Influence of onion seed bacterization on germination and mycosphere microflora of *Sclerotium cepivorum* sclerotia

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Diallyldisulfide (DADS) and onion seedlings grown from bacterized seeds significantly enhanced the germination of *Sclerotium cepivorum* sclerotia in muck soil compared with germination in the absence of seedlings or in the presence of seedlings grown from surface-sterilized nonbacterized seeds. Germination was greater for sclerotia on the soil surface than for buried sclerotia. When used for seed bacterization, *Bacillus subtilis* strain B-2 and four other bacterial strains obtained from rhizospheres of field-grown onions differed in their abilities to enhance germination of sclerotia. Strains B-2, UI-2, and B caused significantly greater germination of sclerotia than did strains UI-1 and W. Sclerotia in soil containing onion seedlings bacterized with B-2, UI-2, and B significantly supported reduced general indigenous bacterial and fungal populations. Treatment of soil with DADS reduced general indigenous bacterial but not fungal populations associated with sclerotia. There were significant inverse correlations between the proportions of sclerotia germinating and populations of bacteria and fungi associated with sclerotia of *S. cepivorum*.

Key words: onion white rot, sclerotia, seed bacterization, mycosphere microflora, germination.

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Le disulfure blallylique (DADS) et les plantules d'oignons issues de graines soumises à une bactérisation ont significativement rehaussé la germination des sclérotes de *Sclerotium cepivorum* (agent de la pourriture blanche de l'oignon) dans les humisols, comparativement à la germination obtenue en l'absence des plantules d'oignons ou en la présence de telles plantules issues de graines stérilisées en surface et non-inoculées. Les sclérotes localisés à la surface du sol ont mieux germé que ceux qui étaient enfouls. Lorsque des graines ont été inoculées avec la souche B-2 de *Bacillus subtills* ou par quatre autres souches de bactéries provenant de rhizophères de pousses d'oignons cultivés au champ, ces souches ont différé dans leur aptitude à rehausser la germination des sclérotes. Cette germination des sclérotes a été significativement plus élevée en présence des souches B-2, UI-2 et B, qu'en présence des souches UI-1 et W. Dans les sols comportant des plantules d'oignons dont les graines ont été soumises à une bactérisation avec les souches B-2, UI-2 et B, les sclérotes ont toléré significativement les populations bactériennes et fongiques indigènes réduites, de façon générale. Le traitement du sol avec du disulfure blallylique a généralement réduit les populations bactériennes, mais non les fongiques, associées aux sclérotes. Des corrélations inverses significatives ont été dégagées entre les proportions de sclérotes en germination et les populations bactériennes et fongiques associées aux sclérotes de *S. cepivorum*.

Mots clés : pourriture blanche de l'oignon, sclérotes, bactérisation des graines, microflore des mycosphères, germination.

[Traduit par la rédaction]

Introduction

Sclerotium cepivorum Berk. causes white rot of *Allium* spp. Sclerotia of the fungus are held in a dormant condition by fungistasis in nonsterile soil (Allen and Young 1968; Coley-Smith *et al.* 1967; King and Coley-Smith 1969) and are stimulated to germinate by aqueous extracts and exudates of plants of the genus *Allium* (Coley-Smith 1960; Coley-Smith and Holt 1966). The active stimulants include various volatile alkyl and allyl mercaptans, sulfides, and disulfides (Coley-Smith and King 1969).

Coley-Smith and King (1969) hypothesized that sulfoxide compounds exuded by intact onion roots into the rhizosphere are metabolized to sulfides and other volatile compounds by soil bacteria. Ikeshoji (1984) suggested that

rhizosphere bacteria are actively involved in the release of sulfide precursors from root cells as well as in the metabolism of these precursors to volatile compounds. Soil microflora, including *Bacillus* spp., metabolize nonvolatile alkyl and alkenyl cysteine sulfoxides to volatile sulphur-containing compounds that stimulate germination of *S. cepivorum* sclerotia in nonsterile soil (Coley-Smith *et al.* 1968; Coley-Smith and Cooke 1971; Coley-Smith and King 1969; King and Coley-Smith 1969).

The preceding evidence suggests that certain bacteria may play a role in the germination response of *S. cepivorum* sclerotia. Utkhede and Rahe (1980, 1983) studied the control of onion white rot by seed bacterization with several strains of *B. subtilis* recovered from sclerotia of *S. cepivorum* on naturally infected onions. Among these strains, *B. subtilis* B-2 provided significant control of onion

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white rot. In addition, when *B. subtilis* B-2 and other rhizobacterial strains (UI-2, UI-1, and B) isolated from the rhizospheres of commercial onions were bacterized onto the seed, the growth of onion seedlings was significantly increased (Reddy and Rahe 1989*a*, 1989*b*). Seed bacterization with the same strains also significantly reduced indigenous bacterial and fungal populations in the onion seedling rhizosphere (Reddy and Rahe 1989*b*). The ability of these bacteria to significantly modify the indigenous microflora associated with the onion seedling rhizosphere suggests that seed bacterization may affect rhizosphere microflora and plant growth and health. These results encouraged us to evaluate the effect of bacterized and nonbacterized treatments and diallyldisulphide (DADS), a known synthetic germination stimulant on germination of *S. cepivorum* sclerotia and on general indigenous bacterial and fungal populations associated with sclerotia.

A preliminary report was published earlier (Reddy and Rahe 1985).

Materials and methods

Source of sclerotia and bacterial strains

Sclerotia of *S. cepivorum* were collected from naturally infected commercial onions grown in muck soil near Cloverdale, B.C. Sclerotia were kept in muck soil at 17°C in the dark in a polyethylene bag until needed. *Bacillus subtilis* strain B-2 isolated from *S. cepivorum* sclerotia from naturally infected onions grown on muck soil near Cloverdale, B.C. (Utkhede and Rahe 1980) and four rhizobacteria (UI-2, UI-1, B, and W) isolated from the rhizospheres of commercial onions (cv. Autumn Spice, Stokes Seeds, St. Catharines, Ont.) growing in muck soil in Cloverdale, B.C. (Reddy and Rahe 1989*b*). For isolation, serial dilutions were made from the washings of onion roots and plated onto Thorton's agar (TA) (Johnson and Curl 1972). Plates were incubated at 25°C on a 16-h light – 8-h dark cycle for 3–5 days. The four isolates were representative of colony types recovered in greatest numbers. From these, strains tolerant of the combination of 300 μg/mL of streptomycin sulphate (S) (Sigma Chemical Co., St. Louis, Missouri), 100 μg/mL of cycloheximide (C) (Sigma), and 30 μg/mL of benomyl (B) (Plant Products, Bramalea, Ont.) in full-strength potato dextrose agar (PDA) (SCB–PDA) were selected as previously described (Reddy and Rahe 1989*a*). Standard procedures (Harrigan and MacCance 1966) were followed for biochemical tests (starch hydrolysis, nitrate reduction, and indole and sulfide production) to compare parental strains with their respective marked strains. Abilities of parental and marked strains to inhibit mycelial growth of *S. cepivorum* in dual culture were compared. This was done by streaking a test bacterial strain 2.5 cm from the edge of a 9 cm diameter Petri plate containing PDA, 24 h before plating a 5 mm diameter core of PDA containing, actively growing mycelium of *S. cepivorum*. The plates were kept at 25°C in the dark for 15 days and evaluated subjectively for possible differences in the nature and magnitude of the inhibition zones between the test bacterial strains and *S. cepivorum*.

Bacterized and nonbacterized onion seed treatments

Seeds of onion cv. Autumn Spice were surface sterilized by soaking in 0.1% HgCl$_2$ solution for 5 min and rinsed with 70% ethanol once and sterile deionized water four times. A fresh bacterial culture of each strain as indicated above was grown for 24 h at 25°C in 250 mL sterile tryptic soy broth (TSB) on a reciprocating shaker (80 rpm). Bacteria were pelleted by centrifugation for 20 min at 6000 × *g*. Bacterial cells were then washed (twice) in 0.1 M MgSO$_4$ buffer and the density of the suspension was adjusted to contain log 9.5 cfu/mL. Surface-sterilized onion seeds were soaked in bacterial suspensions for 10 min at 25°C (5 mL/10 g seed). Seeds were then dried in flowing sterile air for

1–2 h. The bacterial density on the seeds after soaking was determined. Five seeds per bacteria were placed in 10 mL of sterile 0.1 M MgSO$_4$ and magnetically stirred for 5 min. Serial dilutions of the washing bacterial suspensions were plated onto SCB–PDA in triplicate. After 72 h of incubation at 25°C, colonies were counted. Seed inoculation resulted in approximately log 6.17 cfu/seed. Surface-sterilized onion seeds treated similarly with sterile 0.1 M MgSO$_4$ served as nonbacterized controls.

Soil treatments

Muck soil, collected from an onion field near Cloverdale, was used for all experiments. In all cases composite soil samples were collected from the top 15 cm and the soil was used immediately. The soil was thoroughly mixed and screened through a 2-mm mesh sieve. Experiments were conducted in sterilized and nonsterilized muck soil. In experiments requiring sterilized soil, samples of the above soil were placed in polypropylene trays (30 × 20 × 13 cm), covered with aluminum foil, and autoclaved for 1 h at 121°C on each of 3 consecutive days. No microorganisms were detected when the autoclaved soil was plated onto PDA and incubated at 25°C for 14 days on a 16-h light – 8-h dark cycle. The moisture content of the soil was adjusted and maintained at approximately 70% throughout the experiments. DADS (ICN Pharmaceuticals, Inc., Life Sciences Group, Plain View, N.Y.) was added to sterilized or nonsterilized muck soil at 25 μL/100 g of soil and mixed thoroughly. Sterilized and nonsterilized soil not treated with DADS were used as controls.

Sclerotical germination bioassay

Three series of experiments were conducted to assess the germination of *S. cepivorum* sclerotia. There were four main treatments in each experiment. The treatments included (*i*) onion seeds treated with bacteria and planted in soil, (*ii*) onion seeds not treated with bacteria and planted in soil, (*iii*) unseeded but soil treated with DADS, and (*iv*) unseeded and soil not treated with DADS. Treatment effects were tested on the germination of *S. cepivorum* sclerotia placed either on the surface of, or buried in, soil. The various treatments were contained in disposable rectangular plastic pots (depth = 5.5 cm) of approximately 110-g capacity. Each pot contained approximately 75 g of soil. Twenty sclerotia of *S. cepivorum* were placed either 3 cm below the soil surface in a small nylon mesh (0.210 mm size) bag (buried treatment) or arranged in a 4 × 5 grid pattern on a 1 cm diameter nylon mesh (0.210 mm size) disc on the soil (surface treatment). Twenty onion seeds were sown in each pot receiving the bacterized or nonbacterized treatments. Unseeded pots with soil treated with DADS or without DADS served as controls. Each treatment consisted of three pots in an individual wide-mouth glass jar (approximately 13 × 13 × 23 cm) covered with four layers of cheesecloth. There were four jars for each treatment (= 12 pots/treatment) in all experiments. Jars were incubated in a controlled environment chamber at 17°C under fluorescent light with a 14-h photoperiod. Sterile water was added to each pot on a weight basis to replace moisture lost during incubation.

A preliminary experiment revealed that there was little change between 21 and 29 days in the percent germination of sclerotia. Therefore, all germination experiments were terminated at 21 days. Each of the following experiments was conducted twice.

In experiment 1, there were four treatments consisting of soil treated with DADS (DADS), not treated with DADS (C, soil only), onion seeds bacterized with *B. subtilis* strain B-2 (BT), and nonbacterized (NBT). Treatments were evaluated on their ability to influence germination of sclerotia placed on the soil surface.

In experiment 2, treatments are the same as in experiment 1 plus a fifth treatment consisting of two pots containing onion seed bacterized with *B. subtilis* strain B-2 (BT/C) and two pots of unseeded soil not treated with DADS (C/BT) together in the same jar (four pots, two each). The purpose of this treatment was to test the effect of B-2 on control pots placed together in the same environment (same jar). The five treatments were evaluated on ger-
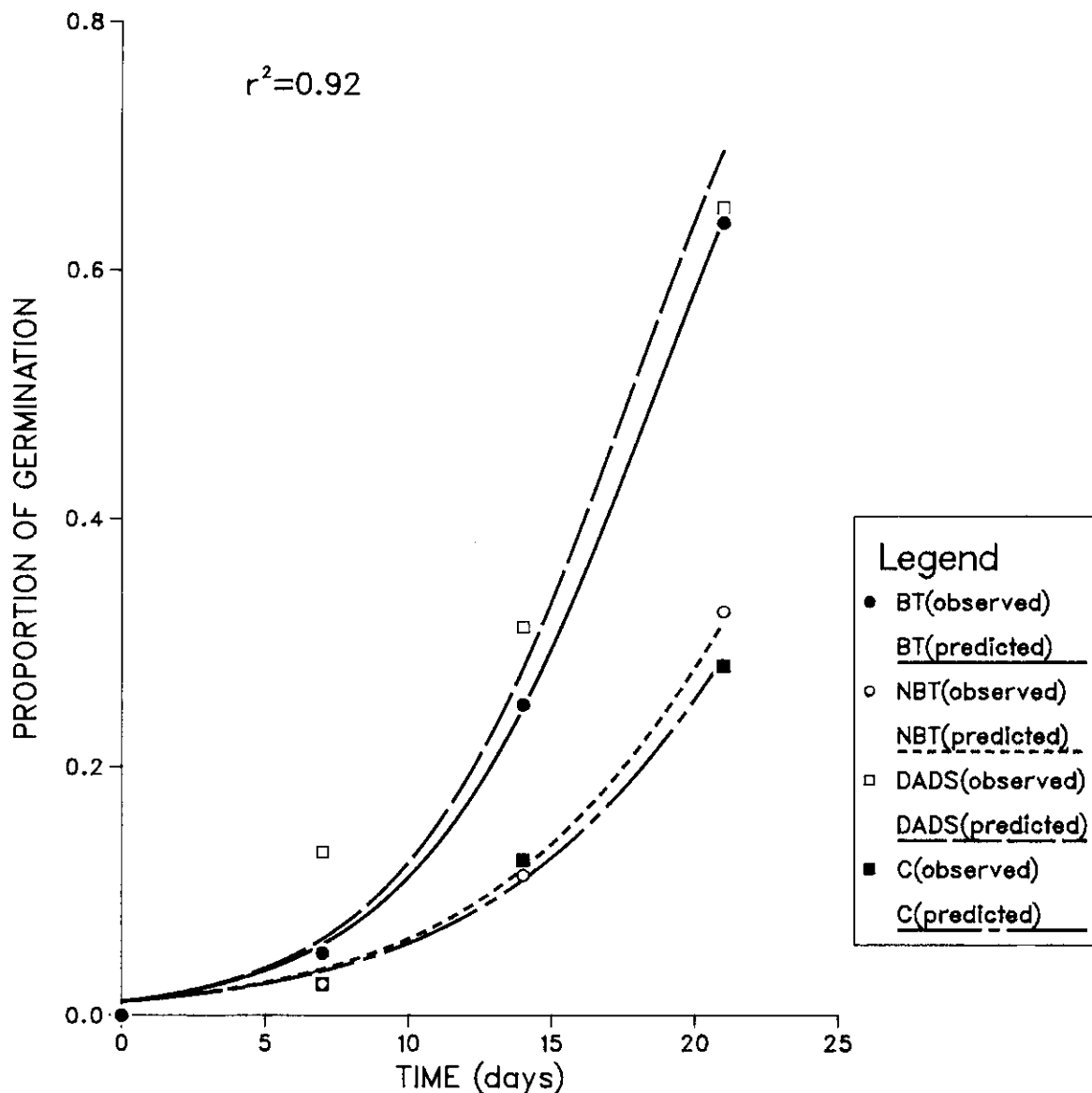
FIG. 1. Effects of diallyldisulfide, bacterized, and nonbacterized surface-sterilized onion seeds on germination of *Sclerotium cepivorum* sclerotia. Sclerotia were placed on the surface of muck soil. BT, seed bacterization with *Bacillus subtilis* B-2; NBT, non-bacterized seed; DADS, soil treated with diallyldisulfide (25 μg/100 g); C, soil not treated with DADS (soil only). Proportion of sclerotial germination was mean of combined data from two experiments each with four replications.

mination of sclerotia placed both on the surface and buried in soil.

In experiment 3, treatments are the same as in experiment 1 plus four additional bacterized treatments including onion seeds treated with strains B, UI-2, UI-1, and W. The objective of this experiment was to compare the effect of strain B-2 on germination of sclerotia with other bacterial strains. Treatment effects were evaluated both on the surface and buried sclerotia.

In each experiment, treatment jars were arranged in a randomized complete block design. One pot was removed from each replicate jar at each sampling period. There were three sampling times in experiment 1 and 2 (7, 14, and 21 days) and two in experiment 3 (14 and 21 days). At each sampling period one nylon mesh disc and one nylon mesh bag (in the case of experiment involving buried sclerotia) were removed aseptically with sterile forceps inside a laminar flow hood from one pot of each replicate jar. Sclerotial germination was determined by viewing sclerotia under a dissecting microscope.

At each sampling period sclerotia scored as germinated were plated onto PDA (four sclerotia per plate) amended with

1000 μg/mL each of chloramphenicol (CP) and streptomycin (S) (Sigma) (CPS-PDA). Plates were incubated in plastic bags in the dark at 17°C and observed for 15 days. Sclerotia-producing colonies typical of *S. cepivorum* were recorded as confirmed germination. Nongerminated sclerotia from the nylon mesh discs and bags were surface sterilized in 0.1% NaOCl for 5 min, washed with six changes of sterile distilled water (SDW), and plated onto PDA (four to five sclerotia per plate). The plates were incubated in the dark at 17°C and observed for 15 days to assess viability.

The data from all the experiments were analyzed by stepwise (Draper and Smith 1981) and logistic regressions (Baker and Nelder 1978). Preliminary analysis indicated that there was no variation with time, so the combined data from repeated experiments were used for further analysis. Estimates from the regression models in the logistic scale were transformed back to the proportion scale and plotted against time for presentation. Significance and interaction effects of various treatments on germination of *S. cepivorum* sclerotia were determined for each experiment by stepwise regression. At each step of the analysis, variables were added to the
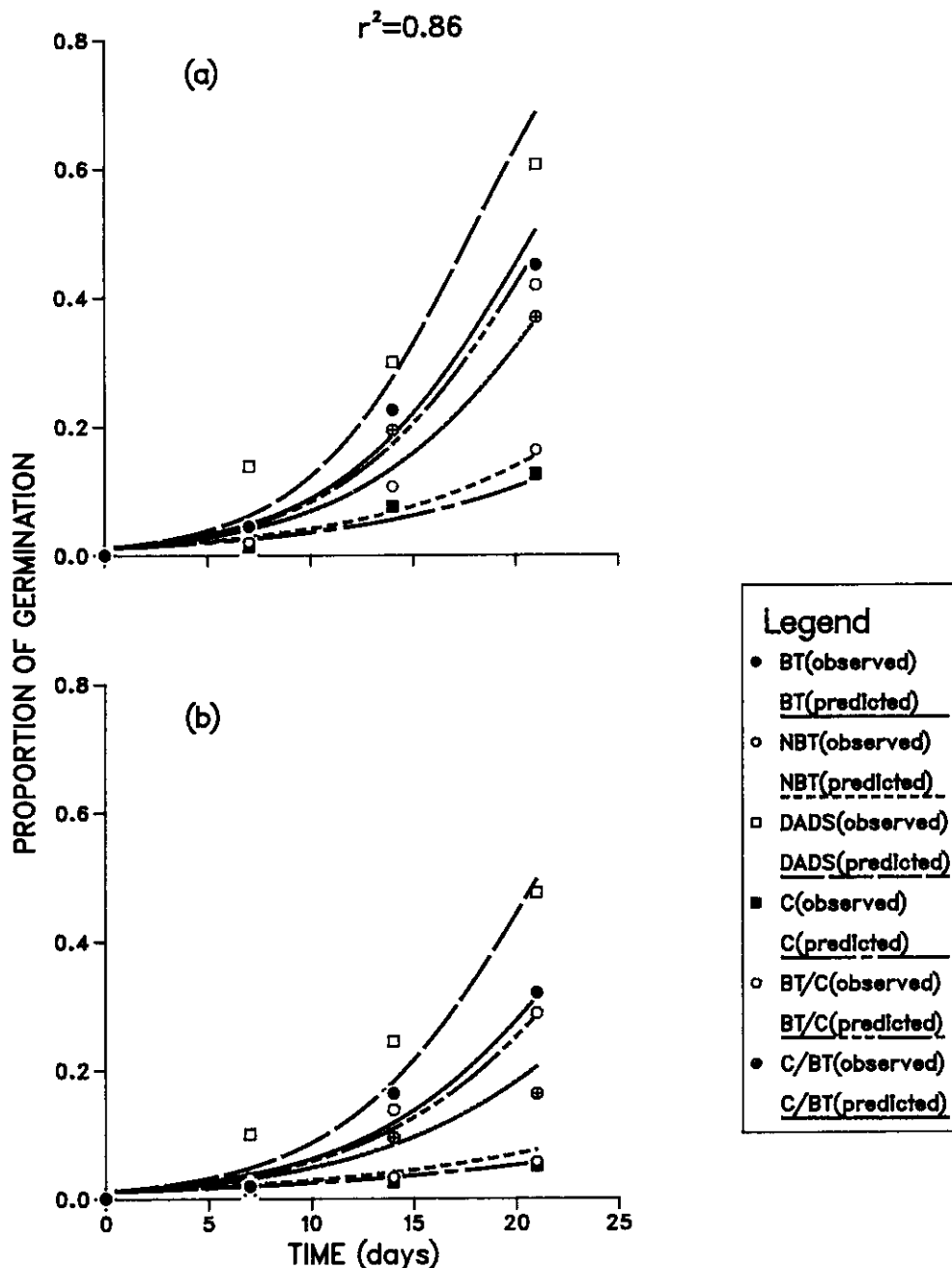
FIG. 2. Effects of diallyldisulfide, bacterized, and nonbacterized surface-sterilized onion seeds on germination of surface (a) and buried (b) sclerotia of *Sclerotium cepivorum*. BT, seed bacterization with *Bacillus subtilis* B-2; NBT, nonbacterized seed; DADS, soil treated with diallyl disulfide (25 μg/100 g); C, soil not treated with DADS (soil only); BT/C (data for BT) and C/BT (data for C), two each of BT and C pots placed together in same jar. Proportion of sclerotial germination was mean of combined data from two experiments each with four replications.

regression model if the *F* statistic was significant at the *P* = 0.05 level. After a variable was added, variables already in the model were removed if they did not produce an *F* statistic that was significant at the *P* = 0.05 level. Mean comparisons were made using the Bonferoni multiple comparison method (Neter *et al.* 1985).

Effect of treatments on populations of general indigenous bacteria and fungi associated with sclerotia

General indigenous bacterial and fungi populations associated with surface and buried sclerotia in experiment 3 were estimated by dilution plating (Johnson and Curl 1972). Thornton's agar (TA) was used for bacteria and rose Bengal agar (RBA) for fungi (Johnson and Curl 1972). Three to five nongerminated sclerotia

from each treatment pot per replicate at each sampling time (including zero time) were rinsed with SDW and put into 1-mL test tubes each containing 0.5 mL SDW. Sclerotia were crushed with a sterile glass rod and the resulting suspension was added to a test tube containing 9.5 mL of SDW. Serial dilutions were made and 0.5 mL aliquots from appropriate dilutions were spread onto each of three RBA plates and 0.1-mL aliquots onto each of three TA plates. Plates were incubated at 24–25°C on a 16-h light – 8-h dark cycle. Bacterial and fungal colonies were counted after 4 and 7 days of incubation, respectively. Populations of bacteria and fungi recovered from both surface and buried sclerotia were estimated and recorded as ln cfu/sclerotium at 0, 14, and 21 days. The experiment was repeated twice. Mean data from the combined
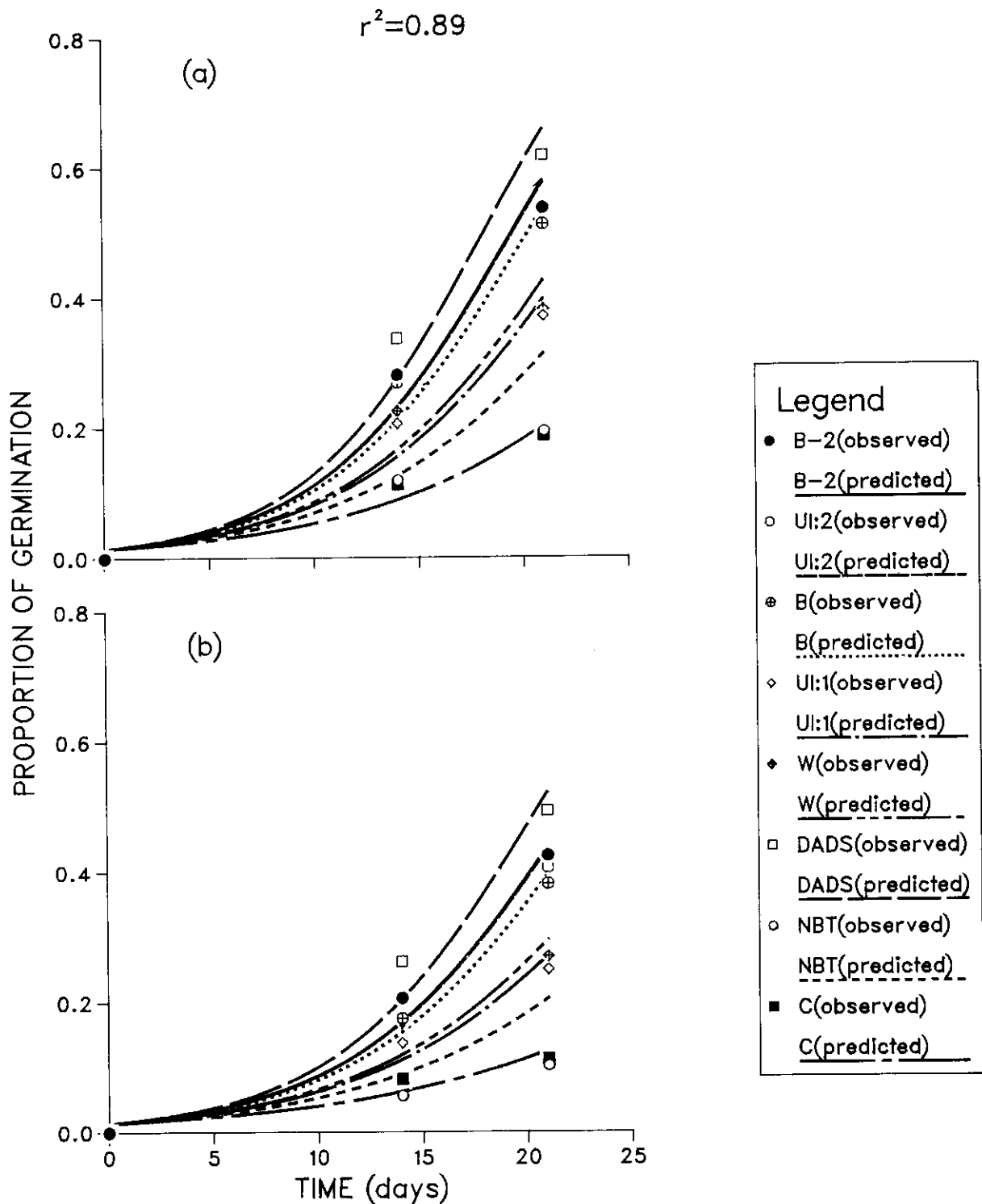
FIG. 3. Effects of diallyldisulfide, bacterized, and nonbacterized surface-sterilized onion seeds on germination of surface (a) and buried (b) sclerotia of *Sclerotium cepivorum*. B-2, UI-1, UI-2, B, and W were strains of bacteria used for seed bacterization; NBT, nonbacterized seed; DADS, soil treated with diallyldisulfide (25 μg/100 g); C, soil not treated with DADS (soil). Proportion of sclerotial germination was mean of combined data from two experiments with four replications each.

experiments were analyzed by stepwise regression and treatments were compared using the Bonferoni multiple comparison (Neter *et al.* 1985).

Results

The marked strains of bacteria expressed colony morphologies, biochemical and cultural characteristics, and antag-

onistic activity against mycelial growth of *S. cepivorum* in dual culture, typical of the respective parental wild types (data not shown).

Germination of sclerotia
Experiment 1

Initial data analysis has shown that there were no significant differences for any given treatment between sterilized
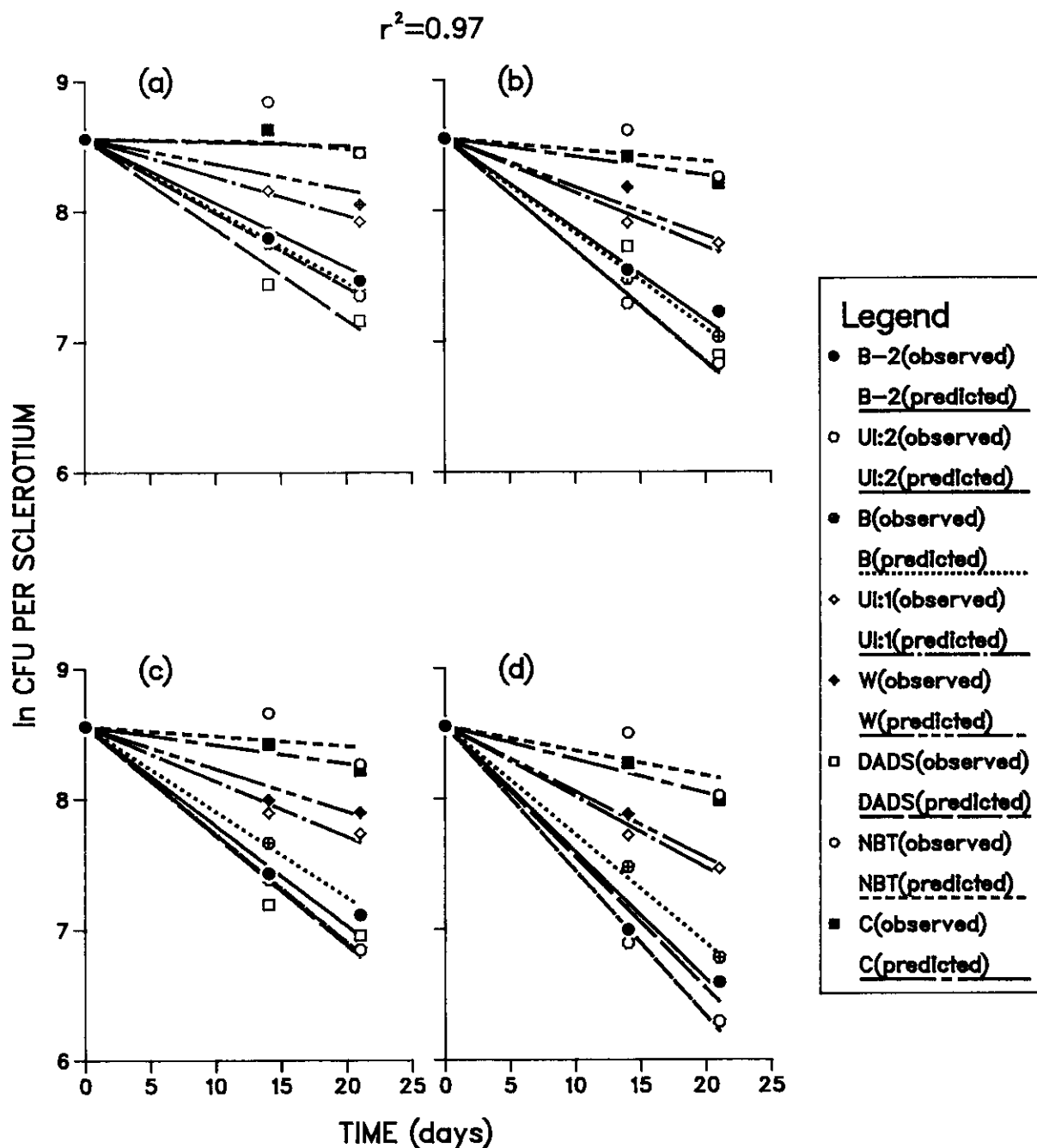
FIG. 4. Effects of diallyldisulfide, bacterized, and nonbacterized surface-sterilized onion seeds on populations of general indigenous bacteria associated with sclerotia of *Sclerotium cepivorum* in experiment 3: (*a*) sclerotia on the surface of sterilized soil; (*b*) sclerotia buried in sterilized soil; (*c*) sclerotia on the surface of nonsterilized soil; and (*d*) sclerotia buried in nonsterilized soil. B-2, UI-1, UI-2, B, and W are the strains of bacteria used for bacterization; NBT, nonbacterized seed; DADS, soil treated with diallyldisulfide (25 $\mu g/100$ g); C, control (soil only).

and nonsterilized soil on sclerotial germination (data not shown). Therefore data shown in Fig. 1 are the mean of combined data from sterilized and nonsterilized soil experiments. Both DADS and onion seed bacterization with *B. subtilis* B-2 significantly ($P = 0.05$) enhanced the germination of *S. cepivorum* sclerotia placed on the soil surface compared with nonbacterization or no DADS.

Experiment 2

As above, data shown in Fig. 2 are the mean of combined data from both the soils. Both DADS and BT significantly ($P = 0.05$) enhanced the germination of surface-placed

sclerotia (Fig. 2*a*) and buried sclerotia (Fig. 2*b*) when compared with C or NBT. The level of germination was significantly ($P = 0.05$) higher for sclerotia on the soil surface than for buried sclerotia (data not shown). Germination of sclerotia in soil without DADS treatment was enhanced significantly when control pots were incubated in the same jar with other pots containing onion seedlings grown from seeds bacterized with *B. subtilis* B-2. This phenomenon was similar for both surface and buried sclerotia.

Experiment 3

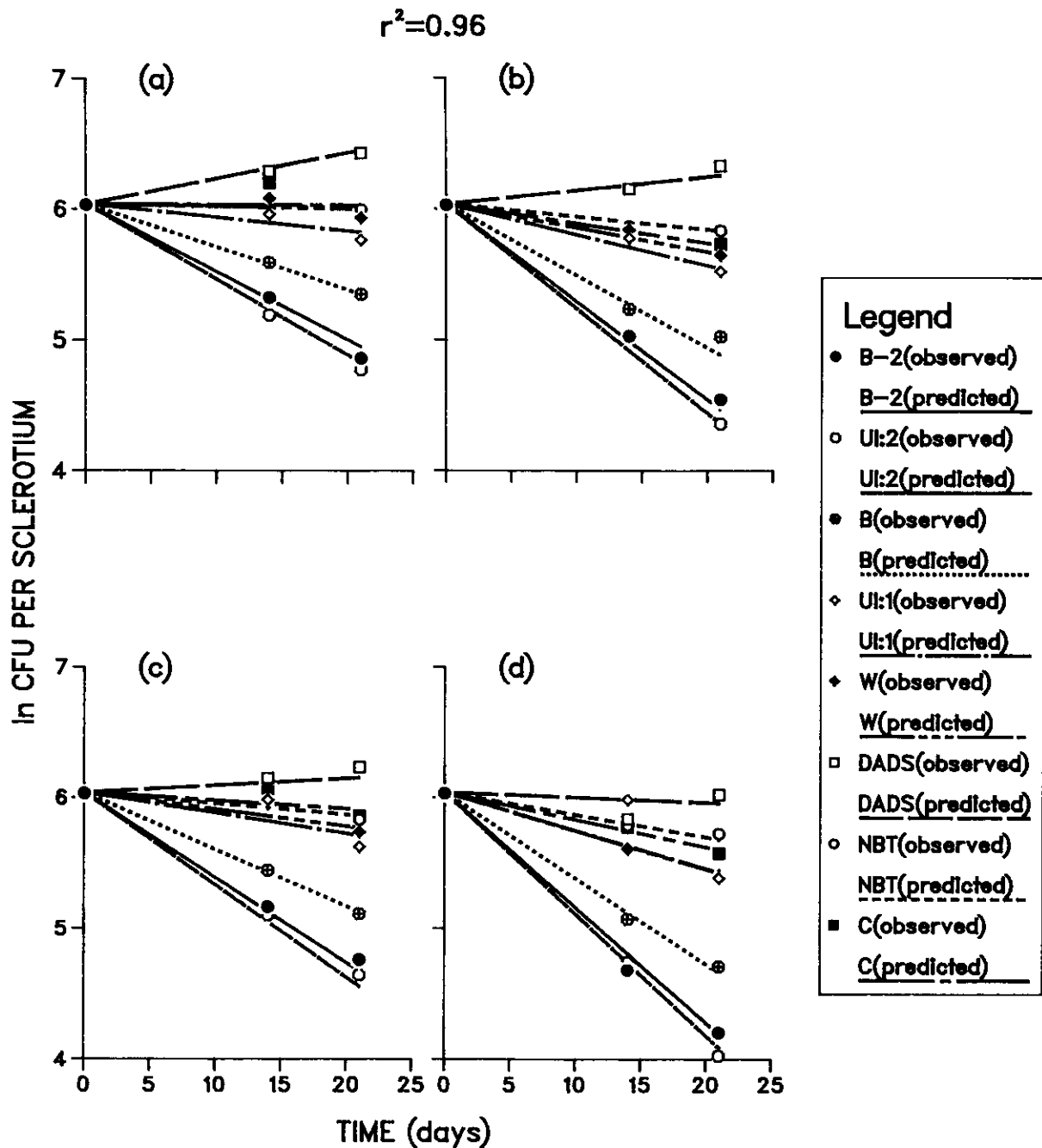Onion seedlings grown from seeds bacterized with four

$r^2 = 0.96$

FIG. 5. Effects of diallyldisulfide, bacterized, and nonbacterized surface-sterilized onion seeds on populations of general indigenous fungi associated with sclerotia of *Sclerotium cepivorum* in experiment 3: (*a*) sclerotia on the surface of sterilized soil; (*b*) sclerotia buried in sterilized soil; (*c*) sclerotia on the surface of nonsterilized soil; and (*d*) sclerotia buried in nonsterilized soil. B-2, UI-1, UI-2, B, and W are strains of bacteria used for bacterization; NBT, nonbacterized seed; DADS, soil treated with diallyldisulfide (25 μg/100 g); C, control (soil only).

different bacteria differed significantly in their ability to enhance germination of sclerotia (Fig. 3). Among bacterial strains tested, B-2, UI-2, and B caused significantly ($P = 0.05$) higher levels of germination than did strains UI-1 and W for both surface or buried sclerotia. The level of germination was significantly higher for sclerotia on the soil surface than for buried sclerotia by these bacterial strains (data not shown).

Sclerotial germination was of the mycelial type; "plug" or "eruptive" germination was rarely seen. Of the 477, 906, and 1420 sclerotia assayed in experiments 1, 2, and 3, respectively, a total of 99% germinated and yielded colonies typical

of *S. cepivorum* on CPS–PDA. No significant differences were found in the proportions of viable sclerotia in the populations of nongerminated sclerotia among the various treatments and controls in both sterilized and nonsterilized soil. Mean proportions of viable sclerotia in nongerminated populations of sclerotia for all treatments in experiments 1, 2, and 3 were 0.94, 0.88, and 0.87, respectively.

Bacteria and fungi associated with sclerotia

Larger numbers of bacteria were associated with sclerotia on the soil surface than with buried sclerotia in both sterilized and nonsterilized soil irrespective of the treatments
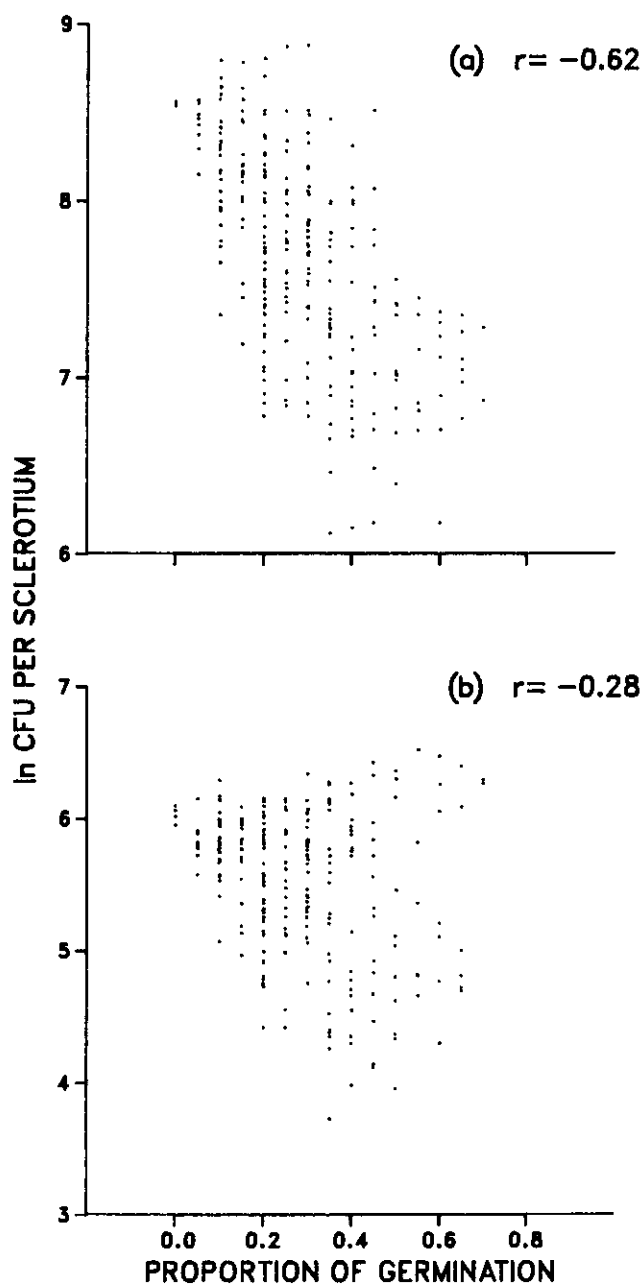
FIG. 6. Significant inverse correlations between the proportions of sclerotia germinating and the populations of associated total indigenous bacteria (a) and fungi (b) in various treatments.

(data not shown). Overall, the reduction of bacterial populations associated with surface or buried sclerotia was significantly ($P = 0.05$) greatest with DADS, B-2, UI-2, and B; intermediate with UI-1 and W; and least with NBT and C, in both sterilized and nonsterilized soils (Fig. 4). No significant differences between DADS, B-2, UI-2, and B; between UI-1 and W; or between NBT and C were observed.

Similar to bacterial populations, large numbers of fungi were associated with sclerotia on the soil surface than with buried sclerotia in both sterilized and nonsterilized soil irrespective of the treatments (Fig. 5). There were also significant treatment differences in the numbers of fungi recovered from sclerotia. The effects of treatments on fungi associated with sclerotia were similar in sterilized and

nonsterilized soils. Indigenous fungal populations on sclerotia were reduced most strongly by seedlings grown from seeds bacterized with B-2 and UI-2 and to a lesser degree by seedlings grown from seeds bacterized with B. Seedlings grown from nonbacterized seeds and from seeds bacterized with UI-1 and W had little effect on fungal populations of sclerotia, whereas DADS significantly increased fungal populations compared with the controls (Fig. 5). There were no significant differences between UI-2 and B-2, nor between UI-1, W, NBT, and C. Significant inverse correlations between the proportions of sclerotia germinating and the populations of associated indigenous bacteria ($r^2 = -0.62$) and fungi ($r^2 = -0.28$) in the various treatments evaluated were found (Fig. 6).

Discussion

The stimulation of germination of S. cepivorum sclerotia and its relationship to disease incidence has been the subject of several studies (Coley-Smith 1960; Coley-Smith and Holt 1966; Coley-Smith and King 1969; Crowe and Hall 1980; Entwistle et al. 1982; Merriman et al. 1981). Our results support the report of King and Coley-Smith (1969) that bacteria can enhance the germination of S. cepivorum. Hough et al. (1981) obtained evidence for a similar role of bacteria in the ovipositional response of the onion maggot, Delia (Hylemya) antiqua, near onion seedlings.

We suggest two mechanisms to explain our findings on the effect of onion seed bacterization on germination of S. cepivorum sclerotia. One possible mechanism is that nonvolatile sulphur compounds in onion root exudates released into the soil may have been metabolized into volatile stimulatory compounds by the introduced bacteria. These compounds may have diffused into or onto the soil and stimulated sclerotia on the surface of, or buried in, soil to germinate. Significantly lower proportions of sclerotia germinated in the presence of onion seedlings grown from nonbacterized, surface-sterilized seeds than from bacterized seeds. Similarly low proportions of germination were observed in the control (soil only) treatment. However, germination was stimulated in the control treatment when control and bacterized treatments shared the same gaseous environment.

Coley-Smith and King (1969) showed that volatile compounds, produced by bacterial degradation of nonvolatile sulphide precursors allyl cysteine and n-propylcysteine, play a major role in the induction of germination of S. cepivorum sclerotia. Without enzymatic activity (Whitaker 1976) or soil bacteria (Ikeshoji 1984), intact onions did not release volatile compounds. Our results substantiate the above reports and offer a clear indication that onion seed bacterization plays a role in the onion root ecosystem as a promoter of sclerotial germination.

A second mechanism that could explain the results obtained in our study is that the enhanced germination of sclerotia associated with seed bacterization was due to the effects of treatments on microflora associated with sclerotia. It is possible that the resident sclerotial microflora had a mycostatic effect on sclerotia and that mycostasis was reduced by certain bacterization treatments and DADS, thus allowing sclerotia to germinate. Reduction of indigenous bacterial and fungal populations associated with surface and buried sclerotia by strains B-2, UI-2, and B and bacterial

populations by DADS was inversely correlated with the proportions of sclerotia germinating in these treatments. These results and this mechanism are consistent with the conclusion of Coley-Smith *et al.* (1967) that *Allium* spp. and their extracts negate or reduce the natural mycostasis that maintains sclerotial dormancy of *S. cepivorum.*

An ability to stimulate germination of sclerotia and biological control activity by the same strain of *B. subtilis* strain B-2 (Utkhede and Rahe 1980) seem contradictory. These two biological activities may be the result of two completely independent mechanisms (i.e., biological control activity via antimetabolites and increased germination of sclerotia via metabolism of onion root exudates) and could be important in white rot disease management. The interrelationship between these two mechanisms warrants further study. Clearly, not all bacteria are equally able to stimulate germination of sclerotia. Significant differences were observed among the various bacterial strains used in our study. Onion seedlings grown from surface-sterilized nonbacterized seeds were not stimulated to germination, even when grown in nonsterilized soil. We find this surprising. Presumably the rhizospheres of these seedlings became naturally colonized. The failure of such seedlings to stimulate sclerotial germination suggests that the microorganisms were different from those used specifically for bacterization. With the exception of the B-2 strain, all bacteria used in our study were selected as representative of bacteria found in the rhizosphere of field-grown onions.

Much has yet to be learned about the dynamics and activities of the onion rhizosphere microflora and about its role in the biology of white rot disease. Clearly, this microflora can play a major role in the stimulation of germination of *S. cepivorum* sclerotia. Knowledge of how to modify or regulate this microflora under field conditions could lead to substantial improvement in the potential for management of onion white rot.

Acknowledgement

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