Studies on Heterokaryosis and Virulence of Rhizoctonia solani

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ABSTRACT

Heterokaryotic field isolates of *Rhizoctonia solani* Kuehn [=*Thanatephorus cucumeris* (Frank) Donk] ('Praticola type' AG-4) interacted with each other to produce new heterokaryons. Naturally occurring genetic markers were employed to show the occurrence of heterokaryosis. The same genetic markers were used to obtain evidence for the formation of heterokaryons between homokaryons. Heterokaryons synthesized from field isolates were avirulent; whereas, heterokaryons synthesized from homokaryons were either as virulent as the parent isolates or were less virulent than the parent isolate with the higher virulence. The basidiospore progeny of all synthesized heterokaryons tested yielded a high percentage of highly virulent cultures even if one parent was weakly virulent. Virulence of *Rhizoctonia solani* appears to be recessive and is determined by multiple factors; avirulence is determined by the cumulative effect of dominant genes.

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Experimental evidence for the occurrence of heterokaryosis in *Rhizoctonia solani* Kuehn [= *Thanatephorus cucumeris* (Frank) Donk] was first reported by Whitney and Parmeter in 1963 (25). Their work was later supported by the findings of Garza-Chapa and Anderson (11), Vest and Anderson (23), McKenzie et al. (15), and Stretton and Flentje (22). While the foregoing information was being developed, it became apparent that the occurrence of heterokaryosis was limited by two factors. The first factor is the anastomosis group to which an isolate belongs. In *R. solani* there are four anastomosis groups (AGs) (16). Isolates belonging to different AGs are unable to anastomose with each other, and thus cannot form heterokaryons. The second factor is that, within each AG, heterokaryon formation appears to be controlled by heterokaryon incompatibility factors (1), H-factors. Only isolates that carry different H-factors are able to form heterokaryons, and isolates that have H-factors in common do not form heterokaryons.

Up until this writing, the work reported on heterokaryosis in *R. solani* has dealt with heterokaryon formation between mycelia derived from single basidiospores; i.e., homokaryons (1, 25). Heterokaryons; e.g., field isolates, have failed to produce new heterokaryons when paired (9, 16). In fact, until recently it was believed that only closely related, compatible homokaryons would form heterokaryons (9).

In the present paper, we show that heterokaryotic field

isolates interact with each other to produce new heterokaryons, remarkably different morphologically and pathogenically from their parent field isolates, and that field isolates form heterokaryons with homokaryons. Also, evidence is presented to show the effects of heterokaryosis on pathogenicity, with special emphasis on the heterokaryons formed between field isolates.

MATERIALS AND METHODS.—Information on field isolates of *R. solani* used in this investigation is given in Table 1. Each culture was derived from a single hyphal tip and possessed the characteristics of the species set forth by Parmeter and Whitney (18). Stock cultures were maintained on potato-dextrose agar (PDA) at 18 C. Cultures of *R. solani* were induced to produce basidiospores by the methods of Flentje et al. (7), or those of Garza-Chapa and Anderson (11). Potato-dextrose agar containing 1% charcoal (PDCA), grade G60, Atlas Powder Co., Wilmington, Delaware, was used for heterokaryon formation (4). In AG-1 and AG-4, patches of dense mycelium, tufts, or a line of dense mycelium (tuft mycelium) formed at the juncture of two compatible cultures were taken to indicate heterokaryon formation (1, 11, 23, 25). Cultures from the tuft mycelium of the different pairings were established by the hyphal-tip method (25).

The field isolates were assigned to anastomosis groups (Table 1) by using the cellophane method of Parmeter et al. (16), or by opposing isolates on sterile slides coated with 1.5% water agar. The tester strains of Parmeter et al. (16) were used.

Tests for virulence of *R. solani* were carried out on five different host plants: cotton (*Gossypium hirsutum* L. 'Acala SJ-1'), red kidney bean (*Phaseolus vulgaris* L. 'Dark Red Kidney'), black eye bean (*Vigna sinensis* T. 'Black Eye 5'), sugar beet (*Beta vulgaris* L. 'USH 9B'), and cabbage (*Brassica oleracea* L. var. *capitata* L. 'Golden Acre'). Pathogenicity tests on cotton, red kidney beans, and black eye beans were made in a plastic seed germinator. Four- to 5-day-old seedlings grown in the greenhouse in Type-C U.C. soil mix (14) were harvested, washed under running tap water, then placed 5 cm apart on plexiglass plates covered with filter paper, three seedlings/plate. A disk of inoculum was placed alongside of each hypocotyl, then covered with tissue paper to reduce drying. The plates, with seedlings attached with the aid of a rubber band, were placed on a Plexiglas rack, three plates/rack, in a crisper (a clear plastic box measuring 12 × 24 × 32 cm) containing 500 ml of Hoagland's solution (12). A second crisper was inverted over the first. Each experiment with 36 seedlings was repeated twice. Virulence of *R. solani* was recorded 3 to 4 days after inoculation as the product of the length and width of the hypocotyl lesion.

The tests for virulence of *R. solani* on sugar beet and cabbage were made on seedlings in petri dishes. Twenty-five seeds, treated with 1% sodium hypochlorite for 1 min and rinsed with sterile distilled water, were distributed 1 cm apart on top of a 4- to 5-day-old colony on 1.5% water agar. After the seeds germinated, a sterile petri dish bottom was substituted for the original lid and taped in place to make room for the seedlings. Results were recorded 6 days after the seeds were placed on the colony of *R. solani*. Each experiment was repeated twice, and each experiment was replicated four times.

The inoculum for all the tests of virulence of *R. solani* was grown on Weinhold's medium A (24) 4 to 5 days at 25 C. A 4-mm diam mycelial disk was used in the water agar or seed-germinator tests. All virulence tests were carried out at 23-25 C under fluorescent lights with intensity 646-753 lx (60-70 ft-c).

RESULTS.—*Heterokaryon formation between field isolates.*—Hyphae of field isolates of *R. solani* belonging

TABLE 1. Field isolates of *Rhizoctonia solani* showing origin, anastomosis groups (AG), and reference to previous reports on the same cultures

Isolate[a,b]	Host and geographic origin	Anastomosis group	Reference to previous report and remarks
43	*Picea glauca* (Moench) Voss, Quebec, Canada	1	Whitney & Parmeter (25) as R-43
65	*Picea* sp.; Canada	1	Parmeter et al. (16), C-65
189	*Brassica cauliflora* Gars.	1	Parmeter et al. (16), ATCC 13248
239	*Glycine max* Merr.; Canada	1	Parmeter et al. (16), S-239
245	*Phaseolus* sp.; Costa Rica	1	Parmeter et al. (16), C-245
30	*Beta vulgaris* L.; California	4	Bolkan
31	*B. vùlgaris;* California	4	Bolkan
41	*Picea glauca;* Canada	4	Parmeter et al. (16), C-41
73	*Beta vulgaris;* California	4	Bolkan
93	*Beta vulgaris;* Australia	4	Parmeter et al. (16), C-93
107	*Pinus banksiana* Lamb; Canada	4	Saksena & Vaartaja (20) as 2351
108	*P. banksiana;* Canada	4	Saksena (19) as 1250
109	*Pinus resinosa* Ait.; Canada	4	Saksena & Vaartaja (20) as 1244
110	*Pinus* sp.; Canada	4	From D. Vaartaja as R2D41
113	*Pinus* sp.; Canada	4	From D. Vaartaja as 2235
132	*Beta vulgaris;* Virginia	4	Parmeter et al. (16); ATCC 10177
142	*B. vulgaris*	4	Parmeter et al. (16), ATCC 14007
208	*Aster* sp.; California	4	Parmeter et al. (16)

[a] 30, 31, and 73 were isolated by H. Bolkan; all other cultures were obtained from J. R. Parmeter, Jr. and retain his designation.

[b] All isolates except 30 and 73 have produced basidiospores one or more times since isolation (Parmeter, *personal communication*). We induced fruiting in 43, 189, 239, 31, 41, 109, 113, 132, 142.

to the same anastomosis group (AG) will usually anastomose when paired. However, heterokaryon formation between field isolates has not been previously reported, except by Bolkan and Butler (3). The field isolates of *R. solani* studied here (Table 2) were obtained from nature; they were shown to be heterokaryotic through single-spore analysis. This determination was based on the fact that basidiospores produced by homokaryons of *R. solani* generally give rise to cultures of uniform morphology. Accordingly, variation in morphology among colonies established from single basidiospores of a field isolate was taken as evidence that the isolate was a heterokaryon. All field isolates listed in Table 2, except 107 and 108, were shown to be heterokaryons by this means. Evidence for the heterokaryotic condition of isolate 108 was obtained from analysis of H-factors (1). Colonies of basidiospore progeny from each field isolate were compared on potato-marmite-dextrose agar (7) or on nitrate-dextrose agar (NDA) (23).

Eighteen field isolates of *R. solani* belonging to AG-1 and AG-4 (Table 1), from different geographical locations were paired in all possible combinations within their respective anastomosis group. Eight pairings (Table 2) developed tuft mycelium (Fig. 1) where the two field isolates met. The rest of the pairings developed no tufts and thus were considered to be incompatible. All cultures derived from the tuft mycelium of different pairings (Table 2) appeared different from either contributing parent isolated, suggesting that the tuft mycelium was heterokaryotic, and that it contained nuclei from each parent isolate.

Two of the field isolates, 142 and 108, carried natural chemical markers useful in showing that the tuft mycelium formed between them is heterokaryotic. Growth of isolate 142 was completely inhibited by lithium chloride at 2.0 g/l, whereas isolate 108 made extensive mycelial growth (Fig. 2). Cultures derived from the tuft mycelium of 142 × 108 were culturally distinct from either of the contributing parent isolates and also made intermediate growth (Fig. 3) on nitrate-dextrose agar containing lithium chloride. This indicated that the latter cultures were heterokaryotic. A second study was made, in which 25 of the putative heterokaryons from the tuft mycelium of 142 × 108 were paired with each parental isolate. No tufts developed, supporting the hypothesis that the putative heterokaryons were true heterokaryons, and that each of them contained at least one H-factor from each parent. The latter conclusion is based on the findings of Anderson et al. (1) that cultures with common H-factors do not form a tuft mycelium.

Additional evidence for the formation of new heterokaryons between field isolates of *R. solani* was obtained by inducing a culture derived from the tuft mycelium of the pairing 108 × 109 to produce basidiospores. Forty-two single-spore cultures were established. These showed great diversity in colony characteristics on potato-marmite-dextrose agar (Fig. 4). The diveristy was striking, and far greater than that of the progeny of any field isolate that we have induced to produce basidiospores, including the parent 109. This diversity is attributed to the inclusion of more than two genetically different nuclei in the cytoplasm of the heterokaryon synthesized from field isolates.

Heterokaryon formation between field isolates and homokaryons.—When it became apparent that field heterokaryons interacted with each other to produce new heterokaryons, it seemed important to investigate the possibility of heterokaryon formation between field isolates and homokaryons. For this purpose, seven field isolates from different geographical locations (Tables 1 and 3) and 10 single-spore cultures established from the progeny of field isolates 31 and 41 (Table 3) were paired in all possible combinations. The formation of tuft mycelium between two isolates was recorded as an indication of heterokaryon formation. Twenty-four pairings developed tuft mycelium where two isolates met (Table 3), thus supporting the hypothesis that heterokaryotic field isolates and homokaryons will interact to produce new heterokaryons when paired.

However, the genetic control of tuft mycelium formation (heterokaryon formation) between some field isolates and homokaryons could not be explained by compatibility factors. For example, field isolate 31 formed tuft mycelium with field isolate 108, indicating that they carry different H-factors (Table 2) but, single basidiospore culture 31-3-8 did not form a tuft mycelium with isolate 108 (Table 3). Similarly, field isolate 41 formed heterokaryons with isolates 109 and 110 (Table 2), but not all basidiospores from the progeny of isolate 41

TABLE 2. Compatibility factors (H-factors) and tuft mycelium formation between field isolates of *Rhizoctonia solani*

	Isolates[a], tuft formation[b] and compatibility factors[c]							
	31	41	107	108	109	110	113	142
	H1 + H2	H2 + H3	H2 + H4 + H6	H4 + H5	H1 + H6	H1 + H6	H1 + H3 + H5	H1 + H2 + H3
31 (H1 + H2)	–							
41 (H2 + H3)	–	–						
107 (H2 + H4 + H6)	–	–	–					
108 (H4 + H5)	+	+	–	–				
109 (H1 + H6)	–	+	–	+	–			
110 (H1 + H6)	–	+	–	+	–	–		
113 (H1 + H3 + H5)	–	–	+	–	–	–	–	
142 (H1 + H2 + H3)	–	–	–	+	–	–	–	–

[a]All isolates belong to anastomosis group 4.
[b]+ = tufts formed; – = no tufts.
[c]The H-factor designations are given arbitrarily and have no relationship to published designations of Anderson et al. (1).

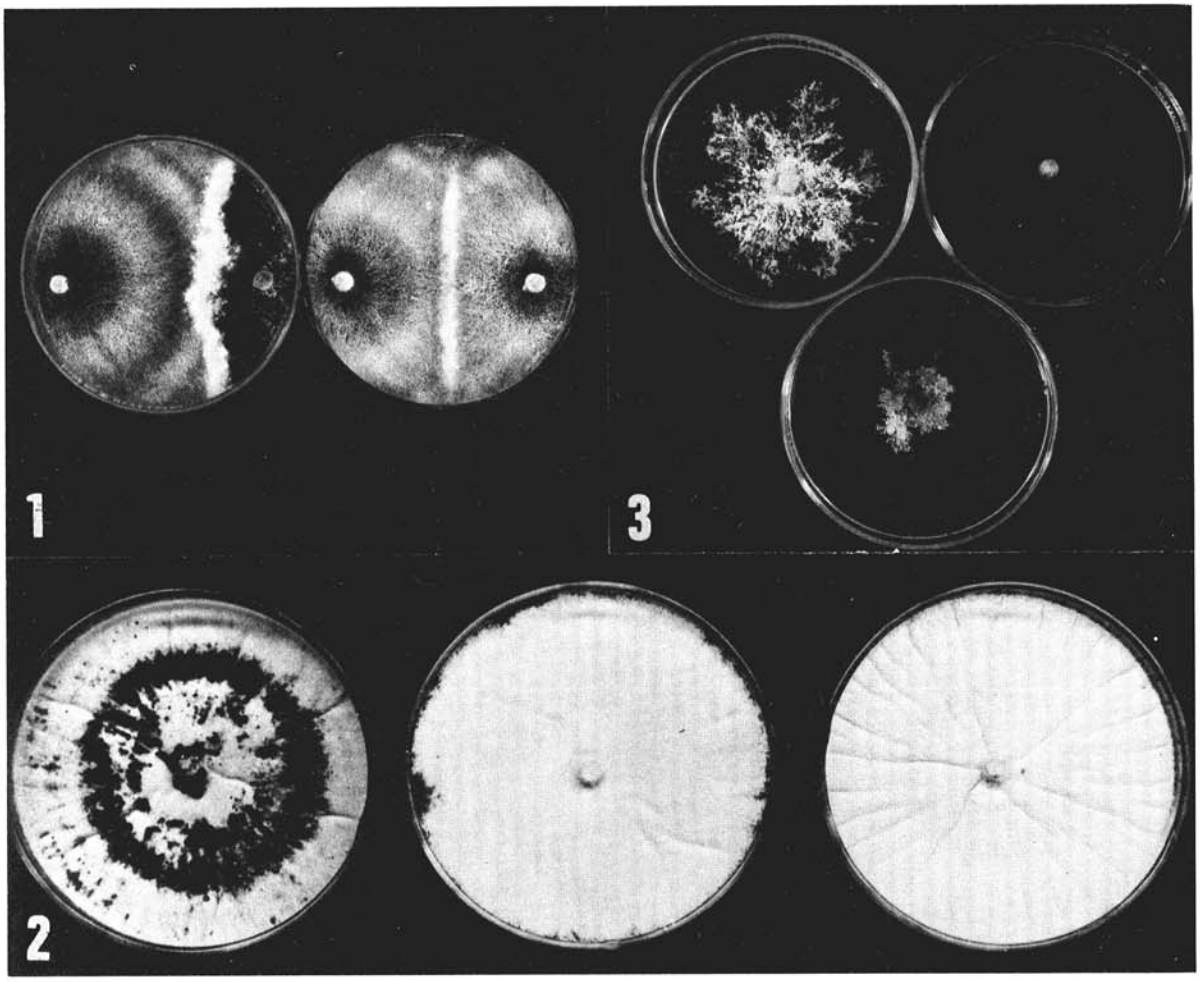

Fig. 1-3. *Rhizoctonia solani.* **1)** Formation of tuft mycelium between field isolates on potato-dextrose-charcoal agar. Left, 108 × 142; right 108 × 31. **2)** Colonies of two field isolates (right, 108; center 142) and a heterokaryon synthesized from them (left) on potato-marmite-dextrose agar. **3)** Colonies on nitrate-dextrose agar containing 2 g/ l lithium chloride. Upper left, field isolate 108; right, field isolate 142; and bottom, a heterokaryon synthesized from 108 and 142.

formed heterokaryons with isolates 109 and 110 (Table 3). This inconsistency of H-factors in relation to heterokaryon formation might have been due to genetic recombinations that developed during the basidiospore production. However, it remains for future research to explore the validity of this idea.

Number of H-factors.—In a recent publication, Anderson et al. (1) reported that field isolates of *R. solani* carry two different H-factors. However, the formation of new heterokaryons between field isolates, and the interaction of field isolates with homokaryons to produce new heterokaryons, suggested that the number of different H-factors in an isolate could be more than two. This hypothesis was tested by pairing 20 single-basidiospore cultures from 108 × 109 in all possible combinations and recording the formation of tuft mycelium. The results are interpreted in terms of the heterokaryon incompatibility factors (H-factors) proposed by Anderson et al. (1).

Four different H-factors were detected. The distribution of the H-factors among the 20 cultures is given in Table 4. A single H-factor was assigned to each isolate, except for 18, 19, and 20, which behaved as a group with two factors, H1 and H3. Each of the 20 single basidiospore cultures (Table 4) was next paired with the parental heterokaryon (108 × 109) and field isolates 108 and 109 from which the heterokaryon was synthesized. The purpose of these pairings was to determine the H-factor make-up of the field isolates 108 and 109, and the 108 × 109 heterokaryon. Eight cultures (1-8, Table 4) developed tuft mycelium when they were paired with field isolate 109; no tufts developed in pairing with field isolate 108 (Table 4). Nine cultures (9-17, Table 4) formed tuft mycelium when paired with isolate 108; no tufts formed with isolate 109 (Table 4). The heterokaryon (108 × 109) did not make tufts with field isolates 108, 109, or with the 20 single-basidiospore cultures (Table 4). Therefore, based on the formation of tufts, field isolate 108 has two H-factors, H1 and H2; field isolate 109 has two different H-factors, H3 and H4, and the parental heterokaryon has

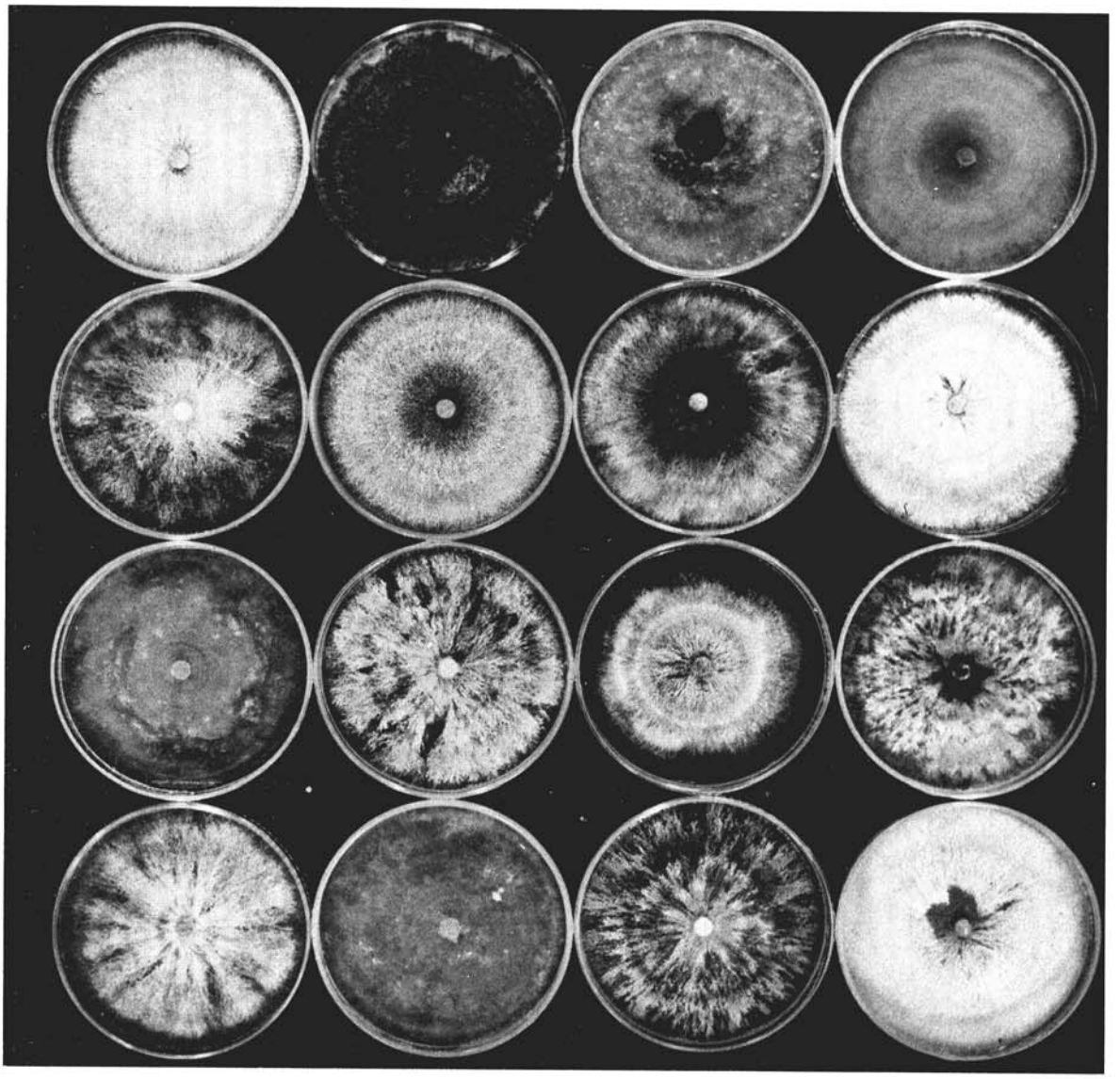

Fig. 4. Basidiospore progeny of a synthesized heterokaryon obtained by pairing field isolates 108 and 109 of *Rhizoctonia solani*. Note the great diversity in cultural characteristics on potato-marmite-dextrose agar.

all four H-factors; i.e., H1, H2, H3, and H4.

To obtain further proof that more than two different H-factors could be brought together within a heterokaryon, three homokaryons were used that carried chemical markers and were also compatible with each other. Isolate 41-12-1 was completely inhibited by lithium chloride at 2.0 g/l (lithium-sensitive) but made extensive mycelial growth in the presence of nickel chloride at 1.0 g/l (nickel-tolerant). Conversely, isolate 31-1 was completely inhibited by nickel chloride at 1.0 g/l (nickel-sensitive), but made extensive mycelial growth in the presence of lithium chloride (lithium-tolerant). Isolate 31-2 was lithium-tolerant and nickel-tolerant at the foregoing levels.

First, isolates 41-12-1 and 31-1 were paired. Following tuft formation, six cultures were derived from the tufts using the hyphal-tip method (25). These six cultures made intermediate growth on nitrate-dextrose agar containing 2.0 g/l lithium chloride (NDA + Li) and on nitrate-dextrose agar containing 1.0 g/l nickel chloride (NDA + Ni) but were completely inhibited when lithium chloride and nickel chloride were both present in the medium (NDA + Li + Ni). This indicates that the six cultures derived from the tuft mycelium of 41-12-1 × 31-1 were heterokaryotic and possessed nuclei from each parent isolate. The heterokaryotic condition of the tuft mycelium, which developed where the two homokaryons met, was substantiated by inducing a culture derived from the tufts of 41-12-1 × 31-1 to produce basidiospores. Ninety-two single-spore cultures were established and

TABLE 3. Tuft mycelium formation between selected field isolates and selected single spore cultures of *Rhizoctonia solani*

Field isolates	Single spore cultures and tuft formation[a]									
	31-3-8	41-6	41-12-1	41-12-2	41-12-3	41-12-4	41-12-5	41-12-6	41-12-7	41-12-8
31	−	+	+	−	+	+	+	+	−	+
41	+	−	−	−	−	−	−	−	−	−
93	+	−	−	+	−	−	−	−	−	−
108	−	+	+	+	+	+	+	+	+	+
109	−	−	+	−	−	−	−	−	−	−
110	−	+	+	−	−	−	−	−	−	−
208	+	−	−	−	−	−	−	−	−	+

[a] + = tufts formed; − = no tufts.

analyzed on the basis of their response to lithium and nickel. Forty-two of the cultures resembled parent isolate 41-12-1 in being lithium-sensitive and nickel-tolerant; 46 of the cultures resembled parent isolate 31-1 in being lithium-tolerant and nickel-sensitive. Four of the progeny cultures resembled parent heterokaryon, 41-12-1 × 31-1, by making intermediate growth on NDA + Li and NDA + Ni but being completely inhibited when nickel and lithium were both present in the medium. The recovery of isolates with characteristics similar to the parent isolates 41-12-1 and 31-1 in the progeny of 41-12-1 × 31-1, confirmed the hypothesis that the cultures derived from the tufts of 41-12-1 × 31-1 were heterokaryotic.

Following the proof that the cultures derived from the tufts of 41-12-1 × 31-1 were heterokaryons, and thus carried two different H-factors, attempts were made to incorporate a third H-factor. For this purpose, each of the six heterokaryons derived from the tuft mycelium of 41-12-1 × 31-1 were paired with nickel-tolerant and lithium-tolerant homokaryon, isolate 31-2. All pairings developed tuft mycelium where the isolates met. Cultures derived from the tuft mycelium of (41-12-1 × 31-1) × 31-2 appeared culturally different from either contributing component, and also made intermediate growth on NDA + Li, NDA + Ni, and NDA + Li + Ni. In a second study, 15 hyphal-tip cultures derived from the tufts of (41-12-1 × 31-1) × 31-2 were paired with each contributing component; i.e., 41-12-1, 31-1, and 31-2. The pairing was done to determine whether each of the foregoing homokaryons has contributed an H-factor to the three-component culture synthesized from them. No tuft mycelium developed between any two isolates paired. Therefore, it is concluded that the cultures derived from the tufts of (41-12-1 × 31-1) × 31-2 were heterokaryons and, in fact, possessed three different H-factors, one from each homokaryon.

The results in Table 2 provide additional evidence that field isolates of *R. solani* could carry more than two H-factors. Based on the tuft formation between paired isolates, field isolates 142, 113, and 107 contain three different H-factors, whereas the rest of the isolates contain only two (Table 2). The data in Table 2 also indicate that the total number of different H-factors in our sample was six.

All heterokaryons synthesized in connection with this study were shown to be stable. No nuclear dissociation was observed through sectoring or by hyphal-tip analysis during 12 mo of continuous culture transfers.

Virulence tests with homokaryons and heterokaryons synthesized from them.—*R. solani* has the capacity to attack a wide range of plants. The fact that field isolates of *R. solani* are usually heterokaryotic may indicate that the ability to attack a broad spectrum of plants could be a result of heterokaryon formation. Garza-Chapa and Anderson (11) and Vest and Anderson (23), in their work on flax seedlings, reported that virulence of *R. solani* may be increased as a result of heterokaryosis. However, proof for the formation of the heterokaryons used in the foregoing work was not presented, and their work was restricted to cultivars of flax. McKenzie et al. (15) studied virulence of *R. solani* to radish seedlings. They paired avirulent mutants; the resulting heterokaryons had the virulence of the wild type. However, the avirulent mutant cultures used to synthesize the wild-type heterokaryon were derived from a single homokaryon, and therefore involved only a single genome.

The present study was undertaken to investigate the potential influence of heterokaryosis on virulence of *R. solani*. For this purpose, eight synthesized heterokaryons and their respective parent homokaryons (Table 5) were tested for virulence on red kidney beans, black eye beans, cotton and cabbage. The results are presented in Table 5. The data show that regardless of the host, the virulence of the synthesized heterokaryons did not exceed the virulence of the parent homokaryon with the higher virulence. In most cases, the synthesized heterokaryon was less virulent than the parent with high virulence (Table 5). A second study was undertaken to explore the segregation for virulence in the basidiospore progeny of a synthesized heterokaryon. Two of the synthesized heterokaryons, 41-1 × 41-3, and 31-1 × 41-12-1, were induced to produce basidiospores. Eighty-three and 92 single-spore cultures from heterokaryons 41-1 × 41-3 and 31-1 × 41-12-1, respectively, were established and tested for virulence on cabbage. The basidiospore progeny of heterokaryon 41-1 × 41-3 segregated for virulence as follows: Five cultures were weakly virulent like parent homokaryon 41-1, 70 cultures were highly virulent like parent homokaryon 41-3, three cultures had similar virulence to the parent heterokaryon 41-1 × 41-3 (Table 5), and five cultures were more virulent than the heterokaryon 41-1 × 41-3. The basidiospore progeny of 31-1 × 41-12-1 segregated for virulence as follows: four cultures were weakly virulent, eight cultures were less virulent than the heterokaryon (31-1 × 41-12-1), and 80 cultures were highly virulent like parent homokaryons 31-

TABLE 4. H-factor compatibility of single basidiospore progeny from the heterokaryon 108 × 109 of *Rhizoctonia solani* and field isolates from which the heterokaryon was synthesized

		H-factors and single basidiospore isolates																			
		H1[a]				H2				H3					H4				H1 + H3		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
H1	1	−	−	−	−																
	2	−	−	−	−																
	3	−	−	−	−																
	4	−	−	−	−																
H2	5	+	+	+	+[b]	−	−	−	−												
	6	+	+	+	+	−	−	−	−												
	7	+	+	+	+	−	−	−	−												
	8	+	+	+	+	−	−	−	−												
H3	9	+	+	+	+	+	+	+	+	−	−	−	−	−							
	10	+	+	+	+	+	+	+	+	−	−	−	−	−							
	11	+	+	+	+	+	+	+	+	−	−	−	−	−							
	12	+	+	+	+	+	+	+	+	−	−	−	−	−							
	13																				
H4	14	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−			
	15	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−			
	16	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−			
	17	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−			
H1 + H3	18	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−
	19	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−
	20	−	−	−	−	+	+	+	+	−	−	−	−	−	+	+	+	+	−	−	−
108 × 109	108	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	−	−	−
	109	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
	109	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a]The H-factor designations are given arbitrarily and have no relationship to published designations of Anderson et al. (1).
[b]+ = tufts formed; − = no tufts.

1 and 41-12-1. The foregoing results suggested that parent homokaryons were genetically dissimilar for virulence and virulence is conditioned by more than one factor.

Virulence tests with field isolates and heterokaryons synthesized from them.—Pathogenicity studies with homokaryons of *R. solani* showed that when two highly virulent isolates are paired, the virulence of the synthesized heterokaryon was either decreased or was the same as the virulence of the parent isolates. However, it was not known if this was the case with the heterokaryons synthesized from field isolates of *R. solani*. In the present study, 75 synthesized heterokaryons and their parent field isolates (Table 6) were tested for virulence on cabbage, sugar beet, red kidney beans, and black eye beans. The results showed that all field isolates were highly virulent on all four hosts (Table 6), but the heterokaryons synthesized from them regardless of the host, were avirulent (Table 6). Virulence tests with field isolates and their synthesized heterokaryons were repeated four times with the same results.

In view of the fact that the parental field isolates were highly virulent, it is difficult to explain on a genetic basis the reason for avirulence of the heterokaryons synthesized from them. Nevertheless, the new heterokaryons contained genes for virulence from each parent and it seems reasonable to make the hypothesis that avirulence was brought about by the presence in the same thallus of parental genomes, and that separation of nuclei during basidiospore formation should restore virulence among the homokaryotic progeny. This hypothesis was tested by inducing one of the synthesized heterokaryons, 108 × 109, to produce basidiospores. Forty-two single-spore cultures from 108 × 109 were established and tested for virulence on cabbage. The basidiospore progeny of 108 × 109 segregated for virulence as follows: four (9.55%) were avirulent, four (9.55%) moderately virulent, and 34 (80.9%) were highly virulent like the parent isolates 108 and 109. This indicates that the factors brought together through heterokaryosis have separated in the progeny of 108 × 109.

An explanation that might account for the observed ratios in the basidiospore progeny of 108 × 109 is the preferential pairing of nuclei during basidisopore

TABLE 5. Virulence of selected homokaryons of *Rhizoctonia solani* and heterokaryons synthesized from them on red kidney beans, black eye beans, cotton and cabbage

Isolates of *R. solani*	Virulence			
	Lesion area (mm^2)			mortality (%)
	Red kidney beans	Black eye beans	Cotton	Cabbage
Homokaryons				
41-1	0.0	0.0	0.0	0
41-3	2.3	12.6	4.7	100
41-5	3.0	7.5	2.0	100
41-10	4.1	19.6	32.2	100
41-14	4.8	11.5	11.2	100
41-12-1	7.5	20.5	--	100
31-1	9.7	37.5	--	100
31-5	7.5	36.5	--	100
31-6	2.3	5.7	--	12.5
Heterokaryons				
41-1 × 41-3	1.5	6.2	2.2	16.5
41-1 × 41-5	2.0	3.1	0.8	12.5
41-5 × 41-10	3.6	15.2	12.2	100
31-1 × 41-14	7.2	21.6	--	100
31-1 × 41-12-1	8.4	30.3	--	100
31-1 × 31-5	8.2	35.2	--	100
31-6 × 41-1	1.2	2.2	--	6.5
31-6 × 41-3	2.2	8.6	--	26.0

formation. If it is assumed that field isolate 108 has nuclei A and B, field isolate 109 has nuclei C and D, and if A and B, or C and D nuclei preferentially pair in basidiospore formation, most of the progeny will show parental virulence. On the other hand, if A and C, A and D, B and C, B and D pair with less frequency and involve genes that condition avirulence then only a few isolates will show low virulence or avirulence.

Pathogenicity tests with isolates from soil.—The synthesis of avirulent heterokaryons from highly virulent field isolates suggested the possibility that such forms are produced in nature and survive in soil as saprobes. In order to test this hypothesis, 15 isolates of *R. solani* were obtained from soil (21). All of the isolates were 'Praticola-type,' AG-4, and possessed the characteristics of the species set forth by Parmeter and Whitney (18). The soil isolates were tested for pathogenicity on cabbage. The results showed that nine of the 15 cultures were avirulent (0% mortality), two were weakly virulent (16.5% mortality), and four were highly virulent (100% mortality). The presence of nine avirulent isolates points out that avirulent types of *R. solani* exist in soil and it is possible that they represent heterokaryons formed from pathogenic field isolates. Attempts to induce the nine avirulent soil isolates to produce basidiospores failed.

DISCUSSION.—Heterokaryon formation between field isolates adds a new dimension to the potential role of heterokaryosis in the adaptation and survival of *R. solani*. Previously it was held that heterokaryons in this species formed only from the pairing of compatible homokaryons or from mutation of existing homokaryon nuclei.

Field isolates of *R. solani* are naturally heterokaryotic; thus heterokaryon formation between compatible pairs produces effects in the immediate new heterokaryon and greatly expands the possibilities for exchange of genetic material at meiosis. Examples of this are seen in the avirulent heterokaryons established from 108 × 142, which form large masses of dark sclerotia (Fig. 2). The parent field isolates 108 and 142 are highly virulent, possess light-colored appressed hyphae and few sclerotia. Also, the basidisopore progeny of heterokaryon 108 × 109 showed unusually great diversity in cultural characteristics (Fig. 4).

The present study indicates that compatibility factors similar to those described by Anderson et al. (1) were involved in the formation of heterokaryons from field isolates. However, compatibility factors controlling tuft mycelium formation between field isolates and homokaryons seems to be different. Our results also demonstrate that field isolates of *R. solani* could carry more than two H-factors. Support for the latter was obtained by analysis of the H-factors in the basidiospore progeny from heterokaryon 108 × 109 and by pairing three compatible homokaryons. The fact that a heterokaryon can possess three or four different H-factors indicates that more than two genetically different nuclei of diverse origins can compatibly exist in a balanced state within a common cytoplasm. In this respect, *R. solani* seems to be unusual among the fungi. Certain homobasidiomycetes such as *Schizophyllum commune* (6) may temporarily incorporate three genotypically different nuclei into a single thallus through anastomosis but *S. commune* is basically a dikaryon and eventually the cells become binucleate. Most of the homobasidiomycetes are dikaryotic with bipolar or tetrapolar incompatibility systems; only 10% are homothallic (2). *Thanatephorus cucumeris*

TABLE 6. Virulence, as percent mortality and lesion area of four field isolates of *Rhizoctonia solani*, and the heterokaryons synthesized from them on cabbage, sugar beet, red kidney beans and black eye beans

Isolates *R. solani*	Virulence			
	Mortality (%)		Lesion area (mm^2)	
	Cabbage	Sugar beet	Red kidney beans	Black eye beans
Field isolates				
31	100	100	42.2	52.4
108	100	100	50.4	50.3
109	100	100	47.2	46.6
142	100	100	40.3	49.8
Heterokaryons[a]				
108 × 142	0	0	0	0
108 × 109	0	0	0	
108 × 31	0	0	0	0

[a]Twenty-five heterokaryons from each combination were tested.

(=*Rhizoctonia solani*), on the other hand, is multinucleate, homothallic, and seems to lack a system that imposes restrictions on the number of genotypically different nuclei/cell. In addition to the foregoing characters, *T. cucumeris* produces predominantly uninucleate basidiospores and lacks clamps; the total of all of these features are found in few fungi. In fact, Boidin (2) recently surveyed the nuclear condition and presence of clamps in 296 species of "Corticiums" in ten families of the Aphyllophorales. Only six, or 2%, have the same pattern as *T. cucumeris*.

Results of studies on pathogenicity revealed that heterokaryons synthesized from homokaryons did not lead to increased virulence in the heterokaryon, and heterokaryons synthesized from field isolates were avirulent. In a recent publication, Flentje (8) reported that virulence in *R. solani* is dominant and conditioned by a large number of genes. However, the present study gave different results. Our findings indicate that virulence is recessive and determined by multiple factors and avirulence is determined by the accumulative effect of dominant factors. The apparent multiple factor and recessive gene control of virulence in *R. solani* was not surprising, because in other basidiomycetes; e.g., the smuts and rusts, it is well known that virulence is usually recessive (17) and determined by multiple factors (10, 13).

The heterokaryons synthesized from field heterokaryons have genes for both virulence and avirulence, but the mechanism controlling their avirulent condition cannot be explained on the basis of the present data; more research is required for a satisfactory answer.

The loss of virulence in heterokaryons formed from field isolates might, at first, seem to be disadvantageous to the species. On the other hand, such heterokaryons produced a large amount of runner hyphae or large masses of sclerotia and thus may be more fitted to survive in the absence of a host than the parent isolates. At the same time, the heterokaryons derived from two field heterokaryons, though avirulent, carry the gene complement for virulence, awaiting only the right conditions to transfer these factors to homokaryons through the basidiospores. Evidence for the latter was obtained experimentally by inducing heterokaryon 108× 109 to produce basidiospores.

The use of charcoal medium (4) in the synthesis of heterokaryons was vital to the present study. No other medium permitted heterokaryons to form between field isolates and the consistent heterokaryon formation between compatible homokaryons allowed the collection of valid data. Charcoal medium was first used by Day and Anagostakis (5) to induce the dikaryon in *Ustilago maydis*.

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