Decomposition of cellulose in soils[1]

JOHANNA C. WENT AND FROUKJE DE JONG

Instituut voor Toegepast Biologisch Onderzoek in de Natuur (Institute for Biological Field Research), Arnhem, The Netherlands

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1. Cellulose decomposition in forest and orchard soils was investigated by studying the breakdown of boiled and washed cellophane in the soils and in vitro.

Decomposition occurred from quick to slow in the order: orchard on clay soil, forest on clay soil, forest on sandy loam, and in the latter in the order: calcareous mull, acid mull and mor.

2. In the different forest soils which were investigated the rate of decomposition was parallel to their water capacity. It slowed down considerably when the water content of the soil decreased, especially after the wilting point was reached.

3. Of the fungi isolated from these soils, those from orchard soil – 5% to 50% *Fusarium* spp. – were among the fastest decomposers of cellulose. This agrees with, and may explain the high rate of decomposition in orchard soil.

4. Decomposition in pure culture is quicker than in soil. As filter-sterilized soil extract checked the decomposition in pure culture, but heat-sterilized soil extract did not, an extractable but heat-sensitive substance may be one retarding factor.

INTRODUCTION

The decomposition of organic matter in different types of soil is one of the research topics of the Institute for Biological Field Research. The present article is concerned with a comparative investigation on *cellulose* breakdown, especially in the litter of two types of forests and in orchard soil.

The soil in one of the experimental forests belongs partly to the mor type and partly to the mull type. *In the mor type*, the accumulated litter of several years forms a distinctly separate layer covering the mineral soil under it. *In the mull type*, the lower layers of litter are mixed with the mineral soil: there is a gradual transition from one component to the other. The mixing of the litter with the mineral soil is due to worms and other small animals. In this

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type of soil the organic material on the top (the fallen leaves) disappears within one year.

The mor type in this forest is found where the water table is low; otherwise one finds the mull type (Minderman, 1960).

Our program included the following items:

1) What is the rate of cellulose decomposition in the litter and in the soil?
2) Which organisms – fungi, bacteria, animals – decompose the cellulose in the soil?
3) Which factors affect the speed of this process?

MATERIAL AND METHODS

Field research was done in:

An oak forest, formerly an oak coppice, in Hackfort, near Vorden, on a loamy mineral soil,

partly of the mor type, pH 3.5 (1)

partly of the mull type, acid, rich in litter, pH 4.0 (2)

"Acid mull, rich in litter" is a mull formation in between mor and acid mull. Here coarse particles of organic matter are mixed with the mineral soil.

partly of the mull type, acid, pH 5.2 (3)

partly of the mull type, calcareous, pH 7.2 (4)

For a description of the soils in this forest, see Minderman (1960).

An old oak forest of the mull type, pH 4.2, on a riverbasin clay soil, in Middachten near Ellecom. (5)

An old orchard, near Beuningen, on a light stream-ridge clay soil (pH 5.4), covered with grass and planted with pear and apple. (6)

A young orchard on the same type of soil (pH 6.4), covered with grass and planted with apple, also near Beuningen. (7)

Rate of decomposition of cellulose in the soil. The process of cellulose decomposition was estimated by inspecting at intervals cellophane sheets sticking to slides which were buried in the soil. The cellophane (number PT 300) from AKU Ltd., Arnhem, was boiled and washed before it was dried on to the slides. The cellophane-covered slides were buried in a vertical position with only the upper 1 cm sticking out of the soil (Tribe, 1957). In every experiment we buried a row of 10 – 30 slides in one plane, to place them against an undisturbed soil surface. At first, slides were taken every week from the soil and examined in the laboratory, later at intervals of 5 – 10 weeks. The results were expressed in numbers, 0 meaning that no cellophane had disappeared, 9 that decomposition was complete.

Microscopical observation of cellophane decomposition in the soil. Cellophane-covered slides could be examined under the microscope when taken from the soil within the first three to four weeks. After 4 weeks too many fungi were growing over the cellophane to study it under the microscope.

For a prolonged observation of the process of decomposition, cellophane-covered glass plates (6 × 11 cm, 1 mm thick) were inserted in the soil. After 3 weeks they were recovered together with the soil against which the cellophane had been pressed. A small cell was constructed with glass plates (Christensen, 1956) allowing observation under a highly magnifying binocular loupe to study the way small animals consumed the cellophane overgrown by fungi and bacteria. The sequence of the organisms decomposing the cellulose was studied by these methods.

Isolation of cellulose-decomposing microorganisms.

1) Dilutions of suspensions made by shaking soil and litter in water were spread on plates of mineral soil extract agar containing 0.7% cellulose; eubacteria, myxobacteria, actinomycetes and occasionally fungi grew out of the soil on to the agar (direct method) (Harmsen, 1946). We did not use the method of de Barjac (1957) for counting the bacteria.

2) Enrichment cultures made on filter paper in a mineral solution were plated out when breakdown of the filterpaper could be seen.

3) Cellophane which had been buried in the soil for two weeks was cut in small pieces of 6 × 4 mm which were placed on cherry agar. Fungi grew out of the cellophane and could be isolated after about two weeks (Tribe, 1957). In some experiments all the pieces of cellophane were used, in other experiments only those parts in which fungal attack of the cellophane was found under the microscope.

Examination of cellophane-decomposing capacity of isolates. The isolates were tested for cellophane decomposition at 28 C, and those, which did decompose the cellophane within 21 days, also at 15 C, 10 C and 5 C.

Three pieces of cellophane, 2 × 2 cm, were placed in each petri dish containing a mineral medium with soil extract, then inoculated with a fungus or a bacterium. After 6 – 30 days the results were read.

Soil-extract agar. In order to study the effect of soil extracts on cellophane decomposition by fungi in pure culture three media with agar were prepared.

Soil extracts were either heat-sterilized or filter-sterilized. The heat-sterilized soil extract was prepared by heating 1 kg of clay soil with 1 liter water at 120 C for 30 min, then adding 0.5 g $CaSO_4$ before filtering, and heat-sterilizing the filtrate. The filter-sterilized soil extract was prepared by extraction at room temperature of 1 kg of clay soil with 1 liter of water overnight, followed by filtering through a bacteriological G5 filter.

We used:

1) Agar with inorganic salts and 5% of heat-sterilized soil extract.
2) Agar with inorganic salts and 50% of heat-sterilized soil extract.
3) Agar with inorganic salts and 50% of filter-sterilized soil extract.

Pieces of sterile cellophane were placed on these agars and inoculated with the fungus under examination.

RESULTS

Cellulose decomposition in the soil

We compared the disappearance of cellophane mounted on slides placed in different types of forest floor and in orchard soils (Went, 1959). Figs. 1 and

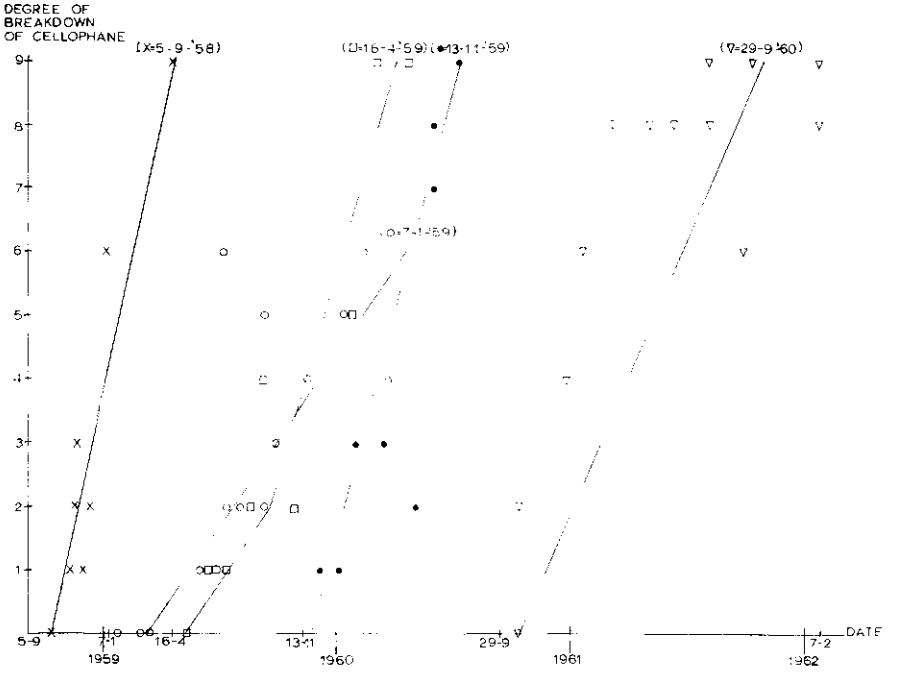

Fig. 1. Breakdown of cellophane in the calcareous mull part of the Hackfort forest soil between September 1958 and February 1962, with a very dry period in 1959 (wilting point reached). The dates are the moment of insertion of the cellophane.

2 give the results in two types of forest floor namely a calcareous mull and a mor. Single experiments were started on 5–9–'58, 7–1–'59, 16–4–'59, 13–11–'59, and 29–9–'60. The breakdown of the cellophane was taken as a measure of the cellulose decomposition in the soil.

Several experiments were continued for more than one year; in some, the

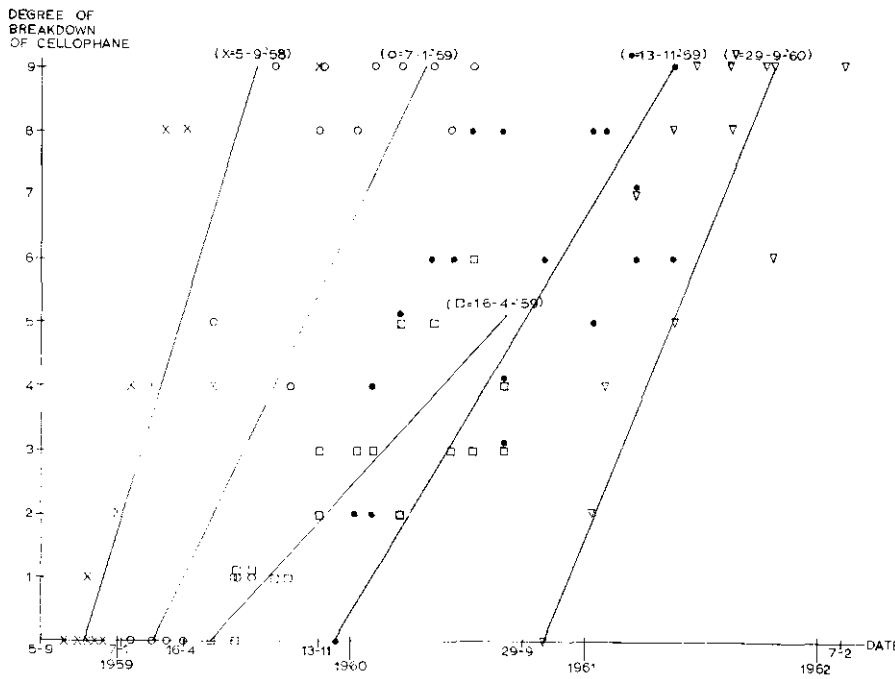

Fig. 2. The same as Fig. 1, but for the mor part of the Hackfort forest.

decomposition of the cellophane was so slow that all the slides had been recovered before the cellophane had been broken down completely.

In the calcareous mull the cellophane disappeared quicker than in the mor as is shown by the comparison of the inclination of the lines in Figs. 1 and 2, but it is always a slow process requiring 20 – 40 weeks, or even more. Exceptional results were obtained with the experiments started on January 7th 1959. From this date onwards the temperature in between the litter and the mineral soil was below zero for one month. In March 1959 a very dry period started which continued until January 1960. In February – April 1960 the rainfall was below normal again. In the calcareous mull part of the forest the wilting point – seldom reached in the Netherlands in these soils – was reached in the middle of May 1959; in the mor, however, only by the end of June. The difference in the drying out of the two forest-floor types has been described by Witkamp (1960, p. 5). Apparently, the cellulose breakdown slowed down when the drought started, and therefore it was inhibited or very much retarded, in the mull earlier than in the mor.

In the experiment started on 16–4–1959, the breakdown of cellulose in the mull returned to normal owing to a few heavy rainfalls in July and in August.

There was not enough rain to restore the usual water content of the mor. During this period the breakdown in the mor was much slower than normal.

The cellophane inserted at the end of 1959 needed less time to decompose than the cellophane inserted during the dry period, and that inserted in September 1960 even less. By then, the rate of decomposition in the mor had returned to normal and in the calcareous mull almost so. The cellophane inserted in January 1961 was decomposing very slowly in the mor, but at a normal rate in the mull. We cannot explain these last differences.

In Fig. 3 the breakdown of cellophane can be followed in 4 different types

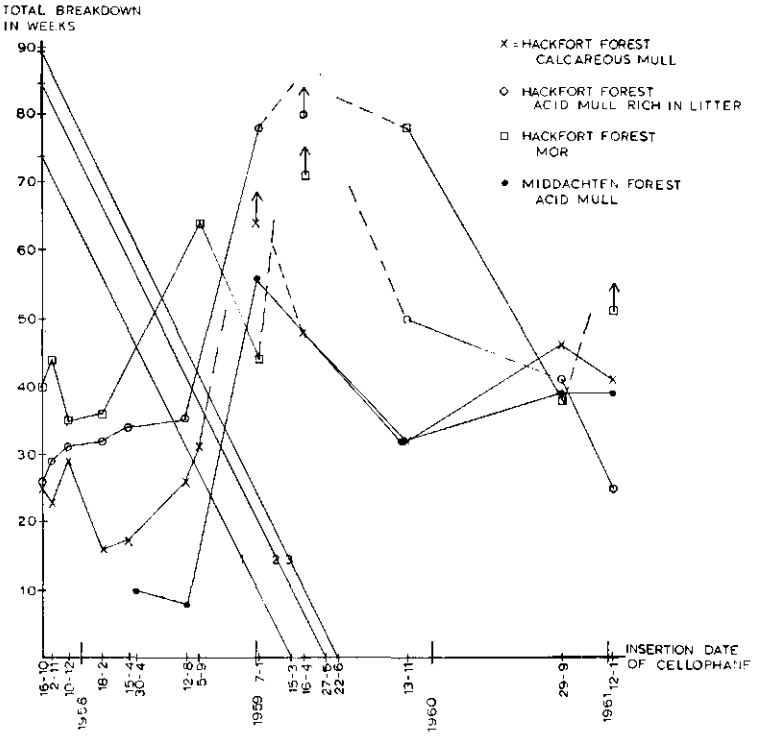

Fig. 3. Lines of total breakdown of cellophane in 4 different types of forest soil in the course of $3\frac{1}{2}$ years.
1: line connecting the points when the dry period in 1959 started. The date on the abscissa corresponds with the number of weeks on the ordinate.
2: line showing the moment when the wilting point in the calcareous mull was reached.
3: line showing the moment when the wilting point in the mor was reached.

of forest floor and over a longer period than in Figs. 1 and 2. In Fig. 3 only the number of weeks necessary for total breakdown is given.

The sequence of the rate of attack from the quickest to the slowest is:

Middachten forest, acid mull; Hackfort forest, calcareous mull; Hackfort forest, acid mull rich in litter; Hackfort forest, mor. In all these forest-floor types the dry period which started in March 1959 caused a great delay in the breakdown. The delay was so great that total decomposition was not reached after 60 – 80 weeks. As this was not foreseen, the number of cellophane slides placed in the soil was insufficient and when the last slide was recovered total decomposition had not been reached. These points are indicated with an arrow, as total decomposition would have been after more weeks than shown in Fig. 3. The lines connecting the points of total decomposition are indicated here by broken lines.

Three straight lines connect the date and the number of weeks after the beginning of the experiment when 1) the dry period started, 2) the wilting point in the calcareous mull was reached (moisture content of the soil below 6.9% w/w), 3) the wilting point in the mor was reached (moisture content of the soil below 13.0% w/w; Witkamp, 1960). It is evident that these lines coincide with retardation in the breakdown of the cellophane.

In orchard soils the cellophane was very quickly decomposed. After 4 weeks, estimations of cellophane breakdown could no longer be made, as the remaining cellophane stuck to the heavy clay soil and could not be recovered.

Golley (1960) also used cellophane to study the cellulose decomposition of different soils. Not all the soils which he investigated gave a good cellulose decay. He could not explain the absence of decomposition in some of the soils. He investigated decomposition in these soils for only 3 – 4 weeks and this may have been too short a period.

Microscopical observation of successive cellulose-decomposing organisms

In the cellophane sheet on slides recovered within 4 weeks after insertion in the soil it was possible to identify foci of attack by fungi, eubacteria and cytophaga's (Fig. 4). In the first few weeks, slides from orchard soils showed more foci of attack by bacteria than slides from forest soils, where fungi dominated from the first. When fungi appeared on the slides from orchard soil, the growth was often striated (Fig. 5, p. 49). This may be due to an attack by *Fusarium*, as a similar picture appeared in a pure culture of *Fusarium* on cellophane. This striation is not the same for different fungi. The direction of the striation is caused by the way the cellophane is fabricated.

Later, the cellophane in forest soils was overgrown by fungi and no separate foci of attack could be recognized. When fungi were covering the cellophane, small animals would start eating these fungi, and in this way gave rise to a great many holes in the cellophane. In the end, no cellophane was left.

Fig. 4. Cellophane recovered from forest soil (mor) attacked by fungi (fanlike growth and hyphae), *Cytophaga*, and slugs or snails. 390 ×.

In the glass cells the following animals were observed eating the fungus-covered cellophane: an oribatid mite (*Rhysotritia duplicata*), collemboles (springtails), enchytraeids (potworms) and fly larvae (*Mycetophilidae*).

The intestinal secretion (gut-juice) of snails and slugs has long been known to contain cellulase. So any eating before the cellophane had been invaded by fungi may be due to these animals (Fig. 6, p. 51).

TABLE 1

Isolations on cellulose agar, *a*, by the direct method, *b*, by inoculation from enrichment medium. Number of isolation dates yielding at least one bacterial isolation.

Material investigated	Soil type	pH KCl	*a*			*b*	
			Number of dates on which isolations were made	Number yielding eubacteria	Number yielding *Cytophaga*	Number of dates on which isolations were made	Number yielding eubacteria and/or *Cytophaga*
Soil	1	7.2	7	6	0	9	8
Soil	2	4.0	7	4	0	9	6
Soil	3	5.2	7	3	0	9	4
Soil	4	3.5	7	2	0	10	2
Litter	1	6.7	14	8	6	15	14
Litter	2	3.0	13	7	7	14	14
Litter	3	5.0	13	5	1	14	14
Litter	4	3.0	14	4	1	15	10

1 = calcareous mull, 2 = acid mull rich in litter, 3 = acid mull, 4 = mor.

Isolations by the direct method and by enrichment culture on filter paper in a mineral solution

Dilution plates of forest soil and litter on cellulose agar as well as enrichment cultures on filter paper yielded mainly bacteria, myxobacteria (cytophaga's) and actinomycetes. The results are presented in Table 1.

It was not feasible to count the bacteria and cytophaga's on cellulose agar. In the lower dilutions fungi would overgrow the agar, and in the higher dilutions the numbers of bacteria and cytophaga's were usually too small to be used for counts to calculate the numbers of these organisms present in the soil. Therefore only the presence or the absence of the organisms in the different isolations is mentioned in Table 1.

1. The table shows that cytophaga's were never isolated from soil on cellulose agar, but often from litter. Enrichment cultures inoculated with litter yielded more bacteria than those inoculated with soil from the same location.

2. From the litter on top of the soils with a quick decomposition (mull) cytophaga's are isolated as often as bacteria; from those with a slower decomposition (mor and mull rich in litter) eubacteria are isolated more frequently.

Isolations from buried cellophane

Fungi attacking cellulose were isolated from cellophane left in the soil for one, two or three weeks.

TABLE 2

Isolation of fungi from cellophane that had been buried for about three weeks in different soils

Stand	Soil type	Isolation date	Number of cellophane squares	% Isolates					
				Trich.	*Pach.*	*Fus.*	*Pen.*	*Mucor*	Other fungi
Forest 1	calc. mull	12–1–61	64	23	0	0	40	3	35
Forest 1	calc. mull	15–3–61	54	30	10	0	35	0	24
Forest 1	acid mull	12–1–61	64	40	2	0	53	1	4
Forest 1	acid mull	15–3–61	66	41	1	0	34	11	14
Forest 1	mor	12–1–61	56	18	38	0	38	2	3
Forest 1	mor	15–3–61	60	35	23	0	34	2	5
Forest 2	acid mull	12–1–61	76	23	0	0	40	3	35
Forest 2	acid mull	15–3–61	72	49	2	0	19	11	20
Orchard 3	mull	17–4–61	54	12	0	11	18	12	47
Orchard 3	muli	21–6–62	78	0	1	25	3	54	17
Orchard 4	mor	17–4–61	72	7	0	15	26	30	21
Orchard 4	mor	21–6–62	78	3	1	50	3	33	11
Orchard 5	mull	17–4–61	60	13	0	5	20	11	51
Orchard 6	mull	21–6–62	78	6	0	43	6	11	35
Orchard 7	mull	17–4–61	66	25	0	19	12	5	38

1: Hackfort oak forest, 2: Middachten oak forest, 3: old orchard, under apple trees, 4: old orchard, under pear trees, 5: old orchard, between trees, 6: young apple orchard planted on old orchard soil, 7: young apple orchard planted on old arable land.
Trich. = *Trichoderma*, *Pach.* = *Pachybasium*, *Fus.* = *Fusarium*, *Pen.* = *Penicillium*.

The cellophane was either cut in small pieces or only such parts were cut out of the sheet where fungal attack was evident under the microscope. Each piece of cellophane was placed on cherry agar. The former method yielded a lesser diversity of fungi, the majority belonging to the genera *Penicillium*, *Trichoderma*, *Pachybasium*, *Mucor* and *Fusarium*. Table 2 gives the numbers of isolations of each of these genera from different soil types, obtained with this method.

Trichoderma and *Penicillium* are more often isolated from forest soils, whether acid or neutral, than from orchard soils, whereas *Fusarium* and *Mucor* were more often isolated from the latter. Isolations were made on two different dates but it is not possible to draw any conclusions about differences in the results without many repetitions of these isolations.

Besides the fungi mentioned in Table 2, the following were isolated from orchard soils by the first method: *Alternaria*, *Cephalosporium*, *Cercosporella*, *Cladosporium*, *Cylindrocladium*, *Dematium*, *Dothiorella*, *Gliocladium*, *Humicola*, *Innatospora*, *Macrosporium*, *Monilia*, picnidial fungi with dark spores,

Fig. 5. Cellophane recovered from orchard soil. 390 ×.

dark picnidial fungi, *Phoma*, *Phomopsis*, *Rhizoctonia*, *Tilachlidium*, *Thozetellopsis*, *Verticillium*, and many sterile fungi.

From forest soils 158 isolations were made with the second method. Thirty-five percent belong to the genera *Trichoderma*, *Pachybasium*, *Penicillium*, and *Mucor*. Thirty per cent do not sporulate. The other 35% belong to the genera: *Alternaria*, *Botrytis*, *Cephalosporium*, *Ceuthospora*, *Chaetomium*, *Coniothyrium*, *Dematium*, *Fusarium*, *Humicola*, *Idriella*, *Myrothecium*, *Phoma*, and *Verticillium*.

Examination of cellulose-decomposing capacity

The isolates were tested for cellulose-decomposing capacity. The results are presented in Table 3. Only those fungi causing total decomposition within 7 weeks at either 5 C, 10 C or 15 C are included in the table.

TABLE 3

Number of strains capable of decomposing cellophane and number of days necessary for complete digestion at different temperatures

Fungus strain	Soil		Number of isolates tested	Number of isolates and number of days after which the cellophane is completely digested at different temperatures					
				15 C		10 C		5 C	
	Forest	Orchard		Numb. isol.	Days	Numb. isol.	Days	Numb. isol.	Days
Fusarium avenaceum		+	2	1	12	1	12	1	22
Fus. culmorum		+	3	3	11–12	3	21–22	3	22–42
Fus. equiseti		+	1	1	12	1	22	1	22
Fus. flocciferum		+	2	2	12–22	1	12	1	22
Fus. graminearum		+	6	6	12–22	4	12–31	4	28–31
Fus. poae		+	5	5	12–22	5	12–42	5	22–31
Fus. sporotrichioïdes		+	5	5	12–22	5	21–42	4	22–42
Fus. spec.		+	10	10	12–30	8	21–28	3	21–31
Tilachlidium		+	2	2	21	2	21	2	28
Alternaria	+	+	3	3	21–22	2	21–22	2	21–31
Cephalosporium	+	+	4	1	12	1	12	1	22
Fusarium sambucinum		+	1	1	11	1	11		
Fus. sublunatum		+	1	1	21	1	28		
Thozetellopsis		+	1	1	21	1	21		
Vermicularia or Ascomycete		+	1	1	21	1	21		
Verticillium effusum?		+	1	1	21	1	35		
Humicola	+	+	9	9	19–21	6	19–35		
Pachybasium	+	+	4	4	20–30	1	30		
Trichoderma	+	+	24	23	12–31	11	22–61		
Botrytis	+		5	1	20–21	1	30–31		
Myrothecium	+		1	1	25	1	54		
Penicillium	+	+	13	2	20–30				
Chaetomium	+		1	1	33				
Coniothyrium	+		2	1	20				
Fusarium arthrosporioïdes	+		1	1	20				
Verticillium	+		2	2	30				

The fungi from the first group decompose cellophane at 5 C in 21 – 42 days (27 isolates out of 43 tested). Six non-sporulating isolates from forest soil and nine from orchard soil also belong to this group but they are not included in the table.

In the second group total decomposition was not reached at 5 C during the period of investigation, but decomposition was complete at 10 C (25 isolates

Fig. 6. Cellophane recovered from forest soil probably attacked by slugs or snails. 390 ×.

out of 48 tested).

In the third group decomposition was only completed at 15 C by 7 out of 19 isolates.

The quickest cellophane-decomposing fungi (first group) were all isolated from orchard soil: *Fusarium* spp., *Tilachlidium* sp., *Cephalosporium* sp. and *Alternaria* sp. The last two species were isolated also from forest soil.

In the second group 8 species were isolated from orchard soil; 3 of these were also isolated from forest soil and two other fungi only from forest soil.

Species from the third group were all isolated from forest soil; *Penicillium* both from forest and orchard soils.

Only two strains of *Penicillium* range among the cellulose decomposers in this table and they are among the slower ones. The other 17 isolates of this genus attack the cellophane but do not decompose it within the period of investigation.

Many strains of *Mucor* were among the isolates, but none decomposed cellophane, nor did *Dematium* sp.

Fan-like growth. Several fungi may develop a "fan-like" growth in the cellophane. This has been described by Tribe (1957) as "rooting branches" and by Gams (1960) as "Submers-Appressorien". We found this type of growth with 50% of our *Fusarium* isolates, with *Alternaria tenuis*, *Trichoderma*, *Pachybasium*, *Phomopsis*, *Monilia*, *Gliocladium*, *Cephalosporium* spp. and with some isolates of *Penicillium*.

Bacteria. Cellvibrio and *Cytophaga* were isolated by the direct method as well as by the enrichment-culture method. *Cellvibrio* was obtained in pure culture, but *Cytophaga* cultures were always contaminated by small numbers of other bacteria. Both organisms grew well when inoculated on pieces of cellophane on mineral-salts soil-extract agar. For making micrographs, the attacked cellophane sheet was washed with running water; this also removed the bacteria. *Cellvibrio* made small round holes in the cellophane (Fig. 7). *Cytophaga* was responsible for transparent areas the form and size of which are reminiscent of the organism itself (Fig. 8). This peculiar form of attack suggests that the *Cytophaga* cells decomposing the cellophane were in direct contact with it.

Bacterial decomposition was slower than breakdown by fungi of the most active group. At 15 C it took 20 – 54 days.

That Henis, Keller and Keynan (1961) and Keynan, Henis and Keller (1961) did not succeed in decomposing cellophane by bacteria may be due to the use of another grade of cellophane or because they did not boil the cellophane before use.

Comparison of the rate of decomposition in nature and in pure culture. Decomposition of cellophane in nature takes from 23 – 48 weeks in the calcareous mull soil, while in pure culture at 5 C the most active species of fungi need only 20 – 30 days. In nature apparently some factors prevent the fungi – the fastest cellulose decomposers isolated from forest soil are *Cephalosporium* and *Alternaria* spp. – from decomposing the cellophane in so short a period. One factor may be that in the soil the cellophane is overgrown by different fungi and bacteria, not all of them fast decomposers. Also, there may be some interaction of fungi and bacteria. In order to test this hypothesis, *Trichoderma koningi* and either a fungus or a bacterium were inoculated simultaneously on opposite corners of a piece of cellophane.

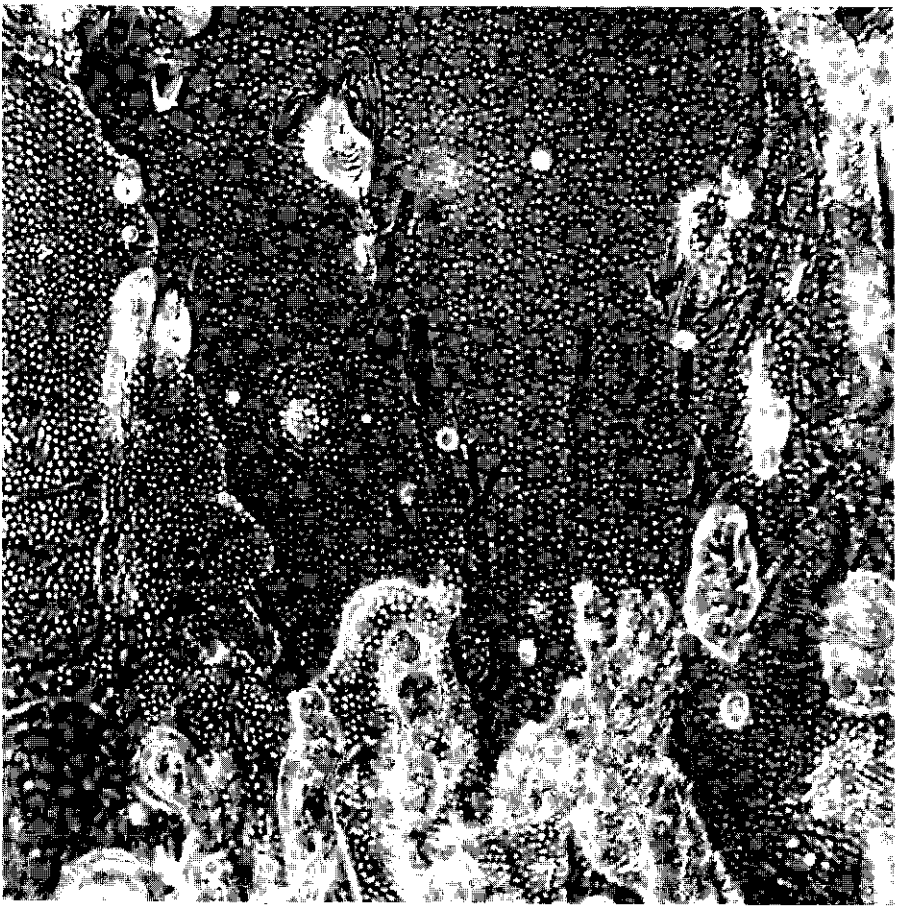

Fig. 7. Cellophane attacked by the combination of a pure culture of *Cellvibrio* (small round holes) and the fungus *Myrothecium*. 350 ×.

We found:

T. koningi + *Chaetomium* is faster than *Trichoderma* alone
T. koningi + *Cytophaga* is faster than *Trichoderma* alone
T. koningi + *Coniothyrium* is as fast as *Trichoderma* alone
T. koningi + *Humicola* is as fast as *Trichoderma* alone
T. koningi + *Alternaria* is much slower than *Trichoderma* alone
T. koningi + *Phoma* is much slower than *Trichoderma* alone
T. koningi + *Cellvibrio* is slower than *Trichoderma* alone

This is the more remarkable since *Alternaria* as well as *Phoma* when in pure culture decomposed the cellophane in 30 – 33 days at 5 C and in 21 – 30

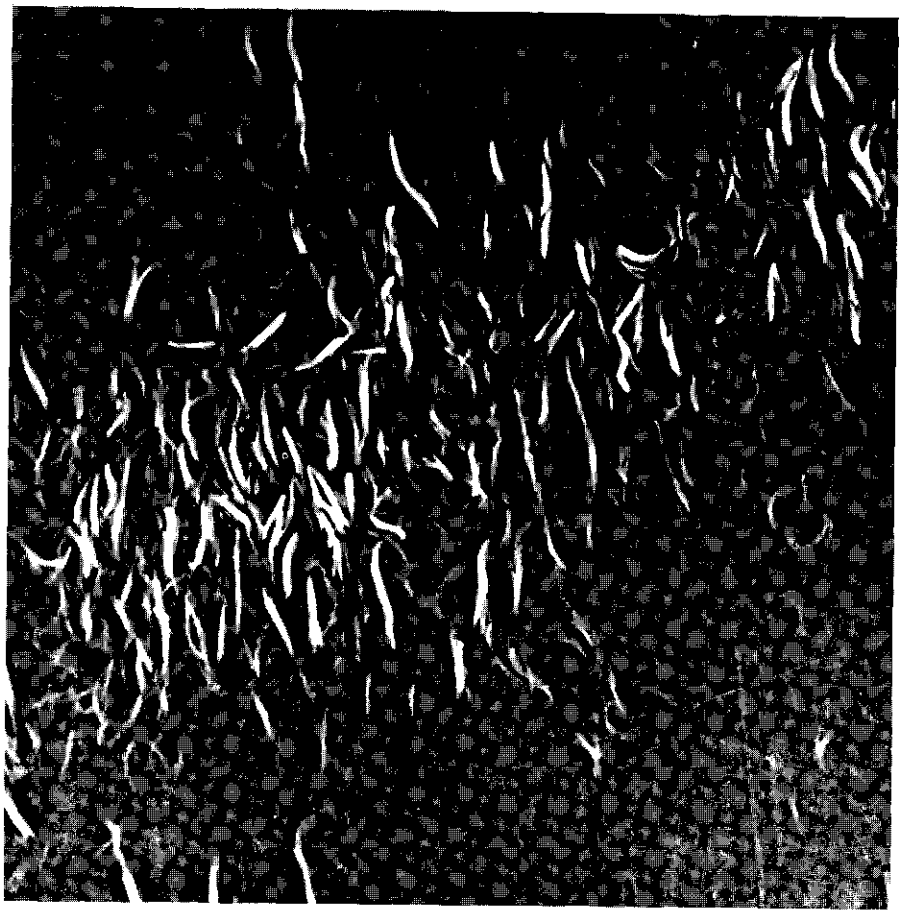

Fig. 8. Cellophane attacked by a pure culture of *Cytophaga*. 1060 ×.

days at 10 C. Apparently the activity of these fungi is inhibited by *Trichoderma* and the activity of *Trichoderma* is also inhibited by them.

Effect of soil extracts. Another inhibiting factor in cellulose decomposition might be some substance in soil. We compared the in-vitro decomposition of pieces of cellophane on mineral salts agar with 5% heat-sterilized soil extract, mineral-salts agar with 50% heat-sterilized soil extract, and agar with 50% filter-sterilized soil extract (Table 4). Ten different fungi decomposed the cellophane in 9 – 35 days at 15 C when either 5 or 50% heat-sterilized soil extract had been added. However, when filter-sterilized soil extract had been added, none of the ten fungi decomposed the cellophane within this period.

TABLE 4

Effect of soil extracts on the decomposition of cellophane by fungi

Number	Name	Number of days necessary for the decomposition of cellophane								
		5% Heat steril. soil extract and inorganic salts			50% Heat steril. soil extract			50% Filtered soil extract		
		5 C	10 C	15 C	5 C	10 C	15 C	5 C	10 C	15 C
1011a	*Phoma* spec.	35	19	19	35	19	19	> 35	> 35	> 35
360 6a	*Fusarium* spec.	35	19	19	35	19	19	> 35	> 35	> 35
360 49a	*Fusarium* spec.	35	19	19	?	19	19	> 35	> 35	> 35
361 26a	?	> 35	35	19	35	35	19	> 35	> 35	> 35
360 28a$_1$	*Fusarium graminearum*	> 35	19	19	> 35	19	19	> 35	> 35	> 35
361 63a	*Vermicularia*?	> 35	19	19	> 35	35	19	> 35	> 35	> 35
366 50a$_1$	?	> 35	35	19	> 35	35	19	> 35	> 35	> 35
1104b	*Cephalosporium ciferri*	> 35	35	35	> 35	35	35	> 35	> 35	> 35
616	*Pachybasium hamatum* var. *candidum*	> 35	35	35	> 35	> 35	35	> 35	> 35	> 35
664b	*Trichoderma koningi*	> 35	35	35	> 35	> 35	35	> 35	> 35	> 35

DISCUSSION

The sequence of the decomposition of cellophane in the Hackfort forest as shown in Fig. 3 corresponds with the water content of the different types of forest floor. The importance of the water content of the soil for the breakdown can also be concluded from the very slow breakdown during the dry period in 1959.

That pH is not a factor in this breakdown is shown by the quick decomposition in the acid mull in comparison with the much slower decomposition in the mor, both with the same pH. In Fig. 3, the quickest decomposition is found in the soil of the Middachten forest, a wet soil, pH 4.2. But a comparison here is less conclusive as the structure of the Middachten forest soil is different from the Hackfort forest soil.

The very quick decomposition in orchard soil can be explained by the quantity of fungi causing rapid decomposition, especially Fusaria, isolated from these soils. The Fusaria which have been isolated, are according to Wollenweber and Reinking (1935) all found as saprophytes of grass or pomatious fruits, with the exception of *Fusarium culmorum*, *F. poae* and *F. sporotrichioides* which are parasites of corn. It can therefore be expected that these fungi will be found in orchard soil covered with grass. From forest soil only very few fungi causing quick decomposition were isolated, which would explain the

slower decomposition. But even fungi from forest soil responsible for slower decomposition should cause a quicker decomposition of the cellophane in nature than that actually found.

This failure to give a quick decomposition may be due to the interaction of several fungi. Decomposition by *Alternaria* was found to be inhibited by *Trichoderma koningi*, and decomposition by *T. koningi* by *Alternaria*.

Another factor responsible for slower decomposition in soil than in vitro may be the presence of inhibiting substances. When heated to 100 C, these substances do not inhibit cellulose decomposition, but when filter-sterilized, they do.

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