# Quantitative Estimation of *Rhizoctonia solani* AG-3 in Soil

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## ABSTRACT

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An improved procedure for quantitative estimation of propagules of *Rhizoctonia solani* anastomosis group 3 (AG-3) in soil was developed. The procedure involved incubating soil pellets on Ko and Hora's medium after both the soil and medium are amended with 5 ppm of prochloraz. After incubation for 24–72 hr, colonies developing from the pellets were examined microscopically for characteristics of *R. solani* and separated into anastomosis groups by examining hyphal anastomosis in paired culture with tester isolates. Isolates of *R. solani* showing hyphal anastomosis with AG-3 tester isolates were further tested for characteristic brown colony color on Stewart's medium. With these techniques, the relative efficiency of recovery of *R. solani* AG-3 from an artificially infested field soil was 75%, and less than 2% of all plated soil pellets yielded interfering contaminant fungi. Without prochloraz in the soil and medium, recovery of *R. solani* AG-3 was reduced 17%, and more than 96% of the soil pellets gave rise to fast-growing contaminant fungi. With prochloraz-amended soil pellets on prochloraz-amended Ko and Hora's medium (KHP), up to 20 propagules of *R. solani*, predominantly AG-3, were recovered per 100 g of potato field soil having a soil moisture of 10–12% (w/w). The assay method permitted recovery of *R. solani* AG-2, AG-4, AG-5, and *R. frazariae* in addition to AG-3 from artificially infested soil.

*Additional keywords*: fungicide, mycelia, sclerotia.

*Rhizoctonia solani* Kühn is a common and complex soilborne plant pathogenic fungus. *R. solani* is currently composed of several anastomosis groups (AG) distinguished by hyphal anastomosis (7,8,21–23). These groups also may differ morphologically, physiologically, and serologically (1,20,30) and in host range, virulence, and distribution in nature (16,22,26).

Propagules (hyphae and sclerotia) of *R. solani* survive in soil independently (3) and in association with organic matter (5,24) and host tissues (29). Propagules can be detected indirectly by entrapping the fungus in host tissues, plant debris, or in other substrates (5,11,14,18,19,25,31). More direct and quantitative methods for recovering *R. solani* involve washing (32), screening (34), or pelleting (13) infested soil. Wet sieving was devised for recovering sclerotia, while pelleting may recover both sclerotia and mycelial fragments.

Weinhold (34) recovered *R. solani* AG-3 from potato soil by wet-screening the soil and culturing the residue on water agar, but the precision of the method was not tested for the efficiency of recovery. Bell and Sumner (2) investigated survival of *R. solani* AG-1, AG-2, AG-3, and AG-4 in artificially infested field soils by pelleting soil on tannic acid-benomyl agar. After 283 days, all anastomosis groups (AG) except AG-3 were recovered. Henis et al (13) found that pelleting soil on Ko and Hora's (KH) medium (18) was more reliable and convenient than soil screening for recovering propagules of *R. solani*. Van Bruggen and Arneson (33) developed an alternative technique to reduce the cost of the multiple-pellet soil sampler described by Henis et al (13). However, neither of these methods separated the recovered isolates into anastomosis groups.

In light of the importance of *R. solani* AG-3 as a dynamic pathogen of potatoes, a sensitive and reliable procedure for its recovery from soil is needed. The objective of this study was to improve the soil pellet technique (13) and Ko and Hora's medium (18) for the recovery of AG-3 in the presence of other, often fast-growing fungi in field soil. A preliminary report of this research has been published (9).

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## MATERIALS AND METHODS

**Stock cultures and tester isolates.** Isolates of *R. solani* and *R. fragariae* were obtained from Idaho field soil and host plants and from ATC collection as previously designated (10). Tester isolates of known *R. solani* AG types were obtained from A. R. Weinhold (Dept. of Plant Pathology, University of California, Berkeley). All collected isolates of *Rhizoctonia* spp. were maintained in chopped potato-soil (CPS) (18) or Czapek's Dox broth (CDB) (16). Individual isolates were stored in tubes of dry sterile soil at 4 C in the dark (6).

**Evaluation of Ko and Hora's (KH) medium amended with fungicide.** In an initial test, three fungicides, benomyl, pentachloronitrobenzene (PCNB), and prochloraz were tested for growth inhibition of *R. solani* AG-1, AG-2, AG-3, and AG-4. The fungicides were added to autoclaved KH medium after partial cooling and before dispensing into petri plates. Each fungicide was added at 1, 5, 10, and 20 $\mu$g or $\mu$l per L of KH medium. The medium without fungicide was used as a control.

Colonies of each AG type were initiated from 4-mm-diameter disks of agar taken from the margin of 4-day-old parent cultures grown on Difco potato-dextrose agar (PDA). A randomized complete block design with two plates per treatment and four replications was used. After incubation at 25 C in the dark for 72 hr, colony diameter was measured and the percentage growth inhibition was calculated in the presence of fungicide relative to the growth on control plates.

Because benomyl and PCNB were markedly inhibitory and prochloraz (1-[*N*-propyl-*N*-[2-[2-4-6-]trichlorophenoxy ethyl] carbamoyl imidazole]; emulsion concentrate, Boots Company Lt., Nottingham, England) least inhibited the growth of *R. solani*, prochloraz was compared further at 0.0, 0.5, 1.0, and 5 $\mu$l per liter to KH medium for its impact on the growth of contaminant fungi from soil. To accomplish this, pellets of a solarized soil that had been collected from an Idaho potato field and then artificially infested with mycelia and sclerotia of *R. solani* AG-3 were placed on the media in petri plates. The pellets were adjusted to 150 mg each and had moisture content that ranged between 10 and 12% (w/w). Pellets (15 per plate, eight plates per replication, and four

replications per treatment) were incubated at 25 C in the dark for 72 hr.

Because 5 μl/L of prochloraz in KH medium was most effective for inhibiting the growth of contaminant soil fungi, KH medium with prochloraz 22 at 5 μl/L was selected for further study and designated KHP medium.

**Modification of the soil pellet method.** The soil-pellet method (13) was modified by adding prochloraz to the soil before pelleting. To accomplish this, 95 g of each fresh soil sample (6 ± 2% moisture, w/w) was screened (2-mm-mesh sieve) and atomized with prochloraz solution, and enough distilled water was added to produce a final prochloraz concentration of 5 μl/kg of soil and a soil moisture level of 10–12% (w/w). In preliminary tests, it was determined that prochloraz levels less than or exceeding 5 μl/kg were less satisfactory. Lower concentrations permitted excessive growth of contaminants and higher concentrations appeared too inhibitory to *Rhizoctonia* spp.

After 1–2 hr for moisture equilibration, the soil was thoroughly mixed, formed into 150-mg pellets with a multiple soil-pellet sampler (13), placed onto fresh KHP medium (15 pellets per plate, 25 plates per sample), and incubated for 72 hr at 25 C in the dark. The perimeter of each soil pellet was examined after 48 and 72 hr of incubation for *Rhizoctonia*-like colonies and for contaminant fungi (fungi other than *Rhizoctonia* that grew faster than 2.5 mm/day). Soil pellets on the medium were best examined when illuminated by a fluorescent light and viewed against a dark background. In some cases, pellets were examined under a stereo microscope at ×20 and ×40 magnification.

**Recovery of *R. solani* AG-types and *R. fragariae* from artificially infested soil.** Soil pellets with and without 5 μl/kg of prochloraz were compared on KHP medium for relative efficiency of recovery of *Rhizoctonia* spp. from field soil. The field soil used was a coarse-textured loam that had been covered with clear plastic for 6 wk before planting to potatoes. This newly collected solarized soil yielded no detectable colonies of *R. solani* when pelleted with or without 5 μl/kg of prochloraz per kilogram onto KHP medium. The soil, therefore, was amended with either a suspension of mycelial fragments of *R. solani* AG-3, AG-4, AG-5, and *R. fragariae*, or soil cultures containing mycelia and sclerotia of *R. solani* AG-3 and AG-4.

Mycelial suspensions of AG-3 and AG-4 were obtained by the Czapek's Dox broth method (16) with some modifications. Mycelial mats of each isolate were grown for 1 wk in 9-cm-diameter petri dishes containing 15 ml of Czapek's Dox broth plus 0.1% yeast extract. The cultures were blended for 20 sec (Waring blender) at low speed and the resulting suspensions dilute with wetting solution (1 ml of Tween 80 per liter of distilled water). The soil cultures were prepared according to the chopped potato soil method (18).

KHP medium was freshly prepared and samples of the solarized field soil were used directly or amended with 5 μl/kg of prochloraz as described above. As a control, propagules of each isolate were aseptically and thoroughly mixed into autoclaved samples of the same field soil (without prochloraz) and pelleted onto plates of PDA containing 50 μg of streptomycin sulphate per milliliter (PDA-S medium). Sufficient quantities of each isolate were added so as to yield 0.5 propagules per soil pellet. The average number of colonies detected after incubation on this control medium was considered 100%. An equivalent quantity of each isolate was added to the prochloraz-amended and unamended samples of field soil and pelleted onto KHP medium (15 pellets per plate, with eight plates per replication, and four replications per isolate).

After incubation for 24–72 hr at 25 C in the dark, plates were examined for *Rhizoctonia*-like colonies. For determinations of relative recovery between treatments, the number of pellets with *Rhizoctonia*-like colonies were counted. The relative efficiency of recovery was determined by dividing the number of colonies recovered from pellets with and without prochloraz on KHP medium by the number of colonies detected on PDA-S medium × 100.

**Recovery of *R. solani* from naturally infested soil.** The characteristics of pellets were further evaluated by using a freshly collected sandy loam field soil (pH 7.4, Aberdeen, ID) that had been in continuous potato culture for 5 yr. During this period, potato plants grown in this soil routinely showed symptoms of infection by *R. solani*. Four soil samples, ten 2- × 15-cm cores of soil per sample, were collected and assayed for *R. solani* within 12 hr after sampling. The samples had gravimetric moisture levels that ranged between 4 and 8% (w/w) and matric potentials (measured with a ceramic plate extractor) that ranged from −1 to −15 bars. A fifth sample of the same soil was examined for the number of recoverable propagules of *Rhizoctonia* spp. when freshly collected and after storing in open containers for 24 and 168 hr at 22 C (room temperature) in diffuse light.

In all of these tests, the soil was amended with 5 μl/kg of prochloraz as described above, pressed into 150-mg pellets, seeded onto plates of KHP medium (15 pellets per plate, four plates per replication, and six replications per sample), and incubated at 25 C in the dark. The perimeter of each soil pellet was examined after 24 and 72 hr of incubation for *Rhizoctonia*-like colonies and for contaminant fungi.

**Identification of anastomosis groups.** All *Rhizoctonia*-like colonies recovered from the potato field soil were transferred onto PDA-S medium for further growth and before evaluation of anastomosis grouping type, the nuclear condition was determined as described by Herr (15). Isolates resembling *R. solani* (multinucleate cells, branching close to hyphal septa, constriction of the branch base, septum in the branch near its base, brown pigmentation, anamorphic) (27) were tested for anastomosis with known AG-types (tester isolates) according to Parmeter et al (26) with some modifications. Pairings were made on autoclaved cellophane strips (3 × 1.5 cm) dipped in soft PDA (13 g/L) and supported on 2% water agar (WA) in 9-cm-diameter petri dishes. Mycelium from the edge of 1-wk-old soil isolates grown on PDA-S medium was placed in the center of the petri dish and separated from three known AG-types by cellophane strips. Isolates were paired initially with AG-2, AG-3, and AG-4. If anastomosis with these AG-types could not be identified microscopically, similar pairings with AG-1 and AG-5 were made. Each isolate of *R. solani* was tested twice to ensure proper classification to AG-type. To provide further supporting evidence for the identity of AG-3 isolates whose hyphae anastomosed with AG-3 tester isolates, each was placed on Stewart's differential medium and checked for brown colony color (10).

**Pathogenicity tests.** The pathogenicity of 18 representative isolates each of AG-3 and AG-4 recovered from the potato field soil was tested by the method of Bolkan and Wenham (4) on potato, radish, and sugar beet seedlings in a greenhouse. In each test, the inoculum consisted of 4-mm-diameter mycelial disks taken from the periphery of colonies grown on PDA for 5 days. Each host plant having four to six leaves was inoculated by placing two mycelial disks against the stem at the soil line and covering the inoculum with a 2-cm layer of an autoclaved mixture of sand and vermiculite (1:1). Controls consisted of seedlings inoculated with disks of sterile PDA.

Fourteen days after inoculation, symptoms of infection by *R. solani* (reddish brown lesions on the stem) were rated on a 1–5 scale (1 = no symptoms, 2 = mild lesion and discoloration, less than 33% of stem circumference altered, 3 = moderate lesion and discoloration, between 33 and 66% of stem circumference altered, 4 = severe lesion and discoloration, over 66% of stem circumference altered, 5 = seedlings damping off). All treatments in each test involved four replications of six seedlings each.

## RESULTS

In agreement with earlier studies (11,14,16,25,34), our initial attempts to recover propagules of *R. solani* from native or artificially infested field soil confirmed that pelleting soil (13) on KH medium (18) was advantageous. However, the selectivity, if not the sensitivity, of this method still appeared unsatisfactory because of concurrent and frequent recovery of fast-growing contaminant fungi in soil samples. *R. solani* AG-3, in particular, was slow growing (30) on KH medium and, therefore, often

TABLE 1. Percentage inhibition[a] of radial growth of *Rhizoctonia solani* AG-types from mycelial disks on Ko and Hora's (KH) medium amended with fungicides

| *R. solani* AG types | Inhibition (%) — Fungicides in KH medium | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Benomyl ($\mu g/L$) | | | | Pentachloronitrobenzene ($\mu g/L$) | | | | Prochloraz ($\mu l/L$) | | | |
| | 1 | 5 | 10 | 20 | 1 | 5 | 10 | 20 | 1 | 5 | 10 | 20 |
| AG-1 | 26 | 94 | 94 | 94 | 66 | 68 | 74 | 76 | 31 | 36 | 45 | 49 |
| AG-2 | 44 | 89 | 89 | 89 | 46 | 46 | 63 | 69 | 33 | 39 | 42 | 46 |
| AG-3 | 58 | 93 | 93 | 93 | 60 | 62 | 69 | 71 | 28 | 40 | 53 | 57 |
| AG-4 | 60 | 94 | 94 | 94 | 71 | 71 | 78 | 81 | 1 | 9 | 19 | 42 |

[a]Means of four replications incubated for 72 hr at 25 C in the dark.

TABLE 2. Source of variation for percentage inhibition of radial growth of *Rhizoctonia solani* AG-types from mycelial plugs on KH medium amended with fungicides

| Source of variation | Polynominal responses of fungicides[a] | | |
|---|---|---|---|
| | Benomyl | PCNB | Prochloraz |
| Concentration | *** | *** | *** |
| Linear | *** | *** | *** |
| Quadratic | *** | * | *** |
| Cubic | *** | NS[b] | ** |
| AG-type | *** | *** | *** |
| Conc × AG type | *** | * | *** |

[a]*, **, and *** significant at the 0.05, 0.01, and 0.001 levels, respectively.
[b]NS = nonsignificant.

TABLE 3. The relative efficiency of recovering *Rhizoctonia* spp.[x] from an artificially infested field soil using Ko and Hora prochloraz (KHP) medium and soil pellets with (SPP) and without (SP) 5 $\mu l/kg$ of prochloraz

| Soil pellets | Propagules recovered (%) | | | | | |
|---|---|---|---|---|---|---|
| | Mycelial fragments | | | | Mycelial fragments and sclerotia | |
| | *R. solani* | | | | *R. solani* | |
| | AG-3 | AG-4 | AG-5 | *R. fragariae* | AG-3 | AG-4 |
| SP | 0 | 11 | 0 | 0 | 18 | 34 |
| SPP[y] | 22 b | 37 ab | 52 a | 19 b | 75 | 88 |

[x]Percentage of propagules recovered using SP and SPP soil pellets on KHP medium relative to recovery from samples of the same soil that were sterilized, equivalently infested, and pelleted on PDA-S medium. Mean percentages from 24 plates (six replicates/treatment and four plates/replicate).
[y]With SPP data, all means followed by different letters are significantly different, $P = 0.05$, according to Duncan's multiple range test.

outgrown by contaminant fungi. This circumstance prompted the use of fungicides in an attempt to improve the selective recovery of *R. solani*.

**Evaluation of fungicide-amended KH media.** In an initial comparison of benomyl, prochloraz, and PCNB fungicides in KH medium, the fungicide that least inhibited the linear growth of *R. solani* AG-1, AG-2, AG-3, and AG-4 was prochloraz (Table 1). When orthogonal polynominals for unequal spacings of fungicide concentrations were fit to these data, linear, quadratic, and cubic effects were all found to be highly significant ($P \leq 0.001$, Table 2). The percentage inhibition with benomyl was shown to plateau at about 90% for concentrations of 5, 10, and 20 $\mu g/L$.

In contrast, treatments with PCNB were very different. With PCNB, only the linear and quadratic effects were shown to be significant (Table 2). For AG-1, a fairly linear inhibition was found for increase between the four concentrations being compared. For AG-2, results suggest a threshold between 1 and 5 $\mu g/L$, while AG-3 and AG-4 indicate a threshold between 5 and 10 $\mu g/L$.

Treatments with different levels of prochloraz showed differential responses over AG. For AG-1 and AG-2, a fairly low linear increase was shown to occur (Table 1). For AG-3, a plateau was noted for the 10 and 20 $\mu g/L$ levels, while very steep linear and quadratic increases were found to occur for AG-4. Concentrations of prochloraz up to 20 $\mu l/L$ of KH medium were normally less inhibitory than either benomyl or PCNB at 1 $\mu l/L$. When prochloraz-amended KH medium was seeded with soil pellets containing propagules of *R. solani* AG-3, prochloraz concentrations approximating 5 $\mu l/L$ permitted the best combination of growth support for *R. solani* AG-3 and growth inhibition of contaminants (Fig. 1). When orthogonal polynominals were fit to the data summarized with lines of best fit in Figure 1, a highly significant linear ($P \leq 0.001$) relationship was associated with the recovery of *R. solani* from soil with different concentrations of prochloraz. With prochloraz, a slight linear increase occurred between 0 and 2.5 $\mu g/L$, then a plateau occurred for the 5 and 10 $\mu g/L$ levels.

In contrast, when the recovery of contaminant fungi were considered, the relationship of prochloraz concentration in Ko and Hora media showed a reverse relationship. Significant linear, quadratic, and cubic relationships were noted ($P \leq 0.001$). The recovery of contaminants steadily decreased as the concentration of prochloraz was increased (Fig. 1B).

**Efficiency of recovery of known populations of *Rhizoctonia* spp.** Background levels of *Rhizoctonia*-like fungi could not be recovered from the solarized field soil plated on KHP medium (regardless of whether soil was amended or not amended with prochloraz). However, when this same solarized soil was amended with known populations of *Rhizoctonia* propagules, significantly more colony-forming units of *Rhizoctonia* spp. were recovered when the soil was amended with prochloraz (Table 3). Without prochloraz in the soil pellets, *R. solani* AG-3, AG-5, and *R. fragariae* were not recoverable in this test. With prochloraz-amended soil pellets, not only were *R. solani* AG-3, AG-5, and *R. fragariae* recoverable, but the relative efficiency of recovery for *R. solani* AG-3 was increased to approximate the recovery efficiency of the faster-growing *R. solani* AG-4.

**Quantitative detection of *R. solani* in naturally infested soil.** The number of colony-forming units of *R. solani* detected in four separate 100-g samples of freshly collected potato field soil was 2.4, 0, 3.0, and 2.4 using soil pellets compared with 20.7, 7.9, 17.7, and 5.4 using prochloraz-amended soil pellets (difference significant at $P = 0.05$). This dramatic advantage with prochloraz, however, was found to decrease (Fig. 2) with increased storage. If pellets were not amended with prochloraz, no response was found as the hours of soil storage increased. However, a negative response occurred as the duration of storage increased for soil pellets amended with prochloraz. The soil pellets with contaminated fungi showed only a slight increase from 97–100% for the nonamended pellets, while a dramatic increase occurred with the prochloraz-amended pellets from 1 to 17%. However, in spite of this increase, the prochloraz-amended pellets remained relatively free of contaminant fungi when compared with the unamended pellets. Interaction relationships between methods compared (prochloraz treatments vs. no treatments) and time of storage were highly significant ($P \leq 0.003$) for the recovery of *R. solani* while the effect of storage on the recovery of fungal contaminants showed significant linear ($P \leq 0.03$) and quadratic relationships ($P \leq 0.007$).

Nearly all soil pellets without prochloraz gave rise to contaminant fungi on KHP medium. However, when the soil pellets contained prochloraz at 5 μl/kg, the development of contaminant fungal colonies was consistently reduced. The increase in contaminant fungi appeared to be related primarily to increases in populations of *Mucor* spp. and *Rhizopus* spp.

**Anastomosis groups and pathogenicity.** Ninety percent of the isolates of *R. solani* recovered from the potato field soil were *R. solani* AG-3, 6% were AG-4 and 4% did not anastomose with the tester AG-types. The identity of the *R. solani* AG-3 isolates was further substantiated by their brown color on Stewart's medium (10)

When representative isolates recovered from soil were tested for pathogenicity on host plants, the mean pathogenicity scores varied somewhat between isolates of the same AG-type. However, the mean pathogenicity scores were significantly different ($P = 0.05$)

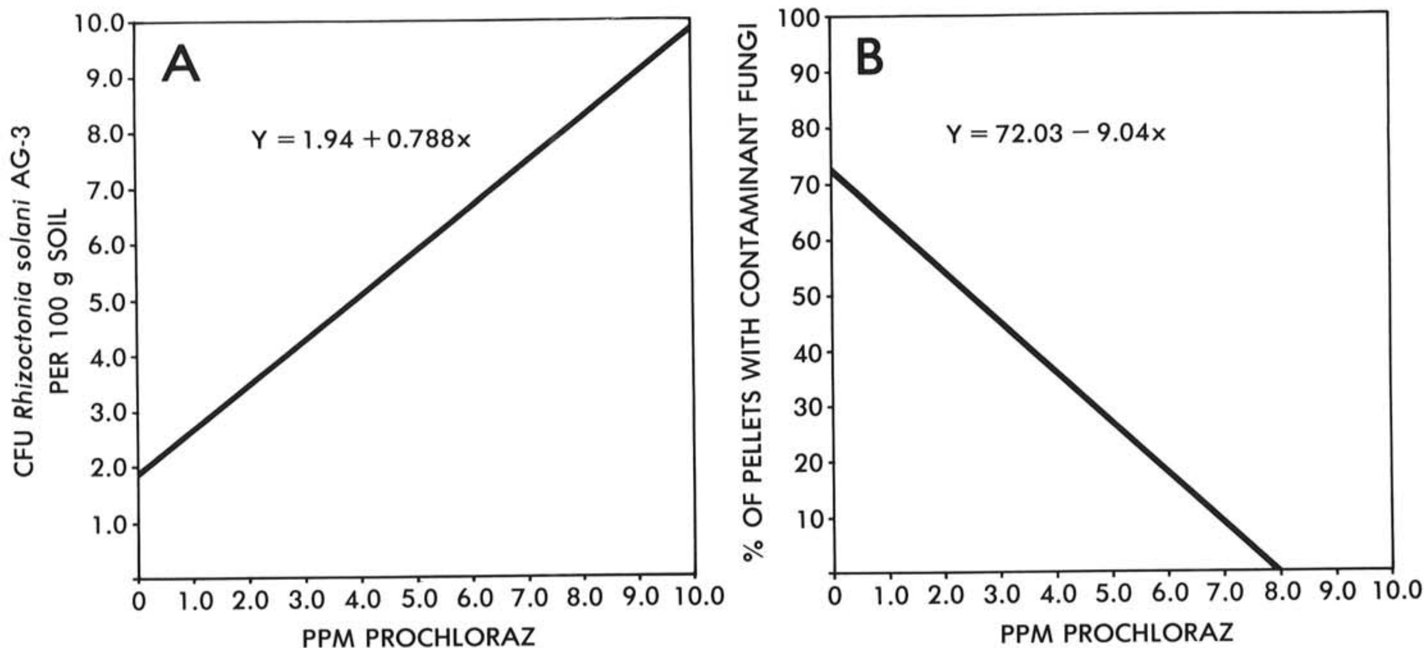


**Fig 1. A,** The effect of different levels of prochloraz in KH medium on the recovery of *Rhizoctonia solani* AG-3. The linear regression between prochloraz concentration and recovery of *R. solani* is highly significant ($P \leq 0.001$, $R^2 = 0.58$). **B,** The effect of different levels of prochloraz in KH medium on the percent contaminant fungi associated with soil pellets. The linear regression between the concentration of prochloraz and pellets with contaminant fungi is highly significant ($P \leq 0.001$, $R^2 = 0.62$.)

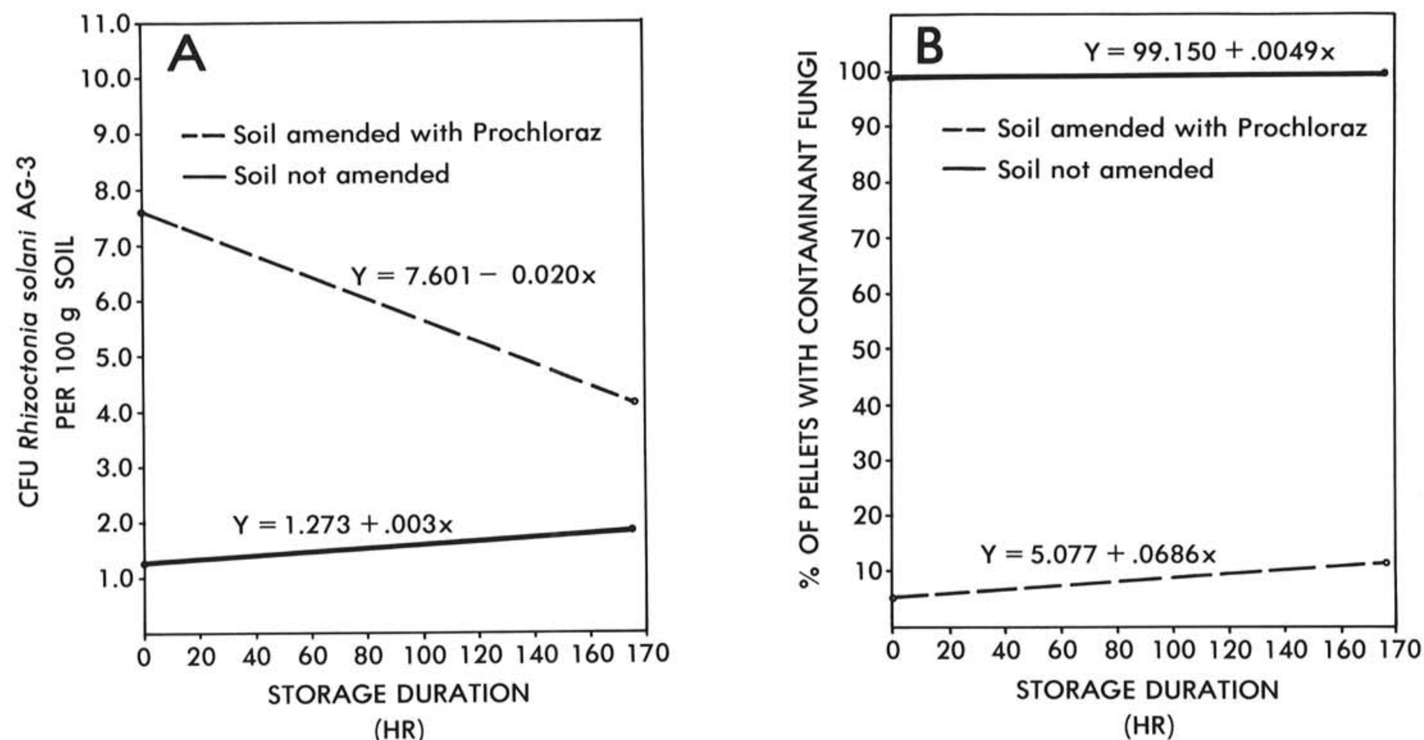


**Fig. 2.** The detection of *R. solani,* **A,** and contaminant fungi, **B,** in naturally infested potato field soil that was stored before pelleting with or without prochloraz then plated on to KHP medium. On unamended soil, the linear relationship between recovery of *R. solani* and storage time was not significant ($R^2 = 0.03$), while for prochloraz-amended soil this relationship was significant ($R^2 = 0.32$). On unamended soil, the linear relationship between percentage of pellets with contaminants and storage time was not significant ($R^2 = 0.20$), while for prochloraz-amended soil this relationship was significant ($R^2 = 0.55$).

between AG-3 and AG-4 isolates (Table 4). The isolates from field soil identified as *R. solani* AG-3, for example, were more pathogenic on potato while the isolates identified as AG-4 were more pathogenic on radish and sugar beet.

## DISCUSSION

Many procedures have been reported in the literature for the quantitative recovery of *R. solani* from soil (13,32–34), but none has been tested for recovering slow-growing isolates such as *R. solani* AG-3. Reported here is a prochloraz modification of the soil pellet technique and the KH medium which improves the recovery of *R. solani* AG-3. The benefit of the prochloraz fungicide at 5 μl/L as an amendment to KH medium (KHP) and at 5 μl/kg in soil pellets lies principally with marked inhibition of fast-growing contaminant fungi in soil. The technique is two to five times more efficient at recovering AG-3 than is the conventional KH medium and soil pellet technique without prochloraz. Amendment of KH medium and soil pellets with prochloraz offers the advantage of recovering slow as well as fast-growing *R. solani* AG-types. Both hyphal and sclerotial propagules were detected. The use of prochloraz may also aid recovery of *R. fragariae* from soil. Like Henis et al (13) who studied a loamy sand soil pelleted on KH medium, we too observed that *R. solani* was overgrown by contaminant organisms after 24 hr when pellets of our field soil were placed on KH medium. In the present study, the growth of isolates of *R. solani* on KHP medium was reduced compared to growth on KH medium, but, by more dramatically inhibiting the growth of contaminants, prochloraz in both the soil and medium permitted the recovery of typical *Rhizoctonia* spp. even after incubating soil pellets for 72 hr.

Davey and Papavizas (11) used different methods to recover active and quiescent propagules of *R. solani* from soil. Because incubation beyond 24 hr does not alter results when our technique is used, it is possible that dormant as well as active propagules of *R. solani* might be recovered. Multinucleate vegetative cells and the *Thanatephorus cucumeris* teleomorph distinguishes *R. solani* from *R. solani*-like (binucleate) isolates (28). In the present study it was assumed that the multinucleate isolates recovered from field soil and that anastomosed with known *R. solani* AG-types would also produce the teleomorph of *T. cucumeris*. A few multinucleate isolates of *R. solani* from the potato field soil failed to anastomose with known AG-types but did develop brown colonies on Stewart's medium (10). Such isolates were presumed to be *R. solani* AG-3. Most conditions of soil storage are extremely artificial and likely to significantly alter the number of recoverable propagules of *Rhizoctonia* spp. Manka, referred to by Johnson and Curl (17), reported that the fungi in soil dried at room temperature were reduced in both number and kind within 14 hr. Papavizas (24) could not recover *R. solani* from an artificially infested soil that was air dried. The present study demonstrated that storing soil at room temperature reduced the number of viable propagules of *R. solani* (Fig. 2). Native populations of *R. solani* in field soils, therefore, may be best represented in freshly collected soil samples.

Pathogenicity tests of AG-3 and AG-4 isolates of *R. solani* recovered from field soil supported the view of previous findings (12,35) that AG-3 isolates are more pathogenic on potato than on other hosts.

TABLE 4. Relative pathogenicity[x] of representative isolates of *Rhizoctonia solani* AG-3 and AG-4 recovered from field soil using the Ko and Hora (KH) medium and prochloraz-amended soil pellet technique

| Seedling hosts | *R. solani* AG-3 (18 isolates)[y] | *R. solani* AG-4 (18 isolates)[y] |
|---|---|---|
| Potato | 2.8 a | 1.7 a |
| Radish | 1.3 b | 2.2 b |
| Sugar beet | 2.0 c | 4.3 c |

[x] Disease symptoms scored as 1 = no symptoms, 2 = mild lesion, 3 = moderate lesion, 4 = severe lesion, 5 = damping off.

[y] Mean value of inoculations resulting from 18 isolates of each AG-type with four replications per isolate. Mean scores in each column followed by different letters are significantly different, at $P = 0.05$, according to Duncan's multiple range test.

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