ASSAY OF RHIZOCTONIA SOLANI IN NATURAL AND ARTIFICIALLY INFESTED SOIL

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ABSTRACT

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By

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The use of seedballs of red table beet in a trapping assay of Rhizoctonia solani in soil is reported for the first time. The new assay consists of burying live seedballs (Cv. Detroit Dark Red--Ferry Morse strain) in the test soil for 2 days at 24 C. After this period the seedballs were removed from the soil, washed vigorously, plated on acidified PDA for 10-12 hours, and observed microscopically for typical Rhizoctonia growth on the individual seedballs. Low levels of Rhizoctonia in soil required more incubation time while certain strains of the organism were adapted to growth at lower temperatures. There was no advantage in using a longer time on the observation plates.

Assay made under a variety of conditions and with soils sampled at several dates indicate that the technique should be very useful as part of a quantitative estimation of inoculum potential in soils of unknown populations. Experiments showed that the method was superior to the previously

described buckwheat stem and immersion tube methods, both in quantity of colonies recovered and in ease of carrying out the assay. The beet seedball assay gave a very high correlation ($r > .99$) between inoculum concentration and disease index when a known pathogenic strain of Rhizoctonia (R-54) was used.

Of seeds from 11 kinds of plants tested for use in the trapping assay only red beet, sugar beet and corn were well colonized by Rhizoctonia (R-54). Corn was also more subject to other fungus contamination. Seeds of tomato, wheat, wax bean, peas, cucumber, soybean, buckwheat and oats were not suitable mainly because of excessive contamination with other organisms.

The beet seedball assay does not differentiate between pathogenic and non-pathogenic strains of Rhizoctonia. Individual isolates vary considerably in their ability to colonize any of the trapping media. A rapid seedling pathogenicity test is needed to accompany the seedball assay. Ten species including alfalfa, cucumber, buckwheat, peas, corn, soybean, wax bean, oats and wheat were compared to radish as a standard. It was confirmed that radish had the best combination of selectivity for Rhizoctonia and resistance to Pythium of the group. Its rapid growth and high germinability are of particular value in index assays for pathogenicity.

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INTRODUCTION

One of the most important soil-borne disease organisms is Rhizoctonia solani Kühn. It incites such diseases as seedling damping off, root rot, stem canker, and storage decay. R. solani has an indefinite number of strains, a wide host range, and wide distribution (6,18,32).

Ordinarily the fungus exists in vegetative form as mycelium and sclerotia but it may sporulate when the perfect stage is induced. It is difficult to isolate by means of the usual dilution plate methods (1,36) that are so useful for detecting freely sporulating species such as Fusarium (3,38). It has thus been necessary to use some form of selective or trapping method for assaying Rhizoctonia in soil. These methods include plant stem segment colonization (9,27,28,29,30,34,35,36,37), immersion tube colonization (20,21,24), debris particle isolation (5,9,27,34), and seed colonization (17,23). Most of these systems have been well explored but comparatively little has been done with seed as a selective substrate for Rhizoctonia in soil. Since red table beet (Cv. Detroit Dark Red) has been shown to be very susceptible to both pre-emergence and post-emergence damping-off by R. solani (10), it was thought that the seed

might be colonized soon after burying in soil. Preliminary trials confirmed this and experiments were designed to test the hypotheses and develop a standardized method for quantitative assay of Rhizoctonia in both natural and artificially infested soils.

LITERATURE REVIEW

The imperfect fungus known commonly as Rhizoctonia solani Kühn is a heterogeneous species with relationships in one or several of the Basidiomycetes depending on the taxonomic definition of R. solani. Synonymy has included:

(Hypochnus filamentosus Pat.)

(H. solani (Pat.) Prill. & Del.)

(Corticium vagum Berk. & Curt.)

(C. vagum Berk & Curt. var. solani Burt.)

(Pellicularia filamentosa (Pat.) Rogers)

Most recently those forms having coarse, relatively fast growing mycelium, sclerotia and typically multinucleate hyphal cells have been designated Thanatephorus cucumeris (Frank) Donk. The fine-mycelial, slower growing, binucleate forms with a less sclerotial or more monilioid cell habit are related to a Ceratobasidium (31). The nuclear condition appears to be the most valid distinction as the other characteristics vary considerably from strain to strain.

The imperfect stage usually has a distinctive mycelium (12). Branches of the sterile mycelium are constricted at point of origin, have a septum close to the point of branching, and often have a right or acute angle branching habit.

Sclerotia and moniloid cells ("thick walled cells" or "chlamydospores") also are characteristic (26,33). The resistant mycelium, sclerotia, and chlamydospores may play an important role in survival of the fungus in or on plant debris (5,27,37) or between or on soil particles (37). Meanwhile, the fungus is able to compete with other soil organisms and saprophytes in the soil (14,29). When susceptible host plants are present under conditions suitable for Rhizoctonia infection these quiescent structures may renew their parasitic activity.

Rhizoctonia spp. induce damping-off, root rot, stem canker, leaf blight, storage decay and other forms of disease on various economic plants throughout much of the world. Because of the economic importance of this fungus, an adequate method for measuring populations in soil is very important and should help in evaluating control methods.

The more adequate methods for isolating and assaying plant pathogens in soil have been comprehensively reviewed and discussed by Menzies (22). He suggested that the buckwheat stem piece colonization method and immersion-tube method are satisfactory for assay of Rhizoctonia populations in soil. Advantage is taken of the fact that Rhizoctonia is a good competitive saprophyte. Parts of stems of certain susceptible crops have been used for isolation and assay of Rhizoctonia in soil (9,27,28,29,30,34,35,36,37). The buckwheat stem piece colonization method was first developed by

Papavizas and Davey (28), and also used extensively by others (9,27,28,29,30,34). The method is very simple and rapid. A number of stem pieces of predetermined size are buried in a definite amount of test soil and incubated there. At the end of a specified time the stem pieces are removed, washed, and plated on a favorable culture medium for observation. Soil temperature, soil type, soil moisture, incubation period, use of sterile or non sterile stem pieces etc. may affect colonization of the stem pieces. Generally, a 2-4 day incubation period, soil moisture at 20-50% W.H.C., and soil temperatures between 20-30 C depending on soil type are optimum for buckwheat stem piece colonization (29). Non autoclaved sterile plant materials, e.g., buckwheat stem pieces, display a particularly pronounced preferential selectivity for Rhizoctonia in soil (34). Rhizoctonia strains also differ in the way they colonize (28,37). Davis (10) indicated that green buckwheat stem segments, aged 10-14.5 weeks, were more efficient than mature dried segments. Colonization frequency did not always correlate with pathogencity on certain host plants. An isolate of Rhizoctonia possessing a high saprophytic ability may be less pathogenic on a certain host plant than another isolate with a lower saprophytic ability as indicated by Papavizas and Davey (28) and Ui and Naiki (37). Other plant stem pieces such as cotton, bean (30,34), Jew's mallow plant (13), and flax (35, 36,37) were also found satisfactory for Rhizoctonia colonization.

The debris particle method (5,9,27,34) used in conjunction with buckwheat stem colonization gave the most complete information since the former reflects quiescent Rhizoctonia in soil and buckwheat colonization the active (9). Unfortunately neither method distinguishes between pathogenic and non-pathogenic strains of R. solani so, for certain purposes, these assays may have to be supplemented with pathogenicity tests (9,22).

Chesters (7) devised a glass immersion tube for isolating active growing fungi in soil. But in practice, Mueller (24) found the method to be inconvenient and difficult. He modified the system with a plastic immersion tube that was more convenient to use than the original and gave less contamination. A selective medium in the immersion tube proved successful for isolating certain specific soil fungi (21). For example, incorporating radish exudates in PDA or water agar significantly increased isolations of R. solani. Frequency of isolation of R. solani with this soil microbiological sampling tube correlated closely with the pre-emergence damping-off of radishes (20). In comparative tests, colonization of the tube medium was always lower but probably more selective than of such substrates as buckwheat stem pieces (9). It was suggested that this high selectivity would be a real advantage in studies on the effect of soil treatment on a particular clone of Rhizoctonia if that clone could be easily recovered by immersion tube (9).

Boosalis and Scharen (5) could detect Aphanomyces euteiches and Rhizoctonia solani by direct microscopic examination of plant debris particles in the soil because of their distinctive morphological characteristics. The sclerotia of R. solani on or within plant debris were readily dislodged during sampling, however, and the method was not considered satisfactory for estimating its inoculum potential in soil. Menzies (22) pointed out that the many non-pathogenic strains in the soil are morphologically indistinguishable from pathogenic strains. A more reliable method for measuring inoculum potential and isolation of R. solani from plant debris, the "debris particle isolation" method, was developed by Boosalis and Scharen (5) and modified by others (9,27,34). Sneh et al. (34) suggested using a gentle water washing instead of a strong jet in order to obtain a better yield of Rhizoctonia. They found a high degree of correlation between visual Rhizoctonia soil infestation levels and colonization potential as assessed by plant segment, immersion tube, and plant debris particle isolation methods.

A host-plant infection assay integrated so many infection factors, other than pathogen population, that Menzies (22) did not consider it generally suitable as an assay method for the pathogen itself. Significant changes may also take place in the population during the time required to grow a plant and obtain disease symptoms especially during a long season. This objection may be only partly overcome by the

use of seedling infection tests but a longer time is required than with other methods (9). Sneh et al. (34) found a close correlation between soil infestation level and disease index but a poorer correlation between soil infestation and percentage of diseased seedlings. Although there were some limitations to the use of a disease index (28) infecting the host plants was best for studies on a variety of isolates. In this way specific pathogenicities on a variety of susceptible plants or species could be determined.

Although the dilution plate method has been widely used for detecting inoculum populations of soil microorganisms in a unit weight of oven dry soil, it has been unsatisfactory for isolating Rhizoctonia and other non-sporulating fungi in soil. According to several reports (1,36), the dilution plate method indicated that Rhizoctonia makes up less than 1% of the total soil fungal microorganisms isolated.

Other methods have been used for identifying and isolating Rhizoctonia from soil. In the soil plate method particles of soil are planted on the surface of a culture medium and observed for growth of typical colonies (36,39), mycelium is kept relatively intact in the soil particles and a better representation is obtained than in soil dilutions. Contact slides (Rossi-Cholodny) (4,36) collect only those organisms that grow onto and adhere to a glass slide but cellulose films (8) may selectively attract some actively growing fungus mycelium. Several other methods such as the plate-profile (1)

and the slide trap (19,36) are related to the immersion tube in principle. The buried profile plates have the particular advantage of locating the level and position in which an isolate was growing in the soil at assay time.

Rhizoctonia infections on seeds had been reported by some workers (2,25). Kendrick and Jackson (17) and Messiean (23) indicated that corn seed could trap Rhizoctonia when buried in soil although the seed may also become colonized by other soil fungi such as Fusarium or Pythium. Apparently there are no reports of other kinds of seed being used in this way to isolate Rhizoctonia.

MATERIALS AND METHODS

Experimental soils

Two types of soil were used in the various assays, a mineral soil and a highly organic "muck" soil. The Conover loam mineral soil was obtained from two locations on the Michigan State University farm--one from a previously uncultivated field plot (A) and the other from a sugar beet plot previously infested with Rhizoctonia (B). These soils were sifted to remove large debris and stored at 30% water holding capacity (W.H.C. = 0.45 g/g oven dry soil) in plastic bags until used in the greenhouse and laboratory experiments. The soil pH was 7.45.

Muck soil was obtained in the Fall of 1967 from Michigan State University's muck experimental farm on the Corey Marsh near Bath, Clinton county. It was stored in a large covered outdoor bin until use. The muck was then sifted and adjusted to about 30% W.H.C. for use in the greenhouse. Basic W.H.C. of a sample of this muck soil was determined to be 3.76 grams of water per gram oven dry soil. The soil pH was 6.4.

Rhizoctonia isolates and cultures

Isolate	Host	Pathogencity	Isolated by
R-45	Pine seedling	Very mildly pathogenic on beets	R. A. Davis
R-47	Pine seedling	Moderately pathogenic on red beet seedlings	R. A. Davis
R-51	Eggplant	Moderately pathogenic on red beet seedlings	R. A. Davis
R-54	Red beet	Severe damping off of red beet	R. A. Davis
R-57	Rutabaga	Crater rot of rutabaga-- severe on radish	K. M. Pan

Plant materials

Seedballs of the red beet (Cv. Detroit Dark Red, Ferry-Morse Seed Co.) were used throughout the experiments. Field crop seeds used for trapping or for pathogenicity tests on seedlings included multigerm sugar beets (Michigan Sugar Co. Acc-2168), monogerm sugar beets (Michigan Sugar Co. 129x133 x 5822-0), alfalfa, oats (Cv. Rodney), wheat (Cv. Genessee) and soybeans (Cv. Harosoy 63). Vegetable crop seeds included beans (Cv. Ferry-Morse's Kinghorn Wax), sweet corn (Cv. Golden cross Bantam F-51), peas (Cv. Alderman), and cucumber (Cv. National Pickling). Buckwheat (Cv. Silver Hulless), tomato (Cv. Fireball), and radish (Cv. Crimson Giant) were also used. Samples of the above seeds were plated on acidified PDA to determine seed-borne Rhizoctonia. None was detected.

Buckwheat (Cv. Silver Hulless) was used to grow plants from which stem segments for trapping assays were cut.

Experimental fungicides

Three fungicides were used in certain experiments: Dexon 5% granular (Chemagro Corp.--p-dimethylamino benzene-diazo sodium sulfonate), Difolatan 80% w.p. (Chevron Chemical Co.--cis-N-(1,1,2,2-tetrachloroethyl)thio-4-cyclohexene-1,2-dicarboximide), and Rohm and Haas 575 50% w.p. (experimental non metallic organic fungicide). Dexon is highly active against Pythium but does not measurably control Rhizoctonia (11). Difolatan and RH 575 are broader in their protection and give noticeable control of damping-off caused by Rhizoctonia as well as the Pythiums (10,11, personal communication D. J. deZeeuw).

Preparation of inocula

Fresh cultures (4-5 days old) of Rhizoctonia on PDA were chopped aseptically with sterile distilled water in a sterilized monel metal Waring Blender for 1 minute. One plate of Rhizoctonia thus suspended in 100 ml water was mixed aseptically in 1 liter of sterilized vermiculite medium (dry 8 mesh vermiculite, 1 liter and potato dextrose broth, 333 ml) in a 2800 ml. Fernbach flask. This Rhizoctonia-vermiculite inoculum (RVI) was ready to be used as soil inoculum at various concentrations after 4-5 days growth at 24 C.

Rhizoctonia assay

The buckwheat stem piece colonization technique for isolating Rhizoctonia from soil (27,28,29,30) was modified slightly for the present work. Segments of 14 week old buckwheat stems (5 mm long) were placed between two layers of a given soil sample in a petri dish (50/dish) and incubated at 24 C for 2 days. At the end of the incubation period the segments were removed and washed to remove soil and loosely adhering debris as subsequently described (Figure 1). The cleaned segments were blotted on paper toweling and plated on acidified PDA for growth of any Rhizoctonia colonies that had been trapped.

The immersion tube colonization technique as described by Mueller (24) and others (20,21) was slightly modified and used to assay Rhizoctonia in soil. Tapered polypropylene centrifuge tubes 12 cm long each with ten 3 mm diameter holes arranged in a spiral were used. The tubes were wrapped with plastic electrician's tape (Johns-Manville No. A-7 or 3 M Co. No. 88), capped and the assemblage autoclaved 20 minutes. After air drying, the sterile tubes were filled aseptically with the assay medium (sterile PDA with 67 ppm rose bengal) to about 1.5 cm from the top. Just before placing the assay tubes in soil, small holes were made with a sterile needle in the tape where it contacted the hardened agar. Four replicate tubes were inserted into each one-liter soil container (cottage cheese box) and the container

covered. After 2 days incubation at approximately 24 C the tubes were removed from the soil and agar surfaces exposed one hole at a time. All exposed agar plugs were removed with a sterile needle and transferred to plates of acidified PDA for growth and observation. Colonies of Rhizoctonia were recorded after 12 hrs growth at 24 C.

To determine colonization of living plants, test soils were placed in either a "half flat",[1] or a plastic cottage cheese box,[2] depending on the needs of the particular experiment. Each half-flat was planted with 100 monogerm sugar beet or radish seeds at uniform depth and kept in the greenhouse for growth. The cottage cheese boxes were large enough for 20 radish seeds and could be used either in the greenhouse, laboratory, or in temperature control tanks at 25 ± 1.5 C. Plants which had damped off were counted daily after emergence had begun. Characteristic symptoms induced by Rhizoctonia were noted and recorded. Plants were then removed from the soil and washed to remove adhering soil. Disease index numbers from 0-5 or 0-4 were assigned according to severity with 0 being no Rhizoctonia symptoms and 5 or 4 representing the maximum infection for a given host. Sugar beet index numbers (0-5) were assigned with regard to speed of pre-emergence and post-emergence damping-off as well as severity

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[1]Half flat: 9.6 x 14 x 3.6 or 488.5 cu. in., or 8.0 liter approximately.

[2]Plastic cottage cheese boxes approximately 1 liter capacity, 14.8 cm tall.

of lesions. In the case of radish, disease index numbers were: 4 = pre-emergence damping-off; 3 = post-emergence damping-off; 2 = the seedling girdled; 1 = a large Rhizoctonia lesion and 0 = a small streak symptom known to be caused by other organisms such as Pythium or without symptoms.

RESULTS

Development of the beet seed trapping assay

The beet trapping assay was devised as a result of efforts to find a convenient, simple, rapid, and accurate method for isolating Rhizoctonia from soil. In preliminary trials 105 seedballs of Detroit Dark Red beet were mixed with 200 ml of Rhizoctonia-inoculated sterile mineral soil and held at greenhouse temperature (20-25 C) or 50 seedballs were placed between two layers of Rhizoctonia-inoculated non-sterilized mineral or muck soil at room temperature (24 C). The seedballs were separated to prevent contact. At the end of a predetermined incubation period the seedballs were sifted from the soil on a 15 mesh screen and briefly washed to remove larger soil particles and plant debris. The seedballs were then transferred to washing sieves made from small frozen orange juice cans covered at one end with 18 mesh plastic screening (Figure 1). The washing sieve (or several of them in parallel) was then placed on 1" OD rubber tube in a 250 ml beaker to allow free downward passage of tap water. Lots of assay seedballs were washed vigorously for 10 minutes.

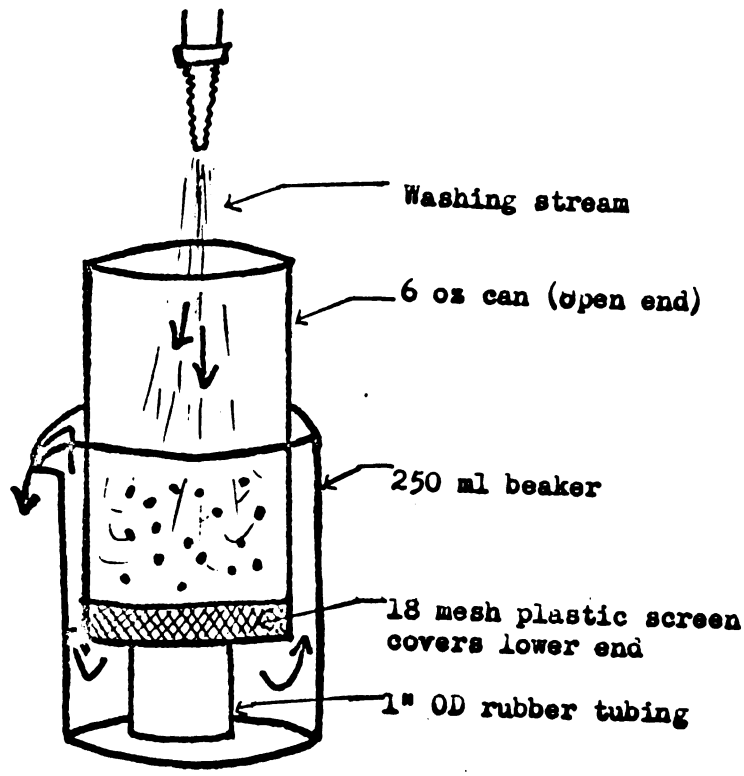

Fig. 1. Washing device for cleaning beet seedballs used in the trapping assay for Rhizoctonia.

After washing, the excess water was blotted from the washed seedballs with clean paper towels. Seedballs were selected at random for plating on each plate of acidified PDA (0.9 ml of 50% lactic acid in 200 ml PDA, pH 4.0). Assay plates were incubated at room temperature (24 C) for 10-12 hrs. Rhizoctonia colonies emerging from the seedballs could then be readily detected with a dissecting or low power microscope (27-125 x). The same technique was also used to evaluate the Rhizoctonia colonization ability of other kinds of seed. The assay was further developed and refined during the course of succeeding work in order to determine its accuracy under various soil conditions.

Recovery of Rhizoctonia from artificially infested sterile soil

The beet seed assay was first used to recover Rhizoctonia from inoculated sterile soil. Nine liters of sterilized mineral soil (source A) was mixed thoroughly with 1 ℓ of RVI (isolate R-54). Parts of this basic 0.1 RVI soil were further diluted after either 0 or 4 days incubation and assayed (Table 1).

A 200 ml portion of each Rhizoctonia-inoculated soil dilution was mixed with 105 beet seedballs in a 3 in. clay pot and incubated in the greenhouse for 2 days. The pots were watered daily. Samples of 100 of the 105 seedballs in each sample were removed, cleaned, plated, and examined for colonization by Rhizoctonia (Table 1).

Table 1. Colonization of red beet seedballs incubated in artificially infested sterile soil.

Inoculum	RVI Concentration[1] and Colonization[2]								
	.100[1]	.050	.025	.0125	.00625	.00312	.00156	.00078	Control
	Percentage								
0-day	100	100	100	97	90	49	--	--	0
4-day	--	--	--	100	100	94	82	53	0

[1]Concentration of the standard Rhizoctonia-vermiculite inoculum (RVI) in the sterile soil mixture.

[2]100 seedballs assayed from each sample of 105.

Rhizoctonia colonized the red beet seedballs readily in the sterile soil-inoculum mixture. The "0-day" soils (diluted and used immediately after initial mixing of inoculum and soil) gave between 90 and 100% colonization at RVI concentrations of 0.00625 or more. At RVI 0.00312 there was nearly 50% colonization. Since no Rhizoctonia was detected in the control soil, it is presumed that the seedballs themselves were Rhizoctonia-free.

Rhizoctonia assayed considerably higher when the soil-inoculum was incubated for 4 days ("4-day soil"). At RVI 0.003 colonization was nearly twice as high as for "0-day" soil (94% vs 49%) (Table 1). The differences at lower concentrations (RVI 0.00156 and 0.00078) were even more pronounced and indicate a more complete establishment of Rhizoctonia in the soil. From these results we may conclude that the beet seedball colonization technique is useful for estimating Rhizoctonia inoculum density or inoculum potential in soil.

In sterilized control soils the seedballs were colonized or contaminated extensively with Stemphylium but none appeared where Rhizoctonia inoculum was high. The Stemphylium apparently could not compete for sites on the seedballs where Rhizoctonia was present because Rhizoctonia usually appears on the PDA plates within 12 hrs. The slower-growing Stamphylium required at least 3-4 days.

Recovery of Rhizoctonia from fungicide-treated, artificially infested sterile soil

Before attempting to assay Rhizoctonia under natural soil conditions it was necessary to determine the effect of a fungicide on the fungus in a controlled situation. A quantity of 4-day inoculum of Rhizoctonia R-54 was diluted with sterile soil to a concentration of .0125 and 2200 ml (2500 g.) aliquots mixed with Difolatan fungicide at 200, 100, 40, 20, 10, 4, and 0 ppm active ingredient. Beet seedballs were incubated in the various soils (105/200 ml/pot) for 2 days and assayed for Rhizoctonia colonization (Table 2).

Table 2. Colonization of red beet seedballs by Rhizoctonia solani in artificially inoculated, fungicide-treated sterile mineral soil.

Fungicide ppm[1]	0	4	10	20	40	100	200
Colonization percent[2]	100	91	80	75	50	42	29

[1]Difolation, Chevron Chemical Co. cis-N-(1,1,2,2-tetrachloroethyl)thio-4-cyclohexene-1,2-dicarboximide. By weight in mineral soil of 1.15 g/ml.

[2]100 seedballs Detroit Red beets (Ferry-Morse Seed Co.) Inoculum concentration of .0125 Rhizoctonia-vermiculite by volume.

As the concentration of Difolatan increased the amount of colonization decreased, reaching 2.9% at 200 ppm. The chemical was phytotoxic and produced severe stunting of

the beet seedlings at 100 and 200 ppm. Under these conditions Difolatan gave only partial control of Rhizoctonia at levels harmless to beets. The LD_{50} of Difolatan on Rhizoctonia in soil was approximately 40 ppm, assuming that colonization of beet seeds is a direct measure of viable pathogenic Rhizoctonia propagules.

The beet seedball assay demonstrated a good control gradient with Difolatan fungicide and should be useful in evaluating the activity of other fungicides against Rhizoctonia as well. To be broadly applicable the assay should be accurate in both natural and artificially infested soils in order to measure degradation or dissipation of fungicide activity over a period of time.

Red beet seedball assay of Rhizoctonia in natural soil

The assay was next used to measure Rhizoctonia in a non-sterilized soil. Mineral soil (source B) was used without sterilization. Fifty seedballs were incubated for 2 days at 24 C in each petri dish containing 100 ml of sample soil. After incubation, 45 of the seedballs were plated for identification of the colonies. Colonization by Rhizoctonia varied with the time of year the soil sample was taken. The November sample, taken before snow fell, gave 83% colonization but there was a drop to 32% after the winter season (March sample). Based on this limited trial and the experience of Banihashemi (3) with Fusarium oxysporum, the technique

appears to be suitable for determining Rhizoctonia infestations under natural competitive conditions. If further work supports this hypothesis it should also be useful in measuring seasonal effects and possibly crop rotation effects as well.

Factors affecting the colonization of red beet seedballs by Rhizoctonia

Many factors may affect the colonization of red beet seedballs by Rhizoctonia. Three which were studied in order to establish a good standard procedure, included length of the incubation period in soil, soil temperature, and the use of sterilized vs. non-sterilized seedballs.

Incubation period: Non sterile mineral soil (source A) was inoculated with RVI of isolate R-54 and a series of dilutions (v/v) were made: 0.2, 0.1, 0.02, 0.01, 0.002, and 0 (control). Six replicate lots of red beet seedballs were incubated in these soils in petri dishes for 12, 24, 48, 72, and 96 hours. High frequency of colonization by Rhizoctonia with relatively little contamination from other organisms was obtained at 24-72 hrs (Table 3). In general, 48 hrs incubation gave maximum colonization, but at the 0.01 dilution 72 hrs was maximum. At high inoculum concentration (0.2 and 0.1) the seedball colonization was maximum at 24-48 hrs but at low concentration (0.02-0.002) the maximum colonization was reached between 48 and 72 hrs. Prolonging the incubation period (72-96 hrs) gave lower percentages of

Table 3. Colonization of red beet seedballs by Rhizoctonia solani in artificially inoculated natural mineral soil. Seeds incubated 12 to 96 hrs at inoculum concentrations from 0.0 to 0.2.

Incubation (hrs)	Inoculum Concentration[1]					
	0.2	0.1	0.2	0.01	0.002	Control
	----------Percent colonization[2]----------					
12	39	19	15	6	1	0
24	87	80	31	8	1	0
48	97	95	66	18	3.5	0
72	83	63	59	22	3	0
96	66	60	38	6	0.5	0

[1]Rhizoctonia-vermiculite inoculum in the soil-inoculum mixture by volume.

[2]Means of 6 replication of 45 seedballs per assay.

colonization. It is suggested that soils of unknown inoculum potential should be assayed at several intervals in the 24-72 hrs incubation period in order to determine their maximum colonization capability.

Soil temperature: Aliquots of 30 day old R-54 inoculated natural muck soil (RVI concentration 0.02) were assayed at incubation temperatures of 16, 20, 24, 28, and 32 C. Six replications of 50 seedballs each were incubated in petri dishes at each temperature. The seedballs were removed after 2 days and assayed on PDA plates for Rhizoctonia colonization (Table 4). Good colonization of the seedballs by Rhizoctonia was obtained in the 16-28 C range. At 32 C the

Table 4. Effect of soil incubation temperature on colonization of red beet seedballs by Rhizoctonia.

	Temperature (C)				
	16	20	24	28	32
	----------Percentage[1]----------				
Colonization	82	87	90	80	26
Standard deviation	6.4	8.0	6.2	7.6	7.0

[1]Based on 6 replications of 45 seedballs/assay.

colonization was minimum (26%) and at 20-24 C it was optimum (87-90%). The optimum temperature of colonization by R-54 in this experiment was similar to its optimum growth temperature on PDA (Figure 2). Standard deviations from the means of seedball colonization were all of the same magnitude varying from 6.2-8.0 regardless of incubation temperature. Colonizations at the temperature of 16, 20, 24, and 28 thus probably overlap and the differences between them are not considered significant.

Sterilized vs. non sterilized seedballs: Some seeds carry fungus propagules that either may interfere with colonization by Rhizoctonia in soil or that could obscure its determination on assay plates. Seedballs of the beet that had been sterilized would not have this complication. The experiment was designed to determine whether sterilization of seed before the assay was advisable. Aliquots of 16 day

old Rhizoctonia-inoculated (R-54) natural muck soil (RVI concentration 0.02) were assayed with (1) autoclaved (20 min), (2) propylene oxide sterilized (1 ml/liter container for 24 hrs), and (3) non-sterilized seedballs. Three replications of 50 seedballs were incubated at 24 C in petri dishes for the standard 2 days. From these 50-seedball lots 45 were plated for colonization assay. Non-sterilized, autoclaved, and propylene oxide-sterilized seedballs were colonized 97.5, 92.5 and 85% respectively (Table 5). Contamination with other soil fungi was highest on the autoclaved, intermediate on non-sterilized and lowest on the propylene oxide-treated seedballs. Since the non-sterilized seedballs had the highest Rhizoctonia colonization they should be more suitable for assaying active Rhizoctonia population in soil. Propylene oxide-treated seedballs, which had the least contamination, would be more suitable if one were mainly attempting to isolate pure cultures of Rhizoctonia from soil.

Table 5. Colonization of sterilized and non-sterilized red beet seedballs by Rhizoctonia. Inoculated muck soil at RVI conc. 0.02.

	Sterilized seedballs		
	Autoclave[1]	Propylene oxide[2]	Non sterilized
	----------Percentage[3]----------		
Colonization	92.5	85	97.5

[1]Autoclaved 20 min at 15 lb.
[2]1 ml/liter of container volume for 24 hrs.
[3]3 replications of 45 seed assayed.

Comparative efficiency of various seeds for *Rhizoctonia* colonization

Beet seedballs may be less suitable than some other seeds for *Rhizoctonia* assay so an experiment was conducted to compare them in this regard. Aliquots of 30 day old *Rhizoctonia*, R-54, inoculated natural muck soil (RVI concentration 0.02) were assayed with eleven types of seed: red beet, sugar beet, corn, soybean, cucumber, buckwheat, oats, wax bean, peas, wheat, and tomato (Table 6). Samples of these seeds had been tested and found free of *Rhizoctonia*. At the end of 2 days incubation the seeds were removed, washed, plated out, and examined as previously described. Red beet, sugar beet, and corn showed the highest percentage of colonization (92%). Cucumber, soybean, oats, and buckwheat were intermediate (65-80%), and wax bean, wheat, tomato, and peas were comparatively poorly colonized by R-54 (Table 6). Red beet, sugar beet, and corn had small variations in colonization frequency (standard deviation 1.7-3.1); cucumber, soybean, wax bean, and peas had large variations (standard deviation 9.2-13.6); and the others were intermediate (Table 6). After 4 days on the acidified PDA, notes were made on contaminating fungi on the assay seeds (Table 6). Soybean, wax bean, and peas showed a large number of *Fusarium* contaminations. *Rhizopus* and/or *Trichoderma* were found contaminating all the seed species but *Alternaria* was somewhat less frequent. There was no advantage in using an extended observation period with any of these seeds.

Table 6. Comparative efficiency of eleven crop seeds for Rhizoctonia colonization assay.

Seed	Number incubated in soil per replication[1]	Number plated per replication[1]	Mean colonization[1]	S.D.[2]	Common contaminants[3]
			-----percentage------		
Red beet (multigerm)	50	45	92	2.2	R,F,T,A
Sugar beet (multigerm)	50	45	92	1.7	R,T,F,A
Corn	15	15	92	3.1	R,F
Cucumber	20	20	80	9.2	R,T,F
Soybean	25	25	72	12.6	F,T
Buckwheat	50	45	67	6.7	T,F,R,A
Oats	50	45	65	7.9	T,A
Tomato	50	45	25	5.2	T,F
Wheat	50	45	23	4.6	R,A
Wax bean	15	15	11	13.6	F,T,R
Peas	15	15	5	13.5	F,T,R

[1]Five replications.

[2]Standard deviation.

[3]Noted on incubated assay seeds after 4 days on acidified agar plates. R = Rhizopus; F = Fusarium; T = Trichoderma; A = Alternaria. Species not determined. Listed in approximate order of frequency.

Correlation of seedball colonization with damping-off of sugar beet caused by Rhizoctonia

It is necessary to determine at what level of seedball colonization a given amount of disease on plants may be produced. A strain of known pathogenicity was chosen for this purpose. A quantity of 20-day old Rhizoctonia R-54 inoculated muck soil (RVI concentration 0.02) was uniformly treated with Dexon fungicide to suppress Pythium spp. and control damping-off induced by them (11). Aliquots of this soil were then treated variously with another fungicide, Rohm and Haas 575, known to be inhibitory or toxic to the Rhizoctonia strain R-54 (Table 7). Part of each fungicide treated soil was used for a pathogenicity test with monogerm sugar beet seed (Table 7). Soil without RH 575 colonized 96.8% of the assay seedballs. As RH 575 was increased the colonization decreased gradually from 40.8 to 2.0%.

For the pathogenicity test, 100 monogerm sugar beet seeds were planted uniformly in each treated soil in greenhouse half-flats. The soil temperatures varied between approximately 20 and 25 C. Cumulative post-emergence damping-off was determined daily and recorded to establish a disease index average for each soil treatment. The index was based on: (5) = presumptive pre-emergence damping-off; (4) = observed post-emergence damping-off on the 1st and 2nd day; (3) = post-emergence damping-off on days 3-4; (2) = post-emergence damping-off on days 5-6; (1) = post-emergence damping-off on days 7-8 or, if living beyond 8

Table 7. *Rhizoctonia* colonization of red beet seedballs compared to pathogenicity on sugar beet seedlings in fungicide-treated natural muck soil.

Fungicide[1]		Seedball	Seedling	Disease
Dexon (ppm)	RH 575 (ppm)	Colonization[2] (percent)	Damp-off[3] (percent)	Index[4] (mean)
90	0	96.8	100	4.89
90	90	40.8	86	2.17
90	180	13.6	30	0.84
90	270	6.4	9	0.14
90	360	2.0	5	0.10

[1]By wt calculated from lbs active/Acre 4" dry muck soil. 90 ppm = approx. 19 lb/A 4".

[2]4 replications of 45 seedballs Detroit Dark Red beets/assay.

[3]100 seeds (monogerm) sugar beets. Includes pre- and post-emergence damping-off.

[4]Index is the mean of: 5 = pre-emergence damp-off; 4 = post-emergence damp-off on days 1-2; 3 = post-emergence damp-off on days 3-4; 2 = post-emergence damp-off on days 5-6; 1 = post-emergence damp-off on days 7-8 or those plants remaining beyond 8 days and having hypocotyl lesioning; 0 = no damp-off or lesioning by *Rhizoctonia*.

days, having hypocotyl lesioning; and (0) = living plants free of *Rhizoctonia* lesions after 8 days. Index numbers for each plant in a given treatment were summed and averaged to give the treatment index (Table 7).

The fungicide RH 575 was inhibitory to *Rhizoctonia* when compared by the seedball colonization, damping-off and disease index criteria. Very clear differences, from the control, were obtained with the lowest concentration (90 ppm or 19 lb/A 4") and the reduction in *Rhizoctonia* was greater with

higher concentrations. Correlations between colonization, damping-off, and disease index were visually evident and particularly significant between seed colonization and disease index where the coefficient was 0.997. This experiment further indicates that the red beet seedball colonization technique is accurate for measuring Rhizoctonia inoculum potential or changes of Rhizoctonia population induced by fungicides.

Susceptibility to Rhizoctonia of seedlings of eleven crops as influenced by inoculation and Dexon treatment

Natural muck soil from the M.S.U. muck farm is heavily infested with Pythium spp. and this remains high when Rhizoctonia inoculum is added. Sugar beet is susceptible to both of these fungi in the seedling stage and it is difficult to distinguish the agent of damping-off precisely in a mixed culture when sugar beet is the test plant. The purpose of this experiment was to find a suitable plant for pathogenicity assay that would be susceptible to Rhizoctonia and resistant to Pythium. Crop plants used for Rhizoctonia pathogenicity assay have been reported by others: radish (20,30), bean (9,28,30), peas (9), tomato (9), wheat (9,30), and soybean (30) for instance.

Rhizoctonia (R-54)-inoculated muck (RVI concentration 0.02, 30 days old), non-incoulated natural muck soil treated with 90 ppm Dexon (19 lb/A 4") and non-treated non-inoculated

muck soils were planted with surface sterilized seeds of 11 crops: radish, alfalfa, cucumber, tomato, buckwheat, peas, corn, soybean, wax bean, oats and wheat. Each of the three soils was segregated in greenhouse flats. Single rows of each crop (15-25 seeds per row, depending on seed size), were seeded uniformly. Final notes on stands and pathogenicity were made 5 days after planting (Table 8).

All seedlings or non-germinated seeds were removed, washed, and examined for symptoms 5 days after planting. Radish, cucumber, tomato, buckwheat, oats, and wheat had distinguishable symptoms in the Rhizoctonia-inoculated, Dexon-treated soil (designated as RID-soil). On the other hand, alfalfa, peas, corn, wax bean and soybean had as severe or more severe symptoms in Pythium soil (designated P-soil) than on RID-soil. They were not sufficiently differential on these soils. Most of the plants investigated had no symptoms useful for the assay on Pythium soil treated with Dexon (designated PD-soil) although some mild symptoms were noted on peas, corn, and wax bean (Table 8).

Radish had clear-cut hypocotyl girdling and damping-off symptoms in RID-soil, but none of these symptoms in the other two soils. Alfalfa damped off severely in both RID and P-soils. A Rhizoctonia streak lesion, brown to black in color, was noted on tomato hypocotyls characteristically only in the RID-soil. Pythium caused a very severe cotyledon decay or hypocotyl lesioning of peas, corn, and wax bean in

Table 8. Hypocotyl and cotyledon symptoms produced by *Rhizoctonia* and *Pythium* infection on 11 crop plants 5 days after planting in several muck soils.

Test plant	Experimental Muck Soil		
	Rhizoctonia inoculated[1] Dexon treated (RID)	*Pythium* soil[2] Dexon treated (PD)	*Pythium* soil[3] no chemical (P)
Radish	Hypocotyl completely girdled or damped off	Healthy	Small streak lesion on hypocotyl
Alfalfa	Hypocotyl completely girdled or damped off	Healthy	Severe damping-off
Cucumber	Large yellowish lesion on hypocotyl	Healthy	Healthy
Tomato	Brown to black streak lesion on hypocotyl	Healthy	Healthy
Buckwheat	Large lesion on hypocotyl	Healthy	Very mild lesion on hypocotyl
Peas	Black lesion on cotyledon, large brown lesion on hypocotyl	Cotyledon black or brown lesion	Cotyledon completely decayed, hypocotyl rotted
Corn	Black lesion on cotyledon	Very mild lesion on cotyledon	Red streak on cotyledon, hypocotyl decayed
Wax bean	Severe girdling of cotyledon and hypocotyl	Mild lesion on cotyledon and hypocotyl	Cotyledon completely decayed, hypocotyl soft decay
Soybean	Large lesion on hypocotyl and cotyledon	Mild lesion on cotyledon	Large lesion on cotyledon
Oats	Small brown lesion on hypocotyl	Healthy	Healthy
Wheat	Mild streak on hypocotyl	Healthy	Healthy

[1]RVI concentration 0.02, 30 days old. Dexon 90 ppm or 19 lb (active)/A 4".

[2]Dexon 90 ppm or 19 lb (active)/A 4".

[3]Natural soil--natural *Pythium* infestation.

P-soil whereas Rhizoctonia was less severe and produced some large cotyledon and hypocotyl lesions on these crops in RID-soil without extensive decay. Soybeans in RID-soil had large lesions on cotyledons and hypocotyl, but in P-soil only on the cotyledon. Oats and wheat had small brown lesions or a mild streak on the hypocotyl when grown in RID-soil.

Several kinds of plants including radish, cucumber, tomato, buckwheat, oats and wheat may be used as indicator plants for detecting Rhizoctonia in muck soil. However, radish is much more susceptible to Rhizoctonia than the other plants and yet relatively unaffected by Pythium. It was decided to use radish as an indicator plant for Rhizoctonia pathogenicity in succeeding experiments.

Comparison of beet seedball colonization and radish disease assays

Since radish was the most useful plant for seedling pathogenicity studies with Rhizoctonia it was decided to compare radish disease production with beet seedball colonization using the same inocula. The object was to establish some index relationship between the two procedures. A 4-day old Rhizoctonia R-54 inoculated natural muck soil (RVI concentration 0.02) was further diluted with untreated muck to give an RVI series of 0.02, 0.015, 0.01, 0.005 and 0 (v/v). Each of these soils was planted with 4 rows of radish, 25 per row, in greenhouse flats and examined daily for 7 days (Table 9). The red beet seedball colonization test consisted

Table 9. *Rhizoctonia* colonization of red beet seedballs compared to radish seedling disease and disease index in various concentrations of artificially inoculated muck soil. A = assays begun 4 days after inoculation. B = same soil aliquots assayed at 14 days.

Initial concentration RVI	A-4 day soil		B-14 day soil		
	Seedballs colonized[1] (percent)	Seedling disease[2] (percent)	Seedballs colonized[1] (percent)	Seedling disease[2] (percent)	Disease index[3] (number)
0.02	95	86	75	69	2.0
0.015	66	84	62	63	1.5
0.01	47	72	42	48	1.13
0.005	27	49	24	25	0.54
Control	0	0	0	0	0.00

[1]4 replications of 45 seedballs/assay.

[2]Based on the control as 100% emergence, 100 seeds/assay.

[3]Means of individual plants as: 4 = pre-emergence damping-off; 3 = damping off within 7 days from planting; 2 = hypocotyl completely girdled at 7 days; 1 = hypocotyl with large lesion at 7 days; 0 = healthy or at most a streak lesion known to be caused by another fungus such as *Pythium*.

of 4 replications of 45 seedballs per assay (Table 9). Increase in Rhizoctonia inoculum concentration was accompanied by an increase in number of diseased plants. Similarly an increase of Rhizoctonia inoculum gave increased colonization of the seedballs.

The same soil series was replanted and assayed a second time 3 days after concluding the previous experiment. In this trial a numerical disease index was also determined for the radish seedlings. Individual plants were rated and averaged as: (4) = pre-emergence damping-off; (3) = post-emergence damping-off within 7th day from planting; (2) = hypocotyl completely girdled at the 7th day; (1) = hypocotyl with a large lesion at the 7th day, and (0) = seedling healthy or at most with a streak lesion known to be caused by another fungus (usually Pythium).

The results of this experiment indicated a high correlation between (1) Rhizoctonia inoculum concentration, (2) red beet seedball colonization, (3) number of seedlings killed and (4) the radish seedling disease index (Table 9). Correlations between inoculum concentration, seedball colonization and disease index were especially high (0.995).

Comparative evaluation of three colonization assays and pathogenicity of 5 Rhizoctonia isolates

Growth rates in culture: Most of the investigations in the present work involve one isolate of Rhizoctonia, R-54. It was decided to include other isolates of different growth

and pathogenic characteristics in order to determine whether the assays were broadly valid. A growth-temperature study of 5 isolates (R-45, R-47, R-51, R-54 and R-57) was made on PDA (pH 6.0).

A standard 4-replicate petri dish experiment was conducted in the dark with the 5 isolates in incubators held at 16, 20, 24, 28 and 32 C. Radial growth was measured daily for 10 days. The mean diameter of each isolate at 2 days is shown in Figure 2.

The optimum growth temperature of R-45, R-47, and R-51 was 28 C but for R-54 and R-57 the optimum was nearer 24 C. Isolate R-51 was the most rapid grower (average of all temperatures) followed by R-47, R-57, and R-54 which were of more intermediate speed (Figure 2). Although all isolates reacted comparatively unfavorably at 32 C, R-57 and R-54 especially slowed down more abruptly. In the case of R-57 the colony diameter at 10 days was nearly the same as at 2 days (10 mm) whereas other isolates allowed to grow that length of time attained either the full plate diameter of 85 mm (R-47, R-51, R-54) or 77 mm in diameter (R-54). Although relatively a slow grower, R-45 was capable of withstanding the full temperature range.

Assay in artificially infested muck soil: Other isolates of Rhizoctonia might not assay with beet seedballs as satisfactorily as R-54. The five isolates (R-45, R-47, R-51, R-54, R-57) were next compared in artificially infested muck

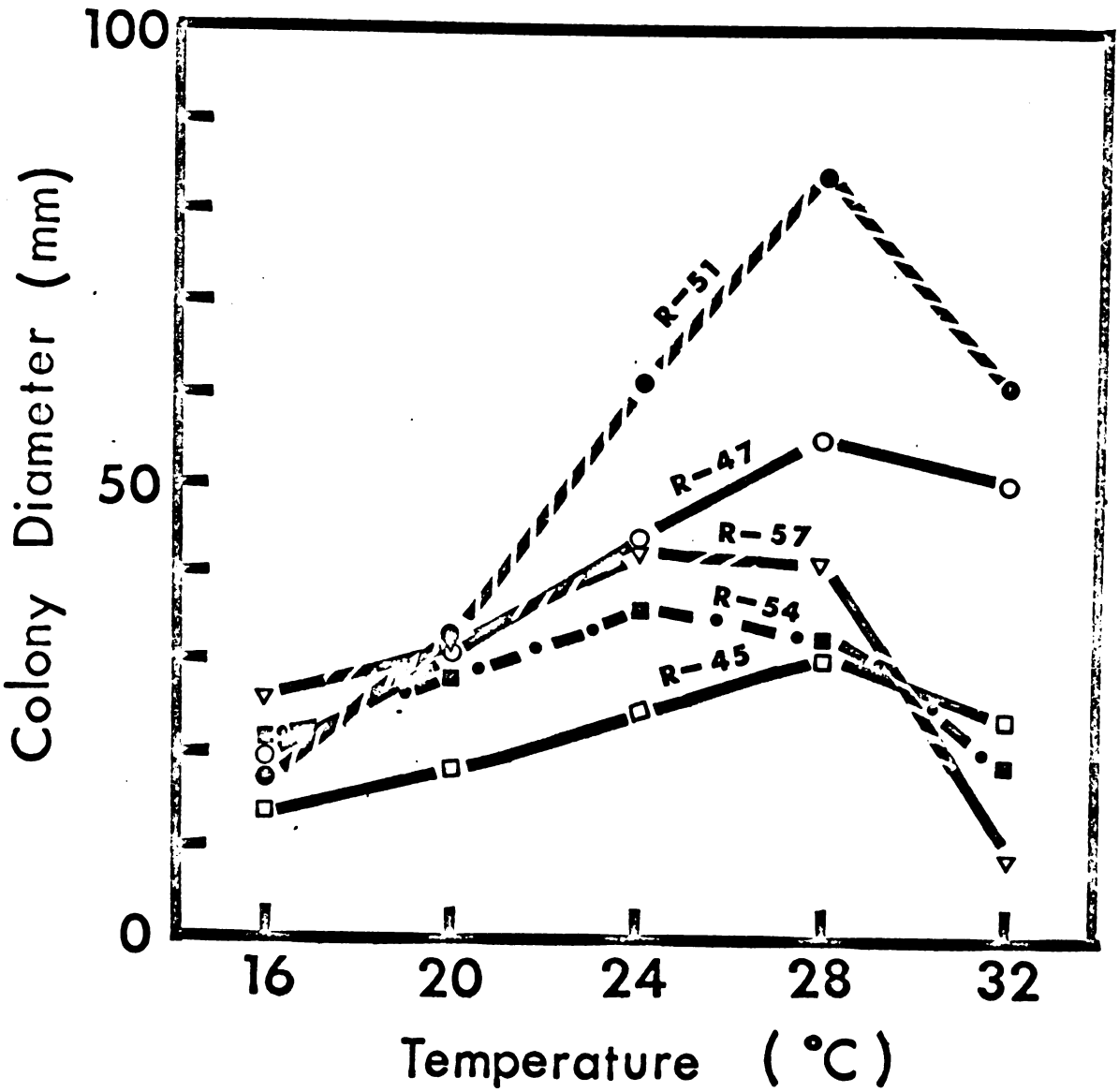

Fig. 2. Radial growth on PDA of five Rhizoctonia isolates (R-45, R-47, R-51, R-54, and R-57) for 2 days at five temperatures.

soil by the beet seedball, radish seedling disease, buckwheat stem segment and immersion tube techniques. The 5 cultures were inoculated in muck soil (RVI concentration 0.03). Part of each soil was used immediately after inoculation (Table 10) and part was incubated in plastic bags in the greenhouse for a time before using in inoculum dilution tests (Table 11).

In the first part of this study, the inoculated muck soils were placed in separate half-flats and each planted with 100 radish seeds for a pathogenicity test. Parts of each of the soils were used at the same time for the red beet seedball colonization assays.

Observations were made daily for radish seedling disease for 10 days after planting and summarized (Table 10). The R-57 and R-54 soils caused very severe radish pre-emergence damping-off. No seedlings emerged from R-57 soil and only 6 from R-54 soil and they damped off immediately after emergence. Isolated R-51 and R-47 were intermediate in pathogenicity. Of these R-51 showed about 33% pre-emergence damping-off, and 63% post-emergence damping-off. Only a few plants (4%) remained healthy. Isolate R-47 caused no pre-emergence damping-off but produced approximately 40% post-emergence damping-off. Isolate R-45 was essentially non-parasitic. It allowed a high final stand, and only a few mild wilt symptoms were observed. These symptoms were unlike any induced by Rhizoctonia, although confirming isolations were not made.

Table 10. Comparative pathogenicity and colonization capability of five *Rhizoctonia* isolates in newly inoculated muck soil. RVI concentration of 0.03.

Isolate	Radish Seedling[1]		Colonization of[2]		
	Emergence (percent)	Stand (percent)	Tubes (percent)	Buckwheat (percent)	Beet seedballs (percent)
R-57	0	0	52	100	100
R-54	6	0	40	100	100
R-51	67	4	17	84	82
R-47	106	60	68	100	100
R-45	98	94	0	0	3.5
Control	100	100	0	0	0

[1]Percent of the control which had an emergence of 84/100 and final stand of 84/100 seeds.

[2]4 replications of 40 agar plugs, 40 buckwheat stem segments, or 40 seedballs per assay.

Table 11. Comparative pathogenicity and colonization capability of 5 Rhizoctonia isolates in inoculated muck soil stored for 7-10 days.

Isolate	Inoculum concn. RVI[1]	Disease index[2] at 25±1.5 C	Disease index[2] at 20-35 C	Colonization[3] of Tubes	Colonization[3] of Buck-wheat	Colonization[3] of Beet seedballs
		-----number-----		-------percent---------		
R-45 7 days	0.03	0	0	0	0	0
	0.015	0	0	0	0	0
	0.0075	0	0	0	0	0
	0.0037	0	0	0	0	0
R-51 7 days	0.03	0.5	0.2	1	20	33
	0.015	0.4	0.27	0	10	16
	0.0075	0.1	0.17	0	5	12
	0.0037	0.2	0.1	0	2	5
Control 7 days	----	0	0	0	0	0
R-54 10 days	0.03	1.8	1.6	10	62	66
	0.015	1.4	0.6	2	27	44
	0.0075	0.8	0.5	1	14	24
	0.0037	0.5	0.35	0	2	10
R-47 10 days	0.03	1.0	1.0	87	97	100
	0.015	1.5	0.85	22	89	100
	0.0075	1.4	0.8	12	60	84
	0.0037	0.4	0.3	2	23	40
R-57 10 days	0.03	0	0	0	0	0
	0.015	0	0	0	0	0
	0.0075	0	0	0	0	0
	0.0037	0	0	0	0	0
Control 10 days	----	0	0	0	0	0

[1]Volume of Rhizoctonia-vermiculite inoculum in soil mixture.

[2]Calculated on means of individual plant ratings as: 4 = pre-emergence damping-off; 3 = damping-off within 10 days of planting; 2 = hypocotyl completely girdled at 10th day; 1 = hypocotyl with large lesion at 10th day; 0 = healthy or at most a streak lesion known to be caused by another fungus such as Pythium.

[3]2 replications of 40 agar plugs, 40 buckwheat stem segments or red beet seedballs/assay.

Four replications each of 40 seedballs, 40 stem pieces or 40 agar plugs were used in the beet seedball, buckwheat stem piece and immersion-tube assays respectively (Table 10). The isolates differed in ability to colonize the immersion tubes. In order of efficiency they were: R-47>R-57>R-54> R-51>R-45. The parallel pathogenicity test ranked: R-57> R-54>R-51>R-47>R-45. This indicates that pathogenicity on radish is not clearly correlated with ability to colonize the agar in immersion tubes under this condition. This same poor correlation held for the other colonization techniques (seedball and buckwheat stem) compared to radish pathogenicity. With isolate R-45 no Rhizoctonia colonies could be detected by either the immersion tube or the buckwheat stem piece techniques. A few of the red beet seedballs were colonized. The seedball and buckwheat stem colonizations were consistently higher than were immersion tubes and much simpler to use in practice. In soils of high Rhizoctonia inoculum there were no obvious differences in amount of colonization on the seedballs or the buckwheat stem pieces by the various isolates. It is concluded that the red beet seedball technique is a more sensitive method for detecting the several Rhizoctonia isolates than the other two techniques.

In the second part of the study inoculated muck soils (RVI concentration 0.03) which had been stored in plastic bags for 7 to 10 days were further diluted with natural muck soil to a RVI concentration series of 0.03, 0.15, 0.0075,

0.00375 and 0 (control). The R-45 and R-51 soils had been stored for 7 days and the R-54, R-47, and R-57 for 10 days before dilution to the experimental concentrations. The same methods were used as had been employed in the first part with non-diluted soil.

Two replicates of 40 seedballs, 40 stem segments or 40 agar plugs were used in the beet seedball, buckwheat stem, or the immersion tube assays respectively with each soil in the series. Each Rhizoctonia inoculated soil was also planted with radish--2 replicates of 20 seeds each--in 100 ml plastic cottage cheese boxes for each growing condition. One of the sets was held at 25±1.5 C in soil temperature tanks and the other in the open greenhouse where the temperature varied between 20-35 C. Damping-off was counted daily and recorded cumulatively. Final notes on disease symptoms and survivors were made 10 days after planting. A disease index was calculated according to disease severity as described in a previous experiment (see Table 9).

Results of the assays and pathogenicity tests are shown in Table 11. Seedling disease on the radish was more severe at 25±1.5 C in the soil tank than at the prevailing greenhouse temperature. The values for disease index vary approximately with inoculum concentration but are rather poorly correlated in the case of R-51 and R-47. The reason is not known but may be because of small samples. Activity of most of the Rhizoctonia decreased following storage and was

reduced to zero with R-57, an ordinarily pathogenic isolate. Isolate R-47, on the other hand, survived storage well and was both moderately pathogenic on radish and also gave good colonization assays by the 3 methods. On the basis of these data it is difficult to explain the low pathogenicity and colonization from the 10 day old R-57 soils (Table 11) compared with its capability in freshly inoculated soil (Table 10). Under the same storage conditions R-54, also basically pathogenic and a good colonizer, remained high in activity. Rhizoctonia R-57 either is a poor saprophyte, is particularly subject to lysis, or is specifically sensitive to storage conditions. Further experimentation will be required to elucidate this point.

There was again a good correlation between inoculum concentration and colonization of 3 substrates with isolates R-47, R-51, and R-54. The colonization assays differed in sensitivity with the clearest results over all given by the red beet seedball method. Nearly as clear results were obtained with buckwheat stems and the poorest with immersion tubes (Table 11). It was shown again that colonization was not necessarily correlated with pathogenicity, but, rather with some other capability of the isolate. For example, R-47 was apparently a good colonizer but was a considerably weaker pathogen than R-54--also a good colonizer.

DISCUSSION

Currently the only adequate selective methods reported for detecting Rhizoctonia populations in soil include stem segment colonization (9,22,34,36) and immersion tube colonization methods (20,22). These methods may measure Rhizoctonia populations in soil quantitatively if used properly, but sometimes they are not convenient. For instance, suitable buckwheat stems 3 months old may be unobtainable and planning for their use is not always convenient. Objections are also to be made for immersion tube techniques. Many tubes are needed and the volume of soil sample needed is also large compared to some other methods. There is often a failure to colonize the individual agar plugs selectively and the plugs must be transferred from tube to plate for observation and identification. The red beet seedball method developed in this study, in contrast, is rapid and convenient as well as apparently selective.

Parallel determinations of colonization of beet seedballs and disease incidence of Rhizoctonia (R-54) on host plants were made at various concentrations of the inoculum in soil. Throughout the experiments results were in good agreement. The highest frequency of Rhizoctonia colonization

of seedballs and highest disease indices were obtained with either high inoculum concentrations in inoculated soil or with low levels of fungicide (Tables 1, 2, 7, 9, 11). Positive correlation of inoculum concentration with seedball colonization or with disease index was very high ($r > 0.99$). Both methods, particularly when used together, give an accurate estimation of Rhizoctonia inoculum potential or changes of Rhizoctonia populations in soil induced by fungicides. The experiments showed that disease index is more reliable than number of diseased plants or percentages for measuring inoculum potential and confirmed the work of others (34).

It should prove possible to measure seasonal changes in populations by the technique and correlate them with infection trials for forecasting purposes. In one case there appeared to be a drop from 83 to 33% colonization over winter. Had this colonization been of the same pathogenic type as R-54 a disease index of approximately 1.0-1.5 would have been obtained on radish or sugar beet seedlings in the spring (Tables 7, 9, 11). Rhizoctonia can survive in natural soil as resistant mycelium, sclerotia, and chlamydospores associated with plant debris particles (5,27,37), but not in silt and clay (27). Banihashemi (3) indicated that Fusarium oxysporum f. melonis is able to survive at 5 C in natural soil as conidia, but the conidia will disappear from inoculated soil within 30 days at temperatures between 5 and 30 C. The beet seedball colonization method is sensitive enough to

detect Rhizoctonia infestation under natural competitive conditions. It should also be useful for measuring crop rotation effects as well.

Although the optimum incubation period for trapping Rhizoctonia by stem segment (29,34) or immersion tube (20) is considered to be 2-4 days, a shorter period of 1-2 days was best in the present work with beet seedballs. The incubation period varied somewhat with inoculum concentration but prolonged incubation led to the same reduction in Rhizoctonia colonization as reported by others (29,34). At low concentrations of inoculum in natural soil (0.02, 0.01, and 0.002) colonization lagged and increased most rapidly between 24 and 48 hrs. The time lag probably reflects the small amount of active inoculum present in the soil during the first and most critical period for seedball colonization. This would indicate that the low initial inoculum began to multiply rapidly but did not have time enough to cause as much colonization as produced by initially higher levels. The beet seedballs began germination at about 48 hrs at 24 C and 30% W.H.C. and natural openings and susceptible tissue may be more available for colonization at that time than later. Part of the lower colonization at 72-96 hrs may result from antibiosis (14,15) between other soil microorganisms and Rhizoctonia already established on the seedballs. Many of the soil microorganisms are competitive saprophytes (14) and prolonging the incubation period would allow them to physically obscure Rhizoctonia

colonies on the assay plate to some extent as well. It is concluded that soils of unknown inoculum potential should be assayed at several intervals in the 24-72 hrs incubation period in order to determine their maximum colonization capability. Papavizas and Davey (29) and Ui et al. (35) found differences in stem segment colonization with soils of different basic temperatures and this depended on presence or absence of temperature--adapted strains of Rhizoctonia. Results reported in this work confirmed that beet seedball colonization probably also depends on the strains of Rhizoctonia present in the test soil. Pathogenicity of the various isolates on radish seedlings differed also with temperature (Table 11).

Few plant seeds proved to be suitable for Rhizoctonia assay in soil and this may explain the small literature on the subject. Although Messiean (23) reported that corn seed was colonized by Rhizoctonia he obtained a very low rate (4%) and also had heavy contamination with other soil fungi. In the present work, colonization of corn seed by Rhizoctonia (R-54) was very high (92%). Differences in corn variety, strain of the Rhizoctonia and inoculum density in soil were probably accountable for the discrepancies. Buckwheat stem segment colonization may be affected by soil type, soil temperature, Rhizoctonia strains etc. as indicated by Papavizas and Davey (28,29) and seed colonization by Rhizoctonia may also be influenced by all of these factors.

A particular seed crop that is susceptible to certain Rhizoctonia strains might measure that strain in soil.

When 11 crop plants were compared to determine the specific pathogenicity of Rhizoctonia with and without the presence of Pythium only radish gave clear-cut differences (Table 8).

Houston (16) indicated that the majority of 25 Rhizoctonia isolates that he studied had an optimum temperature of 28 C with some at 25 C. Our Rhizoctonia isolates R-51, R-47, and R-45 also had a 28 C optimum on PDA, but R-54 and R-57 grew best at 24 C. Ability to grow saprophytically on PDA was not necessarily correlated with pathogenicity. A very pathogenic strain (R-57) is particularly sensitive to temperature being almost completely suppressed at 32 C. It also was a poor saprophyte, at least in the local muck soil. Another pathogenic form (R-54) was a relatively good saprophyte in muck soil.

Presumably the frequency of colonization as determined by immersion tube, buckwheat stem segment and beet seedballs indicated relative saprophytic ability of the fungus. In theory also saprophytic ability of Rhizoctonia is closely correlated with pathogenicity (20,27,34) but only 3 isolates used in this work (R-54, R-51, and R-45) fit the case. The present work confirmed other reports (28,37) that some poor saprophytes were more pathogenic than certain other strains of high saprophyte capability and that the two characteristics do not correlate.

A good correlation between inoculum concentration and colonization of 3 substrates with isolates R-47, R-51, and R-54 was obtained. The colonization assays differed in sensitivity with the clearest results over all given by the red beet seedball method, next with buckwheat stems and the least clear with immersion tubes. Colonization was not necessarily correlated with pathogenicity but depended on the isolate's capabilities. For example, R-47 was apparently a good colonizer but was a considerably weaker pathogen than R-54--also a good colonizer.

Probably no single assay can be found that would measure only pathogenic propagules of Rhizoctonia in soils of many compositions and situations. By taking advantage of the beet seedball assay and indexing it with representative isolates on a seedling pathogenicity test on radish, however, one should be able to predict severity of the disease with considerable accuracy compared to existing methods. The method should be widely applicable in both purely ecological studies on soil and measurements of fungicide effectiveness. In particular it is anticipated that loss of specific fungicide activity will be more easily estimated so that treatment dosages may be optimized. The method cannot measure fungicide degradation directly but in some specific cases may give valuable supporting evidence on residues where the breakdown chemistry is known.

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