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THE MICRO-ORGANISMS DECOMPOSING PECTIC SUBSTANCES IN THE DEW RETTING PROCESS OF FLAX [1]

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In contrast to the water retting of flax, the dew retting is a process in which the pectic substances are broken down aerobically. The flax straw is spread out in thin layers in the field, which is mostly of grass or stubble, and left open to the influence of climatic conditions. If necessary it is turned over once or twice. The flax straw is thus exposed to the attack of aerobic microbes, and the process is dependent on the moisture and temperature conditions resulting from the weather during the retting period. Under these circumstances no control of the process is possible. This is another point of contrast, especially with the warm water retting process, which can be controlled to a considerable extent.

Whereas the organisms causing warm water retting are largely known, our knowledge of those active in dew retting is poor.

In earlier publications (BEHRENS, 1902 a, RUSCHMANN, 1923) *Rhizopus nigricans* Ehrenb and *Rhizopus hiemalis* Wehmer, and *Cladosporium herbarum* (Link) Fr. were estimated as being the most important fungi in dew-retting. BEHRENS (1902 b), criticising HAUMANN (1902) stated that bacteria are of little significance in dew retting. RUSCHMANN considered *Cladosporium herbarum* to be the most important retting fungus: it suppressed the growth of *Rhizopus* and *Mucor* species and the presence of other fungi, yeasts or bacteria seemed

1) Received for publication in January 9, 1956.

to have no significance. In a publication by RUSCHMANN and BARTRAM (1940) attention was paid to *Alternaria* tenuis as a retting fungus.

In a paper by H. L. JENSEN (1941) mention is made of experiments in dew retting under laboratory conditions at varying temperatures. At low temperatures (10°–12°) *Mucor racemosus* (?) and *Cladosporium herbarum* were the most important fungi. At temperatures of 24°–30° C, *Rhizopus nigricans, Cladosporium herbarum, Alternaria* spp. and *Stachybotrys* sp. were most frequent, and at 37° C, *Rhizopus nigricans* and *Aspergillus* spp. were predominant. Under field conditions in Australia the dew retting of flax appeared to be entirely the result of the action of fungi, *Cladosporium herbarum, Dematium pullulans* De Bary & Loew (*Pullularia pullulans*) and *Alternaria* spp. being the most important. *Mucoraceae* were inhibited by the sunlight. Bacteria such as *B. herbicola* Dügg. and *Bac. mesentericus* were unable to induce retting. A few yeasts, designated as "Torula" were able to ret in test tubes, but appeared unable to ret under field conditions.

In 1952 I started a series of experiments in connection with the presence of micro-organisms decomposing pectic substances on dew retted flax straw. From September 1952 until August 1953 a sample of flax straw was laid out on a grass field every month. At the end of each retting period the sample was used for isolation experiments.

To achieve isolation I employed a special technique in which pectin agar plates were used. These plates were made in the following manner:

a layer of solidified soil extract agar, containing 0.1 % K_2HPO_4, 0.03 % $MgSO_4$, 0.05 % $(NH_4)_2SO_4$, 0.05 % Na_2CO_3, 0.05 % asparagine and 1 ml of a saturated solution of $CaCl_2$ per l, was just covered with a 2 % pectin solution. The pectin had to have a low ester content. Such a pectin is made by "Unipektin" in Zürich and sold as "Rotband Pektin". This is the brand I used with good results. The Ca ions diffusing from the agar into the pectin solution solidify the pectin. In about twelve hours the gel will have solidified and it may be treated just like an agar plate. On these pectin plates suspensions of the retted flax were streaked out. After incubation of two to three days at 25° C the pectin hydrolysing micro-organisms were easily recognised by the liquefaction they caused. It is very easy to count the number of such organisms and isolate species. Counts, however, should be considered critically, especially when fungi are concerned. The extent to which colonies of fungi, yeasts and bacteria are disintegrated depends on the extent to which the suspension is shaken. Consequently the number of colonies counted varies with the method used in disintegrating the material. In my investigations I cut the flax straw into pieces 2 to 3 mm long. A quantity of 1 or ½ g of the material in 100 ml of sterile water was shaken for 5 minutes in a magnetic stirrer. From this suspension dilutions were made and plated on the solid pectin medium. In triplicate counts I found a rather large variation in numbers. For that reason I did not pay great attention to the numbers I counted, but contented myself with obtaining a general impression. More attention was given to the species of pectin splitting micro-organisms. However, caution should be observed in the interpretation of the results. Were the organisms I found able to ret? As I used a rather special apple pectin I could not be sure on this point.

For me it was a happy coincidence that Dr. P. SONNE FREDERIKSEN of the Dansk Hørforskningsinstitut at Viby (Denmark) was working during the same

period with the same flax straw, retted in Holland, I used. From personal information, oral and gained by correspondence, I understood that his results, obtained by applying quite a different technique, were largely the same as my own.

FREDERIKSEN counted and isolated the micro-organisms from dew retted straw on malt extract glucose agar plates and meat extract agar plates. Pure cultures of the organisms obtained from these plates were tested for their retting abilities in test tubes on sterilized flax straw. The most characteristic difference in our results lay in the total numbers found and was probably caused by the fact that FREDERIKSEN disintegrated his material to a much greater extent. His total numbers being of the order of a million, were about ten times as great as mine.

The species isolated in the experiments of FREDERIKSEN and myself were, however, largely the same. FREDERIKSEN left the bacteria out of consideration, whereas I found *Pseudomonas fluorescens* Migula to be a strong and frequently occurring pectin decomposer.

The majority of active micro-organisms belong to the fungi and asporogenic yeasts. Among the fungi *Cladosporium herbarum* is the most frequent; *Pullularia pullulans* may occur in rather large numbers, especially in spring and early summer. Other fungi, such as *Penicillium* spp., *Alternaria* spp. and *Phoma* spp. are met with, though in smaller numbers.

Among the yeasts I isolated, *Rhodotorula glutinis, Rhodotorula* (new sp.) and *Cryptococcus albidus* were active pectin hydrolysing organisms.

From FREDERIKSEN I received 10 strains, mostly isolated from Danish dew retted flax straw, designated Nos. 2, 16, 38, 72, 111, 136, 148, 149, 164, and 172.

In testing these strains for pectin hydrolyses on my pectin plates, I found numbers 16, 111, 136, 149, 164 and 172 to be positive; the others were negative. As far as was possible I tried to determine the ten strains. Numbers 111 and 136 were found to be fungi, 136 being *Pullularia pullulans.* These two strains were sent to the Central laboratory for Fungus Cultures at Baarn, where they were ascertained to be *Cephalosporium cifferri* Verona and *Pullularia pullulans* respectively. As regards the others, strains 2, 16, 38, and 72, all colourless, belonged to the genus *Cryptococcus.* Number 148 was a *Sporobolomyces,* most probably *Sp. roseus;* the numbers 149, 164 and 172 were *Rhodotorula* species. These eight strains were sent to the Yeats Department of the Centraal Laboratorium voor Schimmelcultures at Delft, where they were determined as follows:

2) *Cryptococcus neoformans* (Sanfelice) Vuillemin.

16) *Cryptococcus albidus* (Saito) Skinner. Of the colourless strains this was the only one that hydrolysed pectin.

38) *Cryptococcus laurentii* (Kufferath) Skinner.

72) Identical with strain 38.

148) *Sporobolomyces roseus,* Kluyver et Van Niel.

149) A new species of *Rhodotorula,* forming starch and hydrolysing pectin. I proposed to designate this species as *Rhodotorula lini* (nov. spec.). Correspondence with Dr. FREDERIKSEN and the Yeast Department at Delft, however, showed that *Rhodotorula macerans* (nov. spec.) was a better name for this organism.

164) *Rhodotorula glutinis* (Frees) Harrison.
172) Largely identical with str. 149.

As regards the pectin hydrolysing property of these yeasts, only the strains 16, 149, 164 and 172 were found to be positive both in Delft and in my own experiments, numbers 2, 38, 72 and 148 being negative in this respect.

I was rather surprised to find yeasts in such large numbers on the retted flax straw. Yeasts may constitute up to 80% of the total flora of yeasts and fungi, especially during the winter season. These findings were corroborated by FREDERIKSEN, who sent me a table giving the percentages of micro-organisms he found during the year.

Table 1 Survey of the frequency of Fungi and Yeasts on Dew retted Flax Straw samples from Holland.

Name of micro-org.	Period of retting											
	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
	Percentage of isolated micro-organisms											
Not specif. . .	—	—	2.6	9.7	0.5	—	—	—	—	4.0	—	—
Clad. herb. . .	14.7	4.0	17.9	9.7	13.2	5.6	4.7	46.3	1.9	12.0	17.8	22.9
Penic spp. . . .	—	20.0	2.7	0.4	1.0	—	0.4	1.1	—	4.0	—	37.5
Rhod. spp. . .	10.3	—	4.5	8.0	14.8	8.3	2.0	2.1	15.1	12.0	19.8	4.2
Crypt. spp. . .	70.6	40.0	40.2	64.2	57.8	70.1	63.4	3.2	47.2	36.0	24.8	14.6
Pull. pull. . . .	—	—	20.5	14.6	7.8	13.2	10.2	20.0	28.3	32.0	36.6	6.3
Alt. spp. . . .	3.0	—	—	—	0.5	—	1.3	4.2	—	—	—	10.4
Phoma spp. . .	—	—	11.6	3.1	3.9	2.8	—	—	7.5	—	—	—
Ster. myc. . .	1.4	—	—	—	—	—	7.5	—	—	—	—	2.1
Fus. spp. . . .	—	—	—	—	0.5	—	—	17.8	—	—	—	2.1
Absidia sp. . .	—	—	—	—	—	—	—	—	—	—	1.0	—
Mucor spp. . .	—	—	—	—	—	—	—	—	—	—	—	2.0

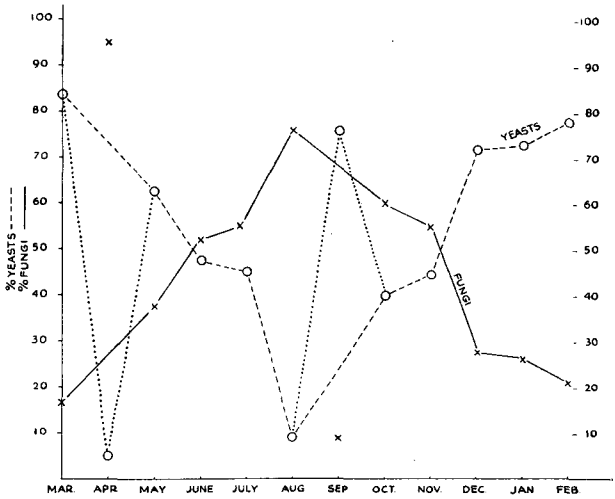

FIG. 1 PERCENTAGES OF FUNGI AND YEASTS IN THE PECTIN-DECOMPOSING FLORA ON DEW RETTED FLAX STRAW.

If these data are collected in a graph in which the percentages of fungi and of yeasts are shown together, the result is as given in Figure 1.

Except in the months of April and September the yeasts prevail during spring, autumn and winter, whereas in summer the percentages of fungi are higher.

It was of interest to have information about the weather, and the phenological data, during these two months. From the Royal Netherlands Institute of Meteorology at De Bilt I received the following details:

September 1952 was an abnormally cold month. The mean temperature, as calculated from hourly observations, was 11.5° C, which made this September the coldest but one in a period of about 250 years. The mean maximum temperature in September is normally 19.3° C, whereas September 1952 had a mean maximum temperature of 15.8° C. Comparatively speaking the nights were a little less cold. Normally, the mean minimum temperature in September is 9.5° C, but in September 1952 it was 6.9° C. The second and third decades of this month in particular were very cold.

The most important phenological observation in autumn is that of the yellowing of the leaves of the horsechestnut. In the autumn of 1952 this yellowing was observed at Wageningen as early as 24 September, whereas the normal date is 13 October. These data consequently accord rather well with the abnormal percentages of yeasts and fungi I found on the retted flax straw.

In April 1953, however, these weather characteristics were fairly normal. Though the mean temperature was somewhat high, the difference from the normal temperature was rather small (0.2° C). One striking feature, however, was the large amount of sunshine in the period from 19 to 23 April (more than 10 hrs a day). Phenological dates did not differ much from the normal ones, though they were some days early.

	Normal	1953
Unfolding of the leaves of the horsechestnut	April 14	April 12
Flowering of the pansy	„ 2	March 29
Flowering of the marsh marigold	„ 15	April 10
Flowering of the cardamine	„ 17	„ 14

It is doubtful whether these small differences are sufficient to account for the abnormal percentage of yeasts and fungi I found. But there could be other factors, such as precipitation and relative humidity of the air that might be important. The data for these factors are given in the following table:

	Precipitation mm	Relative humidity
1952		
Aug.	87.4	78.6
Sep.	39.4	82.3
Oct.	71.1	85.5
1953		
March	12.9	80.49
April	65.8	67.27
May	24.9	67.47

From this table it can be seen that the rainfall was rather high in April 1953 and low in September 1952. The relative humidity was low in April 1953 and not abnormal in September 1952. I would not go so far as to conclude that the rainfall was decisive in causing the abnormalities I found in the development of yeasts and fungi during these two months; but, in combination with other factors, it may have been of influence.

My conclusion is, that in Holland *Cladosporium herbarum* is the most important retting organism during summer, whereas *Cryptococcus albidus, Rhodotorula glutinis* and *Rhodotorula macerans* are doing the larger part of the retting during the winter season. *Pullularia pullulans* may be of some importance in spring and autumn. Some bacteria such as *Pseudomonas fluorescens* may be important throughout the year. As *Cladosporium* and some other fungi give unfavourable colour to the fibre and attack cellulose the winter season should be recommendated for dew retting.

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REVIEWS

635.25

BANGA, O.: Uienveredeling met gebruikmaking van inteelt en herstel door heterosis. (Improvement of onions through inbreeding and recovery by heterosis). *Mededeling Instituut voor de veredeling van Tuinbouwgewassen* no. 66, 1955, 6 pp.

Modern plant improvement is based on the insight that the breeding value of a plant can be judged by its progeny rather than by the plant itself. The gaining of this insight meant the change over from mass selection to single plant selection. An unavoidable result of single plant selection is the weakening of the progeny as a result of inbreeding. This result may, however, be eliminated by making use of the heterosis effect. This general principle underlies the improvement scheme of the onion set forth in this article. A great number of inbred strains has to be produced. Trial crossings show what combination of strains gives the best results. For the production of commercial seed the use of male sterile plants is recommended. In a discussion of breeding behaviour after crossing it is stated how male sterile plants can be produced and kept. After a brief treatment of the favourable results attained with heterosis varieties of onions in the U.S.A. attention is given to the special problem of the Dutch onion breeders; to produce a variety that can stand handling before and during transport.

AUTHOR'S SUMMARY.

635.132

BANGA, O.: Carrot yield analysis. *Mededeling Instituut voor de veredeling van Tuinbouwgewassen* no. 67, 1955, 10 pp. Also: *Euphytica* 4 (1955) 116–126.

1. Judging carrots on genotypial differences in yield is made difficult by many non-genetic factors affecting yield.

2. The yield of a plot of carrots may be imagined as the result of the interaction of the total number of plants per plot, the percentage of usable carrots out of this total number of plants and the average root weight of the usable carrots.

3. The total number of plants per plot