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IMPORTANCE OF PROTOZOA IN THE DYNAMIC CHANGES OF THE RHIZOSPHERE

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Abstract

The autor tries to determine on the basis of his earlier and more recent findings the role of 282 species of protozoa demonstrated from the rhizosphere in the dynamic processes at the surface of the roots (rhizoplane) and around the roots. The population of the rhizosphere by microorganisms, as well as the role of the various factors are demonstrated by experiments. The migration, distribution, quantitative and qualitative changes of the protozoa and the interactions of the various factors are dealt with.

Introduction

It was scarcely one and a half centuries ago that EHRENBERG (1837) demonstrated active protozoa from soil on which he poured water. Following this besides the bacteria the protozoa of the soil also came into the limelight. In spite of this, it was only in 1952 that the finding valid for bacteria (HILTNER, 1904) that the organisms are much more abudant in the soil around the roots of the plants than farther away from them could be proven for the protozoa too (BICZÓK, 1953). The concept of the rhizosphere was born; by this the soil zone influenced by the microflora was understood (HILTNER, 1904). As "the coexistence between the plants and the microorganisms living in the soil can be best observed in the living-space next to the roots" (FEHÉR, 1954), this zone could be regarded as the rhizosphere. The boundary of this is determined by the range of action of the root secretion or the bacteria induced by it. Within this an outer and an inner rhizosphere and a root surface or rhizoplane are recently distinguished (ROVIRA and DAVEY, 1974). It is impossible to draw a boundary between these because the biocoenosis of this sub-biotope is an open system, in which the microorganisms, the root secretion and the complex abiotic factors are in an intricate interaction, and thus often bring about important changes in relatively short time. In these dynamic changes the protozoa have their own place, role, and importance. In the following we try to discuss this problem mainly on the basis of laboratory experiments with the purpose of suggesting useful ideas to researchers working in this field.

Materials and Methods

There are no reliable methods for the investigation of the soils and within them of the microorganisms of the rhizosphere. Cholodny's method (1934) modified by ROSSI (on sterile slides placed in vertical soil slits protozoa besides bacteria and fungi can be observed after two weeks after suitable staining, washing and drying) is rough and the species are not easily identifiable. This method

gives no information just on the microorganisms of the root surface in the rhizosphere. Brodsky's method (1937) (an 8 mm diameter and 35 mm long glass tube filled with wool soaked with hay brew and sunk into the soil) was, owing to the afore-mentioned causes, not reliable. We deemed it better to introduce the end pieces of the root under examination into a 5 cm long, vertically placed vial, in which the sterile water was closed by cotton-wool. On the 6–8 cm deep roots of the pea a small number of *Oicomaonas mutabilis, Bodo* sp., *Amoeba beryllifera, Euglypha alveolata, Hartmanella hyalina, Colpoda cucullus, C. inflata, C. steinii,* on the roots of the strawberry *Oicomonas termo, Amoeba botryllis, A. fasciculata, Diaphanosoma arcuata* and *Colpoda inflata* were identified. The finding drew attention to the importance of the rhizoplane and the necessity of the use of laboratory methods.

The essence of the laboratory methods used by the present author was described earlier (Biczók, 1953–1959). Among them an important role is given to direct procedures, continuous investigations of the various cultures. In order to approach some basic problems the autor often turned to the study of active and cysted protozoa inoculated into sterile soils and living in an ambiance soaked by root extract. The O_2 consumption near the rhizosphere, around the roots and the O_2 consumption of the roots themselves influenced by the microorganisms were measured by the conventional Warburg technicque. Thus valuable comparable data were collected concerning the differences in the activity of the different parts. The results were expressed by time unit per gram values in/ul.

The direct methods are highly appreciated even today. In addition to their relatively high value they can be used even now with the smallest error percentage. This is why Rovira and Davey (1974) emphasize that direct examination of the roots by light microscope provides valuable information for the understanding of the ecology of the microorganisms of the rhizoplane. One such method is that of Geltzer (1961). The method is based on joint raising of plants and soil microorganisms on glass plates covered with a film of organic medium.

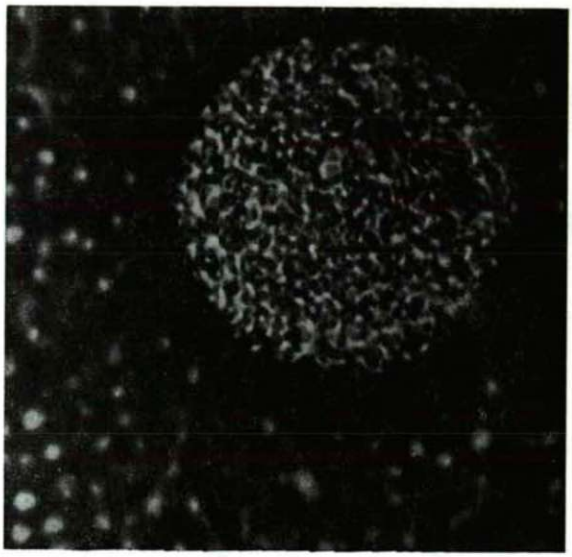
Quantitative investigation of the microorganisms of the soil is a very difficult task. Cutler's method worked out in the twenties, is still used for this purpose (Darbyshire, 1966). The active protozoa of the sample were killed by 2% hydrochloric acid. The number of remaining cysts was subtracted from the total number of protozoa and this gave the number of active protozoa. For the liberation of the microorganisms adhering to the soil particles and roots usually mechanical methods are used (Singh, 1955). Singh's method was modified by Darbyshire (1966), who shook the samples for 5 minutes at 20°C in an incubator shaker. According to my observations such a procedure activated a large number of the cysts (Biczók, 1957) and this throws doubt on the value of the method. For studying the interactions of the microorganisms we often use bacteriumcultures into which protozoa have been inoculated. Using negative nigrosine staining and taking into consideration the amount of water escaping on drying, we got to know not only the quantitative conditions, but also the morphology of the microorganisms of the culture (Figs. 1a and b). Part ot the Hypotricha protozoa, however perish. Their number and quality must be checked by direcf examination of the culture, by fixing and staining methods.

Results and conclusions

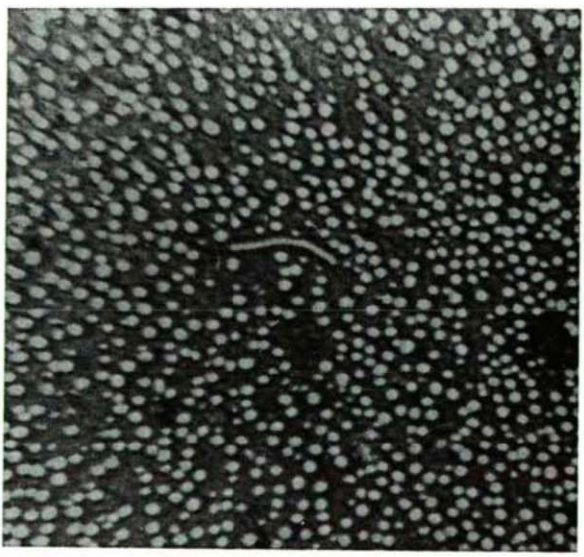
1. Development of the rhizosphere

The development of the rhizosphere is a subject which involves many disputable questions, at least as concerns the protozoa. This is partly due to the fact that the research of the infusoria of the rhizosphere has but a short history, although in recent times more and more researchers turn to the research of these (Biczók, 1952–1965; Darbishyre, 1966; Decheva, 1966; Decloitre, 1975; Geltzer, 1961; 1963; Nikoljuk, 1956; 1968; Rovira, and Davey, 1974; Varga, 1958 etc).

There are three approaches to the problem. (a) Analysis of the 282 species of protozoa of the rhizosphere (66 species of flagellates, 106 species of rhysopods and 110 species of ciliates) demonstrated by me. (b) Examination of the microorganism of the seeds that find their way into the soil. (c) Inoculation of soil and fresh-water protozoa into steril soil and studying of their behavior.



a



b

Fig. 1. Microorganisms in the root extract of (a) *Oenothera biennis* and (b) *Aristolochia clematiti* on the 12-th day (In Fig. b encysting *Colpoda fastigata*. Negative nigrosine staining).

Of the flagellates the following were most frequent and numerous in the rhizosphere of the different plants: *Bodo edax, B. globosus, Oicomonas mutabilis, O. termo* and *Scytomonas pusilla.* Of the rhysopods: *Amoeba gorgonia, A. verrucosa, Dactylosphaerium radiosum, Vahlkampfia limax, Naegleria gruberi, Cryptodifflugia oviformis, Euglypha alveolata, E. laevis, Sphenoderia dentata, Trinema enchelis, T. lineare.* Of the ciliates: *Colpoda cucullus, C. fastigata, C. inflata, C. steinii, Glaucoma scintillans, Platiophrya lata, Uronema marinum, Cyclidium glaucoma.*

The species listed above can be found among the 250 species demonstrated from the soil by SANDON (1927) as well as among the soil-dwelling species found by VARGA. Although several researchers consider the unicellular organisms that occur here soil-dwellers ("Bodenbewohnenden"; LEMINGER 1972), we must suppose that a considerable part of the protozoon population of the soil comes from other biotopes (46% i.e. almost one half of the 282 species live in decayed matter, waters rich in bacteria and pools and only 18% of them are known from the soil). It is very probable that adaptation through thousands of years has made it possible for most of these species to be active in the soil for shorter or longer time. First of all the good many species listed above come into question. It must, however, be taken into consideration, that most of the protozoa have a wide ecological valence. *Colpoda cucullus* and steinii for example thrives well in the antarctic region (SUDZUKI and SHIMOIZUMI, 1957), *Cyclidium glaucoma* in waters of 41–51 °C temperature (ISSEL, 1910), *Euglypha alveolata* in dirty waters very poor in O_2 (LACKEY, 1938) and *Cyclidium glaucoma* in media made almost anaerobic by a gas binding O_2 (NIKITINSKY, 1930). But it must also be taken into consideration that the protozoa active in the rhizosphere are also exposed to stress effects, which in the course of millions of years have led to cyst formation. The protozoa that could not develop this ability are completely absent in the sub-biotope discussed here. Besides VARGA others also emphasized as a possibility of adaptation that the protozoa in the soil are smaller than those in fresh water (KEVAN, 1972). We must also be aware of the fact that active and in cultures encysted protozoa isolated from the rhizoplanes thrive in the culture liquid, and there are considerable differences of size between members of the successive generations.

From the point of view of the development of the rhizosphere it is important that the seeds which get into soil, e.g. the wheat grain, carry a large mass of microorganisms with them (BICZÓK, 1956). The majority of the cultured species are known from the soil and the rhizosphere. On the other hand the phytoflagellates were nearly completely lacking. This fact suggested that the seeds were infected from the soil. SZABÓ (1968) made similar investigations on the microbial level with seeds of *Robinia pseudacacia.* According to his investigations infection took place already in the hull. The picked seeds were infected wit bacteria depending on the circumstances and part of these bacteria have nothing to do with the rhizoplane flora. Root symbiotes may occur among them; the others die in the soil.

More convincing proofs could be expected from the inoculation of the protozoa into sterile soils. I carried out several such investigations.

(a) From a *Colpoda fastigata* clone culture taken from the soil I transferred cysts together bacteria to different sterile soils or more axactly into a hollow made in the middle of their surface. The soil samples closed in by glass plates were saturated with root bren from below. It appeared that the activated animals passed through the 3.5 cm thick garden soil in 8 days, through the 4.5 cm thick layer in

10 days, and trough 12 and 18 cm thick layers in 31 and 35 days respectively. Their movement in the soil became observable after fixation with a mixture of sublimate: formalin (9:1) of the active (partly encysted) forms which adhered to the glass plates when they were taken out periodically. Thus not only the movement, but also the great proliferation of the protozoa in the soil became evident. The active state gradually decreased, but lasted several months. The dynamic movements and changes of state appeared as functions of the changes of the bacteria.

(b) The inoculated materials stemmed from the culture of moss from sodic soil and from the plankton of a small pond near Szeged. The sterile garden soil filtered both materials and selected their microorganisms. The results of these investigations are remarkable. The protozoa isolated from the moss, the small pond and the rhizosphere significantly differed from each other. On the other hand the species (part of which could not be demonstrated in active form either from the moss or from the pond) passing through the sterile soil and demonstrable from the culture liquid or the glass plates are those that are well known from the rhizosphere (Those activated from moss: *Oicomonas termo, Bodo globosus, Bodo* sp., *Naegleria gruberi, Dimastigamoeba soli, Vahlkampfia limax, Colpoda maupasi, C. steinii, Trichopelma sphagnetorum.* From the water of the pond: *Bodo* sp., *Cercobodo* sp., *Oicomonas termo, Naegleria gruberi, Cyclidium glaucoma, Tetrahymena pyriformis, Colpoda steinii,* and some *Hypotricha*).

These methods can be refined (in soil filtrates, with known mono- or polybacterial material using several glass plates). Even so it can be determined which protozoa are nearest to the true soil-dwelling protozoa or are just those (euedafic) and which are not those (euriedafic). Besides this, they show the possible dispersion or proliferation of the protozoa in the rhizosphere. The spreading of the microorganisms in time, which is demonstrable at the bottom of the experimental vessels and on the glass plates, is regular. This regularity can be expressed by the quotient of the distance covered in the soil (S) and the time needed for it (T) (BICZÓK, 1959). In my opinion the earlier term diffusion quotient (DQ) should more correctly be changed to dispersion quotient:

$$DQ = S/T$$

This formula depends on many factors (the structure, water content, temperature, O_2 content of the soil, the presence of material from roots, the amount and composition of the microorganisms, etc). According to my experiments made so far, this applies to both horizontal and vertical dispersion, which excludes among others such a supposition that the migrating gravitational water or solutions could have a decisive role in the penetration of the microorganisms into the soil and their appearance in the rhizoplane.

2. The Rhizosphere Effect

In the research of the rhizosphere the study of the microorganisms of the rhizoplane is in the centre. This is where most bacteria, protozoa and fungi live, metabolism, material and energy exchange are most active. The basis of this is expressed by Katznelson's R/S ratio (1946), which is nothing else than the quotient of the number of microbes in the rhizosphere (R) and in the soil outside the former (S).

In the case of wheat this is 4:1. This quotient can evidently be applied to the protozoa as well. In a closer approach such a connection may be sought between the microorganisms washed off from the roots together with particles of soil and the microorganisms firmly adhering to roots. The latter group is much richer. This is why investigation of the rhizoplane studying of its fauna, is a thankful task (BICZÓK, 1956).

The rhizosphere effect manifests itself most clearly perhaps trough the root secretions, as these are the primary energy source for the microbes and partly the protozoa, too, for according to the findings of various authors, in the exudate of *Triticum aestivum* for example 11 kinds of sugar, 19 different kinds of amino acids, 10 organic acids, 3 nucleotides, flavone and just as many enzymes can be found. Part of these are important in the maintenance of exenic cultures. The amount, quality and balance of the substances mentioned vary according to the plant species, but also according to age. But the exudative substances of the rotting, decaying roots or root cells also change. In older plants the coline of the roots is an important substance (e.g. in the *Bromus*) as it inhibits the growth of microorganisms. It is possible that such an inhibitor — like substance is the explanation of the great difference between the protozoon populations of the young wheat roots and the stubble — field root cultures: Table 1.

Table 1

	Young wheat roots	Stubble-field wheat roots
Flagellates	1,244,500	12,200
Rhysopods	88,717	640
Ciliates	1,364	247 per cu cm

The major processes (allelopathy) induced by the substances excreted by the plant roots play directly or indirectly (first of all through the bacteria) a regulatory role in many respects in the rhizosphere. On the basis of observations made on protozoon cultures of root extracts, which may be regarded as crude models, this conclusion can be drawn (BICZÓK, 1955b). Fig. 2 shows the complex interactions taking place in root extract of Colpoda fastigata from which it is not difficult to read out the stimulating and inhibiting effects, the function of inhibitors which in certain cultures cause massive encystment. Perhaps this makes it understandable why there are so many cysts in the rhyzoplane, why the cultures of carefully washed roots are repopulated so soon and contain — even though temporarily — a large number of microorganism species and individuals.

Encystment is in many respect a function of the rhizosphere effect. It is a process which take place not with clocklike regularity. It is the result of stress exercised by the presence of damaging factors, the depletion or lack of substances essential for life. The cysted state is lasting protection against these (protective cyst) but in part of the cyst it also means reproduction (reproductive cyst). Preservation of the species is thus better ensured. This kind of reproduction is common in the soil, and always results in smaller forms. We must think of this when we bring up as argument for the soil-dwelling nature of certain protozoa the fact that they are smaller-sized than usual (KEVAN, 1962).

Cystment is a result of soil-dwelling way of life and it is not impossible that it

was evolved to a higher level by the rhizosphere effect. Therefore species incapable of developing a cyst are absent in the soil, the rhizoplane. It might be mentioned in addition that encystment occurs also in the culture liquids; dilution of the culture liquids leads to excystment (The concentration of damaging substances is decreased). Root extracts often cause excystment. From this it follows that the periodicity of cystment is not necessary; it only seems to be so because the evoking factors occur periodically in certain cases.

From the effect of the root extract their specificity could be inferred (1955b) and the fact that the exudates of the roots differ from species to species of plants and thus also the microflora and therefore the protozoon populations of the rhizosphere of different plant species too. Fig. 2 justifies this conclusion. More convincing was the fact that in the rhizosphere in sterile soil of different, mutually infected seeds significantly different protozoon faunas developed.

3. The quantity and quality of the protozoa in the rhizosphere

The importance of the rhizosphere effect can be assessed by the quantitative and qualitative composition of the protozoa; by the complex processes in which besides the bacteria and protozoa with a smaller but not negligible number of species and individuals the fungi (HEAL, 1963), nematodes (ANDRÁSSY, 1953), rotatoria, occasionally acarina, tardigrada and algae (NOVICOVA, 1968), take part.

The members of the rhizosphere coenosis are in constant interaction. It is difficult to gain an insight into the complex chain of these interactions because it contains as its components physical and chemical factors of the soil, meteorological as well as biological effects.

Among the chemical factors I mention the pH examined also by myself because its extreme values are a serious limiting factor for the microorganisms. According to LACKEY (1938) *Pleuromonas jaculans* can bear even a pH 2.2-, *Actynophrys* a pH 3-, *Chlamidomonas* and *Urostrycha* a pH 1.8. In my laboratory some protozoa of the rhizosphere (e.g. *Amoeba verrucosa, Trinema lineare*) could bear even the strongly alkaline value of 10, but more of them tolerated the acid pH 4 (*Vahlkampfia limax, Trinema enchelis,* the *Colpoda,* especially the *fastigata* and *steinii* species, as well as *Tachiosoma pellionella* and *Trichopelma sphagnetorum*). According to my observations the majority of the flagellates tolerated these extreme values well. The findings of VARGA (1933) indicate narrover limits of tolerance.

Besides the pH the water content, the O_2 and the temperature are important factors of the dynamic processes of the rhizosphere. These are factors that precondition each other. Our finding that in the lower-lying wet area of the meadow examined by us the number of one-celled organisms is very small, that there are half as many testacea as in the higherlying areas (BICZÓK, 1954; 1955a) practically means that the O_2-consuming decay processes have become increased in this area. Frequent water covering is anyway unfavourable from the point of view of soil respiration, just as much as a higher temperature, which reduces the O_2 of the soil and at the same time increases the O_2 demand of the microorganisms. It is true that 25% of our 106 rhizopods demonstrated from the rhizosphere are satisfied also with less O_2, 21% of them also with O_2 in traces, 17% 110 ciliates has a decreased O_2 demand, 11% of them are near to the anaerobic state, yet the above-mentioned variation is not indifferent because many species may die or encysted.

Meteorological influences play an important role in the quantitative and qualitative development of the protozoa. The classical investigations of KISS (1951) clearly proved the weather — sensitivity of the neuston and seston organisms: the fact that the swarming of certain microorganisms, their increased vegetative and reproduktive processes take place under prefront influence, and the ionization following radiation and electric effects play an important role in this. The proliferation of microorganisms in the rainwater pools in the town of Szeged was interpreted similarly by GELEI and SZABADOS (1950). We have also pointed out this phenomenon in connection with the rhizosphere of the wheat (Fig. 3). There we described not only the development of the proliferation, but also the fluctuation and succession phenomena, as well as the nutrition biological aspects of the various populations (BICZÓK, 1953; 1956).

The major seasonal changes in the number of the protozoa are of a different character. Accordind to HEAL (1964) a maximum of the quantity of the moss — inhabiting testacea can be observed between May and October. In the case of the protozoa of the soil, the maximum number was reached in November and December; from then on the March there was a sharply decreasing tendency, then in August there was a new maximum (FEHÉR and VARGA, 1944). In the rhizosphere of the wheat the number of the protozoa was highest in November; then their number increased again in January and April. The causer of it is understandabla: the condition in November after germination is favourable for microbial activity, and so is early spring. The summer minimum is a sign of a decrease in root activity.

Many dynamic changes are connected with the peculiar character of the relationship between the bacteria and the protozoa. 40% of the 282 kinds of protozoa found in the rhizosphere eat only bacteria. Nearly as many eat other things, but also bacteria. We may therefore rightly say that "bacteria find favourable conditions for development in the rhizosphere, where they reproduce intensively and where they attract protozoa" (GELTZER, 1963). There are bacteriologists who under — estimate this problem, all the more because in comparison with the enormous number of the microbes the number of the protozoa is insignificant in the rhizosphere. But for example a single ciliate is many times larger than a bacterium. This makes their presence important. An example may be the generally 63 μ long cylindrical swarmer of *Pyxidium asymmetricum* nov.sp. found in the rhizoplane (BICZÓK, 1956). The animal and its spindle — like digesting vacuola could well be modelled, and so on the basis of the consumption of bacteria observed under oil immersion we found that in a day it consumed 60,000 bacteria and in approximately a week nearly 400,000, which corresponded to the volume or mass of the animal.

Studying of the bacteria — protozoa relationship produced many theoretical suppositions and experimental facts, which were completed by the investigation of the role of fungi. Connected with these investigations is the finding that there is an inverse relationship between the number of the protozoa and that of the bacteria (CUTLER and SANDON, 1921). This seems to be influenced by some phenomena. e.g. where the microscopic fungi, actinomycetes were abudant, the development of the amoebas was inhibited (GELTZER, 1963). These fungi and certain bacteria have often a protistocidic, sometimes a stimulating effect (GELTZER, 1969). It seems that the bacteria effect the development of the protozoa: *Azotobacter chroococcum* influences them intensively, the nitrifiers less strongly, the cellulose decomposers very slightly, the ammonifiers not at all (NIKOLJUK, 1963). With this is connected

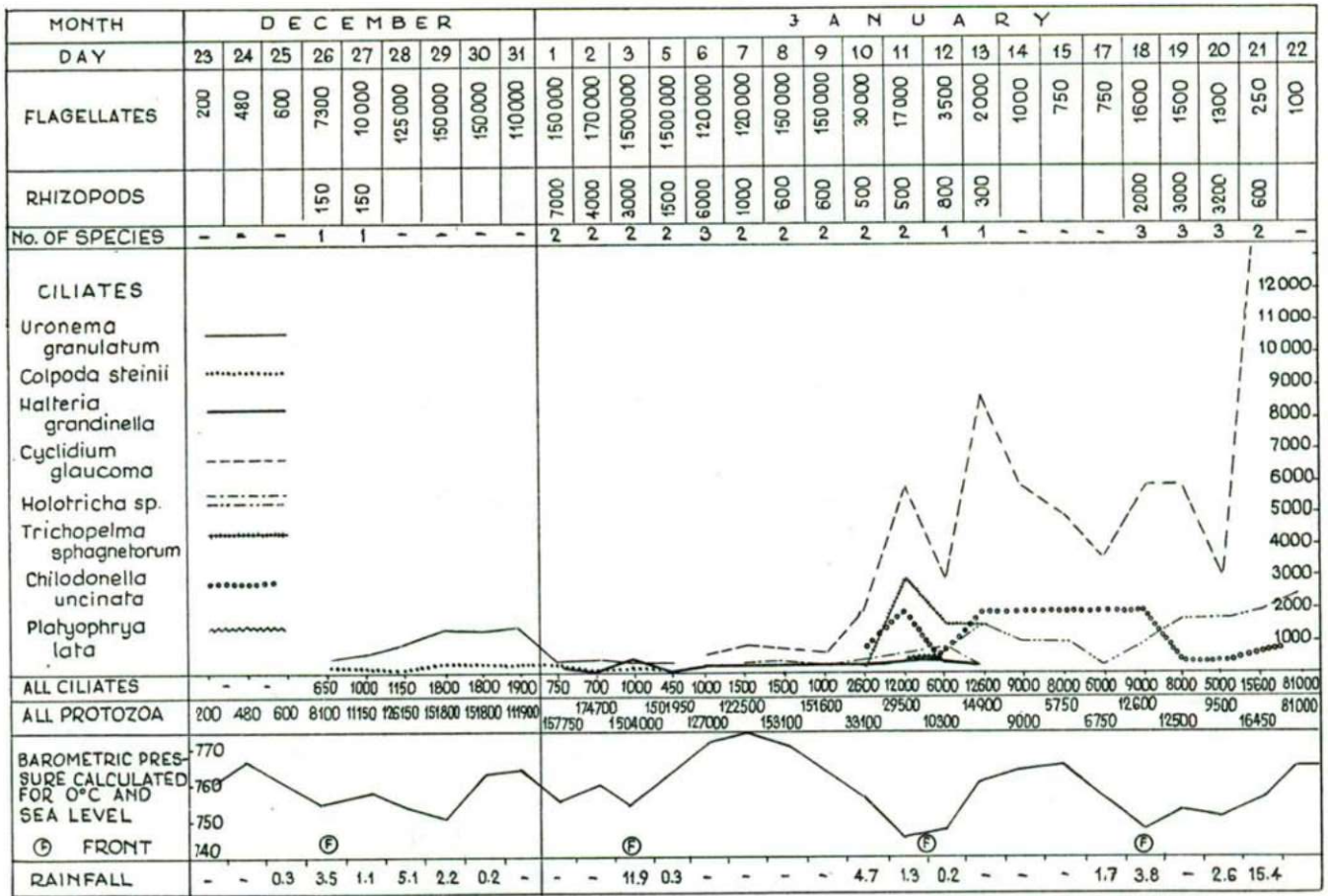


Fig. 3. Quantitative and qualitative changes in protozoa of the rhizosphere of the wheat caused by meteorological influence in December 1951 and January 1952.

the fact that the amoebas digest certain bacteriaw ith ease, others with difficulty (SINGH, 1941). Similar is the situation with certain ciliates (BURBANCK, 1942).

The inhibitions and stimulations resulting from the mutual influences can be followed by the observation of the increase (TELEGDI—KOVÁTS, 1932) or decrease of the CO_2 production, just as the carbon-dioxide production gives information on the activity of the microorganisms in the soil. This is why we investigated the O_2 consumption of the rhizoplane, the soil next to it, and the soil outside the rhizosphere (expressing 1 hour's consumption in μl/g) between 1969 and 1972. It was found that the average consumption of the soil outside the rhizosphere was 2.4 μl, that of the soil next to the roots 3.1 μl, and that of the rhizoplane 38.5 μl/g. The results show clearly why we must concentrate our attention on the research of the rhizoplane. It is worth while to mention that the minimum of consumption was observed in August and December, its maximum in June. This is not connected with the seasonal change of the amount of the microorganisms.

4. The Effect of the Protozoa on plants

Among the complex mutual influences a central question is that influence the protozoa exercise on the roots or the whole plant. This is the most difficult and least cleared question. It is a reasonable supposition that by eating bacteria that are harmful for the development of plants the protozoa influence the development of plants (IWAO HINO, 1926). This is just as barren a hypothesis as that of RUSSEL and HUTCHINSONS' (1909), according to which the infusoria accumulating in soil considerably reduce the population of bacteria, and this results in tiring of the soil. The number of the protozoa is small for this, and the active state of the majority of them is of shorter duration than that of the bacteria. The presumable effect of the metabolites of the protozoa (GELLÉRT, 1958) their soil-preparing role the direct influence of their biologically active substances on the plants (NIKOLJUK, 1954) are questions that deserve further research. But those species, indicators must also be investigated on the basis of which the influence of the protozoa on the plants appears. It is noteworthy that the rhizoplane sometimes contains very many cysts, sometimes chiefly around erosions; it is possible that certain active forms are parasites of the roots.

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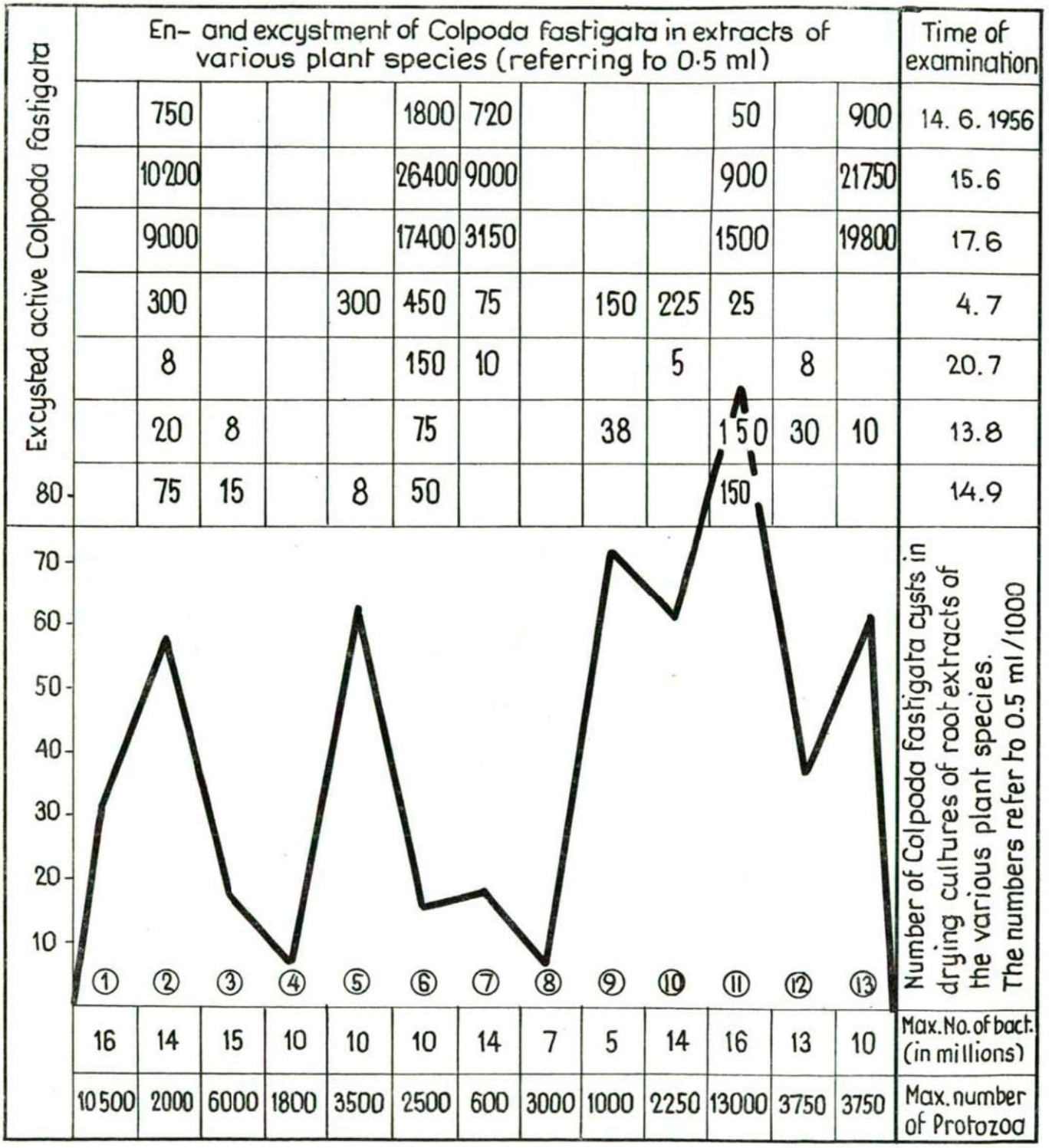

Fig. 2. Cystment of Colpoda fastigata in extracts of various plant species. Explanation: Root extracts of *Achillea millefolium* (1), *Allium angulosum* (2), *Aristolochia clematitis* (3), *Datura strammonium* (4), *Cichorium intybus* (5), *Daucus carota* (6), *Mentha longifolia* (7), *Ononis spinosa* (8), *Nonea pulla* (9), *Reseda lutea* (10), *Salvia nemorosa* (11), *Solanum dulcamare* (12), *Verbena offcinalis* (13).

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