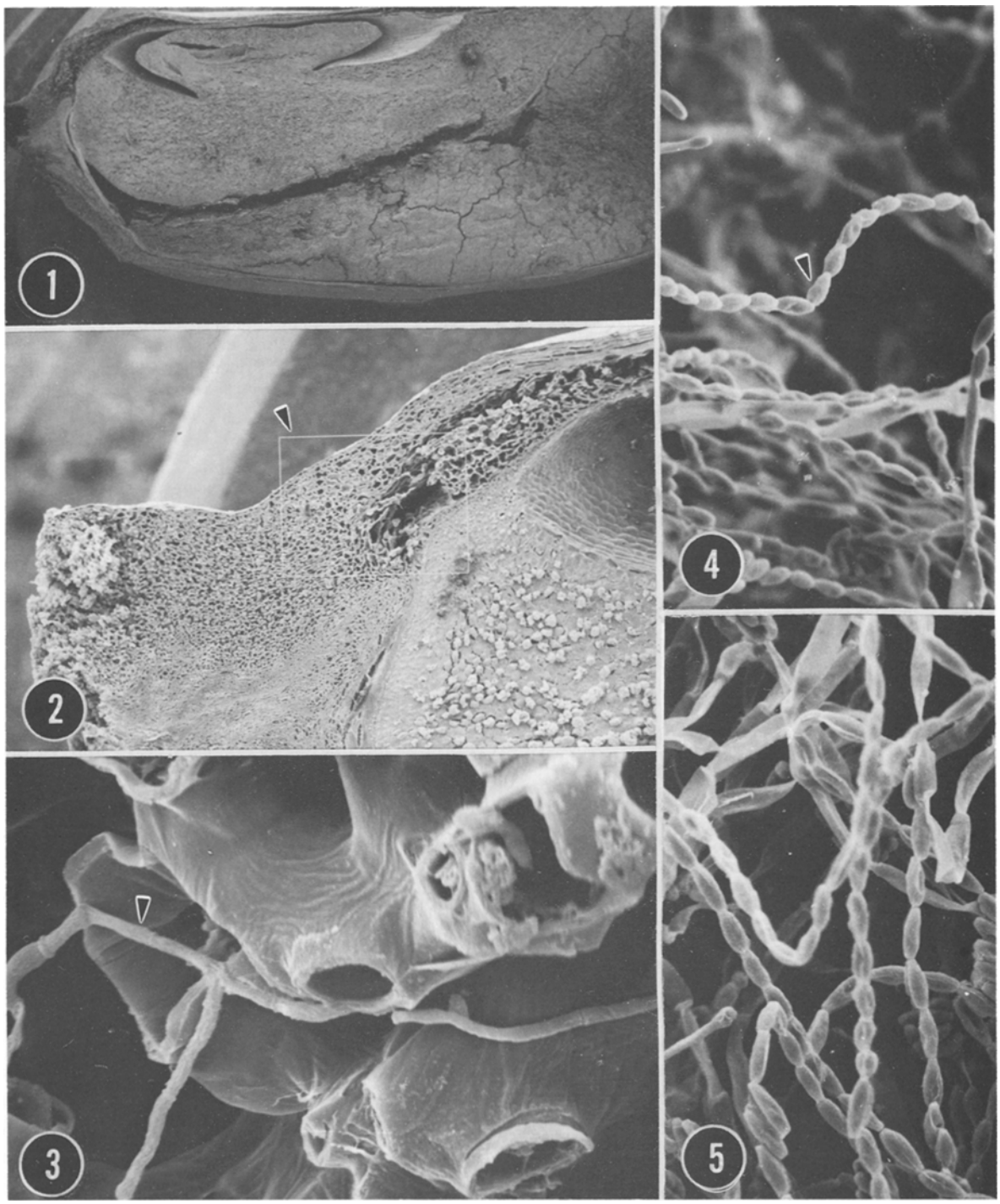

Plate. Scanning electron micrographs showing location of *Fusarium moniliforme* (arrows) within a longitudinal section of a corn kernel, Figs. 1–3. Fig. 4 shows the remaining half of the same kernel after two days incubation on potato dextrose agar media; note the production of long chains of conidia (arrow) indicative of *F. moniliforme*. Fig. 5 shows similar conidia from a known isolate of *F. moniliforme* cultured on potato dextrose agar.

important part in over wintering and inoculum dissemination. Growth and sporulation of the fungus in soil on dead plant parts suggest that *F. moniliforme* is also saprophytic [1, 7].

Since most kernels are infected, the potential for animal toxicity is enhanced under improper storage conditions. Both high kernel moisture and cool temperatures have been reported important for the production of one class of mycotoxins, the fusarins [10], produced by *F. moniliforme*. No information is available that would indicate that mycotoxins are produced by this fungus during the postharvest stage of corn development.

The significance of plant growth regulators

Plant hormones are important to the regulation of plant growth. Much is known about the occurrence, interactions and biosynthesis of plant growth substances. Although plant growth regulators were isolated from parasitic fungi long before they were found in higher plants, little is known about the involvement of plant growth regulators in host-parasite relationships and disease development.

Fusarium moniliforme produces gibberellins and auxins [11, 12]. On rice the fungus causes an overgrowth of stems which can also be achieved by topical application of gibberellins. However, other plants infected with the same fungus do not show symptoms of overgrowth. Oats infected with a gibberellin-producing or a nonproducing strain of *F. moniliforme* indicated that the gibberellin-producing strain produced more hyphae within the host tissue than the nonproducing strain [12, 21]. These data suggest that gibberellin can increase the growth of *F. moniliforme* which might indirectly affect pathogenicity. Therefore, this growth regulator may be associated with production of animal toxin, if by no other reason than an increase in fungal biomass.

Table 1. Effects of fusaric acid on corn seed germination.

Corn cultivar[1]	Fusaric acid, M				
	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	% germination decrease[2]				
1	91a	94a	70b	15c	1d
2	93a	95a	30b	10c	3d
3	90a	91a	40b	20b	1c
4	95a	96a	85b	20c	2d
5	99a	90a	50b	30c	2d
6	98a	91a	45b	10c	1d
7	99a	89a	65b	40b	3c
8	99a	89a	75b	30c	4d
9	99a	90a	80b	10c	2d

[1] These cultivars include both field and sweet corn cultivars.
[2] Each percentage decrease is the mean of three experiments; means within each cultivar (row) followed by the same letter are not significantly different ($p < 0.05$).

Phytotoxicity of *F. moniliforme* metabolites to corn

Fusaric acid. Fusaric acid (5-*n*-butylpyridine-2-carboxylic acid) is produced by *F. moniliforme* and by other Fusaria, especially those in the section Elegans [13]. Fusaric acid and its derivative, dehydrofusaric acid, are produced in large quantities and have been isolated from tissues of *Fusarium*-infected plants [13]. The application of fusaric acid to plants results in increased water loss and leakage of other compounds from plant tissue because of damage to cell membranes [13]. This toxin has also been shown to chelate heavy metals, particularly iron [14], resulting in an inhibition of enzymes such as iron porphyrin oxidase [15]. The final result is reduction in plant respiration. In addition to iron, fusaric acid chelates with copper, cobalt, nickel, zinc and manganese [16].

If the mode of action of fusaric acid is to affect the plant respiratory system, then it should influence germination of seed which is characterized by a high rate of respiration. To test this hypothesis, nine cultivars of corn were germinated on filter paper saturated with 10 ml of a solution of fusaric acid in 10 mM phosphate buffer, pH 6.5. Germination of all nine cultivars were completely inhibited by the two high concentrations,

5.6×10^{-2} M and 5.6×10^{-3} M (Table 1). The effects of fusaric acid at 5.6×10^{-4} M differed according to the cultivar of corn. At this concentration some corn cultivars developed only coleoptiles, while others developed coleoptiles and aborted roots. Concentrations of fusaric acid less than 10^{-5} M had no effect on the germination of all cultivars. The effects of fusaric acid on corn seedlings followed a similar concentration. Its effects on leaves of corn seedlings showed disruption of the cellular membrane at the concentrations between 10^{-3} M and 10^{-4} M, but at the low concentration, 10^{-5} M and 10^{-6} M, only the membranes of the mitochondria and chloroplast were damaged.

Fusarin C. The mutagenic substance, fusarin C, is also produced by several isolates of *F. moniliforme* [10]. The production of fusarin C by *F. moniliforme* has only been documented in inoculated shelled corn, but it has been detected in other cereals, as well as soybean [17]. Fusarin C is very unstable and attempts at demonstrating its activity on corn might reflect this property. Nevertheless, a study was designed to determine its phytotoxicity, with and without dimethyl sulfoxide, on corn seedlings leaves and seed germination. Fusarin C did not produce any detectable phytopathological symptoms (Bacon and Hinton, unpublished).

Moniliformin. Moniliformin (1-hydroxycyclobut-1-ene-3,4 dione) has been shown to be phytotoxic on corn and tobacco, with corn being more sensitive [18]. The minimum concentration, administered topically, required to produce phytotoxicity in corn was high, 0.02 mg per plant. Infusion studies might have yielded similar information but at reduced concentrations. However, since only a few isolates of *F. moniliforme* produce moniliformin, it is probably of no consequence in corn infected with *F. moniliforme*.

The fumonisins. The final group of mycotoxins, the fumonisins, consists of long-chain alkylamines with 2 tricarboxylic acid moieties attached. Fumonisin B_1 is considered the most biologically active. Fumonisin B_1 is structurally similar to the tomato host specific toxins (AL) produced by *Alternaria alternata* f. sp. *lycopersici* [19]. The AL toxins have been isolated from tomato tissue infected with *A. alternata*, and it has been shown that the primary amine of the AL molecule is required for activity [20].

Two cultivars of corn, T1 and FLS, were used to determine the phytotoxicity of fumonisin B_1. Fumonisin B_1 was administered to corn either in a 10 mM phosphate buffer solution, pH 6.5, into which seedlings were suspended or infused through leaf tissue of seedlings grown in sterile sand. Fumonisin test solutions were infused or replaced with fresh fumonisin solutions every two days. Control treated seedlings received phosphate buffer solution only.

After one week, one half of the seedlings was harvested. Both treated seedlings and controls had similar weights, leaf width and root-to-shoot ratios. The remaining seedlings were placed in phosphate buffer without fumonisin for another week. There were no detectable effects of fumonisin B_1 on corn seedlings in the concentration range of 10^{-2} M to 10^{-6} M. Therefore either fumonisin B_1 was not absorbed into the plant, the concentration was too low, or the two corn cultivars used in the study were resistant.

Biocontrol potential with rhizobacteria

According to Toussoun [11], the concept of biological control of soil-borne fungi by microorganisms was studied some 70 years ago by Hartley who proposed to control damping off in pine seedlings by using other microorganisms. At about the same time, investigations into the nature of *Fusarium*-suppressive soils were initiated by Knudson but the results were not published. To date, *Fusarium moniliforme* has not been included as a species controlled by suppressive soils.

There are numerous bacterial species used to control pathogenic soil-borne fungi [22]. The pre-

cise mechanism by which bacteria control disease producing organisms includes antibiosis, growth-promoting substances and nutrient competition. To successfully use a biocontrol agent against *F. moniliforme*, all interactions between the microbial antagonist, the fungus, the corn plant, and the environment must be considered. Although some bacteria possess the ability to control a pathogen, they may also be deleterious [23]. A potential biocontrol agent should always be screened for this characteristic as well as others.

The biocontrol agent should be suitable for applications to the corn kernel before planting, preferably as a spore. The biocontrol agent and the corn seedling will become established together. Since the level of biocontrol will operate at the indigenous population of soil organisms, the two most common strategies for the establishment of the microbial antagonist in the rhizosphere are: (1) to inundate the indigenous microflora with large numbers of antagonists, or (2) to reduce the quantity or alter the composition of indigenous microflora to favor the establishment of the antagonist. Further, the biocontrol agent must be capable of colonizing the corn root system and inhibiting or reducing growth of *F. moniliforme*. To be a successful biocontrol agent, the antagonistic bacterium must be able to: (1) attach or agglutinate to the root system, and rapidly grow, spread, and survive on the root system, (2) occupy areas on roots which will not interfere with the uptake of nutrients by the plant, and (3) compete with *F. moniliforme* and indigenous microflora for nutrients. The antagonist should also be polyphagous and saprophytic, as nutrient versatility can allow for rapid growth and long-term survival of the antagonist.

Once the bacterium has colonized a favorable niche, it must be able to maintain its position on the root system, either by the production of siderophores [24] that have a higher iron-binding affinity than those of the pathogen, or by the production of antibiotics that suppress growth of competing microorganisms. Of the fungal antagonists used, most are bacteria in the genera *Pseudomonas* and *Bacillus* [23]. Other microorganisms with a potential for biocontrol by antibiotic production include the actinomycetes, such as *Streptomyces*. Actinomycetes are also better able to tolerate desiccation than most bacteria [22].

An additional desirable characteristic of a biocontrol agent, although not mandatory, is its ability to stimulate plant growth. Colonization of plant roots by plant growth-promoting rhizobacterium, such as *Azospirillum*, increases biomass and yield because of the production of indole acetic acid or cytokinins by the bacterium. In the rhizosphere there are also populations of pathogens, or deleterious rhizobacteria, which tend to increase with continual corn cropping practices. These bacteria may reduce the crop yields because of the production of phytotoxic substances such as cyanide, volatile sulfur-containing compounds [16], or excessive amounts of phyto-hormones [22] in the rhizosphere. The plant growth-promoting rhizobacteria may be important controls against these minor pathogens [24].

Conclusion

F. moniliforme is one of the earliest known causes of animal toxicosis and plant diseases. In agriculture, the dilemma we are faced with is that of control. Control is exceedingly important since this fungus can produce several mycotoxins on one of the world's largest classes of food, the cereals, particularly corn. Although, as is the case with all plant pathogenic organisms, the best control is the development of resistant varieties. *Fusarium moniliforme* and the corn plant are no exception. However, before we can produce resistance we must first understand how and where this organism establishes itself within the plant.

References

1. Nyvall RF, Kommedahl T. Saprophytism and survival of *Fusarium moniliforme* in corn stalks. Phytopathology 1970; 60: 1233–5.

2. Kommedahl T, Windels CE. Root-, stalk-, and ear-infecting *Fusarium* species on corn in the USA. In: Nelson PE, Toussoun TA, Cook RJ, eds. *Fusarium*: Diseases, biology and taxonomy. University Park: The Pennsylvania State University Press, 1981: 94–103.
3. Booth C. The genus *Fusarium*. Kew, Surrey (UK): Commonwealth Mycological Institute, 1971.
4. Marasas WFO, Nelson PE, Toussoun TA. Toxigenic *Fusarium* species, identity and mycotoxicology. University Park: The Pennsylvania State University Press, 1984.
5. Headrick M, Pataky JK. Relationships among carbohydrates content of kernels, condition of silks after pollination, and the response of sweet corn inbred lines to infection of kernels by *Fusarium moniliforme*. Phytopathology 1990; 80: 487–94.
6. Warren HL. Comparison of normal and high-lysine maize inbreds for resistance to *Fusarium moniliforme*. Phytopathology 1978; 68: 1331–5.
7. Foley DC. Systemic infection of corn by *Fusarium moniliforme*. Phytopathology 1962; 52: 870–2.
8. Pennypacker BW. Anatomical changes involved in the pathogenesis of plants by *Fusarium*. In: Nelson PE, Toussoun TA, Cook RJ, eds. *Fusarium*: Diseases, biology, and taxonomy. University Park: Pennsylvania State University Press, 1981: 400–8.
9. Bacon CW, Siegel MR. The endophyte of tall fescue. J Prod Agric 1988; 1: 45–55.
10. Farber JM, Sanders GW. Fusarin C production by North American isolates of *Fusarium moniliforme*. Appl Envir. Microbiol 1986; 5: 381–4.
11. Toussoun, TA. *Fusarium*-suppressive soils. In: Bruehl GW, ed. Biology and control of soil-borne plant pathogens. St. Paul, MN: American Phytopathological Society, 1975: 145–51.
12. Zak JC. Pathogenicity of a gibberellin-producing and a nonproducing strain of *Fusarium moniliforme* in oats as determined by a colorimetric assay for *N*-acetyl glucosamine. Mycologia 1976; 68: 151–7.
13. Drysdale RB. The production and significance in phytopathology of toxins produced by species of *Fusarium*. In: Moss MO, Smith J, eds. The applied mycology of *Fusarium*. New York: Cambridge University Press, 1984: 95–105.
14. Malini S. Heavy metal chelates of fusaric acid: In vitro spectrophotometry. Phytopath Z 1966; 57: 221–31.
15. Tamari K, Kaji J. Studies on the mechanism of the growth inhibitory action of fusarinic acid on plants. J Bacteriol 1954; 41: 143–65.
16. Banwart WL, Bremner JM. Formation of volatile sulfur compounds by microbial decomposition of sulfur-containing amino acids in soils. Soil Biol Biochem 1975; 7: 359–64.
17. Bacon CW, Marijanovic DR, Norred WP, Hinton DM. Production of fusarin C on cereal and soybean by *Fusarium moniliforme*. Appl Environ Microbiol 1989; 55: 2745–8.
18. Cole RJ, Kieksey JW, Cutler HG, Doupnik BL, Peckham JC. Toxin from *Fusarium moniliforme*: Effects on plants and animals. Science 1973; 179: 1324–6.
19. Bottini AT, Gilchrist DG. A 1-aminodimethylheptadecapentol from *Alternaria alternata* f.sp. *lycopersici*. Tetra Let 1981; 22: 2719–22.
20. Siler DJ, Gilchrist DG. Properties of host specific toxins produced by *Alternaria alternata* f. sp. *lycopersici* in culture and in tomato plants. Physiol Plant Pathol 1983; 23: 265–74.
21. Thakur MS, Vyas KM. Production of plant growth regulators by some *Fusarium* species. Folia Microbiol 1983; 28: 124–9.
22. Schroth MN, Loper JE, Hildebrand DC. Bacteria as biocontrol agents of plant disease. In: Klug MJ, ed. Current perspectives in microbial ecology. Washington, DC: American Society for Microbiology, 1983: 362–9.
23. Hemming BC. Bacteria as antagonists in biological control of plant pathogens. In: Baker RR, Dunn PE, eds. New directions in biological control: Alternatives for suppressing agricultural pests and diseases. New York: Alan R. Liss, 1990: 223–42.
24. Swinburne JR. Iron, siderophores, and plant disease. New York: Plenum Press, 1986.