The Role of the H Locus in Heterokaryosis in Rhizoctonia solani

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ABSTRACT

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Rhizoctonia solani grew as button-like colonies on a medium containing L-sorbose and polygalacturonic acid. Consequently, three auxotrophs were detected and isolated from basidiospores irradiated with ultraviolet light. These were used to determine the function of the two closely linked genes (together called *H* factor) that control heterokaryon formation in *R. solani*. When different monoauxotrophs were paired on minimal agar, strains with either the same *H* factor (*H*=) or different *H* factors (*H*≠) yielded heterokaryons. The *H*≠ heterokaryons were considerably more stable than the *H*= heterokaryons. When fruited, *H*≠ heterokaryons showed strict outcrossing between the two paired strains. The *H*= heterokaryons yielded progeny from both outcrossed and selfed basidia. The *H* locus, therefore, functions like incompatibility loci in other basidiomycetes, and controls the stability and outbreeding of the heterokaryon.

Additional key words: auxotrophic mutations, *Thanatephorus cucumeris,* restrictive growth medium.

Heterokaryosis has been demonstrated among natural isolates of three of the four anastomosis groups (AG) of *Rhizoctonia solani* Kühn (*Thanatephorus cucumeris* Donk) (1, 2, 5, 8, 22). In AG1 and AG4 a nuclear gene locus, called *H,* is associated with the heterokaryosis (1, 8). As many as 17 different *H* factors may exist in natural populations (1). A natural heterokaryon usually has only two *H* factors (8, 9), but Bolkan and Butler (2) formed heterokaryons in the laboratory with up to four *H* factors.

The role of the *H* locus in heterokaryosis is unclear. Its presence is revealed when two single-basidiospore strains with different *H* factors are paired on agar media. A tuft of heterokaryotic mycelium forms along their line of contact (1, 8, 21). Cultures with the same *H* factor do not form the tuft. Even though most natural isolates of *R. solani* are heterokaryotic and carry two *H* factors, heterokaryosis is not essential for survival (14), basidiospore production (5, 22), or pathogenicity (2, 5, 8).

Detection of heterokaryons has been based mostly on the tuft reaction or on differences in morphology between the heterokaryon and its components (8, 13). Forced formation of heterokaryons of *Rhizoctonia* in culture has been reported only by Dodman (4). He made heterokaryons between complementing auxotrophic strains, but did not give details of his results. Also, Dodman used isolates of AG2, in which the *H* locus has not been demonstrated.

In this report, we describe formation of heterokaryons between isolates of *R. solani* carrying complementing auxotrophic markers. The isolates belonged to AG4. Pairings between isolates with the same and different *H* factors were made. A role for the *H* factor is proposed. In addition, a new plating medium for *R. solani* basidiospores is described.

MATERIALS AND METHODS

Fungal strains.—A strain of *Rhizoctonia solani* was recovered from soil of a cotton field near College Station, Texas. This isolate formed basidiospores readily and was assigned to AG4 using the procedure of Parmeter et al. (16). Two *H* factors, arbitrarily designated *H1* and *H2,* were recovered among the basidiospore progeny. Mutant strains were derived from the heterokaryotic wild isolate.

Media.—Minimal medium (MM) consisted of a salts solution with asparagine (11), a utilizable carbon source, at a concentration of 0.5 to 1.0%, and 2.0% agar. For restricted growth of the fungus, L-sorbose (0.5 to 1.0%) also was added. Complete medium (CM) contained 2 g casein hydrolysate, 10 ml vitamin solution (10), and 10 ml of nucleoside solution in each liter of minimal medium. The nucleoside solution contained adenosine, cytidine, uridine, and thymidine each at 1 mg/ml and guanosine at 0.5 mg/ml.

Potato-dextrose agar plus 0.5% yeast extract (PDYA) was used for basidiospore production and stock culture. Determinations of *H* factor were made on minimal agar medium with 1% glucose as carbon source plus 0.1% yeast extract and 1% activated charcoal.

Spore production.—Basidiospores were produced using a modification of the procedure of Stretton et al. (19). An isolate was inoculated at several points on a 9-cm diameter plate of PDYA and incubated at 25 C for 4 days. The plate then was overlaid with approximately 30 g of steam-sterilized soil wetted to a mud. The overlaid culture was kept uncovered and moist at approximately 24 C. Maximum basidiospore yield was obtained after 8-11 days.

The sporulating culture was inverted over a plate of sterile water agar for 1 hour. Sterile water (1.5 ml) then was added to the agar surface, which was rubbed with a flamed glass rod in order to suspend the basidiospores. The spore suspension was drawn off and its concentration determined with a hemocytometer. The sporulating cultures were not inverted directly over water, because the released spores tended to clump on the surface. Addition of Tween-80 to the water did not prevent clumping.

Mutant induction.—For agar-plate irradiations, approximately 2,000-3,000 basidiospores were spread on each of several 9-cm diameter plates of CM containing 0.5% polygalacturonic acid (pH 5) and 0.5% L-sorbose. The agar surfaces of the plates then were exposed to ultraviolet light (UV), maximum emission at 254 nm, at an intensity of 6,000 ergs/sec/cm^2 for 25 seconds. The irradiated plates were incubated in darkness at 24 C until discrete colonies were visible. Colonies distinctly separated from each other then were transferred to fresh CM at 25 C and to MM with 0.5% sucrose and 1% sorbose at 35 C. Colonies that grew on CM at 25 C but not on MM at 35 C were identified either as auxotrophs, temperature-sensitive auxotrophs (auxotrophic only at 35 C), or temperature-sensitive lethals (no growth on any medium at 35 C). Colonies with aberrant morphologies at 35 C also were noted.

For spore suspension irradiations, 6 ml of a suspension of basidiospores (1-2 $\times$ 10^5/ml) were put in a 5-cm diameter glass petri dish and stirred slowly with a magnetic stirring bar. The suspension was then exposed to UV at an intensity of 6,000 ergs/sec/cm^2 for 48 seconds. Irradiated spores were spread on CM and further treated as described for agar-plate irradiations.

Heterokaryon studies.—Heterokaryons were formed at 25 C on MM with 1% glucose. A block of mycelium (2 mm on a side) from a homokaryotic, monoauxotrophic strain was placed against a similar block from a strain with a complementary monoauxotrophic requirement. For comparison, blocks of each monoauxotroph were incubated individually on MM. Growth from the paired blocks was transferred to fresh MM, and single hyphal tips (1 mm) were cut from the edge of this culture. A hyphal tip that grew on MM was selected as a stock culture of a putative heterokaryon.

The stability of heterokaryons was determined by hyphal tip analysis at 25 C. The heterokaryon was grown for 1 day on MM with 1% glucose. Single hyphal tips, 0.75 mm long, were cut from the periphery of the colony and transferred to MM with 0.5% polygalacturonic acid (pH 5) and 0.5% L-sorbose, or to this same medium supplemented with the requirements of the two heterokaryon components. Survival of the tips on these media was noted. Colonies on supplemented MM then were transferred to MM (0.5% sucrose and 1% L-sorbose) supplemented singly with each component's requirement.

Progeny analysis.—Basidiospores from several heterokaryons were spread on CM (0.5% polygalacturonic acid, pH 5, and 0.5% L-sorbose). Colonies were picked from the medium at random, and auxotrophic requirements were determined. Some progeny also were assayed for type of *H* factor.

RESULTS

The effect of sorbose.—Growth of *Rhizoctonia solani* was restricted by L-sorbose, but the degree of restriction depended on the relative amount and kind of utilizable carbon source in combination with L-sorbose. Only a slight restriction of growth occurred in the presence of a high concentration (1.8%) of L-sorbose and a low concentration (0.3%) of glucose. In contrast, growth of *R. solani* was reduced to small button-like colonies on a

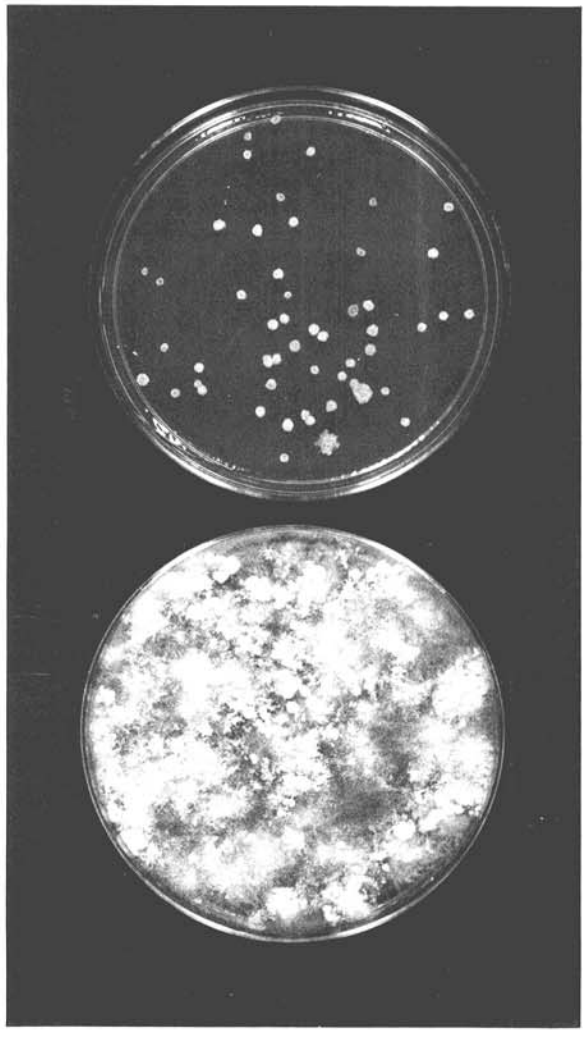

Fig. 1. Colonies from *Rhizoctonia solani* basidiospores spread on minimal agar medium with 1% glucose (bottom), and on minimal agar medium with 0.5% polygalacturonic acid and 0.5% L-sorbose (top). The plates were incubated at 25 C for 6 days.

medium that contained 0.5% L-sorbose and 0.5% polygalacturonic acid, pH 5 (Fig. 1). Colonies that originated directly from spores or from mass mycelial transfers were equally restricted by L-sorbose.

Various sugars and other carbohydrates at 1% were combined with 1% L-sorbose to determine their usefulness in a restrictive medium. The sugars glucose, fructose, sucrose, maltose, cellobiose, glycerol, and lactose were sequentially more favorable for restriction by sorbose. Colony restriction by sorbose with 1% glycerol was comparable to that with 0.5% polygalacturonic acid; over 100 discrete colonies grew without overlap on one plate of either of these media.

The fungus sometimes overcame the effect of L-sorbose after extended periods of incubation on media with sorbose plus maltose, sucrose, or glucose. Moreover, single basidiospores that were treated with UV showed wide variation in sensitivity to sorbose in the presence of the sugars. Colonies from basidiospores on the medium that contained sorbose and polygalacturonic acid generally remained small and button-like.

Mutant yields.—A UV dose of 150,000 ergs/cm^2 killed 90% of the spores subjected to irradiation on agar plates. In suspension, the spores were somewhat more resistant; 180,000 ergs/cm^2 killed only approximately 75% of them, but 288,000 ergs/cm^2 killed 98%.

Mutant yields from one irradiation experiment to the next were variable but generally low (Table 1). Three auxotrophs, designated *ino-1* (requires inositol), *cho-1* (requires choline), and *cas-1* (grows with casein hydrolysate but not with individual amino acids), were recovered as homokaryons with an *H2* factor.

The original auxotrophs *ino-1* and *cas-1* readily formed heterokaryons when paired with the wild homokaryotic *H1* strain. Basidiospore progenies from these heterokaryons included each auxotroph combined with *H1* as well as with *H2*. These autotroph-*H1* factor strains along with the original mutants were subsequently used to study forced heterokaryons.

Heterokaryons.—Table 2 lists the heterokaryons formed in this study. These included three different auxotroph combinations, and each combination included both pairings between two homokaryons with the same *H* factor (*H*= heterokaryons) and two with different *H* factors (*H*≠ heterokaryons). Usually, *H*= heterokaryons formed more slowly and less regularly than *H*≠ heterokaryons. However, both types of heterokaryons involving *ino-1* and *cas-1* formed with equal ease.

The heterokaryons were readily maintained by mass transfer to minimal medium (1% glucose). Almost all hyphal tips from the periphery of the heterokaryotic colony were viable and grew on supplemented minimal medium. However, many tips were not prototrophic. The nonprototrophic tips had the auxotrophic requirement of

TABLE 1. Yields of mutants from basidiospores of *Rhizoctonia solani* irradiated with ultraviolet light (254 nm)

Exp. no.	Irradiation procedure[a]	Survival (%)	No. of survivors tested for mutants	Mutant types[b]
1	Agar plate	6	218	3 m
2	Agar plate	8	1,568	3 a
3	Agar plate	11	2,540	1 a, 1 tsa, 1 tsl, 2 m
4	Spore susp.	2	2,175	2 tsl, 1 m

[a]Basidiospores were exposed to UV light with an intensity of 6,000 ergs/sec/cm^2 for 25 seconds on agar plates or for 48 seconds in suspension.

[b]Legend of abbreviations: m = morphological mutant, a = auxotroph, tsa = temperature-sensitive auxotroph, tsl = temperature-sensitive lethal.

TABLE 2. Hyphal tip analyses of heterokaryons formed between complementary monoauxotrophic strains of *Rhizoctonia solani* with *H* = and *H*≠ factors on minimal agar medium[a]

Heterokaryon components[b]	Hyphal tips surviving on medium (%)		Requirements of the auxotrophic tips[d]
	Minimal	Supplemented minimal[c]	
ino-1 H1 + *cas-1 H1*	62	98	10 *ino*, 34 *cas*
ino-1 H2 + *cas-1 H2*	70	94	14 *ino*, 13 *cas*
ino-1 H1 + *cas-1 H2*	93	99	2 *ino*, 8 *cas*
ino-1 H2 + *cas-1 H1*	95	98	5 *ino*, 3 *cas*
cho-1 H2 + *ino-1 H2*	38	90	39 *cho*, 23 *ino*
cho-1 H2 + *ino-1 H1*	72	97	16 *cho*, 27 *ino*
cho-1 H2 + *cas-1 H2*	15	96	0 *cho*, 71 *cas*
cho-1 H2 + *cas-1 H1*	47	94	41 *cho*, 7 *cas*

[a]At least 110 hyphal tips from each heterokaryon were sampled. The *H*= indicates *R. solani* strains in which both *H* factors are the same; *H*≠ indicates strains in which the *H* factors are different.

[b]Symbol meanings: *ino* = inositol requirement, *cas* = casein requirement, *cho* = choline requirement, *H1* and *H2* = two different *H* factors from wild heterokaryotic parent isolate.

[c]The minimal medium was supplemented with both requirements of the two auxotrophic heterokaryon components.

[d]Hyphal tips that grew on supplemented minimal medium[c], were subtransferred to minimal medium supplemented singly with requirements of the heterokaryon components to identify the specific auxotrophic requirement.

one of the heterokaryon components (Table 2). The frequency of auxotrophic tips varied among the particular combinations of auxotrophs. Moreover, within each combination the $H\neq$ heterokaryons had fewer auxotrophic tips than the $H=$ heterokaryons. Both heterokaryon components were recovered among the auxotrophic tips but frequently in unequal numbers. Eight tips that required casein and two tips that required inositol were selected from $H\neq$ heterokaryons between *ino-1* and *cas-1*. In mating tests on MM plus charcoal, these tips had the same *H* factor as the original auxotrophic strains used to make the heterokaryon.

The four heterokaryons that involved the *ino-1* + *cas-1* combination formed basidiospores. Some sporulated more readily than others, but the ability to sporulate was not related to the *H*-factor combinations. With regard to the two auxotrophic requirements, each heterokaryon gave the two parental combinations (*ino-1* or *cas-1* alone) and two nonparental combinations (*ino-1* + *cas-1* or prototrophy) among the basidiospore progeny. In the two $H\neq$ heterokaryons, the nonparental combinations were as frequent as the parental combinations (Table 3). In contrast, the $H=$ heterokaryon progeny were predominantly parental combinations.

In repeated attempts, none of the individual auxotrophic homokaryons produced basidiospores.

DISCUSSION

The rapid, diffuse growth of *Rhizoctonia solani* was greatly restricted on media that contained L-sorbose. The effectiveness of the sorbose depended on the utilizable carbon source combined with it. Glucose allowed the least restriction by sorbose, and polygalacturonic acid (pH 5) or glycerol allowed the most. Dodman (4) also used sorbose to restrict growth of *R. solani*. He did not achieve good restriction, however, because he used glucose in combination with the sorbose. We found that with 0.5% polygalacturonic acid (pH 5) only 0.5% sorbose was needed to restrict the fungal growth to button-like colonies.

The sorbose-polygalacturonic acid-amended medium allowed conventional procedures for detecting and isolating mutants of *R. solani* after mutagen treatment. The yield of mutants from basidiospores that survived ultraviolet treatment was low. Other workers also reported a dearth of stable induced mutants in *R. solani* (4, 14). Our mutants included auxotrophs, temperature-sensitive auxotrophs, temperature-sensitive lethals, and morphological variants. New selection techniques should be sought for auxotrophs that rarely were isolated by our methods. Moreover, the variation in mutant yields among irradiation experiments suggests that procedures need to be modified.

The only report of heterokaryon formation between complementary auxotrophs of *R. solani* was made by Dodman (4). His strains belonged to AG2, in which no *H* locus has been identified. In our studies, pairings of complementary auxotrophs of AG4 yielded prototrophic colonies. Single hyphal tips from these colonies were often prototrophic which indicated the presence of nuclei from both auxotrophs. The individual components of the pairing were reisolated from cultures started from the prototrophic tips. Moreover, progeny from basidiospores produced by the paired cultures were recombinant for the auxotrophic traits of both components. These findings indicate strongly that the prototrophic colonies were heterokaryotic.

Forcing heterokaryons with complementary auxotrophic mutations permitted the formation of both $H=$ and $H\neq$ heterokaryons. Garza-Chapa and Anderson (8) previously demonstrated hyphal anastomosis between homokaryons that carried the same *H* factor. Our findings confirm their assertion that the *H* locus is not essential for hyphal anastomosis or for the establishment of a heterokaryotic colony. The presence of two different *H* factors in a culture also is not necessary for survival in the soil (15), for basidiospore production (1, 8), or for pathogenicity (2, 8).

The frequency of prototrophic hyphal tips from the periphery of a heterokaryotic colony was used as a measure of the colony's stability. Prototrophic tips were considered to be heterokaryotic. Auxotrophic tips from these same colonies invariably carried single requirements of either one of the heterokaryon components. Within each of the three different pairings of auxotrophs, the $H\neq$ heterokaryons had a higher frequency of heterokaryotic hyphal tips than the $H=$ heterokaryons. In other words, the $H\neq$ heterokaryons were more stable.

Hyphal cells of *R. solani* are multinucleate. Flentje and coworkers (6) showed that the nuclei in the hyphal tip cells divide conjugately. Equal numbers of new nuclei

TABLE 3. Basidiospore progeny from $H=$ and $H\neq$ heterokaryons[a] formed between different auxotrophic strains of *Rhizoctonia solani*

Heterokaryon components[b]	No. of progeny with nutrient requirements		*P* value for 1:1
	Parental[c]	Nonparental[c]	
ino-1 H1 + *cas-1 H1*	123	53	<0.001
ino-1 H2 + *cas-1 H2*	197	105	<0.001
ino-1 H1 + *cas-1 H2*	278	296	.5-.3
ino-1 H2 + *cas-2 H1*	137	144	.7-.6

[a]The symbol $H=$ indicates *R. solani* strains in which both *H* factors are the same; the $H\neq$ indicates strains in which the *H* factors are different.

[b]Symbol meanings: *ino* = inositol requirement, *cas* = casein requirement, *H1* and *H2* = two different *H* factors from wild heterokaryotic parent strain.

[c]Parental types required inositol or casein; nonparental types required both inositol and casein or were prototrophic.

then migrate into the old and newly developing cells. The distribution of the nuclei during this migration is unknown, but it would be of importance if the dividing cell was heterokaryotic. If different nuclear types were distributed at random, some of the newly formed tip cells could be homokaryotic by chance. The frequency of homokaryotic tips then would depend on the number of nuclei per cell and the ratio of the different nuclear types. A heterokaryon with a preponderance of one nuclear type would produce a high frequency of homokaryotic tips, which reflect the disparate nuclear ratio. In our studies, the most unstable heterokaryons also yielded a preponderance of one nuclear type among the homokaryotic tips. Homokaryotic tips from the most stable heterokaryons had more nearly equal frequencies of each nuclear type.

The new nuclei in a dividing tip cell may not be distributed at random. The greater stability of the $H\neq$ heterokaryons, compared to the $H=$ ones, indicates a tendency for nuclei with unlike *H* factor to pair in the hyphae. As a consequence, the developing tip cells of $H\neq$ heterokaryons would be more likely to receive both types of nuclei and therefore to be heterokaryotic. If this is the case, the *H* locus of *R. solani* resembles incompatibility loci in other basidiomycetes. In *Schizophyllum commune,* two unlinked incompatibility loci, *A* and *B*, control the formation and stability of the dikaryon, a heterokaryon in which each cell contains only two nuclei and the nuclei carry unlike *A* and *B* factors. These loci are not essential for hyphal anastomosis or basidiospore production, but they do control nuclear migration, nuclear pairing, and conjugate division (17). Two incompatibility loci (*A* and *B*) also have been demonstrated in *Coprinus lagopus* (20). In *Polyporus palustris* incompatibility is controlled by one locus, called *A*(7). Like the *H* locus of *R. solani,* the incompatibility loci in these other basidiomycetes are multifactorial. Moreover, the *A* and *B* loci in *S. commune,* and the *A* locus in *C. lagopus*, are actually two closely linked genes (3, 12), as is the *H* locus (1).

Progeny analyses of heterokaryons also indicate that the *H* locus regulates nuclear pairing. Anderson and coworkers (1) obtained basidiospores from several $H\neq$ heterokaryons and for each found 1:1:1:1 segregation ratios for the two parental and two nonparental gene pairs. Those ratios indicated that there was strict outbreeding between the two components of the heterokaryons. Any selfing of either of the components would lead to an excess of parental gene combinations. In contrast, heterokaryons of AG2 strains with no known *H* factor yielded progeny showing strict outbreeding (4) or some selfing (13, 18).

Progeny analysis of heterokaryons between *ino-1* and *cas-1* of AG4 showed that each auxotrophic mutation behaved like a single nuclear gene lesion and that the two mutations were unlinked. The equal frequencies of parental and nonparental gene combinations in the $H\neq$ heterokaryons also indicated strict outbreeding; i.e., all basidia carried nuclei with unlike *H* factors before producing basidiospores. In contrast, the $H=$ heterokaryons formed a preponderance of progeny with parental gene combinations. In other words, many basidia originally contained two similar nuclei. The *H* locus, therefore, apparently encourages pairing of unlike nuclei and regulates outbreeding of the fungus, which previously was suggested by Anderson and coworkers (1).

The greater stability of $H\neq$ heterokaryons explains the finding that all natural isolates of *R. solani* (AG1 and AG4) are heterokaryotic and carry more than one *H* factor. In mixed populations the $H\neq$ heterokaryon quickly would tend to replace the $H=$ heterokaryon as they grew in the soil.

LITERATURE CITED

1. ANDERSON, N. A., H. M. STRETTON, J. V. GROTH, and N. T. FLENTJE. 1972. Genetics of heterokaryosis in Thanatephorus cucumeris. Phytopathology 62:1057-1065.
2. BOLKAN, H. A., and E. E. BUTLER. 1974. Studies on heterokaryosis and virulence of Rhizoctonia solani. Phytopathology 64:513-522.
3. DAY, P. R. 1960. The structure of the A mating type locus in Coprinus lagopus. Genetics 45:641-650.
4. DODMAN, R. L. 1972. Heterokaryon formation and genetic recombination between auxotrophic and morphological mutants of Thanatephorus cucumeris. Austr. J. Biol. Sci. 25:739-748.
5. FLENTJE, N. T., and H. M. STRETTON. 1964. Mechanisms of variation in Thanatephorus cucumeris and T. practicola. Austr. J. Biol. Sci. 17:686-704.
6. FLENTJE, N. T., H. M. STRETTON, and E. J. HAWN. 1963. Nuclear distribution and behavior throughout the life cycles of Thanatephorus, Waitea and Ceratobasidium species. Austr. J. Biol. Sci. 16:450-467.
7. FLEXER, A. S. 1969. Bipolar incompatibility in the hymenomycete Polyporus palustris. Am. J. Bot. 56:410-417.
8. GARZA-CHAPA, R., and N. A. ANDERSON. 1966. Behavior of single-basidiospore isolates and heterokaryons of Rhizoctonia solani from flax. Phytopathology 56:1260-1268.
9. GROTH, J. V., and N. A. ANDERSON. 1969. Heterokaryon incompatibility in Rhizoctonia solani. Phytopathology 59:1028 (Abstr.).
10. HOLLIDAY, R. 1961. The genetics of Ustilago maydis. Genet. Res. 2:204-230.
11. HUNTER, R. E. 1974. Inactivation of pectic enzymes by polyphenols in cotton seedlings of different ages infected with Rhizoctonia solani. Physiol. Plant Pathol. 4:151-159.
12. KOLTIN, Y., J. R. RAPER, and G. SIMCHEN. 1967. The genetic structure of the incompatibility factors of Schizophyllum commune: the B factor. Proc. Nat. Acad. Sci. USA 57:55-62.
13. MC KENZIE, A. R., N. T. FLENTJE, H. M. STRETTON, and M. J. MAYO. 1969. Heterokaryon formation and genetic recombination within one isolate of Thanatephorus cucumeris. Austr. J. Biol. Sci. 22:895-904.
14. MEYER, R. W., and J. R. PARMETER, JR. 1968. Changes in chemical tolerance associated with heterokaryosis in Thanatephorus cucumeris. Phytopathology 58:472-475.
15. OLSEN, C. M., N. T. FLENTJE, and K. F. BAKER. 1967. Comparative survival of monobasidial cultures of Thanatephorus cucumeris in soil. Phytopathology 57:598-601.
16. PARMETER, J. R., JR., R. T. SHERWOOD, and W. D. PLATT. 1969. Anastomosis grouping among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-1278.
17. RAPER, J. R. 1966. Genetics of sexuality in high fungi. Ronald Press, New York. 283 p.

18. STRETTON, H. M., and N. T. FLENTJE. 1972. Inter-isolate heterokaryosis in Thanatephorus cucumeris. I. Between isolates of similar pathogenicity. Austr. J. Biol. Sci. 25:293-303.
19. STRETTON, H. M., A. R. MC KENZIE, K. F. BAKER, and N. T. FLENTJE. 1964. Formation of the basidial stage of some isolates of Rhizoctonia. Phytopathology 54:1093-1095.
20. SWIEZYNSKI, K. M., and P. R. DAY. 1960. Heterokaryon formation in Coprinus lagopus. Genet. Res. 1:114-128.
21. VEST, G., and N. A. ANDERSON. 1968. Studies on heterokaryosis and virulence of Rhizoctonia solani isolates from flax. Phytopathology 58:802-807.
22. WHITNEY, H. S., and J. R. PARMETER, JR. 1963. Synthesis of heterokaryons in Rhizoctonia solani Kühn. Can. J. Bot. 41:879-886.