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Antagonistic effects of some soil fungi on *Fomes annosus* in laboratory experiments

Antagonistiska effekter mot Fomes annosus hos några marksvampar i laboratorieförsök

by

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Introduction

The heavy damage of economic importance which hit the Swedish forestry by the root rot fungus *Fomes annosus* (Fr.) Cke has given rise to important research in order to widen the knowledge of the pathogene. Up to 1951, however, basic parts of the dissemination biology of the fungus were so incompletely known that the efforts to resist the disease were limited to some silvicultural measures, for example a change of tree species at the time of regeneration or strong sanitation cuttings. None of the measures was a biological success or economically acceptable. With Risbeth's (1951) theory on the dissemination of the disease by a spore invasion of fresh stump surfaces, an intensive period of research began in order to control *F. annosus* (Yde-Andersen 1961, Driver 1963, Paludan 1963). The fresh stump surfaces were treated with chemicals such as creosote oil, urea, borax or sodium nitrite. The latter has been used extensively because it inhibits *F. annosus* selectively in a low concentration and in addition it is tolerated by saprophytic wood decomposing fungi (Gundersen 1967). In England this type of control has been increasingly replaced by a biological one. By applying a spore suspension of *Peniophora gigantea* (Fr.) Massee on the stump surface, *F. annosus* is conquered, and a faster decay of the whole stump is attained by comparing treatment with chemicals (Rishbeth 1959 *a*, Meredith 1960). This biological control is, however, limited to forest stands without any root rot established, which means for Swedish conditions first rotation Norway spruce or Scots pine on virgin soils. The total area of this type of soil for all cultivated species is per year less than 1 ‰ of the total productive forest land in Sweden (Bärring 1967).

With the intense search for new antibiotics during the late 1940's an increasing interest in the antagonistic phenomenon among microorganisms in forest soils followed. A considerable number of isolations and cultures have been made in order to find some microorganisms which would be capable of both inhibiting the growth of *F. annosus* and persisting in forest soils (Björkman 1949, Rennerfelt 1949, Nissen 1954—56 and Molin 1957).

Rennerfelt (1949) investigated 7 different groups of fungi and bacteria regarding their antagonistic effect against *F. annosus*. An inhibition could be recognized by 1 phycomycete, 1 litter-decomposing fungus, 31 mould fungi and 15 species of bacteria out of a total of 118 species investigated. Of "non-

moulds" *Coniophora puteana* (SCHUM.) KARST. and *Clitocybe nebularis* (BATSCH) FR. caused zones of inhibition that were less than 5 mm.

BEYER & KLINGSTRÖM (1964) isolated and identified *Scytalidium aurantiacum* K et B from heartwood of spruce strongly decayed by *F. annosus*. It was evident from the CO_2-measurements on blocks from the heartwood that the root rot fungus had inhibited the respiratory activity while *Scytalidium* was still respiring.

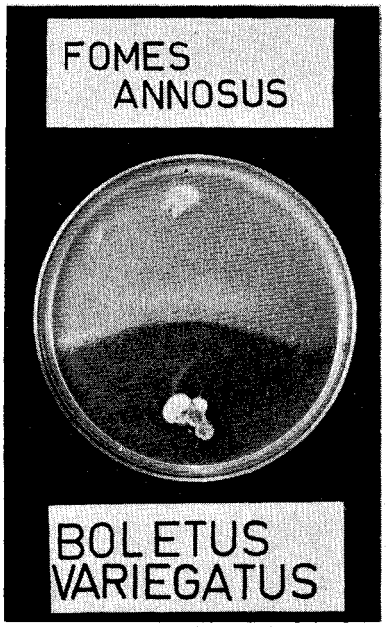
Although a large number of bacteria, actinomycetes, fungi imperfecti and ascomycetes, antagonistic to *F. annosus*, have been studied fairly closely, very little is known about the effect of the mycorrhiza-forming fungi on the root rot fungus. BJÖRKMAN (1956, p. 279) mentions that the formation of mycorrhiza probably protects the host plant against attacks from pathogenic microorganisms.

MOREAU & SCHAEFFER (1959) consider that the rate of infection is connected with the mycorrhiza formation on the spruce roots, which is unsatisfyingly developed in the Jura at other pH-value than 5.1—5.9. ORLOS & DOMINIK 1960) maintain that the violent attacks of *F. annosus* on former farm land depends on the absence of suitable preventive mycorrhiza fungi. Experimental documentation is, however, not stated.

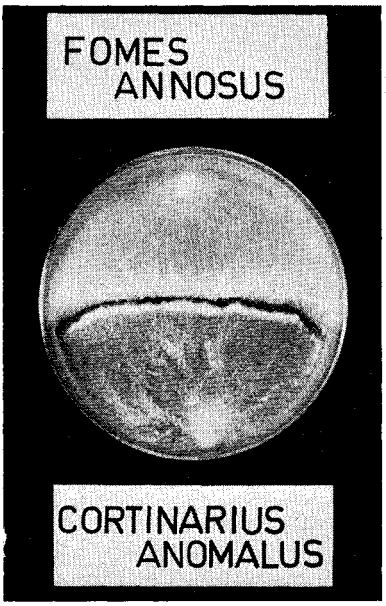
Based on these hypotheses a screening test was performed with the soil fungi included in the collection of the Department of Forest Botany, autumn 1963. Owing to the rapid enlargement of the collection the results have only just become available, and a number of common Scandinavian mycorrhiza-forming fungi are investigated with regard to antagonistic effect against *F. annosus*. The investigations made on the antagonism of mycorrhiza fungi against other microorganisms have touched the problem of *F. annosus* only in papers by RYPÁČEK (1963) and MARX (1967) and without any intention of studying this rot fungus in particular. The investigation reported below was performed as a screening test with the mycorrhiza-forming fungi in the collection of the Department of Forest Botany paying attention only to their effect against *F. annosus*.

Material and methods

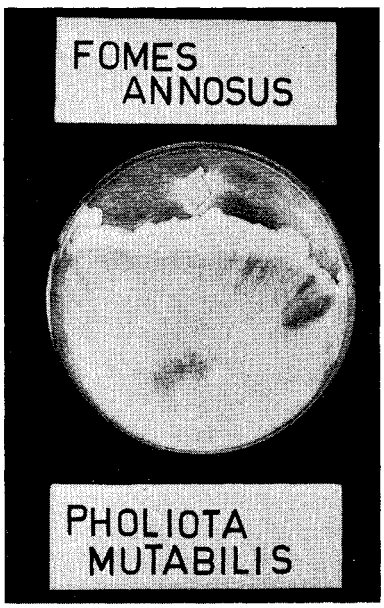
Petri dishes of plastic with malt extract agar = MA (2.5 % malt extract 1.5% agar in tap water) were inoculated at the edge of the dish with inocula 5×5 mm in size from the actual mycorrhiza-forming fungus from a slant MA-culture. At the opposite edge *F. annosus*, No. Sä 1, was inoculated. In



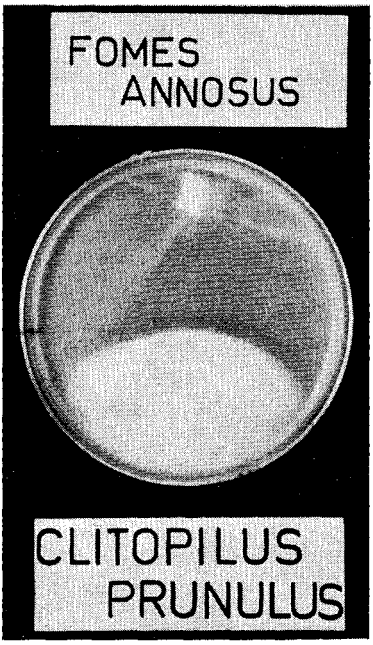
a. The root rot fungus strongly inhibited



b. Non-aggression



c. Invasion



d. Inverted invasion.

Fig. 1. Some types of influence between mycorrhiza fungi and *F. annosus*.

Species	Collection No	The influence on Fomes annosus				Radius growth, mm		Drawings
		Inhibition zone 1.	Nonaggression 2.	Invasion 3.	Inverted Invasion 4.	F. annosus	Soil fungus	
Amanita muscaria (L. ex Fr.) Pers. ex Hooker	C 3	– (1960)	+	–	–	51	9	
» » (») » »	C 4	2 (1963)	–	–	–	47	5	
» » (») » »	C 256	– (1964)	+	–	–	60	4	
» pantherina (D.C. ex Fr) Schum.	I 62	2	–	–	–	47	6	
» rubescens (Pers. ex Fr.) S.F. Gray	C 6	•	–	–	+	50	13	
» » (») »	C 13	–	–	–	+	47	4	
» vaginata (Bull ex Fr.) Vitt	I 27	3	–	–	–	42	12	
Boletus bovinus (L. ex Fr.) O. Kuntze	C 14	32 (1958)	–	–	–	12	9	
» » (») »	C 15	– (1964)	+	–	–	57	8	
» » (») »	C 238	– (1966)	+	–	–	60	8	
» » (») »	C 252	1 (1966)	–	–	–	39	13	
» » (») »	C 42	– (1967)	+	–	–	53	10	
» edulis Bull. ex Fr.	C 17	12 (1955)	–	–	–	19	16	
» » »	I 56	– (1964)	–	–	+	53	13	
» » »	C 93	8 (1967)	–	–	+	41	14	
» » »	C 263	– (1966)	+	–	–	32	22	
» » »	C 18	2	–	–	–	37	13	
» elegans (Klotzsch) Sing.	C 26	–	–	–	+	57	2	
» » »	I 4	5	–	–	–	42	10	
» » »	I 38	4	–	–	–	43	9	
» granulatus (L. ex Fr.) O. Kuntze	C 19	– (1965)	+	–	–	42	12	
» » »	I 53	10 (1963)	–	–	–	38	14	
» » »	C 240	– (1964)	+	–	–	52	7	
» » »	C 37	– (1965)	+	–	–	59	7	
» luteus (L. ex Fr.) S.F. Gray	I 60	– (1964)	+	–	–	35	14	
» » »	I 22	– (1964)	–	–	+	70	11	
» » »	I 3	– (1964)	+	–	–	37	15	
» » »	C 243	– (1964)	–	+	–	44	21	
» » »	C 260	– (1966)	+	–	–	42	7	
» » »	C 81	– (1967)	+	–	–	40	13	
» scaber (Bull. ex Fr.) S.F. Gray	C 28	16	–	–	–	20	19	
» subtomentosus (L. ex Fr.) Quél.	I 55	4 (1963)	–	–	–	25	27	
» » »	C 30	– (1963)	–	–	+	42	5	
» » »	C 31	– (1963)	–	–	+	61	5	
» » »	C 29	– (1963)	–	–	+	60	6	
» » »	C 118	3 (1967)	–	–	–	49	12	
» variegatus (Swartz ex Fr.) O. Kuntze	C 258	24 (1965)	–	–	–	20	7	
» » »	C 36	20 (1954)	–	–	–	31	2	
» » »	I 35	11 (1963)	–	–	–	28	7	
» » »	C 52	12 (1967)	–	–	–	35	7	
» » »	C 35	3 (1966)	–	–	–	35	4	
» piperatus (Bull. ex Fr.) O. Kuntze	C 277	–	–	–	–	50	1	
» versipellis (Secr.) Sing.	C 39	–	–	–	+	55	2	
» viscidus (Secr.) Snell	C 264	–	–	–	+	52	1	
Bovista plumbea Pers.	C 271	–	–	–	+	46	7	
Clitocybe aurantiaca (Fr. ex Wulf) Studer	C 245	3	–	–	–	37	11	
» »								
» nebularis (Batsch) Fr.	C 41	3	–	–	–	43	15	
» »	C 43	3	–	–	–	48	18	
» rivulosa (Pers. ex Fr.) Kumm.	C 44	6	–	–	–	30	14	

Fig. 2. Different types of influence and antagonism concerning 85 isolates of soil fungi and *F. annosus*.

Species	Collection No	The influence on Fomes annosus				Radius growth, mm		Drawings
		Inhibition zone 1.	Nonaggression 2.	Invasion 3.	Inverted Invasion 4.	F. annosus	Soil fungus	
Clitopilus prunulus (Scop. ex Fr.) Kumm.	C 45	–	+	–	–	31	22	
Collybia aeema (Fr.) Quél.	C 46	2	–	–	–	31	14	
» velutipes (Curt.) Quél.	C 47	1	–	–	–	40	7	
Cortinarius anomalus (Fr. ex Fr.) Fr.	I 58	3	–	–	–	40	8	
Hebeloma crustulini forme (Bull. ex St. Am) Quél.	C 87	–	+	–	–	54	10	
Lactarius delicioeus (L. ex Fr.) S.F. Gray	I 47	–	–	–	+	48	8	
» torminosus (Schaeff. ex Fr) S.F. Gray	C 95	–	–	–	+	49	9	
» » »	I 42	–	+	–	–	55	9	
Lycoperdon perlatum Batsch	C 106	–	–	–	+	35	25	
Isol. Monotropa hypopitys L.	D 37	43 (1960)	–	–	–	15	11	
» » »	D 40	10 (1959)	–	–	–	29	11	
» » »	I 46	–	–	+	–	23	27	
» » »	D 35	– (1959)	–	+	–	34	30	
» » »	I 44	–	+	–	–	2	54	
» » »	D 39	– (1958)	–	+	–	25	29	
» » »	D 38	– (1958)	+	–	–	30	23	
Paxillus involutus (Batsch ex Fr.) Fr.	C 122	–	–	–	+	70	5	
Pholiota mutabilis (Schaeff.) Fr.	C 139	–	–	+	–	14	63	
» »	C 262	–	–	+	–	11	43	
» sp.	I 8	–	–	–	+	21	30	
» squarrosa (Müll.) Fr.	C 145	1	–	–	–	56	8	
Psalliota arvensis Schaeff.	C 141	–	–	–	+	48	14	
» »	C 268	–	+	–	–	49	7	
» campestris (L.) Fr.	C 267	–	+	–	–	50	7	
» pratensis (Schaeff. ex Fr.) Quél.	C 203	–	+	–	–	40	10	
Rhizopogon roseolus (Corda) Hollos	C 204	5	–	–	–	35	15	
» » »	C 236	–	+	–	–	44	13	
Thelephora terrestris Fr.	C 227	3	–	–	–	49	18	
Tricholoma fumosum (Berk.) Kühn.	C 60	–	–	–	+	47	7	
» imbricatum (Fr.) Kumm.	C 229	–	+	–	–	67	2	
» » »	C 223	–	+	–	–	56	1	
» nudum (Bull. ex Fr.) Cooke	C 250	2	–	–	–	49	15	
» » »	C 225	2	–	–	–	45	19	
» pessundatum (Fr.) Quél.	C 226	5	–	–	–	48	12	
Cenococcum graniforme (Sow.) Ferd. & Winge	D 10	–	–	–	+	55	13	
» » »	D 11	–	+	–	–	47	16	

the main the inoculations took place simultaneously, but some very slow growing mycorrhiza fungi got an advantage of 3 to 10 days. The final calculations were made when an equilibrium of growth was reached. The growth was determined in mm radius, as was also the zone of inhibition. This was described as a clear zone without any mycelium. The attempt to describe the inhibiting effects, that is used below, concentrates on how *F. annosus* is affected by the mycorrhiza-forming fungi. In the first column of figure 2 titled "Inhibition zone", the uninvaded bright zone is calculated in mm. It is always the permanent final situation between the two microorganisms that is noted. In column 2, called "Nonaggression", it is assumed that both fungi grew unarrested till they met border to border. At this confrontation further growth ceased reciprocally. The third column was titled "Invasion". In a few cases it happened that the mycorrhiza fungus was not arrested by *F. annosus* but grew up to and invaded it. *F. annosus* seemed to stagnate and

gradually also the mycorrhiza fungus finished further progress. The last type of effect recognized in this material is called "Inverted invasion" considering the situation when *F. annosus* was capable of growing over the mycorrhiza fungus before it was arrested. The mycorrhiza fungus stopped growing at a pronounced earlier time.

The four types of affection, described above, are demonstrated in figure 1. The calculations made are collected in figure 2 together with an outline for every number.

Results

There are two facts worthy of attention. First the widespread ability among the mycorrhiza fungi to arrest the development of *F. annosus*. Out of 85 isolates screened 32, or 40 % left a more or less broad zone between themselves and the rot fungus. Secondly there was a variation in the effect against *F. annosus*, that was great both inside the genus and the species. Considering this variable antagonism against *F. annosus*, comparable material is lacking in literature.

A comparison between the genus with three or more species represented indicates that no isolate of *Psalliota* and *Lactarius* had an antibiotic effect against *F. annosus*, but that in the genus *Clitocybe* all species exerted antagonism against the root rot fungus. Within *Boletus* which is frequently represented in this material, 16 isolates out of a total of 37 had an influence on *F. annosus*.

By comparing the species a considerable variation is obtainable regarding the antagonism. All the 5 isolates of *Boletus variegatus* are capable of arresting *F. annosus*. The inhibition zone varies from 3 to 24 mm of width, while none of the 6 isolates of *Boletus luteus* L. is able to arrest the rot fungus. This "all or nothing" is adequate for the few isolates of *Amanita rubescens* Fr., *Tricholoma imbricatum* Fr. and *Cenococcum graniforme* (Sow.) Ferd. et Winge of which none influence the growth of *F. annosus*. The opposite conditions are valid for *Clitocybe nebularis*, *C. aurantiaca* (Fr. ex Wulf.) Studer and *Tricholoma nudum* (Bull.) Fr. i.e. all isolates arrest *F. annosus*. More frequently, however, there are both antagonists and nonantagonists to the root rot fungus within a species as a rule. As examples *Boletus bovinus* L., *B. edulis* L., *B. granulatus* (L). Fr., *B. subtomentosus* (L.) Fr. and isolates of *Monotropa hypopitys* L. could be mentioned. In *B. bovinus* two isolates exert inhibition zones of 32 and 1 mm broadness whilst three isolates are ineffective. Three

isolates of *B. edulis* arrest *F. annosus* with zones 12, 8 and 2 mm broad respectively, whilst two of them lack the inhibiting effect. In the same way two isolates of *M. hypopitys* cause bright zones of 43 and 10 mm, whilst three of them occupy *F. annosus* and finally two stop their growth on contact with the latter.

The zones of inhibition appearing vary in width from 1 to 43 mm. The majority of antagonism, measurable in this way against *F. annosus* may be denoted as weak and within the class 0 to 5 mm clear zone, 64% of the whole material will fall. An inhibition of 6 to 10 mm is performed by 12% of the fungi and a stronger one with clear zones from 11 to 20 mm is exerted by 15%. Of greatest interest are the strong inhibitors, *Boletus variegatus*, 24 mm, *B. bovinus*, 32 mm and the isolate of *M. hypopitys* 43 mm. The first mentioned belongs to the species, all isolates of which caused inhibition of *F. annosus* with the following values 24, 20, 12, 11 and 3 mm. Comparable values for *B. bovinus* are 32, and 1 mm. Three isolates lack inhibitory properties. The isolates from *M. hypopitys* are described earlier. Amongst them the strongest inhibition was calculated for D 37 which left a zone of 43 mm free from mycelia.

It may be reported here that a comparison was made concerning the antagonism against *F. annosus* and the age of the mycorrhiza fungus. No apparent correlation could be established and the most effective antagonist belonged to the oldest isolates of the collection.

The experiences from the plate tests with *F. annosus* and some mycorrhiza fungi were supplemented by an experiment in order to characterize the diffusing antagonistic substances. The experiment was arranged so that from the edge of an arrested culture of *F. annosus* recultivations were made to fresh MA-plates. After a short time of adaptation *F. annosus* started growing and developed normally with all samples tested. This indicates that the antagonistic substances are of a fungistatic type, i.e. they inhibit the further growth of the treated organism, in contrast to the fungicides, which strongly change or kill the organism exposed.

In all screening experiments made with about 80 mycorrhizal and soil fungi reported above, a single *F. annosus* isolated from Norway spruce and denoted Sä 1, has been used. In order to widen the knowledge of how different isolates of the root rot fungus behave when exposed to the antagonism caused by some mycorrhiza-forming fungi, the very active D 37, C 14 and C 258 (Fig. 2) were selected. The screening was modified for practical purposes so that on every MA-plate the mycorrhiza fungus was inoculated in the centre of the dish, and at the edges, and contrary to each other two different isolates of *F. annosus* were inoculated. The isolates are mostly fresh, collected in stands and field trials with spruce in Sweden and

Mark	*F. annosus* isolated from			Tree species	Year of isolation
Ek	Ekshärad	S county,	S	*Picea abies*	1964
Fp	Frodeparken	N »	»	»	1965
G	Garpenberg	W »	»	»	1964
Garp	Garpenberg	W »	»	»	1965
PON	Garpenberg	W »	»	»	1965
Ru	Garpenberg	W »	»	»	1964
Ku	Kulbäcksliden	AC »	»	»	1964
Rö	Röskär, Bogesund	B »	»	»	1965
Sä	Stensängen, Bogesund	B »	»	»	1961
Tö	Tönnersjöheden	N »	»	»	1965
Ånh	Ånhammar	D »	»	»	1964
Öva	Överammer	Z »	»	»	1965
M	Högby Mo	E »	»	*Pinus silvestris*	1961
Git	Git, Tirol		AU	»	1962
Ge	Athens, Georgia		USA	*Pinus elliottii*	1964
Fi	Different localities		SF	Different species	1966

Finland during 1961—1967. They are cultivated in 22°C in darkness, and harvested after 20 days. The calculations made on the inhibition probably were influenced by this way of processing; thus the inhibition zone against Sä 1 was strongly reduced, compared with the earlier reported method. This was a general rule for the three mycorrhiza fungi selected. The contemporary inoculation of a relatively rapid growing rot fungus and a slow growing mycorrhiza fungus may also have been responsible for a lag phase appearing in both growth and toxin diffusion for the latter.

It is evident from figure 3 that the antagonism, caused by some mycorrhiza fungi and earlier manifested with the isolate Sä 1 of *F. annosus*, is of a general type. A single exception has been observed in this material including 85 isolates. No. Ek IS: 99 is arrested by D 37, isolated from *M. hypopitys*, only "edge to edge" but contrary to the isolate from *M. hypopitys*, this isolate of the root rot fungus was markedly inhibited—10 mm—by C 14. In the Finnish material of *F. annosus* there are two isolates with opposite conditions, *i.e.* C 14 exerts no inhibition, but D 37 does.

The variation within the local isolates is obvious. In the material from Röskär near Stockholm, the least influence that an isolate tolerated was a presence of C 14 and C 258 on 6 mm distance and of D 37 on 2 mm. The corresponding values of a sensitive isolate count 15, 14 and 9 mm respectively. The three mycorrhiza-forming fungi selected, influence the isolates of the root rot fungus quite differently, as is demonstrated in the frequency curves of figure 3 including material from Sweden (Röskär) and Finland.

In the Swedish material (Röskär), C 258 acted as the strongest antagonist while the differences between C 14 and D 37 were rather slight. In the Finnish

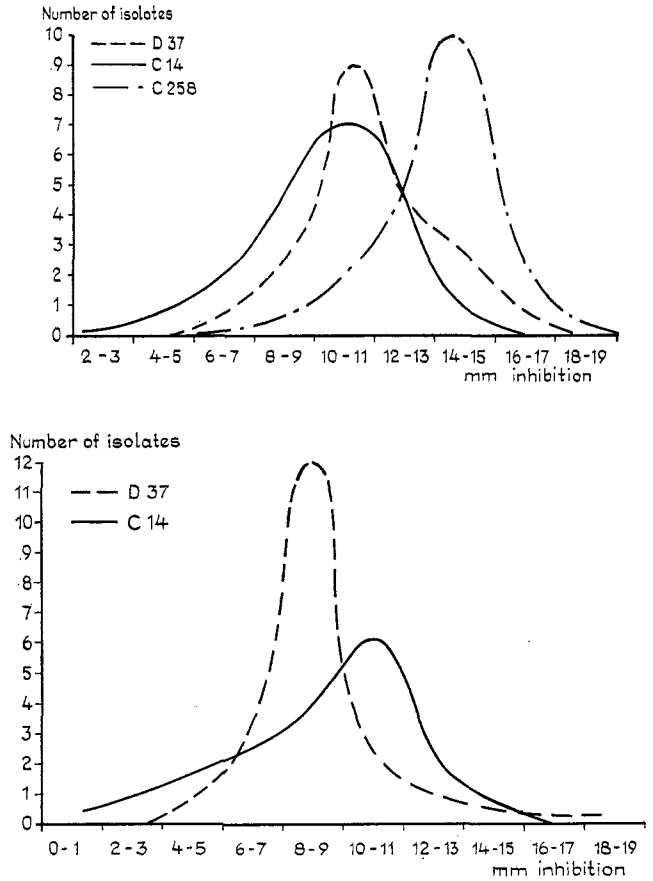


Fig. 3. The variation of isolates of *F. annosus* from a Swedish and some Finnish stands regarding the sensitivity to some antagonistic mycorrhiza fungi.

material where a comparison of the activity between C 14 and D 37 is performed more pronounced differences are, on the contrary, recognized regarding antagonism against *F. annosus*.

The experiments reported were all obtained on solid media. In order to widen the knowledge of the antagonism a final experiment was performed in nutrient solution. It was arranged as a culture of D 37 and C 14 in a SHIVE solution in darkness at a temperature of 20°C. After 5, 10, 20 and 31 days respectively the experiment was interrupted and the mycelium of 4 flasks was calculated as dry weight. The sterile filtered nutrient solution was again inoculated, this time with *F. annosus* and grown under the conditions above. After a further 20 days the experiment was finished and the mycelial weights of *F. annosus* determined.

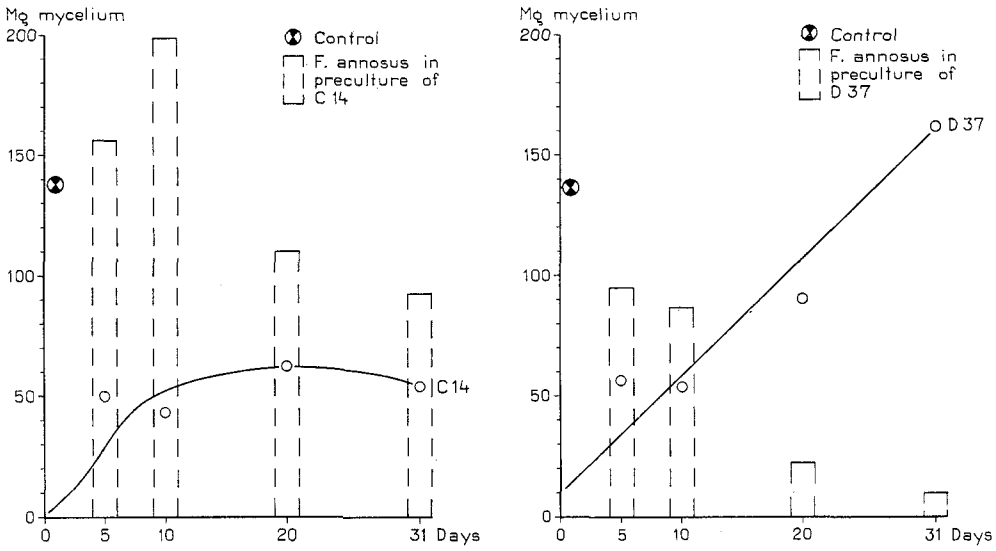


Fig. 4. Growth of *F. annosus* in nutrient solutions pre-cultivated with antagonistic mycorrhiza fungi, and of controls.

In figure 4 the dry weights of *F. annosus*, after growth in solutions precultivated with mycorrhiza fungi are dotted, as well as in the controls. It is observed that also in this medium some toxic substances is exuded into the nutrient solution. This has a depressive influence on *F. annosus*, and is faster acting and gives a stronger final inhibition with D 37 than with the other mycorrhiza fungi investigated.

However only the concentration of glucose of the nutrient solutions was controlled and found satisfactorily high during the experiment time. In addition to the inhibiting effects of the antagonistic fungi there could be a lack of mineral nutrients in cultures with rapid developing fungi affecting the growth of *F. annosus*.

The inhibiting effect which a.o. D 37 exerted against *F. annosus* at a contemporary inoculation of both fungi, could be calculated as an unoccupied bright zone. This clear zone once established, remained intact during the whole observation time, which sometimes was synonymous with a drying out of the substratum. However, very little was known of the importance of a successive inoculation on the intensity of antagonism, i.e. the quantitative aspect of the phenomenon. It was evident, as reported, that the inhibition became total and there was reason to assume that a reciprocal and equal influence was established, i.e. that also *F. annosus* had exuded substances of a toxic kind into the agar. Such substances acting on a general biological scale are reported by Persson (1957) and Bassett *et al.* (1967) by growing *F.*

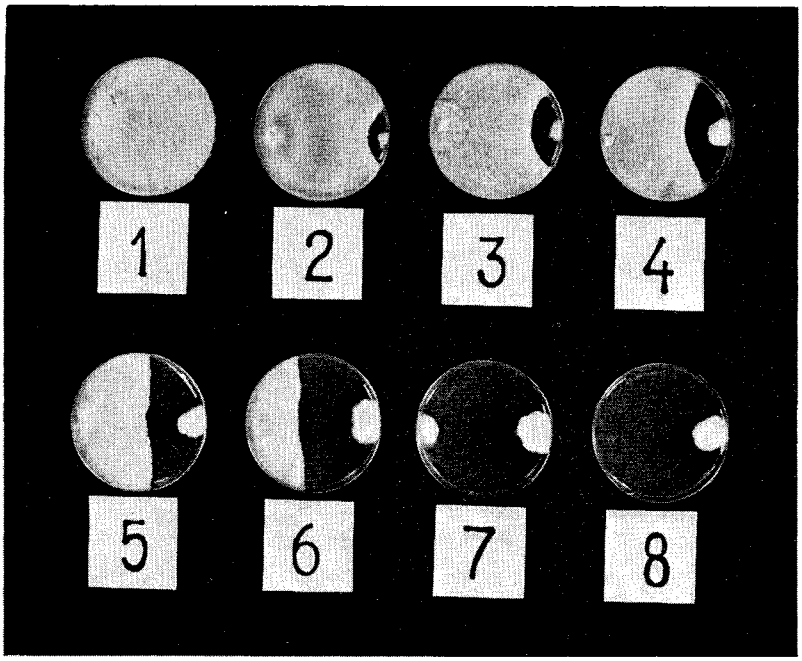


Fig. 5. The influence of the time factor on the antagonism.
F. annosus inoculated left, and D 37 right in the Petri dishes.
1. *F. annosus* after 12 days of growth
2. The zone of inhibition with *F. annosus* 8 days in advantage
3. " " " " " " 4 " " "
4. " " " " " " and D 37 inoculated simultaneously
5. " " " " " D 37 8 days in advantage
6. " " " " " " 16 " " "
7. " " " " " " 32 " " "
8. D 37 after 32 days of growth.

annosus in nutrient solutions. On the other hand, the arrested growth of the mycorrhiza fungus could be a result of that "staling" effect as already DUCLAUX 1898 (WAKSMAN 1947, p. 46) observed, i.e. in a precultivated nutrient solution, a reinoculation of the same species ceased. Such iso-antagonism also could be suspected as causing the cease in growth of ageing D 37 on MA-agar, though the space was abundant and the nutrient status seemed sufficient. To get an opinion of such a relative antagonism between *F. annosus* and D. 37, the two fungi were inoculated on MA-plates at different intervals. Thus *F. annosus* got an advantage of 4 and 8 days respectively before D 37 was inoculated at the edge of the Petri dish. In the next step the two fungi were inoculated simultaneously, and thereafter D 37 got an advantage of 8, 16 and 32 days respectively. Plates with single cultures of D 37 and *F. annosus* served as controls. As a quantitative measure of the inhibition, the unoccupied area

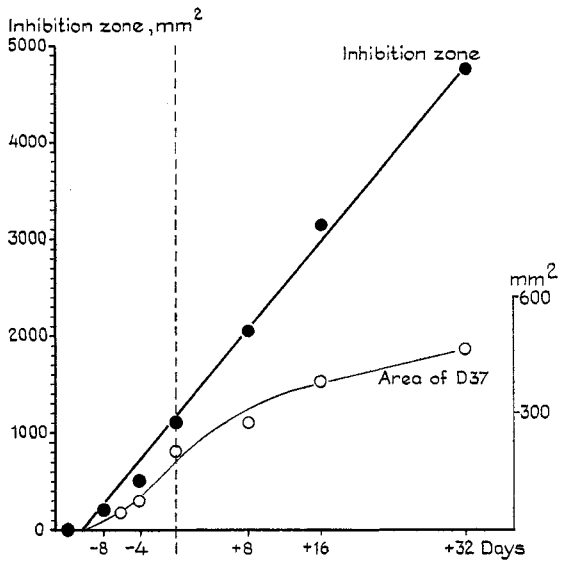


Fig. 6. Growth curve of D 37 and the quantitative inhibition of *F. annosus* at successive inoculations on malt extract agar.

between the two fungi was calculated. With this method a numerical checking of the toxin concentration, exceeding the treshold value for inhibiting *F. annosus*, is obtained. Nothing is yet known of its true value.

From figure 6 a linear correlation seems to exist between the time factor and the strength of inhibition within the interval chosen. A further advantage of D 37 would, however, change the curve towards the limiting values, i.e. the total area of the Petri dish minus the area of D 37. The growth curve of D 37 and the toxin curve are similar in the first phases. With an advantage in time to D 37 the growth curve, however, soon changes from the exponential to the autolytic phase. Instead of an occupied area of expected 900 mm² at 32 days with linear growth, the observed area was about 450 mm². Despite the differences in the rate of the curves discussed above, there exists a positive correlation between growth of D 37 and toxin production.

The question whether *F. annosus* under present conditions is able to influence the mycorrhiza fungus is open to doubt. In those Petri dishes where D 37 was grown singly for 32+12 days, the mycelial areas never exceeded those of D 37 where *F. annosus* and D 37 grew together.

To summarize, it is evident that the observed influence of D 37 on *F. annosus* is a biased antagonism against the root rot fungus, but also of an isoantagonistic character regarding the mycorrhiza fungus itself.

Discussion

There are few principles reported in estimating the antagonism among fungi, in contrast to those of the research of medical antibiotics. No such urgent needs have existed in phytopathology or soil microbiology to measure comparable quantities of biologically active substances as in the antibiotic therapy. Usually it has been sufficient to demonstrate whether an antagonism has developed or not. Besides, there are several investigations where the antagonism is noted as a side effect or a curiosity in the attempts to isolate and cultivate microorganisms. WAKSMAN (1947) mentions a couple of different methods in order to assign qualitatively antagonistic phenomena on solid and semisolid substrata but does not take into account the quantitative aspect. RENNERFELT (1949) investigating the inhibiting effect of a great many fungi and bacteria on *F. annosus* divided the antagonism into four groups regarding the intensity of inhibition.

1) "The two fungi growing against each other without inhibition".
2 a) "The other fungus overgrows".
2 b) "The other fungus is inhibited".
3) "*F. annosus* overgrows".
4 a) "*F. annosus* inhibited < 5 mm".
4 b) "*F. annosvs* inhibited > 5 mm".

The protective properties of mycorrhiza fungi is discussed by ZAK (1964, p. 384) based on the screening of the effect of some *Boletus* on a root pathogene, such as *Phytophthora cinnamomi* RANDS (BRYAN 1960). RYPÁČEK (1963) was studying the inhibition between some wood destroying fungi and *Boletus variegatus* (Sw.) FR. The author declares that an inhibition zone of 1 mm could be ascertained between this fungus and *F. annosus* in an agar disc test. The purpose of the investigation was, however, not to look for a biological control of the root rot fungus. The very attractive theory of inoculating the seedling with a fit symbiotic microorganism antagonistic to *F. annosus*, was generally expressed by ZAK (1964). MARX (1967) reports the effect of five ectotrophic mycorrhiza-forming fungi on ten root pathogenic fungi, a.o. *F. annosus*. Only one mycorrhiza fungus, *Leucopaxillus cerealis* var. *piceina* (LASH) SING. had a weak inhibiting activity against the root rot fungus. With the investigations of MARX (1967) and ŠAŠEK (1967) concerning the protective effect of *Leucopaxillus cerealis* var. *piceina* on *P. cinnamomi* and a.o. *Tricholoma saponaceum* (FR.) KUMM. on some damping off fungi, it was for the first time experimentally checked that a mycorrhizal symbiosis

established with a complete mantle and the Hartig net eliminated damping off on seedlings of two species of pine. MARX (1967) also could demonstrate the presence of the antibiotic diatretyne nitrile in *L. cerealis* var. *piceina* grown in some substrata including root extracts, humus extracts and peat.

The arsenal of microorganisms for biological control of *Fomes* annosus, was in principle widened with the introduction of some active mycorrhiza-forming fungi. In the present study some strongly antagonistic species were discovered using the cross plate technique. The toxic property seems to be of a general nature regarding *F. annosus*. Out of 85 isolates of the root rot fungus, none was unaffected by exposure to the three most active mycorrhiza fungi. Another new observation was the variation within the species and between the species. Thus, no *Boletus luteus* isolate inhibited the growth of *F. annosus* on the one side, on the other side all isolates of *B. variegatus* caused inhibition. Between these two extremes the other species and isolates were grouped. Finally there were no remarkable differences in activity against *F. annosus* between the true mycorrhiza-forming fungi and the rest of the soil fungi investigated.

Summary

85 isolates of soil fungi, part of which may be characterized as mycorrhiza fungi, were included in a screening test against the root rot fungus *Fomes annosus*. It was arrested by 40% of the number and an unoccupied, bright and measurable zone then divided the two microorganisms. Some different types of influence were observed and interpreted. A wide antagonistic spectrum was demonstrated both within and between the species. One isolate of the mycorrhiza fungus *Boletus bovinus*, one of *B. variegatus* and one from roots of *Monotropa hypopitys* inhibited *F. annosus* most strongly. The inhibition established was of a fungistatic type.

In order to widen the knowledge of the variation in sensitivity of *F. annosus*, 85 isolates mainly from field trials in Norway spruce were selected and screened to three strongly active mycorrhiza-forming fungi. As a rule all root rot isolates were highly sensitive to all three antagonists. Only three exceptions were obtained.

The same kind of pronounced sensibility against toxins produced was demonstrated, when *F. annosus* was grown in nutrient solutions precultivated with some active mycorrhiza fungi. Finally the importance of inoculation time on the antagonism was studied on solid media. A strong correlation exists between the time advantage and the toxic level obtained.

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Sammanfattning

Antagonistiska effekter mot Fomes annosus (Fr.) Cke hos några marksvampar i laboratorieförsök.

85 isolat av marksvampar, av vilka flertalet kan betecknas som mykorrhizasvampar, undersöktes på sin förmåga att påverka *F. annosus* i testförfarande på fast närsubstrat. 40 % av dem kunde hejda rotrötesvampen och lämnade en mätbar, oinvaderad zon av växlande bredd mellan de båda mikroorganismerna. Några olika typer av påverkan kunde iakttagas och beskrivas. Stor variation i antagonistisk effekt påvisades såväl inom arten som mellan arterna. Ett isolat av mykorrhizasvampen *Boletus bovinus*, ett av *B. variegatus* och ett från rötter från *Monotropa hypopitys* hämmade kraftigast *F. annosus*. Den hämning som uppstod var av fungistatisk typ.

För att vidga kännedomen hur *F. annosus* påverkas utvaldes 85 isolat, huvudsakligen från granprovytor i Sverige, och testades mot de tre mest aktiva mykorrhizasvamparna. I intet fall förelåg någon generell tolerans mot de tre prövade antagonisterna. I stället visade *F. annosus* en genomgående hög känslighet mot dessa.

Samma höga känslighet mot toxiska substanser erhölls när *F. annosus* odlades i närlösningar som tidigare hyst de ovan nämnda mykorrhizasvamparna.

Slutligen undersöktes betydelsen av tidpunkten för den successiva inokuleringen av de antagonistiska organismerna på fasta substrat. Ett lineärt samband mellan försprång i tid och toxisk nivå synes föreligga.