Biocontrol of Corn Root Infection in the Field by Seed Treatment with Antagonists

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Paper No. 8718, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Accepted for publication 30 September 1974.

ABSTRACT

Kernels of corn *(Zea mays)* of three hybrids were coated with *Bacillus subtilis, Chaetomium globosum,* or captan, and planted in the field in three successive years. Stands increased 9 and 14 days after planting and at season's end for all hybrids with *Chaetomium* and captan treatments and for one hybrid with *B. subtilis* treatment. Treatments hastened attainment of an approximate 95% stand by 1-3 days depending on hybrid and treatment. Stalk rot and breakage were less with the organism- and captan-coated, than with noncoated, kernels. The stalk rot pathogen most frequently isolated was *Fusarium roseum* 'Graminearum'. Grain yields per treatment were higher for kernels coated with captan in all 3 years, for those coated with *C. globosum* for 2 of 3 years, and for those coated with *B. subtilis,* only 1 year, than for kernels not coated.

Phytopathology 65:296-300

Bacillus subtilis protected barley from seedling blight caused by *Helminthosporium sativum* in the field (1) and *Chaetomium globosum* protected oats from Victoria blight caused by *H. victoriae,* and protected flax, particularly mechanically damaged flaxseed, from Fusarium wilt (12). In both of these studies at Minnesota, inoculum was added to soil, not to grains or seeds.

Other isolates of these two antagonistic organisms, when applied to kernels of corn, not only inhibited seed-borne storage and field fungi (9), but protected corn from seedling blight in greenhouse and field tests (4, 7). Other organisms showed promise of being effective antagonists based on greenhouse experiments with corn (8, 9). The current status of biological control has been reviewed recently by Baker and Cook (2) and Papavizas (11), and the mechanisms of control by Baker (3) and Mitchell (10).

Our objective was to ascertain the effect of coating kernels of corn with *Bacillus subtilis* (Cohn) Prazmowski and *Chaetomium globosum* Kunze ex Fr. on seedling and final stands, stalk rot, and yields in the field, in comparison with the standard chemical seed treatment, captan.

MATERIALS AND METHODS.—In general, materials and methods were the same as those in our earlier paper (4). The three hybrids of dent corn tested (*Zea mays* L. 'Minhybrid 508', 'Minhybrid 5302', and 'Minhybrid 6302') were chosen because of their history of differing susceptibility to root lodging: Min 5302 was resistant, Min 6302 was susceptible, and Min 508 was intermediate.

Cultures of *Bacillus subtilis* and *Chaetomium globosum* were isolated originally from soil. They were cultured in 125-ml flasks containing 50 ml potato-dextrose broth at about 22 C. Cultures of *B. subtilis* were increased on a rotary shaker, but those of *C. globosum* were increased in stationary culture. Two-day-old cultures of *B. subtilis,* and 10-day-old cultures of *C. globosum,* were used for coating kernels.

Spores and mycelial masses of *C. globosum* were collected by filtering each culture produced in flasks through filter paper under a partial vacuum. The fungal biomass was removed from the filter paper, suspended in sterile water, and minced in a blender for 10 seconds at low speed. This suspension was divided into two portions: (i) one portion was filtered again through filter paper under partial vacuum and the spores and hyphae were collected on the filter paper, dried at 22 C, then stored in bottles until needed, and (ii) one portion was kept in suspension ready for use directly as inoculum.

When kernels were wet-treated with *B. subtilis* or *C. globosum,* 150 kernels per hybrid were immersed for 5 minutes in the 50 ml culture suspension within 125-ml flasks. The wet-treated kernels were placed on filter paper in petri dishes, dried at 22 C, and stored in envelopes until sown. When kernels were dry-treated with *C. globosum,* they were wetted with sterile water, then coated with dry spores.

Kernels coated with organisms were compared with those coated with captan (*N*-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide). Kernels of all treatments were sown at the St. Paul farm in a silt loam soil. Corn ears were harvested by hand, shelled, and the grain was weighed. Yields were calculated at a 15% moisture content. Stalk rot indices were determined on a scale of 1 to 4 as described by Christensen and Wilcoxson (5). Stalk breakage was ascertained by deflecting the stalk at about ear height to a vertical displacement of about 30 cm and recording breakage if this occurred at the lowermost internode.

Field trials in 1968 and 1969 were based on two dates of planting each year with three replicates per hybrid and treatment (10 plants per replicate) at each date. In 1970, results were based on nine replicates of 120 kernels sown per replicate per hybrid and treatment.

Data on soil temperature and rainfall were obtained from the U.S. Environmental Sciences Service Administration, Climatological Data for Minnesota.

RESULTS.—*Effect on plant stands.*—Seedlings began to emerge in the field 9 to 12 days after planting, depending on the season. Delay in emergence 2 weeks after planting was the greatest in 1968 (lowest value in bar of Fig. 1), least in 1969 (highest value of bar), and intermediate in 1970 (middle value of bar). The two antagonists were about as effective as captan in improving 2-week-old or final stands (Fig. 1). Even though stands at 2 weeks were low for 1968 (35-40%) for all treatments, the final stands were the highest that year (90% or more). The highest percentage stand initially was in 1969, and this resulted in the second-highest final stand. In 1970, the initial stands were intermediate and in all treatments, but *C. globosum* gave the lowest final stands. Environmental conditions that delayed emergence apparently did not affect final stands. Seed treatments for those years did not improve stands appreciably over nontreated kernels, although slight increases were noted for captan and *Chaetomium globosum*-treated kernels. The differences in 1970, though small, were significant at $P = 0.01$ (Tukey's HSD procedure). Differences for the other two years were significant at $P = 0.05$ or 0.10 for two of three hybrids (the ones previously shown to be most susceptible to root lodging).

In 1970, seedlings began to emerge on the ninth day after planting for each of the three hybrids, but the percentage emergence varied with hybrid and treatment (Fig. 2). Captan and *C. globosum* seed treatments improved stands of all hybrids ($P = 0.01$); however *B. subtilis* was not effective. Another measure of effectiveness was the number of days delay in total emergence; this was determined as the number of days for stands to come within five plants of the final stand. Nontreated kernels of each hybrid required 14 days to reach five plants from final stand and captan and *Chaetomium*-treated kernels required 11-13 days (Fig. 3). Apparently, a delay of 1-3 days in reaching near-final stands (five plants from final stand), as well as the percentage stand at the first day of emergence or at 2 weeks, does have an effect on yield of grain (Table 1). The values for "five plants from final stand" were presented (as well as the final stand) because emergence was fairly regular and consistent until about the last five plants which emerged in a pattern that was erratic and not attributable to the treatments studied. Thus, stand at that point seemed to be a better measure of the effects of treatment than did final stand (although the latter are shown).

Effect on stalk rot and breakage.—Previous work over a period of several years had established that Minhybrid 6302 was susceptible to root lodging, and that Minhybrid 5302 was relatively resistant, whereas Minhybrid 508 was usually intermediate. These ratings were confirmed (at least for 5302 and 6302) and it was apparent also that application of organisms or captan to kernels was more effective for the hybrids more susceptible to root and stalk lodging (Min 508 and 6302), but the differences were not significant ($P = 0.05$) (Fig. 4).

The results shown for hybrids in Fig. 4 are illustrated in terms of the stalk rot index (rating scale, 1-4); however when the averages are compiled for the three hybrids in terms of percentage of stalks showing some degree of stalk rot, or, in which stalks broke when displaced from the vertical, captan and the antagonists had a generally beneficial effect in reducing incidence (Fig. 5). Apparently *Chaetomium globosum* was at least as effective as captan in preventing stalk rot or breakage.

Since these were field studies with no inoculation of stalks, and since it was desirable to know the pathogens involved, the stalk fragments were placed on two kinds of agar media: acidified potato-dextrose agar, and pentachloronitrobenzene-peptone agar. The percentage stalks infected with fungi based on isolation using the two

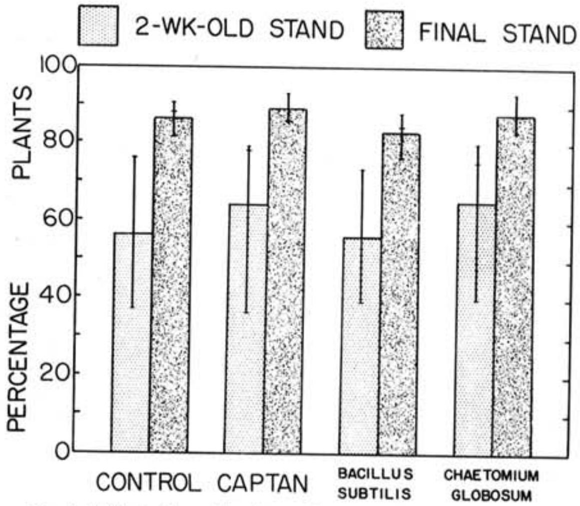

Fig. 1. Effect of application of captan, *Bacillus subtilis*, or *Chaetomium globosum* to corn kernels: effects on stand 2 weeks after planting and at the end of the season for a 3-year period (1968-70). Values in 1968 and 1969 are based on 180 plants (three hybrids, three replicates, 10 plants/replicate, two planting dates) and values for 1970 on at least 2,700 plants (three hybrids, nine replicates, 100-120 plants/replicate). Range in values are for the 3 years.

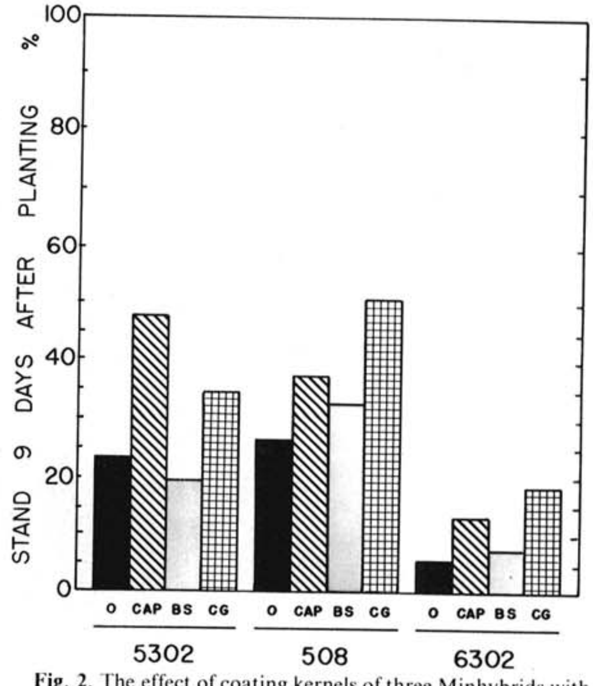

Fig. 2. The effect of coating kernels of three Minhybrids with captan (CAP), *Bacillus subtilis* (BS), and *Chaetomium globosum* (CG), and compared with noncoated kernels (0), on stand of corn 9 days after planting in the field in 1970. Results are averages of nine replicates of 120 kernels planted per replicate for each treatment and hybrid.

kinds of media were (respectively) as follows: *Fusarium roseum* (Lk.) emend. Snyd. & Hans. 'Graminearum', 61 and 68%; *F. roseum* 'Avenaceum', 26 and 29%; *F.*

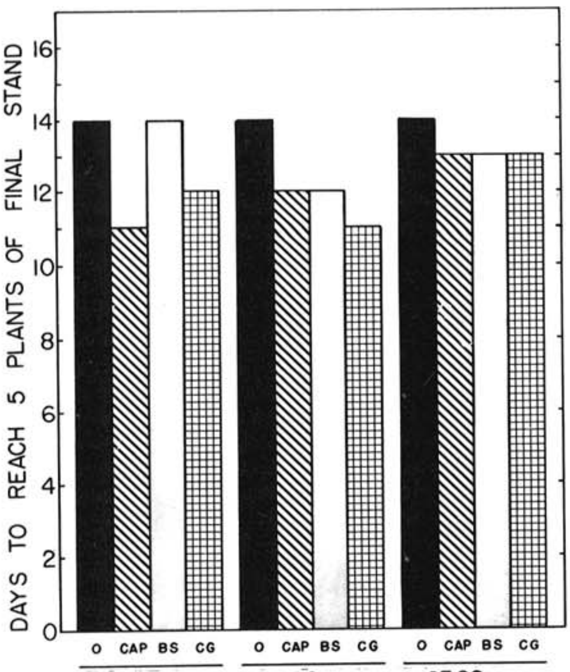

Fig. 3. Effect of coating kernels of three Minhybrids with captan (CAP), *Bacillus subtilis* (BS), and *Chaetomium globosum* (CG), and compared with noncoated kernels (0), in terms of the number of days for emergence to come within five plants of the final stand in the field during 1970. Results are averages of nine replicates of 120 kernels planted per replicate for each treatment and hybrid.

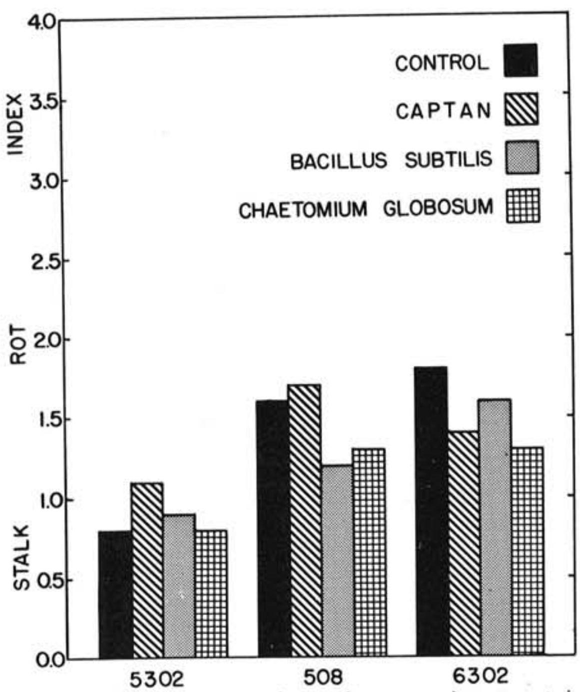

Fig. 4. Effect of captan and organisms as seed treatments in three Minhybrids (5302, 508, and 6302) of corn in the field in terms of the index of stalk rot from natural infection in 1969. Results are averages of 30 plants per treatment and hybrid.

oxysporum Schl. emend. Snyd. & Hans., 30 and 26%; *F. tricinctum* Cda. emend. Snyd. & Hans., 22 and 10%; *F. moniliforme* Sheld. emend. Snyd. & Hans., 4 and 7%; *Nigrospora oryzae* (Berk. & Br.) Petch, 26 and 13%; *Alternaria tenuis* Auct., 9 and 0%; and *Cephalosporium* sp., 0 and 4%. Isolations were made from 25 stalks, 10 tissue fragments per stalk.

Effect on yield.—Captan and *C. globosum* improved the average yield of three hybrids in at least 2 or 3 years' test (Table 1). *Bacillus subtilis* had a negative or no effect in 1968 and 1970, but improved yields significantly in 1969 when *C. globosum* had no significant effect.

In 1968 and 1969, *C. globosum* was applied either by immersing kernels in a spore suspension or by rolling kernels in a dry preparation of hyphae and spores, but there was no appreciable difference between the two methods of application. Consequently in 1970, kernels were immersed briefly in the suspension a few days before kernels were planted.

In 1968, in one trial, *Chaetomium*-treated kernels of Min 6302 gave yield increases that were significant at $P = 0.01$, but in a second trial only at $P = 0.10$. Also, captan-treated kernels significantly increased yields in one hybrid (Min 6302), but *B. subtilis* significantly increased yield for another hybrid (Min 5302), both $P = 0.05$. In 1970, both captan and *C. globosum*-treated kernels gave significant increases ($P = 0.01$) in yield for all three hybrids. In general, in 1968 and 1969, increases in yield over nontreated kernels for the resistant (Min 5302) and susceptible (Min 6302) hybrids, respectively, were 6 and 8% for treatments with *B. subtilis,* 6 and 4% for treatments with *C. globosum,* and 5 and 10% for treatments with captan. Thus a greater response from seed treatment was usually realized from treatments of a susceptible than a resistant hybrid, but the differences were not always consistent or large, and so were not presented in Table 1.

Soil temperature, soil moisture, or both may also influence susceptibility of hybrids to stalk rot. All treatments and hybrids in 1968 averaged 7.6% increase in yield over no treatment, but in a planting 2 weeks later in the same season this averaged only 3.1% increase in yield. The reverse was true in 1969; then the earlier planting averaged only 2.6% yield increase whereas 2 weeks later an 8.9% increase of treated over nontreated kernels was found. Differences in soil temperature and moisture at the two different times of planting in each year may have accounted for these differences.

In 1968, soil temperature at a 5-cm depth averaged about 3 C higher than soil temperature in 1969 (18 vs. 15 C) and the rainfall was 3.5 cm less during comparable 2-week periods (24 April-7 May) after planting (0.8 vs. 4.3 cm). The warmer, drier soil in 1968 may have made *C. globosum* more effective than *B. subtilis* as a seed treatment. In 1969, in wetter soil during the same period, *B. subtilis* was more effective than *C. globosum*. In 1970, soil temperature during the same period as in 1968-69 was the highest (20 C), and the rainfall was intermediate (3.9 cm), which may have rendered *B. subtilis* ineffective as well as reduced the response from the other two treatments (Table 1). Thus, there may be a relationship between the effectiveness of some organisms as seed treatments and soil moisture (measured as rainfall) and temperature, and moisture may be more critical for *B. subtilis* than for *C. globosum*. However, this hypothesis needs further experimentation.

DISCUSSION.—This work, together with our previous work (4, 8), demonstrated the feasibility of biological control for root diseases of corn in the field by the coating of kernels with antagonistic microorganisms. This is biological control whether a restricted view is taken in which confrontation of antagonists and pathogen occurs or whether a more general view is taken as represented in the discussion by Baker and Cook (2). Corn roots were protected from *Fusarium roseum* f. sp. *cerealis* 'Graminearum' in inoculation of soil in the greenhouse (7, 8) and from naturally occurring pathogens in soil in the field both in earlier work (4) and in this paper, and this protection not only improved stands but yields of grain in the 3 years of field tests.

The reduced emergence, reduced stands after 2 weeks and at season's end, as well as the increase in stalk rot and breakage from lack of seed treatment, were modest but the cumulative effect of all these losses gave lower yields that varied some with hybrid and season.

To estimate the worth of organisms as seed treatments, it is necessary to compare them with captan, the fungicide most frequently used for treating corn kernels. Organisms usually were effective when captan also was effective, especially among the root rot-susceptible hybrids. However, there was more inconsistency in behavior of antagonists than of captan. Clearly, there are factors that affect the efficacy of organisms that do not affect captan. Temperature and moisture of soil are two important factors to be considered.

Cook and Papendick (6) noted the reduced activity and mobility of bacteria in dry soil, and a concomitant increase in root rot of wheat caused by *Fusarium roseum* 'Culmorum'. Our results showed also that in relatively dry soils, *Bacillus subtilis* was not as effective as it was in moist soils, based on rainfall data in the 3 years of the yield test in the field. *Chaetomium globosum* seemed to function as a seed treatment over a wider range of soil moistures than did *B. subtilis*. It is possible that the bacterium might be more effective in moist soils and the fungus in dry soils, and that the combination of the two organisms could give protection over a wider range of soil moistures, and perhaps temperatures, than afforded by either one alone. Such treatments merit further study and, in fact, such studies are currently being made at Minnesota.

Undoubtedly the wide use of captan as a seed treatment is attributed to its reliability, ease of application, and low cost of application. But captan is not always a good seed treatment. Under prolonged conditions of low soil temperature and high soil moisture, organisms proved superior to captan in reducing root infection, probably because of multiplication of organisms and their growth from the kernel to the root surface (4).

The effectiveness of captan was due also to the fact that technology of seed treatment with chemicals has been developed over decades and the appropriate formulations and techniques of application have been well worked out. The technology of coating kernels with organisms has not been pursued with the same intensity of effort, except perhaps for the applications of nitrogen-fixing bacteria to seeds of legumes. Further experimentation is needed to develop the technology of such procedures for seed treatments.

Thus, there is need for research on (i) types of coating materials as carriers of antagonists, (ii) moisture and temperature effects, (iii) duration of storage after treatment, (iv) application to seed of several organisms in sequence or as mixtures, (v) animal toxicity of coated kernels, and (vi) adverse environmental effects. When such studies are completed, the use of organisms for coating corn kernels could be commercially feasible. It may even be possible to use both fungicides and organisms together in the protection of seedlings from disease.

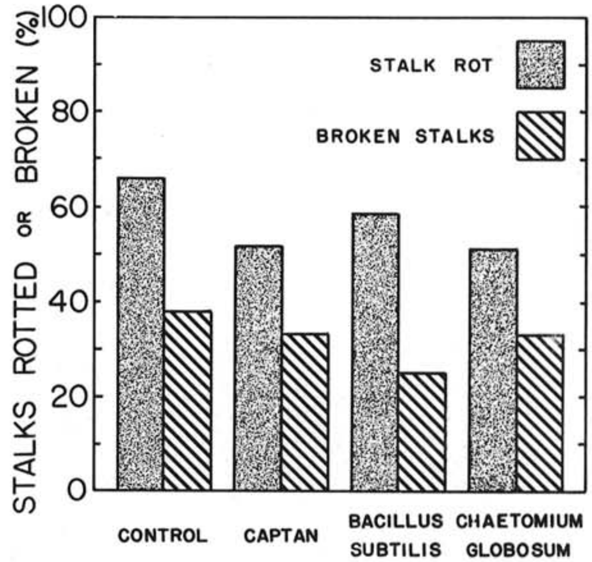

Fig. 5. Effect of captan and organisms as seed treatments in three Minhybrids (5302, 508, and 6302) of corn in the field in terms of percentage of plants with stalk rot and of plants that broke when deflected 30 cm from the vertical. Results are averages in 1969 of 60 plants per treatment and are based on natural infection.

TABLE 1. Effect of coating kernels with captan, *Bacillus subtilis*, or *Chaetomium globosum* on yield of corn in the field for 3 years

Kernels coated with:	Increase in yield over control/year (%)		
	1968[a]	1969[a]	1970[b]
Captan	7.7	6.0	3.3
B. subtilis	1.8	8.3	−5.0
C. globosum	6.6	1.8	3.5

[a]Each value is an average of 180 plants (three hybrids, three replicates, 10 plants/replicate, two planting dates).

[b]Each value is an average of at least 2,700 plants (three hybrids, nine replicates, 100-120 plants/replicate).

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