STUDIES IN THE PHYSIOLOGY OF THE FUNGI

VIII. Mixed Cultures

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Some consideration has been given in previous literature to the behavior of fungi in mixed cultures, but this has been sufficiently reviewed by Harder,[1] whose work along the line seems to be the most complete. His conclusions, however, are based merely upon observations on the rate of growth and color production in the medium and mycelium. The purpose of his work was to determine whether the inhibition or stimulation of growth, as the case may be, might not be the result of the depletion of the available carbohydrates in the medium or a change in the hydrogen ion concentration.

In the present work the following fungi were used: *Lenzites vialis* Pk., *Merulius pinastri* (Fr.) Burt, *Daedalea quercina* (L.) Fr., *Trametes Peckii* Kalchbr., *Pleurotus sapidus* Kalchbr., *Merulius lacrymans* (Wulf.) Fr., *Lentinus lepideus* Fr., *Daedalea confragosa* (Bolt.) Fr., *Coniophora cerebella* Pers., *Polystictus versicolor* (L.) Fr., *Isaria* sp., *Polyporus lucidus* (Leys.) Fr., *Polystictus hirsutus* Fr., *Aspergillus glaucus*,[2] *A. niger* Van Tieg., *A. fumigatus* Brizi, *A. versicolor* Tiraboschi, and *A. Sydowi* Bainier and Sartory.

All the fungi were grown upon 2 per cent potato agar plates prepared in the manner previously described.[3] After growth

[1] Harder, R. Uber das Verhalten von Basidiomyceten und Ascomyceten in Mischkulturen. Naturwiss. Zeitschr. f. Forst- u. Landw. 9: 129–160. *pl. 3–4. f. 1–2.* 1911.

[2] Thanks are due to Dr. Charles Thom for the determination of the *Fungi Imperfecti* included in this list.

[3] Zeller, S. M., Schmitz, H., and Duggar, B. M. Studies in the physiology of the fungi. VII. Growth of wood-destroying fungi on liquid media. Ann. Mo. Bot. Gard. 6: 137–142. 1919.

TABLE I

Fungus	Organisms stimulating	Organisms inhibiting	Organisms overgrowing	Organisms not influencing
L. vialis	D. quercina*	T. Peckii*, P. sapidus*, Isaria*, A. niger†, A. Sydowi*	T. Peckii, P. versicolor, P. hirsutus	L. vialis, L. lepideus, D. confragosa
M. pinastri		D. quercina†, T. Peckii*, L. lepideus*, D. confragosa†, C. cerebella†, P. versicolor*, P. lucidus*, P. hirsutus*, A. fumigatus†, A. Sydowi*	M. pinastri, T. Peckii, P. sapidus, A. niger	M. pinastri
D. quercina	M. lacrymans*, D. confragosa*, P. lucidus*, P. hirsutus*	M. pinastri†, L. lepideus*, C. cerebella*, Isaria*, A. niger*	T. Peckii, P. sapidus, P. versicolor	L. vialis, D. quercina
T. Peckii	D. quercina*, A. fumigatus*, A. glaucus*	D. confragosa*, Isaria*, A. glaucus*, A. versicolor†, A. Sydowi*	P. sapidus	
P. sapidus		L. vialis*, L. lepideus†, P. versicolor†, P. lucidus*, P. hirsutus†	D. quercina, M. lacrymans	P. sapidus
M. lacrymans		L. lepideus†, P. versicolor†, P. lucidus†, A. fumigatus†	M. pinastri, T. Peckii	
L. lepideus		M. pinastri*, P. sapidus†, P. lucidus†	T. Peckii, P. versicolor	L. vialis
D. confragosa	M. lacrymans†	M. pinastri†, T. Peckii†, P. lucidus†, P. hirsutus†	P. sapidus	L. vialis, A. fumigatus
C. cerebella	L. vialis,* M. lacrymans†	M. pinastri†, D. quercina†, P. versicolor*, A. glaucus*, A. niger*	T. Peckii	

P. versicolor	T. Peckii*	A. versicolor†, A. Sydowi†, M. pinastri*, P. sapidus†, A. niger*, M. lacrymans†, D. confragosa†, C. cerebella†*, P. lucidus†, P. hirsutus†, A. fumigatus*	D. quercina	P. versicolor, A. glaucus
Isaria sp.?		M. pinastri†, A. niger†	P. sapidus, P. versicolor, P. lucidus, P. hirsutus	Isaria
P. lucidus	M. lacrymans*, Isaria*, A. niger*	L. vialis†, M. pinastri*, P. sapidus*, M. lacrymans†, L. lepideus†, D. confragosa†, C. cerebella*, P. versicolor†, A. glaucus†, A. versicolor†	T. Peckii, A. niger	P. lucidus
P. hirsutus		M. pinastri*, P. sapidus†, D. confragosa†, P. versicolor†, A. versicolor*, A. Sydowi*, A. glaucus*	L. vialis, T. Peckii, M. lacrymans, B. cerebella, P. lucidus	P. hirsutus
A. glaucus		T. Peckii*, C. cerebella*, P. hirsutus*, A. glaucus†, A. niger†, A. fumigatus*, A. versicolor†	L. vialis, T. Peckii, P. sapidus, P. lucidus, P. hirsutus	A. fumigatus, A. Sydowi
A. niger		A. versicolor*, A. Sydowi†, D. quercina*, M. lacrymans†, D. confragosa*, C. cerebella*, P. lucidus*, P. hirsutus*, A. glaucus†, A. fumigatus*	T. Peckii, P. sapidus	
A. fumigatus		M. pinastri†, M. lacrymans†, A. glaucus*, A. versicolor†, A. Sydowi†	T. Peckii, Isaria, P. lucidus	D. confragosa, A. fumigatus
A. versicolor		P. hirsutus*, A. fumigatus†, A. glaucus†	P. sapidus, A. Sydowi	P. versicolor, A. glaucus
A. Sydowi		P. hirsutus*, A. niger†, A. fumigatus†	P. sapidus, Isaria, P. lucidus, A. versicolor	

*=after contact; † =before contact.

of the fungi the plates were cut into small squares (about 8 mm. square), which were used as inocula. Agar plates made in a similar manner were each inoculated with three of the fungi in such a way as to have all possible combinations of each fungus. From these plates the reciprocal influence of growth was determined. The results of the plate cultures are shown in table 1.

The outstanding feature of these results is the preponderance of inhibition of growth of one fungus before and after contact with another. In some cases this inhibition took place when the two colonies were still a considerable distance apart; in others only when they came into close proximity with each other. Figures 5 and 2 respectively of pl. 4 illustrate this feature. In those cases where inhibition occurred after contact the condition is shown by a straight line unless one fungus has a much more rapid growth than the other. Figures 8 and 11 illustrate this point. It often happened that one fungus on the plate grew much more rapidly than the other two, cutting off contact between them. Therefore, all possible combinations could not be recorded in the table.

There were not as many instances where one fungous colony grew over another as there were of inhibition of growth. In some cases of the former type one colony was completely covered, and the shape of the submerged colony determined that of the colony of the invading fungus, as, for example, in the case of *Pleurotus sapidus* growing over *Aspergillus glaucus* and *A. Sydowi* (see figs. 3 and 6). In these cases the growth of *Pleurotus sapidus* is greatly accelerated as soon as it reaches the colonies. In other cases the growth of the invading fungus was comparatively slow, as, for example, when *Pleurotus sapidus* invaded a colony of *Aspergillus niger* (see fig. 9). At first both of these fungi were mutually inhibited and then *Pleurotus sapidus* gradually advanced. A peculiarity of this special case is the fact that the spores of *Aspergillus niger* disappeared in the invaded section. It could not be determined whether these spores germinated or were digested.

When two colonies of the same fungus came into contact there was usually no influence of the one colony on the other; that is,

the mycelium of the two thoroughly intermixed, as fig. 12 in the plate shows for *Merulius pinastri*. An exception to this general condition is illustrated when two colonies of *Aspergillus niger* grew together. At first there was an inhibition of growth as shown by a straight line formed by the margins of the two colonies. Later, however, the two colonies generally intermixed. As table 1 shows, there was often an intermixing of the mycelium of two different species. This may be explained by the theory advanced by Clark[1] that many deleterious substances, which at certain concentrations retard growth, later cause great acceleration of mycelial development in the retarded cultures.

Cases of stimulation of growth when two colonies came into contact were comparatively rare, while cases of stimulation before contact occurred seldom indeed. However, examples of both these types were observed. In many cases it is hard to distinguish between true stimulation and a mere heaping up of the mycelium due to mechanical hindrance. Figures 7 and 10 of the plate show a stimulation of *Trametes Peckii* in contact with *Daedalea quercina*. At first there was a heaping up of the mycelium, and this appeared to be a great stimulation of growth. However, this may equally well be considered a mere increase in the amount of aërial mycelium due to a mechanical hindrance of the surface of the medium. A peculiar case of stimulated growth of *Daedalea confragosa* is shown in fig. 1. It is not certain whether this is caused by the presence of *Merulius lacrymans* or some other factor. However, there does not seem to be any valid reason why a stimulation by diffusion should not be expected as much as inhibition of growth by diffusion, as where *Lentinus lepideus* and *Aspergillus glaucus* are mutually inhibited before contact. In the latter case the colonies never came together. A slight stimulation of growth of *Polyporus lucidus* in the neighborhood of *Isaria* is shown in fig. 4.

It was noticed that the sporulation of certain of the *Fungi Imperfecti* was influenced by the growth of other fungi. For example, there seemed to be an increase in size and number

[1] Clark, J. F. On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**: 289–327, 378–404. 1899.

of the heads of conidiospores of *Aspergillus Sydowi* when in contact with *Merulius pinastri*, and the same is true of *Aspergillus niger* in contact with *A. glaucus*.

As previously mentioned, hydrogen ion concentrations of solutions were determined after fungi had grown on them for two weeks, and the results obtained indicate that there is no definite relation between the active acidity produced by these fungi and their ability to inhibit or stimulate the growth of another. For example, in fig. 3, *Pleurotus sapidus* grew over *Aspergillus glaucus* very rapidly, but was entirely inhibited by *A. versicolor* on the solid agar medium. In the solutions *Pleurotus sapidus* produced an active acidity of P_H 5.4, while *Aspergillus glaucus* and *A. versicolor* changed the active acidity to about the degree P_H 6.6 and P_H 6.4, respectively; also, *Trametes Peckii* grew over both *Daedalea quercina* and *Aspergillus fumigatus*, although the change in active acidity produced by *Daedalea quercina* was P_H 3.0 and that produced by *Aspergillus fumigatus* was P_H 6.6. Many such examples could be cited by comparing with table 1 the following active acidities produced by the fungi: *Lenzites vialis*, P_H 5.0; *Merulius pinastri*, P_H 7.0; *Daedalea quercina*, P_H 3.0; *Trametes Peckii*, P_H 4.2; *Pleurotus sapidus*, P_H 5.4; *Merulius lacrymans*, P_H 5.0; *Lentinus lepideus*, P_H 5.4; *Daedalea confragosa*, P_H 5.8; *Coniophora cerebella*, P_H 5.4; *Polystictus versicolor*, P_H 5.4; *Isaria* sp., P_H 6.8; *Polyporus lucidus*, P_H 5.4; *Polystictus hirsutus*, P_H 5.2; *Aspergillus glaucus*, P_H 6.6; *A. niger*, P_H 5.8; *A. fumigatus*, P_H 6.6; *A. versicolor*, P_H 6.4; and *A. Sydowi*, P_H 6.8. The control solution upon which no fungi had grown had an active acidity of P_H 5.4. Of course, there are some instances where similar effects could be correlated with similar changes in hydrogen ion concentration; for instance, *Trametes Peckii* is similarly influenced by both *Aspergillus fumigatus* and *A. glaucus*.

The fungi were also grown on a nutrient solution containing the same ingredients as the agar previously mentioned. Since certain of the *Basidiomycetes* used do not grow well upon liquid media it was found desirable to add to the cultures sufficient quartz sand, free from all soluble substance, so that a slope of sand could be formed above the surface of the solution out into

which the solution diffused. All of the *Basidiomycetes* grew well upon these sand slopes, and the solution was easily drained from the sand at the end of the period of culture. After two weeks' growth of the fungi the hydrogen ion concentration of the solutions was determined according to the methods previously cited.[1]

In cases where there was a marked stimulation or inhibition of growth between two fungi on the plates, these fungi were then grown on similar sand slopes. After they had made considerable growth the solution was filtered off, sterilized, and prepared for inoculation with the reciprocal fungus. Controls of these solutions were kept uninoculated. The amount of growth of the second inoculation was determined by the dry weight of the fungous mat and the amount of sugar remaining in the solutions estimated. The latter was accomplished by reducing equal amounts of the solutions with equal amounts of Fehling's solution and estimating visually the amounts of copper oxide. The distinctions were so evident that quantitative determinations were unnecessary.

The dry weight of mycelium produced in each case and an estimation of the amount of sugar remaining in the solution after growth of the first and second fungus are shown in table II.

In some cases it would seem that the carbohydrate content of the nutrient solution upon which a fungus had previously grown might have been the limiting factor for growth. In others, however, this is not true; for example, when *L. vialis* follows *A. niger* there is very little growth, although the carbohydrate content was high, while in the control solution upon which no fungus had grown *L. vialis* made considerable growth and used a greater part of the sugar in the solution. This would tend to indicate that *A. niger* in its metabolism may have secreted some substance which was toxic to the growth of *L. vialis*. It is of course quite probable that such toxic substances were formed in many more instances but were destroyed in the process of autoclaving between the first and second inoculation.

This in general agrees with the conclusions reached by Fulton[2] that fungi in their growth show a more marked tendency to grow

[1] Zeller, Schmitz, and Duggar, *l. c.*

[2] Fulton, H. R. Chemotropism of fungi. Bot. Gaz. 41: 81–108. 1906.

TABLE II

RELATION OF THE AMOUNT OF GROWTH TO THE QUANTITY OF SUGAR REMAINING IN THE SOLUTION

Fungus	Grown on solution after	Dry weight of mycelium (gms.)	Relative amounts of remaining sugars
L. vialis	A. niger	.080	Much
	A. Sydowi	.062	Trace
	Control	.221	Medium
M. pinastri	T. Peckii	.115	Trace
	A. niger	.040	Trace
	Control	.119	None
T. Peckii	M. lacrymans	.154	Much
	P. sapidus	.335	Much
	Isaria	.140	Medium
	A. niger	.084	Trace
	A. fumigatus	.118	Medium
	A. glaucus	.126	Medium
	Control	.221	Much
P. sapidus	A. niger	.302	None
	A. glaucus	.180	Trace
	A. Sydowi	.309	Trace
	Control	.353	Much
M. lacrymans	T. Peckii	.178	Trace
	D. confragosa	.100	Trace
	Control	.230	Much
L. lepideus	T. Peckii	.094	Medium
	A. niger	.073	Trace
	A. fumigatus	.100	Trace
	Control	.111	Much
D. confragosa	M. lacrymans	.290	Trace
	Control	.231	Much
P. versicolor	P. sapidus	.372	Trace
	A. glaucus	.085	None
	A. Sydowi	.088	Trace
	Control	.266	None
P. lucidus	Isaria	.117	Trace
A. niger	P. sapidus	.270	Medium
	M. lacrymans	.240	Trace
	D. confragosa	.141	Trace
	C. cerebella	.270	Trace
	A. fumigatus	.078	None
	Control	.270	Trace
A. fumigatus	C. cerebella	.156	Much
	Control	.158	Trace

out and *away from* the medium influenced by their own growth metabolism than to grow *towards* a diffusion center, whether this center contains nutritive or deleterious materials. This may also be the condition produced in stale cultures.

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