INTRASPECIFIC AND INTERSPECIFIC AVERSION IN DIPLODIA[1]

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INTRODUCTION

In the course of an extensive plating program involving the identification of fungi associated with the ear-rot diseases of dent corn, strains[3] of *Diplodia zeae* (Schw.) Lév.[4] were observed which in culture manifest intraspecific aversion, a phenomenon infrequently reported in fungi. It was observed that when adjacent colonies of *D. zeae* appeared in the culture plates one of two contrasting reactions occurred where the mycelia came together: The hyphae either intermingled freely, with a piling up or "drifting" of the mycelium at the line of juncture, or, more frequently, an antagonistic reaction took place in which growth at the tips ceased and was apparently followed by a dying back of the mycelium. This was followed by a darkening of the agar between the averting colonies, delimiting them cleanly from each other (fig. 1). The aversion phenomenon occurred upon all culture media tried, including potato-dextrose agar, oatmeal agar, and Coons', Richards', and Czapeks' media. Aside from the fundamental aspects of the problem, further studies on aversion in this economically important fungus seemed justified on the basis of practical considerations, especially in relation to pathogenicity studies and inoculation technique. The present paper includes the results obtained in studies involving (1) stability of strains of *D. zeae* for aversion reactions, (2) number and distribution of strains, and (3) inoculation experiments with single strains and mixtures of strains of *D. zeae* and *D. macrospora*.

OCCURRENCE OF INTRASPECIFIC AVERSION IN FUNGI

A study of the literature reveals a growing list of cases in which aversion has been observed within specific lines of fungi. The cases cited below are those which the writer believes bear analogy to the situation in *Diplodia* to the extent that the manifestation of the phenomenon appears to be dependent upon genetic differences in the cultures involved. Cayley (*3*)[5], in 1923, first directed particular attention to this phenomenon in her studies with *Diaporthe perniciosa*. In a later paper Cayley (*4*) cites, as additional cases, those described by Brunswik in *Coprinus fimetarius* and in *C. friesii;* by Vandendries in *C. micaceus, C. radians*, and *Panaeolus complanatus;* by Nakata in *Sclerotium rolfsii;* and by Mounce in *Fomes pinicola*. More recently

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[3] The term "strain", as used in the present paper, refers to any *Diplodia* culture or group of cultures that shows aversion to all other *Diplodia* cultures.

[4] The aversion phenomenon also has been observed in *Diplodia macrospora* Earle.

[5] Reference is made by number (italic) to Literature Cited, p. 679.

cases of intraspecific aversion have been reported by Vandendries in *Pleurotus columbinus* (*8*) and *P. ostreatus* (*9*), by Vandendries and Brodie (*10*) in *Lenzites betulina*, and by Brodie (*1*) in *Corticium calceum*.

STABILITY OF AVERSION REACTIONS OF STRAINS OF DIPLODIA ZEAE

Twenty-one cultures of *Diplodia zeae*, including 12 strains, all isolated from rotted kernels of dent corn, were used in tests for studying the stability of the particular aversion reactions. Stability of reaction was maintained in every culture, (1) following mycelial propagation, (2) through successive pycnidial generations, and (3) following reisolations from inoculated corn plants. Two-percent potato-dextrose agar was used in all plating tests on stability.

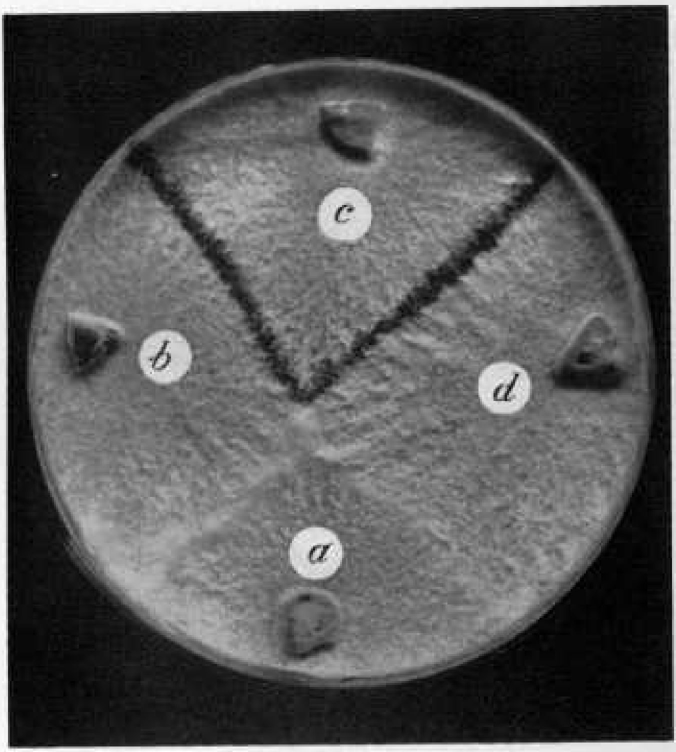

FIGURE 1.—Plate cultures showing aversion between strains of *Diplodia zeae*. Aversion is shown between colony *c* and colonies *b* and *d*. Colonies *a*, *b*, and *d* are of the same strain and show no aversion to one another. Colonies *a* and *c* would show aversion to each other if their mycelia came together. Note the intermingling or "drifting" of the mycelium between colonies *a* and *b* and between *a* and *d*.

The question first studied was whether two cultures of *Diplodia zeae* that show aversion to each other are of the same strain or of different strains. Plating mycelial inocula from any single culture of *D. zeae* at opposite sides in a Petri dish always resulted in a free intermingling of the hyphae, with a piling up or drifting of the mycelia where they met. In contrast, when mycelial inocula from two averting cultures were plated at opposite sides in a Petri dish, the characteristic aversion reaction always occurred. It naturally followed that all isolates of a given strain, wherever obtained reacted alike toward other cultures. For example, if cultures A and B were found to be alike, and if C and D also were alike, then, if further platings showed A and C to be alike, it was invariably found that B and D likewise

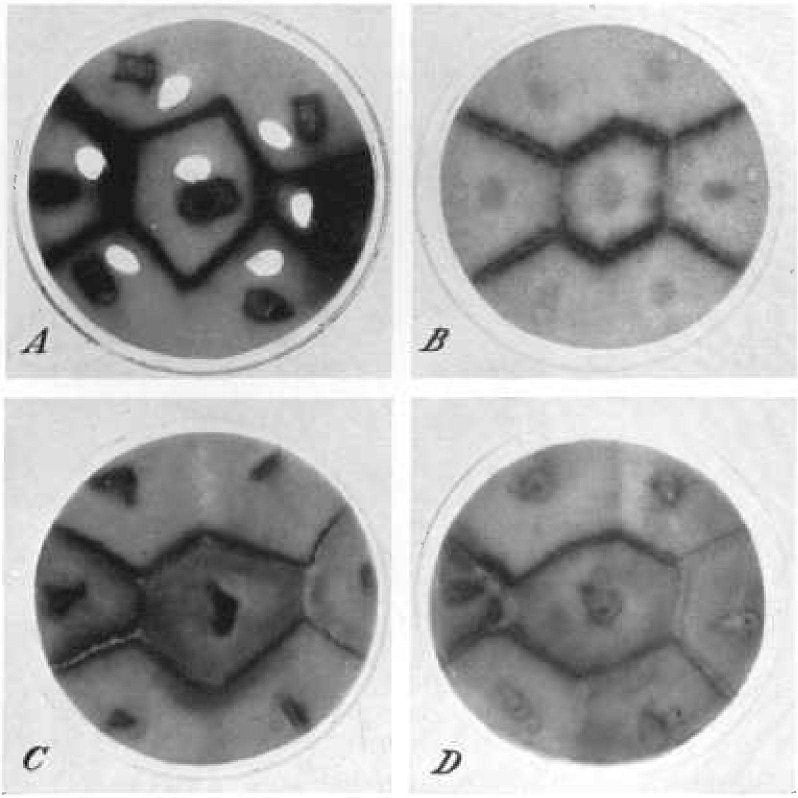

FIGURE 2.—Stability of aversion reactions in strains of *Diplodia zeae* as shown by plate cultures. *A*, Seven original colonies of *Diplodia* growing from corn kernels. *B*, The same cultures following propagation by means of mycelial transfers. *C*, Cultures again combined following three successive pycnidial generations. Monospore cultures were used throughout. *D*, The same cultures again combined following reisolations from inoculated ears of corn. The cultures in *C* were used for pycnospore production for the inoculations, therefore four pycnidial generations actually were involved in these stability studies.

would intermingle, and that any one of the four would react in exactly the same manner as any of the others when plated against any different strain.

STABILITY FOLLOWING MYCELIAL PROPAGATIONS

The 21 cultures used for the studies on stability following mycelial propagation were propagated in groups of 7 in each culture plate. The three groups involved five, four, and three different strains, respectively, which were so arranged on the plates that each group presented its characteristic design as a result of the reactions between the strains. Mycelial transfers to other plates, with the cultures placed in the same relative positions, resulted in every case in the same type of design as that in the original plate (fig. 2, *B*). This evidence of stability was maintained in all cultures through each of three successive transfers.

STABILITY THROUGH SUCCESSIVE PYCNIDIAL GENERATIONS

After the cultures had been propagated three times by means of mycelial transfers, transfers were made to oatmeal agar to induce abundant pycnidial development. Three monosporic cultures were obtained from each of 3 randomly selected pycnidia from each of the 21 cultures. Plating the three cultures from a given pycnidium against one another resulted always in intermingling of the hyphae when the cultures met, indicating a uniformity in reaction for all spores from a given pycnidium. A similar lack of aversion resulted when representative colonies from the three pycnidia from a given culture were plated against one another, indicating that all the pycnidia from that culture were alike. Finally, plating the various cultures in their respective original positions resulted again in the designs originally observed in each plate group (fig. 2, *C*). This procedure was carried through three successive pycnidial generations with no changes in the behavior of any of the progenies, thereby demonstrating the stability of the aversion reactions of the strains through three pycnidial generations.

STABILITY FOLLOWING REISOLATION FROM INOCULATED CORN PLANTS

Cultures from the third pycnidial generation were used for field inoculations to study possible changes in reactions following passage through the host. The seven cultures illustrated in figure 2, *C*, were used in this experiment. A spore suspension from each of the cultures was injected with a hypodermic needle into the tips of five ears in each of three inbred lines of dent corn. Platings of the reisolated cultures against the original stock cultures proved that no changes had occurred in the reactions of any of the strains. When the isolates again were combined on plates in their original relative positions, the same design again was obtained (fig. 2, *D*), thus demonstrating the stability of the strains through the pathogenic phase of their life cycles.

NUMBER AND DISTRIBUTION OF STRAINS

Some information on the extent and distribution of strains of *Diplodia zeae* was obtained from experiments in which randomly selected cultures were paired on plates in all possible combinations. Twenty-five cultures of *D. zeae*, isolated from 25 ears of corn collected from 1 field near Bloomington, Ill., were used for a so-called local test. The results from the platings showed 1 strain to occur 3 times, 2 others to occur twice each, and 18 to occur but once, thus making a total of 21 strains in this random group of 25 cultures. For a regional test 25 cultures of *D. zeae* were obtained from as many widely separated points throughout the Corn Belt. In this experiment only two of the cultures proved to be of the same strain. Observational evidence from plating many hundreds of colonies of *D. zeae* from damaged kernels in terminal market samples of corn indicates further the existence of a multiplicity of strains. Aversion between adjacent colonies has usually been the rule, intermingling being relatively rare.

INOCULATIONS WITH MIXTURES OF STRAINS

Inoculation experiments involving mixtures of strains of *Diplodia zeae* suggested two problems of particular interest: (1) Would aversion function in the host tissues to such an extent that one strain would inhibit the further development of the others and thus singly overrun the host? (2) If this should prove to be true, would it be possible to use such mixtures for determining the relative pathogenicity of strains? In field experimental work, a mixture of several cultures frequently has been used on the assumption that this provided a safeguard against the use of an inferior culture.

EXPERIMENTS IN 1934

Three strains of *Diplodia zeae* isolated from rotted corn kernels from Maryland, Ohio, and Illinois were used in a mixed-strain experiment conducted at Madison, Wis., in 1934. In future references these strains are called nos. 150, 26, and 73, respectively. Two inbred lines of corn, namely, R4, which is resistant to ear infections of *D. zeae*, and Lan, which is susceptible, were inoculated by hypodermic injections of spore suspension into the tips of the ears while in the milk stage of development. Care was taken that the cultures were of the same age and that the inocula used were of approximately equal spore concentrations. Inoculations first were made with single cultures to test for pathogenic differences between the fungus strains, after which equal volumes of the remaining inocula were combined and used for the mixed-strain inoculations. The inoculated ears were harvested about 6 weeks later. It was apparent that the extent of rotting that had developed in the ears was no more severe when the mixture of strains was used than when the strains were used separately. Nor were any decided differences in pathogenicity evident between the three strains used. These relationships held in both the resistant and susceptible inbred lines.

The identity of the fungus strains recovered from the rotted ears was established by means of a series of platings that involved, finally, the pairings of representative cultures from each ear against the stock cultures of the strains used in the experiment. Five kernels, taken spirally from each of the rotted ears, were used as a basis fcr the determination of the strain or strains in the ear. The results from the identification of the reisolated cultures are given in table 1.

TABLE 1.—*Strains of Diplodia zeae recovered from ears of corn inoculated with single strains and with a mixture of these strains at Madison, Wis., 1934*

Strains used for inoculum	Inbred [1] lines inoculated	Ears inoculated	Inoculated ears from which indicated strains were recovered		
			No. 150	No. 73	No. 26
Single strains:		*Number*			
No. 150	R4	15	15		
	Lan	15	15		
No. 73	R4	15		15	
	Lan	15		15	
No. 26	R4	15			15
	Lan	15			15
Mixed strains:					
Nos. 150, 73, and 26	R4	15			14
	Lan	14		[2] 1	14

[1] R4 is resistant and Lan is susceptible to diplodia ear rot.
[2] Tip kernels of 1 ear plated strain 73; the remainder of the ear plated strain 26.

It will be observed from table 1 that the strain recovered from each of the single-strain inoculations was the same as that used in the inoculum. This was to be expected in view of the stability of the aversion reactions of strains of *Diplodia zeae* as established in the earlier experiments. The data also show that in the mixed-strain inoculations, with one exception, strain no. 26 was the only one reisolated, and apparently it had completely inhibited the development of strains nos. 150 and 73, both in the resistant and in the susceptible inbred line of corn.

EXPERIMENTS IN 1935

In view of the consistency with which strain no. 26 inhibited the others in the mixed inoculations in 1934, it was decided to repeat the experiment with certain modifications the following season. As no differentials in reaction between inbred lines and fungus strains were obtained in 1934, it was decided to use but one inbred, Wis. R3, in 1935, and to concentrate on a somewhat more elaborate series of strain mixtures.

A strain of *Diplodia macrospora* was added to the three strains of *D. zeae* previously used for observations on interspecific aversion. In addition to the single-strain inoculations, the following mixtures were used: *D. zeae*, nos. 150 and 73; 150 and 26; 73 and 26; 150, 73, and 26; and *D. macrospora* and *D. zeae* nos. 150, 73, and 26. The methods for inoculation and for identification of cultures recovered were similar to those of the previous season. The results from the experiments are given in table 2.

TABLE 2.—*Strains of Diplodia zeae and D. macrospora recovered from ears of corn of inbred line Wis. R3 inoculated with single strains and various mixtures of these strains and species, at Madison, Wis., 1935*

Species and strains used as inoculum	Ears inoculated	Inoculated ears from which the indicated strains were recovered				
		D. macrospora	*D. zeae* no. 150	*D. zeae* no. 73	*D. zeae* no. 26	Mixture of strains
Single strains:	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
D. macrospora	14	[1] 12	0	0	0	0
D. zeae no. 150	14	0	14	0	0	0
D. zeae no. 73	15	0	0	15	0	0
D. zeae no. 26	11	0	0	0	11	0
Mixed strains:						
D. zeae nos. 150 and 73	15	0	0	15	0	0
D. zeae nos. 150 and 26	13	0	0	0	13	0
D. zeae nos. 73 and 26	15	0	0	1	14	0
D. zeae nos. 150, 73, and 26	36	0	0	0	28	[2] 8
D. macrospora and *D. zeae* nos. 150, 73, and 26	33	0	2	1	24	[3] 6

[1] Of 14 ears inoculated 12 developed ear rot.
[2] Strain no. 26 predominated in 6 of the 8 ears from which more than 1 strain was isolated. Strain no. 73 predominated in 2 of the 8 ears. Strain no. 150 was isolated from tip kernels in only 1 ear.
[3] *D. zeae* no. 73 predominated in 4 of the 6 ears from which more than 1 strain was isolated. *D. zeae* no. 150 predominated in 2 of the 6 ears. *D. zeae* no. 26 was isolated from all 6 of the ears. *D. zeae* nos. 73, 150, and 26 were all isolated from 1 ear. *D. macrospora* was absent in all ears.

Except for *Diplodia macrospora,* which appeared decidedly less pathogenic under the conditions of this experiment, no differences in extent of ear rot were evident in any of the inoculations, either in the comparisons between strains or between the various mixtures of strains. The data in the table show that the cultures recovered from the single-strain inoculations were the same as those used in the inoculum, again demonstrating stability following passage through the host.

Of the three strains of *Diplodia zeae*, no. 73 alone was recovered from the 15 ears inoculated with a mixture of nos. 73 and 150. Apparently it had completely inhibited the development of the latter. In a similar manner strain no. 26 completely inhibited the development of strain no. 150 in all of the 13 ears inoculated with a mixture of the two strains. Strain no. 26 inhibited strain no. 73 in 14 out of 15 ears inoculated with this mixture and was itself inhibited by strain no. 73 in the 1 remaining ear. Thus a definite sequence in inhibitory effects occurred among these three strains, no. 73 inhibiting no. 150, and no. 26 inhibiting both no. 73 and no. 150.

Strain no. 26 in most instances maintained its inhibitory powers in the mixture involving all three strains. It alone was recovered from 28 of the 36 ears inoculated with this mixture. In addition to no. 26, other strains also were recovered from each of the remaining eight ears of this group. Strain no. 26 predominated on six of these ears (it alone was plated in the region between the middle and the butt of the ear); strain no. 73 predominated on two of the ears; and strain no. 150 was recovered from tip kernels of only one ear.

The results from the mixture involving the three strains of *Diplodia zeae* and one strain of *D. macrospora* again showed *D. zeae* no. 26 to be the dominating one. It alone was recovered from 24 of the 33 ears in this group. *D. zeae* no. 150 was the only one recovered from two ears, and *D. zeae* no. 73 was the only one recovered from one ear. *D. zeae* no. 26 also was recovered from each of the remaining six ears that yielded more than one strain of the fungus. *D. zeae* no. 73 predominated on four of these ears, and *D. zeae* no. 150 predominated on the remaining two ears. In no case was *D. macrospora* recovered.

These experiments demonstrated that *Diplodia zeae* no. 26 was able to manifest its inhibitory powers, in the various mixtures of strains used, in two successive seasons and in three inbred lines of corn.

DISCUSSION

The occurrence of intraspecific aversion in *Diplodia* represents an exception to the general rule as stated by Porter (*7*), i. e., that antagonism between fungi becomes more marked as the phylogenetic relationships are widened. It is beyond the scope of the present paper to discuss the hypotheses that have been advanced to explain aversion, but it is desirable to point out an important and basic difference between aversion in *Diplodia* and a phenomenon sometimes observed within specific lines in other fungi. Accepting Brown's definition (*2, p. 106*) of staling as the effect of "those metabolic products of the organism responsible for slowing down or stopping its growth", in the opinion of the author it becomes apparent that aversion in *Diplodia* is not due to staling products. Aversion in *Diplodia* results from the interaction between two physiologically different strains. In some other genera an entirely different situation exists, which might be confused with intraspecific aversion. In *Chaetomium* sp., for example, a very distinct inhibition of growth occurs when two colonies approach each other in culture. However, an identical reaction is observed when a given culture is plated against itself. This, it seems, is either a nutritional response, or, more probably, a reaction closely allied to Brown's conception of staling. In either case it does not establish the existence of distinct physiological differences such as appear to exist in the strains of *Diplodia.*

In the literature dealing with intraspecific aversion the aspect of the problem studied most intensively concerns the relationship between the phenomenon and sex. Among studies on the inheritance of the phenomenon and its complex sexual relationships in Ascomycetes and in Hymenomycetes are papers by Cayley (*3*, *4*), Vandendries (*8*, *9*), and Vandendries and Brodie (*10*).

Little of the previous work on aversion is directly analogous with the studies reported herein with *Diplodia.* The fact that like meets like with intermingling of the mycelia and that aversion results when unlike strains meet has been reported by Cayley (*3*) in a homothallic form of *Diaporthe perniciosa*, by Nakata (*6*) in *Sclerotium rolfsii*, and by Mounce (*5*) in *Fomes pinicola.* A case in which unlike strains sometimes intermingle is described by Cayley (*4*), and this she believes to be a heterothallic form of *Diaporthe.* In this instance mycelia which meet can segregate into averting and nonaverting strains in later perithecial generations.

The uniformity in reaction of all monosporic mycelia from individual pycnidia in *Diplodia zeae* is in agreement with Cayley's (*3*) results with *Diaporthe.*

The multiplicity of strains found in *Diplodia zeae* agrees generally with studies on this aspect by Cayley (*3*), Mounce (*5*), and Nakata (*6*), with *Diaporthe perniciosa*, *Fomes pinicola*, and *Sclerotium rolfsii*, respectively.

No previous accounts of mixed-strain inoculations are known to the writer. Cayley (*4*) raised the question whether the aversion reaction might possibly be inhibited in host tissues owing to absorption of the toxins secreted by the fungus. A case of aversion between colonies of *Corticium calceum* on a piece of pine wood was recently described by Brodie (*1*). He believed this to be the first reported case of the occurrence of the phenomenon in nature.

The results from the mixed-strain inoculations with *Diplodia* indicate that the aversion reaction functions within the host. The consistency with which a strain eventually gets the upper hand and rots the major portion of the ear can hardly be explained on any other basis. The writer does not recall having plated more than one strain of *D. zeae* from naturally infected ears, except possibly where separate infections had occurred, one at the tip and another at the butt end of the ear.

No conclusions could be drawn from the mixed-strain experiments regarding the relationship between ability to inhibit and degree of pathogenicity. Despite the definite sequence in inhibitory powers manifested among the strains in the mixed inoculations, no appreciable differences in extent of ear rot were apparent when the strains were used singly in inoculations. It is quite conceivable, however, that an improved technique for comparing relative pathogenicity might reveal differences not apparent in these experiments. On the other hand, it is possible that physiological studies on the fungus strains might show differences in rates of spore germination and subsequent growth which might be correlated with their so-called inhibitory powers.

The results from the inoculations with mixtures of strains indicate that, for practical purposes, the use of mixed cultures accomplishes little beyond the diluting of the dominating strain used. The method does provide a technique for the selection of a very dominating strain for inoculations in experimental work.

The many strains of *Diplodia zeae*, each of which apparently remains constant or stable for its aversion character, raises a question as to the origin of all these strains. If the capacity of strains to inhibit others is an attribute of *Diplodia* strains generally, should not the strains in a given region have become limited through natural selection to a relatively small number? This situation seemingly does not exist. The evidence reported herein indicates that the differences among the strains are genetic and suggest the possible existence in this species of a sexual stage not yet described.

SUMMARY

The phenomenon of intraspecific aversion, infrequently reported in fungi, has been found in *Diplodia zeae* (Schw.) Lev. and *D. macrospora* Earle, and interspecific aversion has been found between these two species.

The stability of the particular aversion reactions of the different strains of *Diplodia zeae* was maintained, after successive mycelial propagations, through three successive pycnidial generations in culture and through inoculation into ears of corn and subsequent reisolation.

The number of strains of *Diplodia zeae* apparently is very large. Twenty-one different strains were obtained from 25 cultures isolated from as many ears of corn collected in one field near Bloomington, Ill. Among 25 isolates from widely separated points throughout the Corn Belt, 24 strains were obtained.

Field experiments involving inoculations with single strains and with mixtures of strains were conducted at Madison, Wis., in 1934 and 1935. Inoculations with various combinations of three strains of *Diplodia zeae* and a strain of *D. macrospora* were made in three inbred lines of corn. Identification of the strains recovered from the subsequently rotted ears proved that inhibitory effects had occurred so that usually only one strain of the fungus could be reisolated from an ear. It was also found that a definite sequence existed in the inhibitory powers of strains upon one another. So far as tried, *D. zeae* predominated over *D. macrospora*.

No conclusions could be drawn regarding the relationship between the capacity of strains for inhibiting the development of others and their degrees of pathogenicity.

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