Annales Agriculturae Fenniae

Maatalouden
tutkimuskeskuksen
aikakauskirja

Journal of the
Agricultural
Research
Centre

Vol. 21, 3

Annales Agriculturae Fenniae

JULKAISIJA — PUBLISHER

Maatalouden tutkimuskeskus
Agricultural Research Centre

Ilmestyy 4—6 numeroa vuodessa
Issued as 4—6 numbers a year

ISSN 0570 — 1538

ALASARJAT — SECTIONS

Agrogeologia et -chimica — Maa ja lannoitus ISSN 0358-139X
Agricultura — Peltoviljely ISSN 0358-1403
Horticultura — Puutarhaviljely ISSN 0358-1411
Phytopathologia — Kasvitaudit ISSN 0358-142X
Animalia nocentia — Tuhoeläimet ISSN 0517-8436
Animalia domestica — Kotieläimet ISSN 0358-1438

JAKELU JA VAIHTO

Maatalouden tutkimuskeskus, Kirjasto, 31600 Jokioinen

DISTRIBUTION AND EXCHANGE

Agricultural Research Centre, Library, 31600 Jokioinen

ANNALES AGRICULTURAE FENNIAE, VOL. 21: 115—122 (1982)

Seria ANIMALIA DOMESTICA N. 58 — Sarja KOTIELÄIMET n:o 58

WILTED AND UNWILTED GRASS SILAGE FOR YOUNG BULLS

MIKKO KOMMERI, VAPPU KOSSILA and JAAKKO KIVINIEMI

KOMMERI, M., KOSSILA, V. & KIVINIEMI, J. 1982. **Wilted and unwilted grass silage for young bulls.** Ann. Agric. Fenn. 21: 115—122. (Agric. Res. Centre, Inst. Anim. Husb., SF-31600 Jokioinen, Finland.)

Precision-chopped, wilted grass silage was compared to unwilted, flail-harvested grass silage in a two-year experiment (1977—1978). The storage of silage in tower silos or in walled bunker silos was also compared. All the herbages were treated with 5 l/t of preservative AIV-2, which contains formic acid. The digestibility of the silage was determined in experiments on rams. Sixty-four Ay bulls, eight HfAy and eight ChAy crosses (average age 8—14 months) were used in the production experiments.

The grass was cut with a crimper-type mower conditioner in the morning and collected with a precision-chop forage harvester the same day. The DM content of the raw material was higher than 30 % and there was no effluent production from the wilted silage. The quality of all silage was good. Because it was used slowly, the wilted silage was susceptible to post-opening fermentation. On the other hand, normal fermentation was lower and the average DM losses were also lower in wilted silage than in unwilted silage (8,7 and 13,3 % respectively).

In the first year of the experiment, the digestibility of organic matter in unwilted silage (70,2 %) was better ($P < 0,05$) than that of wilted silage (66,5 %). In the second year there were no significant differences in the digestibility or in the palatability between the silages. With a concentrates level of 3 kg, the bulls grew an average of 1 038 g/day on unwilted silage and 974 g/day on wilted silage. For production of 1 kg liveweight the bulls on wilted silage consumed an average of 7,78 kg DM and 6,51 fu. Animals on unwilted silage consumed an average of 7,25 kg DM and 6,05 fu per kg liveweight gain. The difference was nonsignificant ($P < 0,05$).

Index words: wilted, unwilted, grass silage.

INTRODUCTION

One of the main aims of Finnish fodder production is to enhance protein self-sufficiency by increasing the use of silage in ruminant feeding. Although silage production has increased rapidly (five fold during ten years), the area still being used for hay is twice as much as that being used for silage.

One of the main reasons for not wanting to acquire silage harvesting equipment and storage places has been the small size of farms and fact

that they already have hay-making equipment. Because of this, and because of the climatic conditions, almost all of the silage prepared in Finland is unwilted. Tower silos have traditionally been the most popular form of storage.

The Ministry of Agriculture and Forestry and SITRA (Finnish National Fund for Research and Development) have financed this research project to increase the production of silage and to intensify research into the techniques of silage preparation and management so that they can be developed at reasonable costs. The silage-making and beef production experiments described in this report were carried out by the Institute of Animal Husbandry of the Agricultural Research Centre.

MATERIAL AND METHODS

Preparation of silages

In the summer of 1977 the silages were prepared at Jokioinen from the second harvest of the sward (25.—29. 7.). The following summer they were harvested from the first harvest of the same sward (12.—16. 6.). To eliminate the differences between the plant species, stage of development and yield of the field plots all the silages were prepared at the same time from each plot.

The main species used in silage swards were cocksfoot (1/3), timothy (1/5) and meadow fescue (1/5). Fertilizer N was applied to the swards at a rate of 100 kg/ha/harvest. In the spring, an N—P—K fertilizer (20—4—8) was applied, and after the first harvest calcium-ammonium nitrate (27,5 % N) was applied.

Flail-type forage harvesters were used to harvest unwilted silages. During chopping, AIV-2 solution was added to the silage at 5 l/t. Grasses to be wilted were mown in the morning with a crimper-type mower conditioner, and collected with a precision-chop forage harvester, either in the afternoon or evening of the same day. AIV-2 preservative was added to the wilted silage during harvesting with a precision-chop forage harvester at 5 l/t. The tower silos were filled using a grab hoist which was also used when emptying them. The walled bunker silos were filled and consolidated using a four-wheel drive tractor equipped with a front loader. All the silos were sealed with plastic sheet. A 15 cm layer of sawdust was spread on top of the plastic in the walled bunker silos, whereas in the tower silos concrete blocks (350 kg/m^2) were used to compress the silage.

To determine the storage losses from the silage, the raw materials, effluent and silage were weighed and analysed. The Conventional feed analyses were carried out using standard methods. The pH, ash and crude protein contents of the effluent were determined. Besides the normal feed analyses of the silage, the pH of the effluent from fresh silage was determined electronically. The water extracts from the samples were tested quantitatively for acetic acid, propionic acid, butyric acid, valeric acid and isovaleric acid using gas chromatography (HUIDA 1973), for lactic acid (BARKER and SUMMERSON 1941), sugars (SOMOGYI 1945, SALO 1965) and ammonium nitrogen (McCULLOUGH 1967) using a colorimeter and for soluble nitrogen with the Kjeldahl method. The total nitrogen was determined from fresh samples using the Kjeldahl method. The dry matter content of the silages, determined by drying the samples in a drying oven at 105 °C, was corrected by adding all of the butyric, propionic, valeric and isovaleric acid and 80 per cent of the acetic acid values to the dry matter.

The storage losses were determined using the so-called sack method. After a sample had been taken for feed analysis, 15 kg of grass was weighed into damp jute sacks. During feeding, when the sacks were found, the contents were weighed and the chemical composition of the silage was analysed and the losses calculated.

Experiments on animals

The digestibility experiments were carried out with silage each year using the 4 × 4 latin square design. In the first year, the rams in the experiment weighed slightly under 70 kg and in the second year a little over 40 kg. Apart from determining the digestibility of the feeds, the nitrogen balance and the palatability were also determined. The animals received only water, a mineral and trace element mixture and a vitamin mixture in addition to the silage.

Meat production experiments were carried out using a factorial design. During the first year all (32) animals were Ay bulls, but in the second year there were also 8 Friesian-Ay (4♀ + 4♂) and 8 Charolais-Ay crossbreeds (4♂ + 4♀) (48 animals altogether). In addition to the silage, the animals also received barley meal 3 kg/animal/day, a certain amount of mineral and trace element mixture and water ad lib. At the end of the experiments the animals were slaughtered and carcasses were classified in order to determine their quality.

Experimental design

The following experimental design was used in all experiments.

	Type of silo	Type of silage
1	tower	wilted
2	tower	unwilted
3	walled bunker	wilted
4	walled bunker	unwilted

RESULTS AND DISCUSSION

Silage quality and chemical composition

The swards used for the silage making were rather poor in both of the experiment years. The crude fibre content was high in the first year. This was partly due to rain, which prolonged the preparation of the silage.

The grass harvested with the mower conditioner dried quickly. Usually 4—6 hours windrow drying without tedding increased the dry matter content of the raw material from 16,6 % to 32,9 % in the first year, and from 25,3 % to 39,2 % in the second. Although it rained, wilting was successful so that there was no effluent from the wilted silage. With unwilted silage, effluent was produced at the following percentages of the fresh weight of the raw material:

Year	1977	1978
tower silo	21,7	7,3
walled bunker silo	9,8	3,9

Because there was less weight on top of the walled bunker silos, a smaller amount of effluent was produced than in the tower silos. The type of silo had little effect on the constitution of the effluents (Table 2).

Table 1. The average chemical composition of the raw material used for silage.

Year	1977	1978
Dry matter %		
unwilted	16,60	25,33
wilted	32,72	39,16
% in dry matter		
ash	10,21	8,82
crude protein	16,96	15,54
crude fat	3,97	3,55
crude fibre	28,24	24,64
N-free extract	40,62	47,45

Table 2. The analysed values of effluent from unwilted silage.

Year	1977		1978	
	Tower	Walled bunker	Tower	Walled bunker
pH	4,11	4,41	4.33	4,35
dry matter %	4,47	4,64	8,59	7,29
ash %	1,17	1,28	1,64	1,61
crude prot. %	1,09	0,91	1,91	1,56

Until they were opened the silages were well preserved. Because the rate of consumption of the silages was low, there was some post-opening fermentation. The spoiled silages were not used in the experiments and their effect is not shown in Table 3.

Table 3. The average chemical composition of silages used in beef production experiments, 1977 and 1978.

	Tower wilted	Tower unwilted	Walled bunker wilted	Walled bunker unwilted
Dry matter %	33,93[b]	23,58[a]	33,66[b]	22,15[a]
In dry matter % ...				
ash	9,96	9,56	10,20	10,30
crude protein	17,01	16,57	17,20	16,64
crude fat	4,12[a]	5,15[b]	4,16[a]	4,78[b]
crude fibre	27,95	28,85	27,90	28,24
N-free extract	40,96	39,88	40,54	40,04
acetic acid	0,99[a]	1,50[a]	1,04[a]	2,57[b]
propionic acid ...	0,01	0,01	0,01	0,05
butyric acid	0,01	0,00	0,05	0,05
lactic acid	2,18[a]	3,65[ab]	2,29[a]	4,04[b]
sugars (gluc.)	10,48	12,88	11,04	9,55
% in total N				
ammonium-N ...	7,23[b]	3,73[a]	7,25[b]	4,78[a]
soluble-N	55,81[b]	51,97[ab]	51,97[ab]	45,94[a]

$a < b = P < 0,05$

In the wilted silages the DM content was 11 percentage units higher than in the equivalent unwilted silages. Both acetic and lactic acid fermentation was stronger in the unwilted than in the wilted silages. Only in some silage was there evidence of butyric acid fermentation. Wilting raises the concentration of the cell contents in grass and thus increases the osmotic pressure in the cells. This inhibits the activity of certain microbes and decreases the volume of fermentation gases. The activity of butyric acid bacteria is impaired at very high pH values when the osmotic pressure is high enough. On the other hand, lactic acid bacteria are capable of activity under very dry conditions (WIERINGA 1958).

Fermentation differences of the same type and just as distinct as in this experiment have been verified by BUTLER and BAILEY (1973), HONIG and ROHR (1974), and WEISE and HORNING (1975) when storing without additive. In experiments by SKOVBORG and ANDERSEN (1979) the amount of lactic acid fermentation was lower and the amount of ammonium nitrogen in total nitrogen less than in wilted silage (without additive). However, there was a smaller amount of acetic acid in wilted than in unwilted silage. ZIMMER (1969) established that when the dry matter content of silage increases, the content of butyric and acetic acid decreases. He found that the lactic acid content rose in the beginning, and fell when the dry matter content rose over 30 %.

In this experiment, the proportions of ammonium and soluble nitrogen in total nitrogen were clearly higher in wilted than in unwilted silage. Summarizing several experiments, MARSH (1979) verified the fact that wilting is less effective in preventing the breakdown of protein than the breakdown of carbohydrates. Usually the result has been opposite to that obtained in this experiment.

Minerals are lost from unwilted silage together with the effluent, which means the ash content of wilted silage is a little higher than that of unwilted. Because of the stronger fermentation of unwilted silages, the chemical composition is also a little different; a slightly lower crude protein and higher crude fat content than in wilted silages.

Storage losses

The losses due to respiration and shedding on the field were not determined in this experiment. Because of strong winds, the field losses from wilted herbage were considerable. As the silage was prepared quickly, there was little change of respiratory loss, and the effluent losses from the dry raw material were of no significance. There was no effluent production by wilted silages, and the production by unwilted was only 3 % of DM on average (tower 4,2 %, bunker 1,8 %). The average fermentation and effluent losses during 2 years of storage according to the sack samples are shown in Table 4.

Table 4. The average storage losses in 1977 and 1978.

	I Tower wilted	II Tower unwilted	III Walled bunker wilted	IV Walled bunker unwilted
Dry matter %	9,05	13,97	8,44	12,66
Ash	4,59	16,36	2,37	15,46
Org. matter	9,55	13,67	9,04	12,34
Crude protein	5,90	15,54	2,79	13,15
Crude carbohydr. ..	10,92	14,87	11,32	13,65

The fermentation losses were the same in both the tower and walled bunker silos. The slightly lower storage losses in the bunker silos than in the tower silos can be partly explained by the smaller effluent losses. The storage losses determined from the sack samples are slightly smaller than the real losses, because the proportion of spoiled top layer and other silage is not included. There was more spoiled silage from the unwilted walled bunker silo (4,0 % or DM) than from the corresponding tower silos (1,5 %). The highest top layer losses were from wilted tower silage, partly due to the slow rate of feeding during the experiments.

The storage losses determined from the sack samples whilst in the silo were clearly lower from wilted silage than from unwilted silage (8,7 % and 13,3 % of DM respectively). The dry matter losses from the abovementioned silages are considerably lower than the 21,2 % obtained by ETTALA and KOSSILA (1980) using the same method.

The average DM loss was 7,6 % of the entire herbage composition determined from wilted silage in 1978. Correspondingly, the DM loss of unwilted silage was 17,2 %.

The fact that the storage losses of wilted silage were lower than of unwilted silage can be explained, not only by the fermentation being lower than in unwilted silages, but also because there were no effluent losses from wilted silage. Similar results have been published by NASH (1959), ZIMMER (1966), HONIG (1967), WALDO (1977) and SKOVBORG and ANDERSEN (1979). Taking all the storage losses into account, SKOVBORG and ANDERSEN (1979) achieved a larger net DM yield per hectare with wilted than with unwilted silage.

Digestibility of silages and calculated feeding value

The rams preferred unwilted to wilted silage. Those receiving wilted silage drank 1—2 l of water/day more than those receiving unwilted. In the first year, the nitrogen balances were better when feeding unwilted silage, and also the digestibility of organic matter was three to four percentage units better on unwilted silage. The average digestibility of organic matter with wilted silage was 66,5 % and with unwilted silage 70,2 %. Similar differences were not established in the second year. In fact, the digestibility of silage stored unwilted in tower silos was lowest. On average the digestibility and bulk value (1,42 kg DM/fu) on unwilted silage was slightly better than that of wilted silage (1,46 kg DM/fu).

MARCH (1979) summarized that only in 10 out of 40 experiments had wilting improved the digestibility of silages. The average DM digestibility of wilted silage (68,5 %) was 1,5 percentage units lower than the digestibility on unwilted silage (70,0 %). The digestibility of protein was better with unwilted silage nine times out of thirteen.

Table 5. The average digestibility, N-balance and feed value of silages in 1977 and 1978.

	Tower wilted	Tower unwilted	Walled bunker wilted	Walled bunker unwilted
Digestibility %				
organic matter ...	67,22	68,09	67,10	69,46
crude protein	64 49	68 32	67,77	68,43
crude fat	62,07[a]	65,76[b]	60,03[a]	65,83[b]
crude fibre	69,06	69,36	68,35	69,48
N-free extract ...	67,40	67,25	66,85	69,90
crude carbohydr. .	68,29	68,25	67,56	69,92
N-balance g/day ...	1,69	2,73	2,43	3,40
Bulk value kg DM/fu	1,47	1,43	1,46	1,41
g dig. crude prot./kg DM	109 5	112,0	117,5	110,5

a < b = P < 0,05

There were no large differences in the palatability, nitrogen balance, digestibility or feed values between both years in either walled bunker silos or tower silos. The results of the digestibility experiment are shown in Table 5.

Beef production experiment on bulls

In the first experiment, Ay bulls (aged 156—380 days) ate 2,5 kg of barley DM and an average of 3,7 kg of silage DM/day. In the second experiment, where the animals were partly crossbreeds and also about 2 months older than in the first experiment, the amount of silage DM consumed was 5,6 kg/animal/day. The DM consumption of wilted and unwilted silage per 100 kg liveweight was equal (1,39 kg/d).

In MARSH's (1979) review of 31 experiments on feeding only silage to growing cattle, the wilted silage DM consumption was an average of 31 % higher than that of unwilted silage. When concentrate was added to the ration, the benefit achieved by wilting was lowered to 12 %.

In the present study young bulls grew an average of 1000 g/day in both years. The average daily gain of bulls on unwilted silage was 64 g/day faster than that of bulls on wilted silage (1038 g/day and 974 g/day, respectively). SAUE and BREIREM (1969) obtained similar results. According to MARSH (1979) wilting had no effect on growth in the experiments where the animals received additional concentrate, whereas without concentrate the bulls grew faster on wilted silage.

The better weight gain achieved in the present experiment on unwilted silage corresponds with the results from the digestibility experiments. The animals on wilted silage used an average of 7,78 kg DM and 6,51 fu for the production of 1 kg liveweight. Animals on unwilted silage however used their feed more effectively: 7,25 kg DM and 6,05 fu per kilo liveweight gain.

The average killing out percentage of bulls on wilted silage was higher than that of bulls on unwilted silage (48,7 and 48,2 %, respectively). Because of this, there was on average only a 3,2 kg difference in the slaughter weight in the favour of wilted silage. There was no difference between the groups in carcass quality points. Nor was there any significant difference in carcass weight between the animals fed silage from tower silos or walled bunker silos (Table 6).

Table 6. The average liveweight gain, feed consumption and carcass quality of bulls in 1977 and 1978.

	Tower wilted	Tower unwilted	Walled bunker wilted	Walled bunker unwilted
Age (at start of expt.) days	239	238	240	241
Age (at end of expt.) days	412	411	414	414
Liveweight (at start of expt.) kg	261	260	258	260
Liveweight (at end of expt.) kg	431,8	441,4	429,5	441,4
Liveweight gain g/day	980	1 039	967	1 037
Silage kg DM/animal/day	4,72	4,98	4,89	4,82
Total feed consumption kg DM/day	7,32	7,46	7,44	7,38
fu/day	6,14	6,14	6,20	6,24
dig. crude protein g/day	754	767	797	745
Carcass weight	210	213	210	214
Killing out %	48,64	48,10	48,84	48,39
Carcass points total	15,15	15,25	15,41	15,49

REFERENCES

BAKER, S. B. & SUMMERSON, W. H. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138: 535—554.

BUTLER, G. W. & BAILEY, R. W. 1973. Chemistry and biochemistry of herbage. London.

ETTALA, E. & KOSSILA, V. 1980. Organic matter, mineral and nitrogen losses from fresh grass silage during ensiling. Ann. Agric. Fenn. 19: 9—20.

HONIG, H. 1967. Gärvorzang, Verluste und Qualität bei der Konservierung von Mährweidegras mit unter-

schiedlichem Trockensubstanzgehalt. Das Wirtschaftseigene Futter 13: 287—297.

— & ROHR, K. 1974. Biologische Kenndaten verschiedener Konservierungsverfahren. 1. Mitt. Qualität, Verluste und Materialstreuung bei der Konservierung. Das Wirtschaftseigene Futter 20: 265—276.

HUIDA, L. 1973. Quantitative determination of volatile fatty acids from rumen sample and silage by gas-liquid chromatography. J. Scient. Agric. Soc. Finl. 45: 483—488.

MARSH, R. 1979. The effects of wilting on fermentation in the silo and on the nutritive value of silage. Grass and Forage Sci. 34: 1—10.

MCCULLOUGH, H. 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clin. Chem. Acta 17: 297—304.

NASH, M. J. 1959. Partial wilting of grass crops for silage. 2. Experimental silages. J. British Grassl. Soc. 14: 107—116.

SALO, M-L. 1965. Determination of carbohydrate fractions in animal foods and faeces. Acta Agr. Fenn. 105: 1—102.

SAUE, O. & BREIREM, K. 1969. Comparison of formic acid silage with other silages and dried grassland products in feeding experiments. Proc. of the 3rd general meeting of the European Grassland Federation. Braunschweig. p. 282—284.

SKOVBORG, B. & ANDERSEN, P. E. 1979. 7. beretn. Faellesudv. Stat. Planteavls og Husdyrbrungsforsøg. København.

SOMOGYI, M. 1945. A new reagent for the determination of sugars. J. Biol. Chem. 160: 61—68.

WALDO, D. R. 1977. Potential of chemical preservation and improvement of forages. J. Dairy Sci. 60: 306—326.

WEISE, F. & HORNING, H. 1975. Einfluss unterschiedlicher Vorwelkzeiten auf den Gärverlauf bei Wiesenschwingel. Das Wirtschaftseigene Futter 21: 10—24.

WIERINGA, G. W. 1958. The effect of wilting on butyric acid fermentation in silage. Neth. J. Agric. Sci. 6: 204—210.

ZIMMER, E. 1969. Biochemische Grundlagen der Einsäuerung. Proc. of the 3rd general meeting of the European Grassland Federation. Braunschweig. p. 113—125.

Manuscript received February 1982

Mikko Kommeri and Vappu Kossila
Agricultural Research Centre
Institute of Animal Husbandry
SF-31600 Jokioinen, Finland

Jaakko Kiviniemi
Finnish Research Institute of
Engineering in Agriculture and Forestry
SF-03450 Olkkala

SELOSTUS

Esikuivattu ja tuore ruohosäilörehu lihanaudoilla

Mikko Kommeri, Vappu Kossila ja Jaakko Kiviniemi

Maatalouden tutkimuskeskus ja Valtion maatalouskoneiden tutkimuslaitos

Kahden satokauden (1977—1978) kokeissa verrattiin tarkkuussilppurilla korjattuja esikuivattuja ruohosäilörehuja samoista nurmista kelasilppurilla korjattuihin tuoresäilörehuihin. Lisäksi verrattiin tornia ja laakasiiloa säilörehuvarastona. Kaikki rehut valmistettiin muurahaishappoa sisältävällä AIV-2 säilöntäainelisäyksellä, 5 l/tn. Rehuista tehtiin sulavuuskokeet pässeillä. Tuotantokokeissa käytettiin keskimäärin 8—14 kk:n ikäisiä Aysonnimulleja (64 kpl) sekä HfAy (8 kpl) ja ChAy (8 kpl) risteytyksiä.

Aamulla telaniittomurskaimella niitetty ruoho korjattiin tarkkuussilppurilla samana päivänä. Raaka-aineen kuiva-ainepitoisuus nousi yli 30 %:n eikä esikuivatuista rehuista tullut puristemehua. Kaikki säilörehut olivat laadultaan hyviä. Hitaan syötön vuoksi olivat esikuivatut rehut arkoja jälkikäymiselle. Toisaalta varsinainen käyminen oli niissä vähäisempää ja siilovaiheen säilöntätappiot (8,7 % ka:sta) pienemmät kuin tuoreissa säilörehuissa (13,3 % ka:sta).

Ensimmäisenä koevuonna tuoreiden säilörehujen orgaanisen aineen sulavuudet (70,2 %) olivat parempia ($P < 0,05$) kuin esikuivattujen (66,5 %). Toisena koevuonna oa. sulavuudet olivat keskimäärin yhtä hyviä. Myöskään maittavuudessa ei ollut eroja rehujen välillä. Esikuivattujen rehujen hyväksikäyttö kasvuun oli heikompi kuin tuoreiden. Ero ei kuitenkaan ollut merkitsevä ($P < 0,05$). Kolmen kilon väkirehutasolla mullit kasvoivat tuoreilla säilörehuilla keskimäärin 1 038 g ja esikuivatuilla 974 g/pv.

ANNALES AGRICULTURAE FENNIAE, VOL. 21: 123—130 (1982)

Seria PHYTOPATHOLOGIA N. 89 — Sarja KASVITAUDIT n:o 89

FUSARIUMS OF THE POTATO IN FINLAND IV. VARIATION OF TUBER RESISTANCE TO STORAGE DISEASES BEFORE AND AFTER HARVESTING

ESKO SEPPÄNEN

SEPPÄNEN, E. 1982. **Fusariums of the potato in Finland IV. Variation of tuber resistance to storage diseases before and after harvesting.** Ann. Agric. Fenn. 21: 123—130. (Agric. Res. Centre, Inst. Pl. Path. SF-01300 Vantaa 30, Finland.)

The influence of the stage of tuber development and pre-storage conditions on the tuber resistance to *Fusarium sulphureum* and to *Phoma exigua* var. *foveata* were studied by infecting tubers of cvs. Sabina and Pito artificially at different physiological ages and after a short period of pre-storage under different environmental conditions.

The response of these cultivars to both of the fungi was similar. During the earliest harvest times tubers are very susceptible to infection, but their resistance increases during the course of the season. At harvest time the resistance is highest, and decreases during the course of the storage period. Planting time and vine killing had no significant effect on the resistance.

Pre-storage at low RH and extreme temperatures (6 and 24 °C) yielded a great and rapid decrease in resistance. The decrease was most striking in tests with pre-matured tubers. Presumably, the increase in respiration and evaporation of the tubers caused rapid physiological ageing and the subsequent pre-mature decrease in resistance.

Index words: potato, dry rot, gangrene, *Fusarium sulphureum*, *Phoma exigua* var. *foveata*, tuber resistance, growth season, storage conditions.

INTRODUCTION

It is well known that the susceptibility of potato tubers to *Fusarium* and *Phoma* fungi varies in accordance with their growth rhythm and maturity. BOYD (1952 a) measured the resistance of tubers as a percentage of infection level, and concluded that the susceptibility of tubers to *Fusarium solani* var. *coeruleum* was highest just after flowering. JÖNSSON and BÅNG (1979), measuring the tuber resistance as a function of the growth rate of the fungus, obtained a similar result. Towards the end of the season tuber resistance increased, being highest at harvest

time. In his later investigations, BOYD (1967) concluded that an increase in resistance was followed both after vine killing and with natural maturation. Tuber resistance to *Phoma exigua* var. *foveata* increases evenly towards the end of season (FOX and DASHWOOD 1970, JÖNSSON and BÅNG 1979).

The decrease in tuber resistance during the storage period was demonstrated in as early as 1917 by PETHYBRIDGE and LAFFERTY and by a number of workers since then. Naturally, factors like cultivar, fungus and growth conditions may have some influence on the result. Studying the effect of 13 days of prestorage under temperature conditions of 1—2, 10—12 and 20 °C, LANSADE (1949) found that the growth rate of *Fusarium coeruleum* in the tubers was higher the lower the temperature at which the tubers were pre-stored. Another noteworthy result was a lower growth rate of the fungus when the tubers were stored under conditions of high humidity. BOYD (1952 b) measured resistance according to the level of infection and concluded that pre-storage of tubers under a temperature of 3—4 °C did not decrease their resistance to *Fusarium coeruleum*, but pre-storage at —1 °C or at 22 °C did.

There are no reports about the other fungi, such as *Fusarium sulphureum*, *F. avenaceum* and *F. trichothecioides*, each of which is known to be a common pathogen of stored potatoes.

The aim of this study was to extend our knowledge of the general variation in tuber resistance and in particular about the influence of some practical procedures connected with harvesting, such as vine killing and wound healing conditions.

MATERIAL AND METHODS

The trials were carried out on two cultivars, Sabina and Pito, the former being a fairly early and the latter a late cultivar. Both of them have medium resistance to the fungi, *Phoma exigua* var. *foveata* and *Fusarium sulphureum*, used in these tests (SEPPÄNEN 1980, 1981 b). The isolates used must be considered highly pathogenic. Some trials were done with only one cv.

Potatoes were grown according to conventional methods. The soil was coarse sand, fertilized with 80 kg N, 110 kg P and 120 kg K/ha. The seed potatoes used were virus-free stocks originating from the Seed Potato Centre.

All three seasons studied (1979, 1980 and 1981) were fairly favourable for potatoes. In 1979 and 1980, especially during August and September, the temperature was higher and the rainfall less than normal. In 1981 the same period was rather warm but rainy.

To study the effect of the stage of development of the tubers and the effect of storage conditions on the resistance of the tubers we harvested tubers at different ages (also tubers planted at different times) and stored them for different periods of time and under different conditions. After each treatment the tubers were inoculated and incubated as uniformly as possibly.

In each test we used tubers of nearly the same size. Before infection, they were washed and dried well. For infection a wound 5 mm in diameter and 2 mm deep was made with a cork-borer, at the midpoint between the heel and rose ends of the tuber. A mixture of a c. 4-week-old pure culture of the fungus and the remaining agar was used as inoculum. The wounds were filled with the inoculum and left uncovered. The tubers were incubated for 20 days at 12 °C, which were the proposed optimum conditions for each fungus (SEPPÄNEN 1980, 1981 a).

The trials involved 10 (in some trials 20) tubers for each treatment, with 3 replicates. For analysis, the tubers were halved longitudin-

ally through the infection locus and the radial and apical growths were measured. Usually their averages are presented here to indicate the resistance in each stage or after each treatment.

All the results were analyzed using variance analysis and the LSD values were calculated with Tukey-Hartley tables.

RESULTS AND DISCUSSION

Variation in resistance as a function of the stage of development

The dependency of tuber resistance upon the stage of development of the potato was studied by infecting tubers from trials with different planting and harvest times. In 1979 we used three harvest times, in 1980 six and 1981 three. The results (Fig. 1 and Table 1) are sufficient to give an approximate picture of the development of tuber resistance during the season. The scantier results of 1979 and 1981 in general confirm the results obtained in 1980, with the exception of the results of *Fusarium sulphureum* in 1979, which were opposite. The results of pre-storage trials in 1980 also reflect the development of tuber resistance (Table 4 and 5).

During the earliest harvests tubers were very susceptible. During the latter half of August their resistance was significantly higher and remained almost unchanged until the latter half

Table 1. The development of tuber resistance of cvs. Pito and Sabina to *Phoma exigua* var. *foveata* and *Fusarium sulphureum* during growth seasons 1979, '80 and '81. The figures indicate the growth of the fungi in 20 days.

Harvested	Inoculated with: *Phoma exigua* var. *foveata*		*Fusarium sulphureum*	
	Pito	Sabina	Pito	Sabina
1979 13/8 ...	6,9^{b}	—	3,9^{a}	—
3/9 ...	5,6^{a}	—	6,0^{b}	—
17/9 ...	5,2^{a}	—	6,9^{c}	—
F	31,50***		20,60**	
LSD_5 %	0,6		0,4	
1980 24/7 ...	5,8^{c}	6,8^{d}	8,6^{c}	10,8^{e}
18/8 ...	3,3^{a}	3,0^{ab}	6,1^{a}	6,9^{cd}
28/8 ...	2,6^{a}	2,8^{a}	6,2^{a}	7,2^{d}
12/9 ...	4,2^{b}	3,2^{ab}	7,2^{ab}	5,9^{a}
19/9 ...	3,1^{a}	3,7^{bc}	6,3^{a}	6,7^{bc}
29/9 ...	4,1^{b}	4,0^{c}	7,9^{bc}	6,4^{b}
F	32,21***	53,05***	11,44***	27,93***
LSD_5 %	0,6	0,6	1,0	0,3
1981 12/8 ...	—	3,1^{b}	—	5,8^{b}
7/9 ...	2,0	2,1^{a}	2/9 5,2^{b}	4,3^{a}
17/9 ...	2,2	3,0^{b}	3,3^{a}	4,9^{a}
F	6,00	144,78***	50,68**	15,33**
LSD_5 %	—	0,2	0,7	0,7

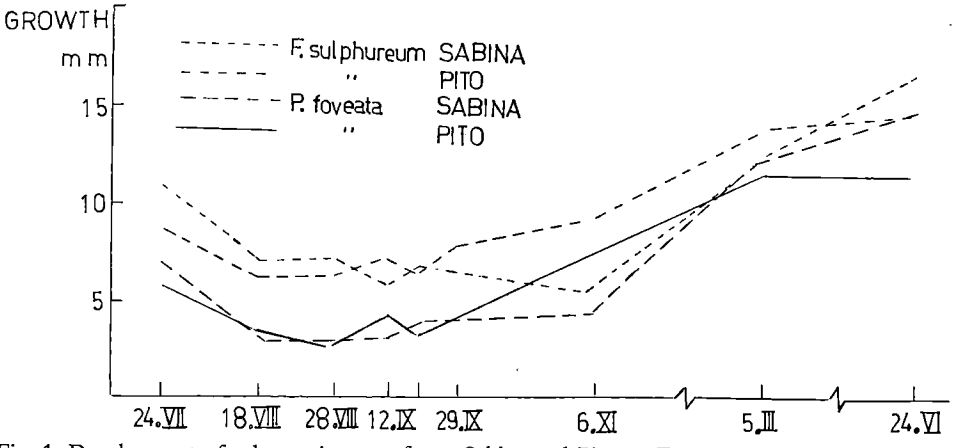


Fig. 1. Development of tuber resistance of cvs. Sabina and Pito to *Fusarium sulphureum* and *Phoma exigua* var. *foveata* during the season of 1980 and the storage period. The tests on Nov. 6 and later were made with the tubers harvested Sept. 29. The curves indicate the growth of the fungi in 20 days, all the inoculations and incubations were carried out as uniformly as possible.

of September when the first decrease in resistance was ascertained. Later, during storage, the decrease in resistance continued. The result was almost the same with both cultivars and both fungi. This supports the earlier information about the dependence of tuber resistance to *Phoma exigua* v. *foveata* on the stage of development of the potato. There is no earlier information about *Fusarium sulphureum*. It is worth nothing that tuber resistance to *F. sulphureum* does not vary in the same way as with *Fusarium solani* v. *coeruleum*, which was reported by JÖNSSON and BÅNG (1979): there was no period of very high susceptibility during the early stage of tuber development.

In 1980 and 1981 the importance of date of planting was studied. The first date was the conventional planting time, the second two weeks later and the third four weeks later. The results show that planting time has very little, if any, effect on the tuber resistance at harvest time (Tables 2 and 3). Pair comparisons show that planting time had no influence on more than a half of the tests. The earlier planting, however, caused an increase in resistance more often than the latter one, especially with the later

Table 2. The influence of planting time on the tuber resistance of Pito to *Phoma exigua* var. *foveata* and *Fusarium sulphureum* in 1980. Figures indicate the growth of the fungi in 20 days. 3 × 20 tubers for each test.

Planting time	Inoculated with *F. sulphureum*			
	21/8	10/9	26/9	5/11
30/5	7,7^{c}	4,5^{a}	5,6^{b}	4,6^{a}
13/6	6,2^{b}	4,4^{a}	6,0^{b}	4,1^{a}
27/6	5,6^{b}	4,5^{a}	5,5^{b}	5,9^{b}
F Planting time		5,25*		
Inoculation time ...		72,15***		
Pl × inocul		19,13***		
LSD_5 %		0,6		
	Inoculated with *Phoma e.* v. *foveata*			
30/5	4,7^{c}	3,0^{a}	3,1^{a}	3,3^{a}
13/6	3,9^{b}	2,8^{a}	3,0^{a}	3,0^{a}
27/6	4,9^{c}	3,7^{b}	3,1^{a}	2,8^{a}
F Planting time		17,10***		
Inoculation time ...		119,52***		
Pl × inocul		9,86**		
LSD_5 %		0,4		

Table 3. The influence of planting time on the tuber resistance of Pito and Sabina in 1981. Figures indicate the growth of the fungi in 20 days. 3 × 20 tubers for each test.

Planting time	Inoculated with *Fusarium sulphureum*					
	Sabina			Pito		
	3/9	17/9	5/10	3/9	17/9	5/10
21/5 ..	4,3^{a}	4,9^{ab}	4,9^{ab}	5,2^{bc}	3,3^{a}	4,6^{bc}
4/6 ..	6,3^{c}	4,3^{a}	5,2^{b}	7,0^{d}	4,5^{bc}	4,3^{b}
18/6 ..	6,6^{c}	5,4^{b}	5,3^{b}	5,5^{c}	4,9^{bc}	5,3^{c}
F Planting time ...		21,66***			14,41***	
Inoculation time		16.67***			38,31***	
Pl × inocul		13,82***			9,38**	
LSD_5 %		0,6			0,7	
	Inoculated with *Phoma exigua* var. *foveata*					
	Sabina			Pito		
	7/9	17/9	5/10	7/9	17/9	5/10
21/5 ..	2,1^{a}	3,0^{e}	2,9^{e}	2,0^{a}	2,1^{a}	2,8^{b}
4/6 ..	2,0^{a}	2,5^{bcd}	2,7^{cde}	1,9^{a}	2,8^{b}	3,3^{c}
18/6 ..	2,8^{de}	2,4^{bc}	2,3^{ab}	3,0^{bc}	2,3^{a}	3,1^{bc}
F Planting time ...		10,53***			26,90***	
Inoculation time		22,22***			68,56***	
Pl × inocul		29,91***			23,83***	
LSD_5 %		0,3			0,3	

cv. Pito. Clearly the growth rhythm dominates the tuber resistance so strongly that the possible effect of planting time is masked by it, especially during favourable seasons like those during the trials.

During the storage period the tubers gradually lost their resistance. When tubers harvested at different times were inoculated in November, the tubers harvested early were by far more susceptible than those harvested later (Fig. 3, Table 6). This result was very surprising and can only be explained using pre-storage trials under different conditions of relative humidity.

In 1979, 1980 and 1981 some trials were carried out to elucidate whether or not vine killing has any influence on the tuber resistance, but no effect was discovered.

Effect of environmental factors

We studied the resistance of tubers harvested at different stages of their development and pre-stored for a certain time under different condi-

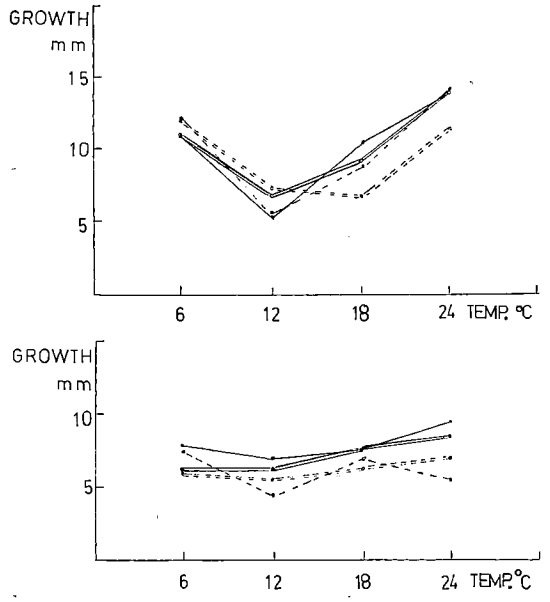


Fig. 2. The growth of *Fusarium sulphureum* and *Phoma exigua* var. *foveata* in tubers of Pito pre-stored for 1 or 2 weeks at different temperatures. The RH conditions were not regulated, according to later records it was about 80 % at the lower temperatures and about 50 % at the higher ones.

——————— harvested Aug. 13 pre-stored 1 week
——————— » » » » 2 »
= = = = = = » Sept. 3 » 1 »
═══════ » » » » 2 »

Table 4. Dependence of tuber resistance of Sabina and Pito to *Fusarium sulphureum* on pre-storage temperature. Humidity conditions were similar (95 ± 5 % RH) at each temperature. The tubers inoculated on 24/11 were harvested 29/9 and stored using conventional methods until 10/11. The figures indicate the growth of the fungus during incubation for 20 days.

Harvest time	Inoculation time	Pre-storage temperature 6 °C	12 °C	18 °C	24 °C
Sabina					
24/7 ..	31/7	11,6^{h}	14,0^{i}	9,3^{g}	12,0^{h}
8/8 ..	15/8	7,4^{def}	7,8^{def}	6,5^{bc}	7,3^{cde}
18/8 ..	27/8	6,9^{cd}	5,7^{ab}	6,4^{bc}	6,7^{bcd}
28/8 ..	5/9	8,2^{ef}	7,0^{cd}	6,6^{bc}	7,8^{def}
12/9 ..	24/9	5,8^{ab}	5,7^{ab}	6,4^{bc}	5,3^{a}
29/9 ..	24/11	7,7^{def}	7,5^{def}	8,3^{ef}	8,5^{f}
F Inoculation time .		296,33***			
Pre-storage		12,21***			
Inocul × pre-stor		16,05***			
LSD_5 %		0,8			
Pito					
24/7 ..	31/7	12,1^{f}	9,0^{de}	8,2^{bcde}	8,6^{cde}
8/8 ..	15/8	7,2^{abc}	7,3^{abc}	6,5^{ab}	5,9^{a}
18/8 ..	27/8	7,8^{abcde}	8,1^{bcde}	7,6^{abcde}	6,3^{ab}
28/8 ..	5/9	8,5^{cde}	6,1^{a}	6,8^{abc}	6,4^{ab}
12/9 ..	24/9	6,3^{ab}	7,2^{abc}	6,3^{ab}	6,6^{ab}
29/9 ..	24/11	9,1^{de}	9,3^{e}	11,1^{f}	10,8^{f}
F Inoculation time .		61,33***			
Pre-storage		8,23***			
Inocul × pre-stor		7,00***			
LSD_5 %		1,2			

tions of temperature and relative humidity (RH). The tubers from different pre-storage trials were infected and incubated in the same way.

Preliminary trials were carried out in 1979, when tubers were pre-stored at four temperatures (6, 12, 18 and 24 °C) one or two weeks before inoculation. Already one week of storage at 6 and 24 °C strongly decreased the tuber resistance (Fig. 2). Humidity was not controlled in these tests. In 1980 the tests were continued under the same temperature conditions but using uniform (95 ± 5 %) RH conditions.

There were some differences in the resistance of tubers pre-stored at different temperatures (Tables 4 and 5), when only the treatments of each harvest time are compared, the main variation being connected with harvest time. Within each harvest time, the extreme temperatures (6 and 24 °C), however, may have some influence on tuber resistance. The results when compared with those in Fig. 3, reveal that the importance of RH during the storage period is obviously greater than that of temperature, at least within the limits of 6 and 24 °C. Comparative tests using low and high conditions of RH at the same temperature confirmed this assumption (Tables 7, 8 and 9). The differences were greatest at temperatures of 6 and 24 °C, which indicates the combined influence of temperature and RH.

The great influence of dry conditions also explained the phenomenon presented earlier (Table 6, Fig. 3), that the tubers from the earlier harvest times lost their resistance much more

Table 5. Dependence of tuber resistance of Sabina and Pito to *Phoma exigua* var. *foveata* on pre-storage temperature. Humidity conditions were similar (95 ± 5 % RH) at each temperature. The tubers inoculated on 24/11 were harvested 29/9 and stored using conventional methods until 10/11. The figures indicate the growth for 20 days.

Harvest time	Inoculation time	Pre-storage temperature 6 °C	12 °C	18 °C	24 °C
Sabina					
24/7	31/7	$4{,}9^{bc}$	$8{,}3^{fg}$	$8{,}3^{fg}$	$8{,}8^{g}$
8/8	15/8	$4{,}0^{ab}$	$3{,}5^{ab}$	$4{,}1^{ab}$	$5{,}1^{bc}$
18/8	27/8	$4{,}1^{ab}$	$4{,}4^{ab}$	$3{,}2^{a}$	$6{,}6^{de}$
28/8	5/9	$3{,}5^{ab}$	$4{,}6^{abc}$	$3{,}3^{a}$	$4{,}2^{ab}$
12/9	24/9	$2{,}8^{a}$	$3{,}0^{a}$	$2{,}9^{a}$	$3{,}5^{ab}$
29/9	14/10	$4{,}9^{bc}$	$3{,}7^{ab}$	$3{,}9^{ab}$	$4{,}7^{abc}$
29/9	24/11	$5{,}9^{cd}$	$7{,}5^{ef}$	$8{,}8^{g}$	$9{,}2^{g}$
F Inoculation time ...		$130{,}50^{***}$			
Pre-storage		$32{,}59^{***}$			
Inocul × pre-stor ..		$8{,}23^{***}$			
LSD_5 %		1,0			
Pito					
24/7	31/7	$4{,}3^{bcd}$	$8{,}1^{f}$	$5{,}3^{de}$	$8{,}2^{f}$
8/8	15/8	$2{,}9^{ab}$	$3{,}2^{ab}$	$3{,}5^{abc}$	$3{,}8^{abc}$
18/8	27/8	$3{,}5^{abc}$	$3{,}7^{abc}$	$3{,}1^{ab}$	$3{,}7^{abc}$
28/8	5/9	$3{,}3^{ab}$	$3{,}2^{ab}$	$2{,}7^{a}$	$3{,}3^{ab}$
12/9	24/9	$2{,}8^{a}$	$3{,}6^{abc}$	$3{,}0^{ab}$	$4{,}8^{cd}$
29/9	14/10	$3{,}8^{abc}$	$3{,}3^{ab}$	$4{,}3^{bcd}$	$5{,}9^{e}$
29/9	24/11	$6{,}2^{e}$	$8{,}2^{f}$	$9{,}2^{f}$	$9{,}1^{f}$
F Inoculation time ...		$196{,}58^{***}$			
Pre-storage		$46{,}98^{***}$			
Inocul × pre-stor ..		$10{,}31^{***}$			
LSD_5 %		0,9			

rapidly than the tubers harvested at the end of the harvest period. The young tubers with thin peel clearly respired more effectively, thus losing something connected with their resistance.

The results confirm the observation of LANSADE (1949) that both the storage temperature and the storage RH influence tuber resistance. In Finnish potato production, the harvest must often be taken in before the maturation of tubers, so that the decrease in tuber resistance may often be important in practice, too.

It is not possible to draw conclusions about the exact reasons for the decrease in tuber resistance on the basis of this material. With regard to the decrease in resistance to *Phoma exigua* v. *foveata*, the literature often mentions that gangrene occurs in the parts of bulk potato stores exposed to cold and »drought». We can pose the question of wheather or not high ventilation might decrease tuber resistance, alone or together with low temperature and RH. It is possible that the ascertained decrease in resistance when tubers were stored under conditions of low RH and extreme temperatures was a consequence of accelerated respiration and evap-

Table 6. Tuber resistance of Sabina and Pito to *Fusarium sulphureum* and *Phoma exigua* var. *foveata* harvested at different times and the decrease in resistance during the storage period. The figures of August 8 inoculated at harvest time are in parenthesis because they are values interpolated from 24/7 and 18/8. The figures indicate the growth of the fungi during incubation for 20 days. Compare Fig. 3.

Harvest time	Inoculated with *Fusarium sulphureum* At harvest time	6/11	5/3	24/6	Inoculated with *Phoma exigua* v. *foveata* At harvest time	6/11	5/3	24/6
Sabina								
8/8	$(8{,}1)^{b}$	$11{,}6^{c}$	$13{,}6^{d}$	$12{,}2^{c}$	$(4{,}7)^{b}$	$11{,}1^{c}$	$11{,}7^{c}$	$15{,}5^{g}$
18/8	$6{,}1^{a}$	$11{,}7^{c}$	$13{,}1^{d}$	$14{,}9^{e}$	$3{,}0^{a}$	$10{,}1^{d}$	$11{,}3^{c}$	$15{,}5^{g}$
12/9	$5{,}9^{a}$	$6{,}1^{a}$	$12{,}9^{cd}$	$12{,}4^{cd}$	$3{,}2^{a}$	$5{,}8^{c}$	$11{,}7^{e}$	$13{,}8^{f}$
30/9	$6{,}4^{a}$	$5{,}5^{a}$	$12{,}2^{c}$	$14{,}8^{e}$	$4{,}0^{ab}$	$4{,}4^{b}$	$12{,}4^{e}$	$16{,}6^{g}$
F Harvest time	$48{,}69^{***}$				F Harvest time	$34{,}78^{***}$		
Inoculation time ...	$445{,}30^{***}$				Inoculation time ..	$1\,049{,}10^{***}$		
Harvest × inocul ..	$33{,}28^{***}$				Harvest × inocul .	$33{,}42^{***}$		
LSD_5 %	1,0				LSD_5 %	1,0		
Pito								
8/8	$(7{,}6)^{a}$	$15{,}3^{ef}$	$16{,}4^{f}$	$13{,}5^{d}$	$(4{,}4)^{ab}$	$9{,}8^{d}$	$8{,}0^{c}$	$5{,}1^{b}$
18/8	$6{,}9^{a}$	$13{,}6^{d}$	$16{,}1^{f}$	$11{,}7^{c}$	$3{,}3^{a}$	$10{,}2^{d}$	$7{,}4^{c}$	$7{,}9^{c}$
12/9	$7{,}2^{a}$	$8{,}5^{b}$	$14{,}7^{e}$	$12{,}7^{cd}$	$4{,}2^{ab}$	$5{,}1^{b}$	$10{,}2^{d}$	$11{,}0^{d}$
30/9	$7{,}9^{ab}$	$9{,}1^{b}$	$13{,}4^{d}$	$14{,}6^{e}$	$4{,}1^{ab}$	$7{,}5^{c}$	$11{,}5^{d}$	$11{,}5^{d}$
F Harvest time	$43{,}86^{***}$				F Harvest time	$16{,}57^{***}$		
Inoculation time ...	$412{,}88^{***}$				Inoculation time ..	$152{,}97^{***}$		
Harvest × inocul ..	$33{,}28^{***}$				Harvest × inocul .	$31{,}81^{***}$		
LSD_5 %	1,0				LSD_5 %	1,2		

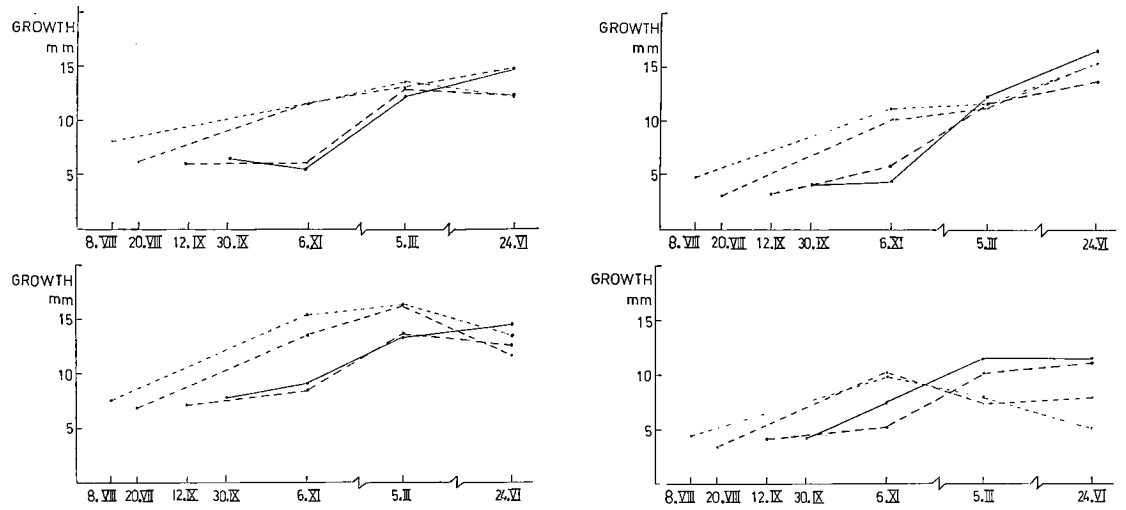


Fig. 3. The growth of *Fusarium sulphureum* (left) and *Phoma exigua* var. *foveata* (right) in tubers of Sabina (above) and Pito (under) harvested at different stages of their development. There are striking differences between early and later harvests in the test on November 6, after which the differences decreased, and even became opposite in Pito.

Table 7. Influence of humidity conditions prevailing during 3 weeks pre-storage on tuber resistance of cvs. Sabina and Pito to *Fusarium sulphureum*. Potatoes were grown and stored until the start of pre-storage using conventional methods. They were inoculated and incubated uniformly. Figures indicate the growth of the fungus in 20 days.

Tubers pre-stored under conditions of °C/% RH	Growth of fungus mm			
	Inoculation time		Inoculation time	
	5/12-80	4/3-81	5/12-80	4/3-81
	Sabina		Pito	
12/95± 5	5,8^{a}	7,3^{ab}	5,1^{a}	7,3^{c}
12/55±10	6,1^{ab}	7,3^{ab}	6,4^{b}	7,0^{bc}
24/95± 5	6,6^{b}	7,0^{a}	4,9^{a}	6,7^{ab}
24/55±10	6,7^{b}	7,5^{b}	6,3^{b}	6,4^{a}
F	4,10*	4,83*	74,30***	9,03**
LSD_5 %	0,6	0,4	0,3	0,5

Table 8. Influence of humidity conditions prevailing during four weeks' pre-storage on tuber resistance of cv. Sabina to *Fusarium sulphureum* and *Phoma exigua* v. *foveata*. Potatoes were harvested on August 12, pre-stored under different environmental conditions until September 8 when inoculated, and incubated uniformly (34 days at 10 ± 2 °C).

Pre-storage conditions °C/% RH	Growth of fungi mm	
	Fusarium sulphureum	*Phoma exigua* v. *foveata*
6/95± 5	7,8^{d}	2,6^{ab}
12/95± 5	6,0^{c}	2,7^{ab}
12/50±10	5,6^{bc}	3,4^{b}
18/95± 5	5,3^{ab}	1,9^{a}
24/95± 5	4,9^{a}	3,4^{b}
24/50±10	8,4^{e}	7,6^{c}
F	55,34***	69,40***
LSD_5 %	0,9	0,6

Table 9. Influence of humidity conditions prevailing during 24 days' pre-storage on tuber resistance of cv. Sabina to *Fusarium sulphureum* and *Phoma exigua* var. *foveata*. Harvested October 5, inoculated, and incubated uniformly (20 days at 12 °C).

Pre-storage conditions °C/% RH	Growth of fungi mm	
	Fusarium sulphureum	*Phoma exigua* v. *foveata*
6/95± 5	5,4^{a}	5,1^{a}
6/75±10	6,3^{b}	7,0^{b}
12/95± 5	4,8^{a}	5,2^{a}
12/75±10	5,3^{a}	5,6^{ab}
18/95± 5	4,6^{a}	6,5^{ab}
18/55± 5	5,0^{a}	9,4^{c}
24/95± 5	5,0^{a}	6,4^{ab}
24/45± 5	6,6^{b}	10,0^{c}
F	23,80***	31,71***
LSD_5 %	0,8	1,0

oration of the tubers. It may merely be a result of rapid ageing of the tubers, but it may involve some other biochemical changes in the tuber. Knowledge of the great importance of RH and temperature or their combined effect is useful when studying the actual reasons for the decrease in tuber resistance.

REFERENCES

BOYD, A. E. W. 1952 a. Dry-rot disease of the potato V. Seasonal and local variations in tuber susceptibility. Ann. Appl. Biol. 39: 330—338.

— 1952 b. Dry-rot disease of the potato VII. The effect of storage temperature upon subsequent susceptibility of tubers. Ann. Appl. Biol. 39: 351—357.

— 1967. The effects of length of the growth period and of nutrition upon potato-tuber susceptibility to dry rot *(Fusarium coeruleum)* Ann. Appl. Biol. 60: 231—240.

FOX, A. R. & DASHWOOD, P. 1970. Some aspects of the biology of *Phoma exigua* var. *foveata* in relation to the control of potato gangrene. Proc. 4th Triennial Conf. EAPR: 190—191.

JÖNSSON, U. & BÅNG, H. 1979. Mottaglighet för torra rötor i potatis. Förändringar under växtperioden. Växtskyddsnotiser 43: 54—57.

LANSADE, M. 1949. Recherches sur la Fusariose ou pourriture sèche de la pomme de terre, *Fusarium coeruleum* (Lib.). Sacc. Bull. Techn. Inf. Ingén. Serv. Agric. 41: 419—432.

PETHYBRIDGE, G. H. & LAFFERTY, H. A. 1917. Further observations on the cause of the common dry-rot of the potato tuber in the British Isles. Sci. Proc. R. Dubl. Soc. 15: 193—222.

SEPPÄNEN, E. 1980. Studies on potato gangrene in Finland. Ann. Agric. Fenn. 19: 173—179.

— 1981 a. Fusariums of the potato in Finland II. On the growth optima of *Fusarium* species in tubers of cv. Bintje. Ann. Agric. Fenn. 20: 157—176.

— 1981 b. Fusariums of the potato in Finland III. Varietal resistance of potato tubers to some *Fusarium* species. Ann. Agric. Fenn. 20: 177—183.

Manuscript received April 1982

Esko Seppänen
Agricultural Research Centre
Institute of Plant Pathology
SF-01300 Vantaa 30, Finland

SELOSTUS

Perunan mukuloiden varastotaudinkestävyyden vaihtelusta

ESKO SEPPÄNEN

Maatalouden tutkimuskeskus

Tutkimuksessa selvitettiin perunan mukuloiden kestävyyttä varastotauteja aiheuttavia *Phoma exigua* var. *foveata* ja *Fusarium sulphureum* sieniä vastaan. Tarkoituksena oli osoittaa, missä määrin Pidon ja Sabinan mukuloiden kestävyys vaihtelee niiden fysiologisen kehityksen aikana, eri lämpötiloissa ja erilaisissa kosteusoloissa suoritetun esivarastoinnin aikana sekä vaikuttaako istutusaika ja varsiston hävittäminen kestävyyteen.

Kestävyys kumpaakin sientä vastaan vaihteli melko yhdenmukaisesti. Nuoret mukulat olivat hyvin alttiita. Kestävyys parani mukuloiden kehittyessä mutta heikkeni uudelleen varastointikauden alusta lähtien.

Mukuloiden esivarastointi alhaisessa (6 °C) ja korkeassa (24 °C) lämpötilassa sekä alhaisessa kosteudessa heikensi kestävyyttä merkitsevästi. Vaikutus näkyi selvemmin aikaisissa nostoissa, jolloin mukuloiden tuleentuminen oli kesken. Oletettavasti mukuloiden hengityksen ja haihtumisen kiihtyminen nopeuttaa niiden fysiologista vanhenemista ja samalla heikentää kestävyyttä.

Istutusaika ja varsiston hävittäminen eivät kokeissa vaikuttaneet olennaisesti mukuloiden kestävyyteen.

ANNALES AGRICULTURAE FENNIAE, VOL. 21: 131—136 (1982)

SERIA AGROGEOLOGIA ET -CHIMICA N. 111 — Sarja MAA JA LANNOITUS n:o 111

DISTRIBUTION OF NITRATE IN RED BEET ROOTS AND LEAVES FERTILIZED WITH UREA OR AMMONIUM NITRATE

HEIKKI KALLIO, MAARIT KYYRÖ, AINO-MAIJA EVERS and JOHAN KORKMAN

KALLIO, H., KYYRÖ, M., EVERS, A-M. & KORKMAN, J. 1982. **Distribution of nitrate in red beet roots and leaves fertilized with urea or ammonium nitrate.** Ann. Agric. Fenn. 21: 131—136. (Dept. Chem. and Biochem., Lab. Food Chem., Univ. Turku, SF-20500 Turku 50, Finland.)

Different parts of red beet accumulated nitrogen ions in the same way with both urea and NH_4NO_3 fertilizing. The nitrate content of the root increased from the peel to the marrow. The content in the root tip was also higher than in the whole root. The least nitrate was observed in the leaf lamina. When the total nitrate in beets was reduced by using a nitrification inhibitor (nitrapyrin) the nitrate content decreased analogously in the eight fractions studied. Thus the incorporated nitrapyrin does not seem to affect the nitrate level in beets *per se*.

Index words: red beet, nitrate, distribution of nitrate, nitrification inhibitor.

INTRODUCTION

The capacity of roots to take up nitrate is controlled by a permease. This has been shown, for example in tobacco (HEIMER and FILNER 1970), maize (JACKSON et al. 1973, NEYRA and HAGEMAN 1975), wheat (ASHLEY et al. 1975) and barley (CHANTAROTWONG et al. 1976, RAO and RAINS 1976 a, b). The enzyme is induced by nitrate ions. The cereals have been studied very intensively. Uptake of nitrogen in wheat and maize depends on the size and activity of the roots (GOODMAN 1979) and on the environmental temperature (JACKSON et al. 1976). In maize, light also promotes the transport of nitrogen (BEEVERS et al. 1965). Anaerobic conditions retard the uptake very effectively (JACKSON et al. 1976) and ammonium ions inhibit transport into the roots of barley (RAO and RAINS 1976 a) and wheat (MINOTTI et al. 1969, BRETELER and SMIT 1974).

The environmental factors, the duration and intensity of light in particular, also affect the nitrate content in beets (CANTLIFFE 1972, 1973, MINOTTI and STANKEY 1973). The nitrate content in beets is increased more by nitrate fertilizers than by ammonium fertilizers (LEE et al. 1971, CANTLIFFE 1972, 1973, PECK et al. 1974).

In the roots the nitrate is either reduced, stored or transported to other parts of the plant. It is usually assumed that the total nitrate consists of »storage» nitrate and »metabolizable» nitrate

(HEIMER and FILNER 1971, FERRARI et al. 1973, SHANER and BOYER 1976), of which the metabolizable fraction is more important in the control of nitrate reduction in plant tissues. The content of metabolizable nitrate is higher in young cells than in old (FERRARI et al. 1973, ASLAM and OAKS 1975). In maize the transport of nitrate from the roots to the leaves depends on temperature (SHANER and BOYER 1976). A drop in temperature deactivates nitrate reductase but the effect on the nitrate content in leaves is minor.

In recent years, the cultivation conditions for nitrate accumulating plants have been under special supervision. This is due to the new recommendations and limitations in many countries. The nitrification inhibitor nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) has been shown to reduce the nitrate content in red beets with urea fertilizing (KALLIO et al. 1980), in radishes (MILLS et al. 1976), and in spinach (BENGTSSON 1968, von KICK and MASSEN 1973, von SIEGEL and VOGT 1975, MILLS et al. 1976). To obtain a better understanding of the nitrate accumulation in red beets, the localization of nitrate into different parts of red beet roots and leaves was studied using either urea or ammonium nitrate fertilizers. The level of nitrate in the plants was regulated with the addition of nitrapyrin.

MATERIAL AND METHODS

Material and treatments

A commercial variety, *Rubia Sv.*, of the globe red beet (*Beta vulgaris* L. var. *conditiva*) was used in the study. The experiment was conducted at Kotkaniemi Experimental Farm, Kemira Ltd. (60°22′N, 24°22′E) during the 1980 growing season. Before fertilizing in the spring, the results of soil analyses were as follows: pH 6,0, total N 0,17 %, Ca 1 290 mg · l^{-1}, K 189, P 23, NH_4-N 10,8, and NO_3-N 25,9 mg · l^{-1} (Ca, K and P were extracted with ammonium acetate, pH 4,65 and NH_4-N and NO_3-N with water). There was a total of six trial units with two replicates each. The area of each plot was 5 m^2, and the layout was a randomized plot design. The plots were fertilized with nitrogen applied as either urea or a chlorine-free compound fertilized at a rate of 80 kg of nitrogen · ha^{-1} containing nitrapyrin (2-chloro-6(trichloromethyl)pyridine) at a rate of 0, 5,3 or 8,0 kg · ha^{-1}. The composition of the compound fertilizer was: total N 10 % (NO_3-N 3,8 and NH_4-N 6,2), P 4, K 17, B 0,15, Cu 0,4, Mn 0,7, Mg 2,5, Mo 0,02, Zn 0,03 and Fe 0,01 %.

The urea-treated plots were fertilized with 800 kg · ha^{-1} of a PK compound fertilizer (total N 2 %, P 7,9, soluble P 4,4, K 14,9, B 0,2, Cu 1,5, S 5,0, Fe 0,1, Cl 14,1, Ca 12,9, and Mg 0,1 %). The fertilizers were spread on the surface and turned into the soil.

The seeds were planted on June 3 in rows with the seeds 1,5 cm apart. Conventional farming practice was observed in controlling weeds and pests (Betanal 5,0 l · ha^{-1} and Dimethoate 0,2 kg · ha^{-1} on June 12). The data of harvesting was September 1.

Analytical methods

A 2,5 kg sample of beets from each replicate was weighed, washed and dried, and the roots and leaves cut off and weighed separately and deep-frozen. Each root was divided into six fractions as follows: the root tips (subsample no. 1) were cut off, the roots were peeled (peel, no. 3) and the upper part of the root (4) was cut off horizontally. The rest of the roots were cut cylindrically into three vertical layers along the vascular strands; the first layer (8), the second

layer (7), and the marrow (5), all of identical thickness. The petioles (2) were cut from the leaf laminae (6). The fractions were numbered as in an earlier work by KALLIO and SANDHOLM (1982). The corresponding fractions of beets from each replication were pooled, weighed, finely ground, and deep-frozen for the nitrate analyses. A 5 g thawed beet fraction was shaken for 15 min with boiling distilled water and the nitrate in the extract was determined potentiometrically. The dry weights of the samples were determined by drying them for 16 h at 105 °C.

RESULTS AND DISCUSSION

The proportions of the fresh fractions and the dry matter contents of the whole roots and leaves and fractions are shown in Table 1. All trial units have been included (n = 12) in the table. The dry matter content of the root is highest in the superficial parts, decreasing towards the marrow. The leaf petioles have the highest water content. Nitrapyrin has no effect on the dry matter content of red beets (Table 2) but the values of the roots and leaves are somewhat higher with NH_4NO_3 fertilizing than with urea. However, no statistical examination was possible.

The effect of nitrapyrin on nitrate nitrogen content in the roots and leaves is also shown in Table 2. Together with urea fertilizing 5,3 kg · ha^{-1} of nitrapyrin reduced the nitrate content by 60 % in roots and by 50 % in leaves. With 5,3 kg of nitrapyrin and NH_4NO_3 fertilizing the content in roots was reduced by 40 % and in leaves by 60 %. The corresponding values with 8 kg of nitrification inhibitor were 70, 50, 60 and 80 %, respectively. When the results are compared with those from an earlier study (KALLIO et al. 1980) the effect of the season and the environmental conditions on the nitrate contents is clearly seen. In the earlier work the nitrate content in beets was only about one half of that in the present study.

Nitrapyrin reduced the nitrate content in each fraction of each trial unit except one (Fig. 1). The only exception was the leaf lamina of the urea-fertilized trial unit with 8 kg of nitrapyrin. The contents and changes in nitrate shown in Figure 1 were almost the same with both urea and NH_4NO_3 fertilizing at all inhibitor levels. The values seem to be somewhat lower with NH_4NO_3 than with urea (Table 2). Of the

Table 1. Relative proportions of the fresh red beet fractions and the dry matter content in red beets and fractions.

Fraction of red beet	Dry matter content, (%, n = 12)	Proportions of the fractions[a] (%)
Petiole	9,0±0,8	54±3
Leaf lamina	11,7±0,7	46±3
Root tip	15,5±1,7	2±0
Upper part	17,0±1,2	8±1
Peel	15,9±0,7	6±1
1st layer	14,9±0,9	47±3
2nd layer	14,3±0,8	22±2
Marrow	14,4±1,2	15±2
Whole leaves	10,3±0,8	41±4[b]
Whole root	14,9±0,8	59±4[b]

[a] The proportions (%) of the leaf fractions calculated from the leaf fresh weight and the proportions of the root fractions from the root fresh weight.
[b] Calculated from the whole plants.

Table 2. Dry matter and NO_3-N contents in red beets at different nitrapyrin levels with urea and NH_4NO_3 fertilizing.

Nitrapyrin kg · ha^{-1}	Dry matter (%)				NO_3-N content (% DW)			
	Roots		Leaves		Roots		Leaves	
	Urea	NH_4NO_3	Urea	NH_4NO_3	Urea	NH_4NO_3	Urea	NH_4NO_3
0	13,8	15,3	9,7	11,2	0,22	0,13	0,10	0,12
5,3	14,7	15,3	10,0	10,4	0,08	0,07	0,06	0,05
8,0	14,8	15,5	9,7	10,5	0,07	0,06	0,04	0,03

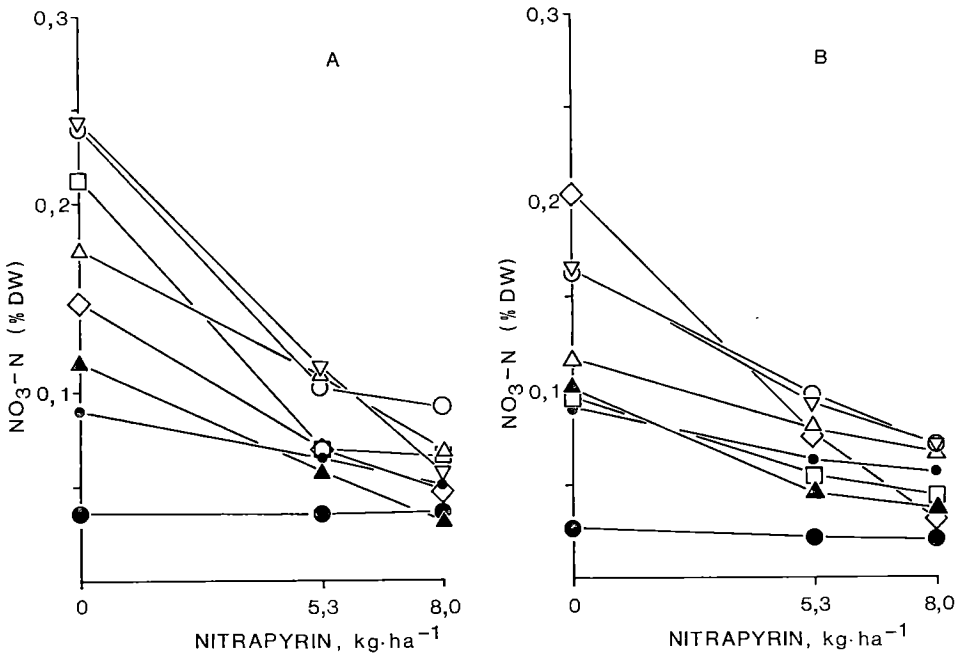


Fig. 1. NO_3-N content of fractions of red beet with urea (A) and ammonium nitrate (B) fertilizing with varying nitrapyrin dosages. Petiole ◇, leaf lamina ●, root tip △, upper part ●, peel ▲, 1st layer □, 2nd layer ○, marrow ▽.

24 fractions studied (eight fractions with three nitrapyrin levels), the nitrate nitrogen was higher in only five of the NH_4NO_3-fertilized beets than in the urea-fertilized fractions (statistically not significant). With both nitrogen fertilizers the nitrate level of the root increases from surface to marrow (Table 3). The root tip also had a higher content than the root, on average. The changes in the nitrate level in red beet did not affect the relative distribution of nitrate in the different parts of the plant. The distribution of nitrapyrin in the different parts of red beet has been analyzed in an earlier study (KALLIO and SANDHOLM 1982). The highest contents of nitrapyrin residues (on a dry weight basis) were found in the root tips and petioles; and the peel contained more of it than the leaf laminae or other parts of the root.

The results of the present study indicate that the small amounts of nitrapyrin incorporated into the plant do not *per se* affect the local nitrate balance between the different parts of the beet. This is evident because the nitrapyrin rationing (5,3 or 8,0 kg · ha^{-1}) did not alter the

Table 3. Proportions (on dry weight basis) of NO_3-N contents in fractions of red beet roots.

Fertilizer	Relative NO_3-N contents[a]						
	Whole root	Marrow	2nd layer	1st layer	Peel	Upper part	Root tip
Urea	100	110±18	125±13	94±8	67±10	66±17	105±19
NH_4NO_3	100	130± 1	133± 4	70±8	70± 8	88±12	108±11

[a] NO_3-N content of the whole root = 100

balance between the fractions. The parts with high reduction of nitrate (*i.e.* marrow, 1st layer and 2nd layer) in this study also contain, according to KALLIO and SANDHOLM (1982), only minor amounts of nitrapyrin residues. Thus in the case of red beet, as stated several times before (GORING 1962 a, b, CAMPBELL and ALEEM 1965, WALKER 1976), the inhibitory effect of nitrapyrin on nitrification is concentrated on the soil micro-organisms.

REFERENCES

ASHLEY, D. A., JACKSON, W. A. & VOLK, R. J. 1975. Nitrate uptake and assimilation by wheat seedlings during initial exposure to nitrate. Plant Physiol. 55: 1102—1106.

ASLAM, M. & OAKS, A. 1975. Effect of glucose on the induction of nitrate reductase in corn roots. Plant Physiol. 56: 634—639.

BEEVERS, L., SCHRADER, L. E., FLESHER, D. & HAGEMAN, R. H. 1965. The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings. Plant Physiol. 40: 691—698.

BENGTSSON, B. L. 1968. Effect of nitrification inhibitor on yield and nitrate content of spinach. Z. Pfl.ernähr. Bodenkd. 121: 1—4.

BRETELER, H. & SMIT, A. L. 1974. Effect of ammonium nutrition on uptake and metabolism of nitrate in wheat. Netherl. J. Agric. Sci. 22: 73—81.

CAMPBELL, N. E. R. & ALEEM, M. J. H. 1965. The effect of 2-chloro-6-(trichloromethyl)pyridine on the chemoautotrophic metabolism of nitrifying bacteria. I. Ammonia and hydroxylamine oxidation by Nitrosomonas. Antonie van Leeuwenhoek 31: 124—136.

CHANTAROTWONG, W., HUFFAKER, R. C., MILLER, B. L. & GRANSTEDT, R. C. 1976. In vivo nitrate reduction in relation to nitrate uptake, nitrate content and in vitro nitrate reductase activity in intact barley seedlings. Plant Physiol. 57: 519—522.

CANTLIFFE, D. J. 1972. Nitrate accumulation in vegetable crops as affected by photoperiod and light duration. J. Amer. Soc. Hort. Sci. 97: 414—418.

— 1973. Nitrate accumulation in table beets and spinach as affected by nitrogen, phosphorus, and potassium nutrition and light intensity. Agron. J. 65: 563—565.

FERRARI, T. E., YODER, O. C. & FILNER, P. 1973. Anaerobic nitrite production by plant cells and tissues: Evidence for two nitrate pools. Plant Physiol. 51: 423—431.

GORING, C. A. I. 1962 a. Control of nitrification by 2-chloro-6-(trichloromethyl)pyridine. Soil Sci. 93: 211—218.

— 1962 b. Control of nitrification of ammonium fertilizers and urea by 2-chloro-6-(trichloromethyl)-pyridine. Soil Sci. 93: 431-439.

GOODMAN, P. J. 1979. Genetic control of inorganic nitrogen assimilation of crop plants. Nitrogen assimilation of plants. (Eds. E. J. Hewitt & C. V. Cutting.) p. 165—176, Academic Press, London & New York.

HEIMER, Y. M. & FILNER, P. 1970. Regulation of the nitrate assimilation pathway of cultured tobacco cells. II. Properties of a variant cell line. Biochim. Biophys. Acta 215: 152—165.

— & FILNER, P. 1971. Regulation of nitrate assimilation pathway in cultured tobacco cells. Biochim. Biophys. Acta 230: 362—372.

JACKSON, W. A., FLESHER, D. & HAGEMAN, R. H. 1973. Nitrate uptake by dark-brown corn seedlings. Some characteristics of apparent induction. Plant Physiol. 51: 120—127.

—, KWICK, K. D., VOLK, R. J. & BUTZ, R. G. 1976. Nitrate influx and efflux by intact wheat seedlings: Effects of prior nitrate nutrition. Planta 132: 149—156.

KALLIO, H., LINKO, R. R., TIKANMÄKI, E. & PUNTARI, I. 1980. Effect of nitrapyrin on nitrapyrin residues and nitrate content in red beet roots fertilized with urea. J. Sci. Food Agric. 31: 701—708.

— & SANDHOLM, J. 1982. Distribution of nitrapyrin (2-chloro-6-(trichloromethyl)pyridine) and 2-chloro-6-(dichloromethyl)pyridine in red beets treated with nitrapyrin. J. Sci. Food. Agric. 33: 451—455.

KICK, H. VON & MASSEN, G. G. 1973. Der Einfluss von Dicyanamid und N-serve in Verbindung mit Ammoniumsulfat als N-dünger auf die Nitrat und Oxalsäure von Spinat *(Spinacia oleracea)*. Z. Pfl.ernähr. Bodenkd. 135: 220—226.

LEE, C. Y., SHALLENBERGER, R. S., DOWNING, D. L., STOEWSAND, G. S. & PECK, N. H. 1971. Nitrate and nitrite nitrogen in fresh, stored and processed table beets and spinach from different levels of field nitrogen fertilisation. J. Sci. Food Agric. 22: 90—92.

MILLS, H. A., BAKER, A. V., MAYNARD, D. N. 1976. Effects of nitrapyrin on nitrate accumulation in spinach. J. Amer. Hort Sci. 101: 202—204.

MINOTTI, P. L., WILLIAMS, D. C. & JACKSON, W. A. 1969. The influence of ammonium on nitrate reduction in wheat seedlings. Planta (Berl.) 86: 267—271.

MINOTTI, P. L. & STANKEY, D. L. 1973. Diurnal variation in the nitrate concentration of beets. Hort. Sci. 8: 33—34.

NEYRA, C. A. & HAGEMAN, R. H. 1975. Nitrate uptake and induction of nitrate reductase in excised corn roots. Plant Physiol. 56: 692—695.

PECK, N. H., CANTLIFFE, D. J., SHALLENBERGER, R. S. & BOURKE, J. B. 1974. Table beet (*Beta vulgaris* L.) and nitrogen. Search Agric. 4: 1—25.

RAO, K. P. & RAINS, D. W. 1976 a. Nitrate absorption by barley. I. Kinetics and energetics. Plant Physiol. 57: 55—58.

— & RAINS, D. W. 1976 b. Nitrate absorption by barley. II. Influence of nitrate reductase activity. Plant Physiol. 57: 59—62.

SHANER, D. L. & BOYER, J. S. 1976. Nitrate reductase activity in maize (*Zea mays* L.) leaves. I. Regulation by nitrate flux. Plant Physiol. 58: 499—504.

SIEGEL, O. VON & VOGT, G. 1975. Über den Einfluss eines Nitrifikationsinhibitors auf die Stickstoffverbindungen des Spinats. Landwirtsch. Forsch. 28: 242—248.

WALKER, N. 1976. Rep. Rothamsted Exp. Stn. Part I, p. 283.

Manuscript received March 1982

Heikki Kallio
Department of Chemistry and Biochemistry
Laboratory of Foodchemistry
University of Turku
SF-20500 Turku 50, Finland
and
Saarioinen Ltd
SF-36420 Sahalahti, Finland

Maarit Kyyrö
Saarioinen Ltd
SF-36420 Sahalahti, Finland

Aino-Maija Evers
Kemira Ltd
SF-02271 Espoo 27, Finland

Johan Korkman
Kemira Ltd
SF-00100 Helsinki 10, Finland

SELOSTUS

Nitraatin jakaantuminen punajuuren juuriin ja lehtiin urea- ja Puutarhan Y 1-lannoituksilla

HEIKKI KALLIO, MAARIT KYYRÖ, AINO-MAIJA EVERS ja JOHAN KORKMAN

Turun Yliopisto, Saarioinen Oy ja Kemira Oy

Punajuuri on nitraattia keräävä kasvi, jolla erityisesti typpilannoitteen määrä, muoto ja lannoituksen ajankohta vaikuttavat nitraattipitoisuuteen. Nitraatin akkumuloitumista punajuuren eri osiin tutkittiin työssä urea- ja Puutarhan Y 1-lannoituksella. Juuren nitraattipitoisuus kasvoi pinnasta ytimeen päin (kuiva-aineesta laskettuna). Myös juuren kärjen pitoisuus oli suurempi kuin juuressa keskimäärin. Pienin nitraatin määrä oli lehtilavoissa. Kun alennettiin punajuuren kokonaisnitraattisisältöä käyttämällä lannoituksen yhteydessä nitrifikaatioinhibiittoria (nitrapyriini), alenivat eri fraktioiden nitraattipitoisuudet analogisesti kummallakin lannoitteella eri inhibiittoritasoilla. Kokeillulla inhibiittorilla voitiin merkitsevästi alentaa punajuuren nitraattipitoisuutta.

ANNALES AGRICULTURAE FENNIAE, VOL. 21: 137—145 (1982)

Seria AGROGEOLOGIA ET -CHIMICA N. 112 — Sarja MAA JA LANNOITUS n:o 112

SURVIVAL OF FECAL INDICATOR BACTERIA IN AN INDUSTRIAL-SCALE COMPOSTING PROCEDURE

Jorma Hirn, Heikki Kallio ja Elisa Tikanmäki

Hirn, J., Kallio, H. & Tikanmäki, E. 1982. **Survival of fecal indicator bacteria in an industrial-scale composting procedure.** Ann. Agric. Fenn. 21: 137—145. (Nat. Veter. Inst., SF-00101 Helsinki 10, Finland.)

The behavior and survival of fecal indicator bacteria (fecal coliform bacteria, fecal streptococci, *Clostridium perfringens*) were observed during various seasons in an industrial-scale composting process of a foodstuff factory garbage disposal system. In the course of the study the prevalence of *Salmonella* in these composts was also investigated.

In the spring and fall composts the logarithmic counts of fecal coliform bacteria ranged from 6,55 to 7,01. In the winter composts the counts were, apparently because of the freezing of the raw material, somewhat lower. A corresponding feature was also seen in fecal streptococci whose counts in the spring/fall composts ranged from 6,18 to 6,54. With *C. perfringens* this feature was not observed; its counts ranged from 4,45 to 4,77.

The numbers of fecal coliform bacteria decreased within a 1-to-2-week waiting period to below the limits of determination. During the first 2 to 4 weeks, however, considerable variations in the numbers of fecal bacteria were seen intermittently. The apparent reason in the winter piles were the considerable variations in temperature increments; in the spring and fall piles the causative factor was perhaps the intermittent proliferation of *Klebsiella pneumoniae* in the surface parts of the piles in particular.

The reduction of fecal streptococci was of the order of 2 to 3 logarithmic counts within 6 to 7 weeks; 6 months after the beginning of the composting the counts were low. The species selection, however, seemed to change essentially within the first few weeks.

The numbers of *C. perfringens* diminished by 1 to 2 logarithmic counts. The reason for the reduction may be the destruction of vegetative cells in the beginning of the study as well as possible germination of spores into vegetative cells, followed by their destruction under the influence of high temperatures.

No *Salmonellae* were seen in the investigated samples. This result was expected, althougt it is evident that they may occasionally occur in the raw materials. The high composting temperature, however, eliminates *Salmonellae.*

On the basis of this study except Ca-test piles, the different types of piles or the different seasons seemed to have had very little variable effect on the numbers of fecal indicator bacteria.

Index words: fecal indicator bacteria, *Salmonella,* composting process, food industry wastes.

INTRODUCTION

The interest in land disposal of wastes from animal husbandry, industries and municipalities has grown rapidly during the past few years. The application of these wastes in soil does not, however, offer a solution to all problems of garbage disposal because there are a lot of both non-pathogenic (WITTHAUER 1980) and pathogenic micro-organisms (RAUTOPURO 1979, World Health Organization 1979, DUDLEY et al. 1980, HIRN 1981). Diseases caused by pathogenic micro-organisms known to exist in animal, industrial, and municipal wastes are of primary importance to public health. Yet, it is often quite difficult to show the existence of pathogenic or potentially pathogenic micro-organisms in garbage or composts because each species or group of micro-organisms should be detected with its specific method, which may be quite laborious indeed. For this reason the hygienic quality and also the health hazards of composted material have been estimated indirectly by the so-called fecal indicator bacteria (HUHTA et al. 1978, LANGELAND 1980).

The behavior of micro-organisms in garbage depends on many different factors. These include, for instance, the temperature, the humidity, the pH, the physical composition of garbage, and the intermicrobial competition (EDMONDS 1976). These factors vary considerably when different wastes are composted with different composting methods.

The purpose of this study was to observe the prevalence and behavior of some fecal indicator bacteria (fecal coliform bacteria, fecal streptococci, *Clostridium perfringens*) and *Salmonella* in compost piles prepared on an industrial scale and of different compositions and structures during different seasons.

MATERIAL AND METHODS

Samples

The samples for the bacteriological study were taken from each pile from three different points at a 50-to-60-cm depth from the surface with a clean shovel, each sample in its own plastic bag. The amount of sample from each sampling point was 150 to 200 g. The samples were frozen, packed in cold boxes, and transported for examination, which was commenced immediately the next morning. The three samples from each pile were pooled together and mixed in the laboratory; the bacteriological examination was done on the pooled samples. Fecal indicator bacteria (fecal coliforms, fecal streptococci, *C. perfringens*) were studied immediately from the pooled samples. The study was repeated weekly up to 6th to 7th week, and six months after the mixing (range 23 to 29 weeks). When the piles were turned or aerated, samples were taken both before and after the procedures. *Salmonella* was studied only at the time of mixing and 1 week after composting. The piles, their raw material, the piling process, and composition have been reported in more detail by KALLIO and TIKANMÄKI (1982).

Bacteriological investigations

To detect fecal coliforms, fecal streptococci, and *C. perfringens* a plate count technique was employed (Standard Methods for the Examination of Water and Wastewater, 1980). The growth medium for fecal coliforms was mFC-agar (Difco Laboratories, Detroit, Mich., USA) (GELDREICH et al. 1965, HIRN 1976), for fecal streptococci Slanetz and Bartley agar (Orion Diagnostica, Espoo, Finland) (SLANETZ and BARTLEY 1957) and for *C. perfringens* tryptose-

sulfite-cycloserine-egg yolk agar (TSCEY) (Difco) (HARMON 1976, HIRN and RAEVUORI 1978). Incubation for fecal coliforms was at 44,5 °C for 24 h, for fecal streptococci at 37 °C for 48 h and for *C. perfringens* at 37 °C for 24 h. *C. perfringens* was incubated anaerobically in GasPak jars equipped with GasPak disposable hydrogen and carbon dioxide generator envelopes (BBL, Cockeysville, Md., USA). The samples were diluted with physiological NaCl solution.

To confirm the typical blue or bluish colonies assumed to be fecal coliform bacteria, some further tests were done with the API 20 E method (Analytical Products Inc., La Balme les Grottes, France). Correspondingly, the identification of *C. perfringens* was confirmed by taking off the TSCEY-agar 100 typical black colonies surrounded by a zone of white precipitate. The colonies were further examined for the following characteristics: Reduction of nitrate, motility, production of acid from lactose, and hydrolysis of gelatine. Serological typing of *C. perfringens* on lactose egg yolk agar (HOBBS et al. 1971) was also done.

The *Salmonella* was examined by transferring 15 g of pooled sample into tetrathionate broth (BBL). Incubation at 43 °C for 24 and 48 h was used. The isolation of the organism was done from bromthymol-blue lactose agar (Merck, Darmstadt, FRG) which was incubated at 37 °C for 24 h. Typical blue colonies were transferred to TSI and urea agars (Orion Diagnostica).

RESULTS AND DISCUSSION

The numbers of *fecal coliform bacteria* in composts mixed in the winter seem to be somewhat smaller (5,43 and 5,90) than in the composts mixed in the spring (6,55 and 6,78) and in the fall (7,01 and 6,58) (Table 1). The concentration of fecal coliform bacteria in the CaO-stabilized piles was < 4,00. This is quite understandable because the pH of the compost during the first weeks was between 12 and 13 (KALLIO and TIKANMÄKI 1982). As a whole, the initial numbers of fecal coliform bacteria are as expected, because their numbers in the sludge may vary widely, ranging from 3,00 to 6,00 (EDMONDS 1976, KERR 1978). The numbers of fecal coliform bacteria in different completed compost mixtures have respectively been of the order of 5,00 to 7,00 (HUHTA et al. 1978, Langeland 1980). In the present study the numbers of fecal coliform bacteria usually decreased within 1 to 2 weeks down to below the limits of the determination methods used. This was expected because the pile temperatures ranged from 65 to 86 °C (KALLIO and TIKANMÄKI 1982). HUHTA et al.'s (1978) and LANGELAND's (1980) studies do not show a corresponding decrease. In their studies the temperatures did not, however, exceed 50 °C.

During the study great variations were observed among different piles, however, in the reduction of the numbers of fecal coliform bacteria. In the winter piles the variation was seen in particular 2 to 3 weeks after the construction of the piles. Then some of the samples were negative and some positive. One reason for the great variation was the very different temperature behavior of the different piles as a result of the low environmental temperature (KALLIO and TIKANMÄKI 1982). In the spring and fall piles a corresponding great variation in temperatures was not observed. On the other hand, intermittent high results which can be interpreted as positive results were seen in both the fall (weeks 2 to 4) and spring (week 2) piles. On the basis of the relatively few (30) identifications and colony morphology this is probably due to a proliferation of *Klebsiella pneumoniae* under favourable conditions (surface

Table 1. The effect of composting time on the means and standard deviations of the logarithmic counts of fecal coliforms in the different piles during the winter, the spring and the fall.

Time (week)	Type of a pile[a]	n	Log. fecal coliform count/g					
			Winter		Spring		Fall[b]	
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
0	A	4	5,43	0,35	6,55	0,30	7,01	0,12
	B	4	5,90	0,24	6,78	0,39		
	C	3					6,58	0,28
	D	2					<4,00	
1	A	4	4,58	0,21	<2,00		6,12	0,12
	B	4	5,34	0,04	<2,00			
	C	3					6,96	0,22
	D	2					<4,00	
2	A	4	3,71*	1,23	( )[c]***		5,22*[c] (6,69)	1,73 (0,26)
	B	4	4,04**	1,05	( )[c]****			
	C	3					5,17 (6,23)	0,53 (0,26)
	D	2					<4,00 (<4,00)	
3	A	4	3,39* (4,73)**	1,31 (1,25)			5,77**	1,44
	B	4	<3,00 (<3,00)					
	C	3					<4,00	
	D	2					<4,00	
4	A	4	<3,00		(<2,00)		(2,33)*	(0,47)
	B	4	<3,00		(<2,00)			
	C	3					(3,00)*	(1,41)
	D	2					(2,00)	
5	A	2	<2,00 (<2,00)					
	B	2	<2,00 (<2,00)					
6	A	2			(<2,00)		(<2,00)	
	B	2			(<2,00)			
	C	3					(<2,00)	
	D	2					(<2,00)	
7	A	2	<2,00 (<2,00)					
	B	2	<2,00 (<2,00)					
26, 29[d]	A	4	2,20**	0,34				
	B	4	2,12**	0,21				

[a] A corresponds to the piles AB_1C_1, AB_1C_2 and C_3 Formula 4; B corresponds to the piles AB_2; C corresponds to the piles C_3 Formula 3; D corresponds to the piles C_3 Formula 2 (KALLIO and TIKANMÄKI 1982).
[b] The means and standard deviations of the fall piles are the means of three samples.
[c] The means and standard deviations within parentheses are the means after turning or aerating of the piles.
[d] The samples were taken from the winter piles after 29 weeks.
* The means and standard deviations are the means of 1 positive and 2—3 negative results (< 2,00 or < 3,00).
** The means and standard deviations are the means of 2 positive and 1—2 negative results (< 2,00 or < 3,00).
*** Two samples > 5,00 and two < 2,00.
**** One sample > 5,00 and three < 2,00.

temperature of piles from 25 to 40 °C; cyclitols in the pine bark waste) (TALBOT and SEIDLER 1979). After a prolonged (> 4 weeks) composting process, however, the logarithmic counts seemed to be comparably low ($\leqslant$ 2,00).

The numbers of *fecal streptococci* in the winter piles (5,69 and 5,89) also seemed to be somewhat lower than in the spring (6,49 and 6,45) and fall (6,54 and 6,18) piles (Table 2). These results agree with those by LANGELAND (1980). Not-

Table 2. The effect of composting time on the means and standard deviations of the logarithmic counts of fecal streptococci in the different piles during the winter, the spring and the fall.

Time (week)	Type of a pile[a]	n	Log. fecal streptococci count/g					
			Winter		Spring		Fall[b]	
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
0	A	4	5,69	0,26	6,49	0,29	6,54	0,15
	B	4	5,89	0,13	6,45	0,20		
	C	3					6,18	0,30
	D	2					<4,00	
1	A	4	5,47	0,32	5,58	0,59	5,29	0,08
	B	4	5,91	0,04	5,83	0,35		
	C	3					5,67	0,21
	D	2					<4,00	
2	A	4	5,97	0,24	6,22 (5,51)[c]	0,39 (0,94)	4,16 (5,65)	0,23 (0,41)
	B	4	4,81	1,10	5,23 (5,13)	0,67 (0,56)		
	C	3					<4,00 (4,99)	(0,72)
	D	2					<4,00 (<4,00)	
3	A	4	5,26 (5,54)	1,15 (0,77)	5,58	1,26	4,46	0,23
	B	4	5,05 (5,34)	0,86 (0,41)	5,20	0,95		
	C	3					4,72	1,01
	D	2					4,74	0,74
4	A	4	5,21	0,90	3,70 (4,94)	0,56 (1,17)	4,18 (5,10)	0,92 (0,07)
	B	4	4,28	1,31	5,21 (5,35)	0,21 (0,39)		
	C	3					4,54 (4,99)	0,41 (0,31)
	D	2					2,93 (2,50)	0,23 (0,50)
5	A	4	3,95 (4,84)	0,29 (0,44)	4,77	1,08	5,18	0,27
	B	4	4,12 (4,61)	0,69 (0,53)	5,32	0,38		
	C	3					5,26	0,45
	D	2					<2,00	
6	A	3			4,02[d] (5,25)[d]	1,02 (0,05)	5,50 (4,44)	1,09 (0,42)
	B	3			5,16[d] (6,46)[d]	0,12 (0,08)		
	C	3					4,42 (4,28)	0,06 (0,21)
	D	2					2,93 (5,79)	0,93 (0,64)
7	A	4	3,68 (4,14)	1,03 (1,17)				
	B	4	3,60 (4,30)	0,88 (0,71)				
23, 26, 29[e]	A	4	<2,00		2,76	0,84	2,20	0,28
	B	4	<2,20		2,57	0,56		
	C	3					<2,00	
	D	2					3,74[f]	1,74

[a] A corresponds to the piles AB_1C_1, AB_1C_2 and C_3 Formula 4; B corresponds to the piles AB_2; C corresponds to the piles C_3 Formula 3; D corresponds to the piles C_3 Formula 2 (KALLIO and TIKANMÄKI 1982).
[b] The means and standard deviations in the fall piles are the means of three samples.
[c] The means and standard deviations within parentheses are the means after turning or aerating of the piles.
[d] The means and standard deviations are the means of two samples.
[e] The samples were taken from the winter piles after 29 weeks, from the spring piles after 23 weeks and from the fall piles after 26 weeks.
[f] The mean and standard deviation is the mean of 5,48 and < 2,00.

withstanding singular exceptions, no *fecal streptococci* were found in CaO-stabilized piles. As a whole, it was established that the number of colonies to be interpreted as positive diminishes during the first 6 to 7 weeks rather evenly by 2 to 3 logarithmic counts. After six months of studying the numbers seem to range from < 2,00 to 3,00. During the course of this study it was

Table 3. The effect of composting time on the means and standard deviations of the logarithmic counts of *C. perfringens* in the different piles during the winter, the spring and the fall.

Time (week)	Type of a pile[a]	n	Log. *C. perfringens* count/g					
			Winter		Spring		Fall[b]	
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
0	A	4	4,45	0,13	4,66	0,07	4,75	0,24
	B	4	4,58	0,16	4,50	0,10		
	C	3					4,77	0,17
	D	2					<2,00	
1	A	4	4,31	0,20	3,77	0,44	3,82	0,46
	B	4	4,36	0,10	3,85	0,22		
	C	3					4,21	0,40
	D	2					<2,00	
2	A	4	4,45	0,33	4,33 (3,35)[c]	0,52 (0,78)	4,15 (4,19)	0,59 (0,28)
	B	4	4,04	0,07	3,33 (3,63)	0,51 (0,38)		
	C	3					4,47 (4,10)	0,30 (0,08)
	D	2					<2,00 (<2,00)	
3	A	4	3,76 (4,07)	0,54 (0,05)	3,26	0,67	4,26[d]	
	B	4	3,60 (3,78)	0,20 (0,13)	2,83	0,84		
	C	3					4,08	0,15
	D	2					<2,00	
4	A	4	3,54	0,20	3,22 (3,71)	0,96 (0,20)	3,91 (4,65)	0,43 (0,12)
	B	4	3,48	0,42	3,34 (3,34)	0,43 (0,49)		
	C	3					3,58 (3,59)	0,27 (0,30)
	D	2					<2,00 (<2,00)	
5	A	4	3,29 (3,61)	0,29 (0,36)	2,91	0,56	3,79	0,21
	B	4	3,45 (3,36)	0,36 (0,54)	3,97	0,20		
	C	3					3,96	0,47
	D	2					<2,00	
6	A	3			3,41[e] (3,28)[e]	0,01 (0,00)	3,70 (4,28)	0,17 (0,51)
	B	3			3,51[e] (3,84)[e]	0,25 (0,28)		
	C	3					3,71 (3,84)	0,10 (0,26)
	D	2					<2,00 (<2,00)	
7	A	4	3,24 (3,40)	0,35 (0,48)				
	B	4	3,69 (3,75)	0,16 (0,18)				
23, 26, 29[f]	A	4	2,88	0,34	2,54	0,32	3,40	0,28
	B	4	3,12	0,32	2,23	0,25		
	C	3					3,32	0,12
	D	2					<2,00	

[a] A corresponds to the piles AB_1C_1, AB_1C_2 and C_3 Formula 4; B corresponds to the piles AB_2; C corresponds to the piles C_3 Formula 3; D corresponds to the piles C_3 Formula 2 (KALLIO and TIKANMÄKI 1982).
[b] The means and standard deviations in the fall piles are the means of three samples.
[c] The means and standard deviations within parentheses are the means after turning or aerating of the piles.
[d] The logarithmic figure is the figure of one sample.
[e] The means and standard deviations are the means of two samples.
[f] The samples were taken from the winter piles after 29 weeks, from the spring piles after 23 weeks and from the fall piles after 26 weeks.

found that, based on colony morphology, the species selection of the colonies which can be interpreted positive on the used plate apparently changed. It is to be assumed that in the initial phases of the study the mesophilic species, the streptococci of group D in particular, comprise the main proportion of the growing bacteria. With the rising temperature they are, however,

destroyed and replaced by thermophilic species. With ongoing composting natural reduction then takes place (GALLER et al. 1978).

The counts of *C. perfringens* were very close to one another in all the composts of the different seasons (from 4,45 to 4,77) (Table 3); no difference between the winter piles on the one hand and the spring/fall composts on the other hand, such as in the numbers of fecal coliforms and fecal streptococci, was seen. This is probably due to the better freeze-resistance of the spores (the raw material was partly frozen) as compared to vegetative cells. In the CaO-stabilized piles the used method did not reveal *C. perfringens* at the concentration of 2,00. During the course of the study, in all different types of composts in all different seasons, a reduction of 1 to 2 logarithmic counts took place within 6 to 7 weeks of composting. After 6 months of composting the counts ranged from 2,23 to 3,40. The reduction seemed to be rather small during the concluding phases of the study. Notice that HUHTA et al. (1978) and LANGELAND (1980) in their studies did not find any reduction in the numbers on *C. perfringens*. One reason for this reduction of the numbers may be the destruction of the vegetative cells (the method used includes no heating phase) immediately after the onset of composting, and perhaps also the germination of spores into vegetative cells caused by the high temperatures in the piles; the cells would then have been destroyed. The strains isolated from the plates (100) were identified in all cases as *C. perfringens*.

No *Salmonellae* were found in the tested samples (48). In spite of the rather small number of samples it is obvious that *Salmonella* does not appear in the raw material used, mainly sludge and poultry house litter, except intermittently (HIRN 1980). The health hazard caused by human pathogenic bacteria, *Salmonella* in particular, is considerably reduced by the high temperatures reached during the composting, 65 to 86 °C (KALLIO and TIKANMÄKI 1982); these temperatures destroy a major proportion of the vegetative cells of pathogenic bacteria (PLATZ 1977 a, b).

On the basis of the determination of the number of fecal indicator bacteria and clarification of the occurrence of *Salmonella* in different compost piles in different seasons it seems obvious that the composting methods used in the study efficiently reduce the numbers of bacteria and essentially minimize health hazard to man or animals which may be caused by pathogenic bacteria.

REFERENCES

DUDLEY, D. J., GUENTZEL, M. N., IBARRA, M. J., MOORE, B. E. & SAGIK, B. P. 1980. Enumeration of potentially pathogenic bacteria from sewage sludges. Appl. Environm. Microbiol. 39: 118—126.

EDMONDS, R. L. 1976. Survival of coliform bacteria in sewage sludge applied to a forest clearcut and potential movement into groundwater. Appl. Environ. Microbiol. 32: 537—546.

GALLER, W. S., DAVEY, C. B., MEYER, W. L. & AIRAN, D. S. 1978. Animal waste composting with carbonaceous material. Environmental Protection Agency, Rep. EPA-600/2-78-154. 95 p.

GELDREICH, E. E., CLARK, H. F., HUFF, C. B. & BEST, L. C. 1965. Fecal coliform organism for the membrane filter technique. J. Amer. Water Works Ass. 57: 208—214.

HARMON, S. M. 1976. Collaborative study of an improved method for the enumeration and confirmation of *Clostridium perfringens* in foods. J. Ass. Off. Anal. 59: 606—612.

HIRN, J. 1976. Fekaalisten koliformibakteerien määrittäminen vedestä. Suom. Eläinlääk.l. 82: 3—9. (English summary.)

— 1980. Indicator bacteria and *Salmonella* in food processing and domestic effluent. J. Water Pollut. Control Fed. 52: 48—52.

— 1981. Lietelannan välityksellä leviävien mikrobiologisten ja parasitologisten zoonoosien aiheuttamat

riskit Suomessa. Suom. Eläinlääk.l. 87: 231—237.
— & RAEVUORI, M. 1978. Tryptose-sulfite-cycloserine-egg yolk agar membrane filter method for the detection of *Clostridium perfringens* in water. Water Res. 12: 451—453.
HOBBS, G., WILLIAM, K. & WILLIS, A. T. 1971. Basic methods for the isolation of clostridia. (In: Isolation of Anaerobes. Eds. Shapton, D. A. & Board, R. G.) Academic Press: 1—23. London.
HUHTA, V., SUNDMAN, V., IKONEN, E., SIVELÄ, S., WARTIOVAARA, T. & VILKAMAA, P. 1978. Jätelietekuorirouhe-seosten maatumisen biologia. Jyväskylän yliopisto, Biologian laitos, Tiedonantoja 11. 124 p.
KALLIO, H. & TIKANMÄKI, E. 1982. Composting of sewage sludge and other wastes of a food plant in Finland. Ann. Agric. Fenn. 21: 91—102.
KERR, R. S. 1978. Sewage disposal on agricultural soils. Chemical and microbiological implications. Vol. II. Microbiological implications. Environmental Protection Agency, Rep. EPA-600/2-78-131b. 93 p.
LANGELAND, G. 1980. Kompostering kan gi kloakkslam av tilfredsstillende mikrobiologisk-hygienisk kvalitet. Vann 1B: 27—31.
PLATZ, S. 1977 a. Untersuchungen über das Verhalten pathogener Mikro-organismen bei den Heissverrottung von Hühnerkot. Zbl. Vet. Med. B 24: 25—34. (English summary.)
— 1977 b. Untersuchungen über den Einfluss thermischer und athermischer Faktoren auf die Tenazität pathogener Bakterien während der Heissverrottung von Hühnerkot. Berl. Münch. Tierärztl. Wschr. 90: 156—159. (English summary.)
RAUTOPURO, H. 1979. Maan jäätymisen vaikutus jätevesilietteen bakteereihin. Vesihallitus, Tiedotus 179. 34 p. (English summary.)
SLANETZ, L. W. & BARTLEY, C. H. 1957. Numbers of enterococci in water, sewage and feces determined by the membrane filter technique with an improved medium. J. Bact. 74: 591—595.
Standard Methods for the Examination of Water and Wastewater. 1980. 15th Ed. American Public Health Association, Washington D.C. 1134 p.
TALBOT, H. W., Jr. & SEIDLER, R. J. 1979. Cyclitol utilization associated with the presence of *Klebsiellae* in botanical environments. Appl. Environ. Microbiol. 37: 909—915.
WITTHAUER, D. P. 1980. Biocoenosis and degradation in model wastewater treatment plants. Eur. J. Appl. Microbiol. 9: 151—163.
World Health Organization. 1979. Human viruses in water, wastewater and soil. World Health Organization, Technical Rep. Series 639. 50 p.

Manuscript received March 1982

Jorma Hirn
National Veterinary Institute
P.O. Box 368
SF-00101 Helsinki 10, Finland

Heikki Kallio
Saarioinen Ltd.
SF-36420 Sahalahti, Finland
Present address:
University of Turku
Department of Chemistry and Biochemistry
SF-20500 Turku 50, Finland

Elisa Tikanmäki
Saarioinen Ltd.
SF-36420 Sahalahti, Finland

SELOSTUS

Fekaalisten indikaattoribakteerien käyttäytyminen teollista mittakaavaa olevassa kompostointiprosessissa

JORMA HIRN, HEIKKI KALLIO ja ELISA TIKANMÄKI

Valtion Eläintieteellinen laitos, Saarioinen Oy ja Turun Yliopisto

Työssä seurattiin fekaalisten indikaattoribakteerien (fekaaliset koliformiset bakteerit, fekaaliset streptokokit, *Clostridium perfringens*) käyttäytymistä ja säilymistä suomalaisen elintarviketeollisuuslaitoksen jätehuoltoon liittyvässä ja teollis-mittaisessa kompostointiprosessissa eri vuodenaikoina. Lisäksi selvitettiin *Salmonella*n esiintymistä komposteissa.

Fekaalisten koliformisten bakteerien logaritmiset lukuarvot valmiiksi sekoitetuissa kevät- ja syyskomposteissa olivat 6,55:n ja 7,01:n välillä. Talvikompostissa vastaavat lukuarvot olivat, ilmeisesti raaka-aineiden jäätymisen vuoksi, hieman alhaisempia. Samanlaiset tulokset saatiin fekaalisilla streptokokeilla. Arvot olivat kevät-syyskomposteissa 6,18—6,54. *C. perfringens*illä ei tätä ilmiötä havaittu, vaan lukuarvot vaihtelivat 4,45—4,77.

Fekaalisten koliformisten bakteerien määrät laskivat 1—2 viikon kuluessa määritysrajojen alapuolelle. 2—4 viikon kuluttua työn aloittamisesta todettiin ajoittain huomattavaa vaihtelua fekaalisten koliformisten bakteerien lukumäärissä. Syynä vaihteluun oli talviaumoissa ilmeisesti lämpötilan nousussa olleet vaihtelut ja kevät- sekä syysaumoissa ajoittainen *Klebsiella pneumoniae*n lisääntyminen etenkin aumojen pintaosissa.

Fekaalisten streptokokkien väheneminen oli 2—3 logaritmisen lukuarvon luokkaa 6—7 viikon aikana. Kuuden kuukauden kuluttua määrät olivat vähäisiä. Lajisto näytti kuitenkin muuttuvan oleellisesti muutaman ensimmäisen koeviikon kuluessa.

*C. perfringens*in määrät vähenivät työn kuluessa 1—2 logaritmisen lukuarvon verran. Syynä vähenemiseen saattoi olla vegetatiivisten solujen tuhoutuminen kokeen alussa sekä itiöiden mahdollinen germinoituminen vegetatiivisiksi soluiksi korkeahkon lämpötilan vaikutuksesta ja edelleen tuhoutuminen.

*Salmonella*a ei tutkituista näytteistä todettu. Tulos oli odotettu. Tosin on ilmeistä, että raaka-aineissa *Salmonella*a saattaa ajoittain esiintyä. Korkea kompostointilämpötila eliminoi *Salmonella*n.

Tutkituilla eri aumatyypeillä ja vuodenajoilla ei näyttänyt suoritetun kokeen perusteella olevan merkittävää vaikutusta fekaalisten indikaattoribakteerien lukumääriin lukuun ottamatta koeaumoja, joihin oli lisätty CaO:a.

ANNALES AGRICULTURAE FENNIAE, VOL. 21: 146—148 (1982)

Seria AGROGEOLOGIA ET -CHIMICA N. 113 — Sarja MAA ja LANNOITUS n:o 113

RESEARCH NOTE

A COMPARISON BETWEEN A DRY-COMBUSTION METHOD AND A RAPID WET-COMBUSTION METHOD FOR DETERMINING SOIL ORGANIC CARBON

JOUKO SIPPOLA

SIPPOLA, J. 1982. **A comparison between a dry-combustion method and a rapid wet-combustion method for determining soil organic carbon.** Ann. Agric. Fenn. 21: 146—148. (Agric. Res. Centre, Inst. Soil Sci., SF-31600 Jokioinen, Finland.)

The dry-combustion method gave 6—7 % higher organic carbon contents than the rapid wet-combustion for clay and coarse mineral soils and 12 % higher for organic soils on an average. The difference is most likely because of incomplete oxidation of organic matter by the rapid wet-combustion method.

The coefficient of variation of the wet method was 7,5 % and that of the dry method 4,7 % on an average. The dry-combustion method was considered to be more precise for organic carbon determination in noncalcareous soils in Finland than the rapid wet-combustion method.

Index words: organic carbon, rapid wet-combustion, dry-combustion, mineral soils, organic soils.

INTRODUCTION

In determining soil organic carbon content methods based on the determination of the change in concentration of the oxidizing reagent are much used because of their fastness and simplicity. Readily oxidizable organic matter is reacting and fresh plant material or inert forms of carbon are not taken into account. The interference by other easily oxidizable substances is possible.

Despite of various modifications (WALKLEY and BLACK 1934, ALTEN et al. 1935) the rapid wet-combustion methods recover organic carbon incompletely and correction factors are needed when total organic carbon is estimated. Standard samples whose organic carbon content is known may also be used but their type of organic matter must be same as those of the analyzed samples (ORPHANOS 1973).

Also dry-combustion methods are developed to determine soil carbon content. Carbonate carbon and other non-organic carbon if present are included. In case of calcareous soils a pretreatment is therefore needed. An important advantage of the dry-combustion over wet-combustion is that little reagents are used.

This investigation was initiated to compare the organic carbon results obtained by a rapid wet-combustion and a dry-combustion method in a sample material consisting of acid soils in Finland.

MATERIAL AND METHODS

The soil samples of the study (Table 1) included all the major soil types of Finland and they were collected all over the country. Air dried through 2 mm sieve ground samples were used for determinations.

The instrument used for organic carbon determinations according to the dry- combustion method was a LECO CR-12. The size of samples was 500 mg and they were covered with 0,5 g Al_2O_3 (Merck 1097) before pushing into oven at 1370 °C. In the instrument the carbon of samples is burned in a stream of oxygen to CO_2 which is determined by infrared detector. The instrument was calibrated using powdered calcium carbonate as standardizing material. The wet-combustion method used was a modified ALTEN et al. (1935) method (TARES and SIPPOLA 1978).

RESULTS AND DISCUSSION

According to the results (Table 1) the dry-combustion method gave higher organic carbon contents than the wet-combustion method. The difference was 6—7 % on the average in the groups of clay and coarse mineral soils and 12 % in organic soils. This difference was most likely due to the incomplete oxidation of organic matter by the wet-combustion method. An indication of a partial reaction of organic carbon despite of the use of external heating to increase reaction is the larger difference between methods in the groups of organic soils than in mineral soils.

The standard soil used in the wet-combustion method was less suitable for organic than for mineral soils since its organic carbon content was only 5,55 %. A better agreement of results would have been expected when separate standards for each of the main soil types could have been used (DROVER and MANNER 1975). The agreement between low organic matter soils, however, was expected to be better.

In the dry-combustion method the inclusion of carbonate carbon causes too high organic carbon values. The soils of the study were, however, acid with an average pH(H_2O) of 5,5. The carbon in the normal rates of liming from 5 to 10 t/ha of limestone is less than 1 % of the total carbon in these soils. The coefficients of variation of determination (Table 1) are larger than this and therefore evenly applied liming will not affect the results significantly. The variation coefficient for all soils of the wet-combustion method was 7,5 % compared to 4,7 % of the dry-combustion method (Table 1).

Table 1. Organic carbon contents of air dry soil, determined by dry- and wet-combustion methods and their coefficients of variation.

	n	Org. C % Method of combustion			Coefficient of variation % Method of combustion	
		Dry	Wet	Difference	Dry	Wet
Clay and silt	55	3,88	3,61	0,27***	2,7	4,8
Heavy clay ...	5	4,73	4,24	0,49**	1,7	2,6
Silty clay	15	4,01	3,80	0,21**	2,1	3,1
Sandy clay ...	8	4,62	4,31	0,31***	1,6	2,3
Silt	27	3,44	3,18	0,26***	3,6	5,9
Coarse mineral soils	82	4,33	4,08	0,25***	6,2	7,4
Finer finesand .	17	4,29	3,99	0,30***	3,5	2,9
Finesand	35	4,21	3,98	0,23***	7,1	4,6
Sand	3	5,46	5,08	0,38	12,3	4,7
Glacial till	27	4,38	4,15	0,23*	4,4	11,2
Organic soils ...	46	32,59	29,09	3,50***	3,1	5,0
Mould	12	17,63	15,53	2,10***	2,5	5,0
Carex peat ...	32	37,55	33,50	4,05***	3,1	4,9
Sphagnum peat	2	42,85	39,70	3,15	0,5	4,1
All soils	183	11,30	10,20	1,10***	4,7	7,5

***p<0,001, **p<0,01, *p<0,05

Large variability of results was found in groups of sand and glacial till soils. This may be caused by inhomogeneity of samples of coarse texture and organic matter. Low coefficients of variation were observed in groups of clay soils.

The dry-combustion method gives reproducible results and the operation of the instrument is fast. When the error caused by free lime is taken care of, reliable results for soil organic carbon are obtained.

REFERENCES

ALTEN, F., WANDROWSKY, B. & KNIPPENBERG, E. 1935. Beitrag zur Humusbildung. Erg. Agriculturchem. 4: 61—69.

DROVER, D. P. & MANNER, H. 1975. A comparison between the Walkley-Black and a dry-combustion method for determining organic carbon in a humic brown soil, Papua, New Guinea. Comm. Soil Sci. Plant Anal. 6: 495—500.

ORPHANOS, P. I. 1973. On the determination of soil carbon. Plant and Soil 39: 706—708.

TARES, T. & SIPPOLA, J. 1978. Changes in pH, in electrical conductivity and in the extractable amounts of mineral elements in soil, and the utilization and losses of the elements in some field experiments. Acta Agric. Scand. Suppl. 20: 90—115.

WALKLEY, A. & BLACK, I. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29—38.

Manuscript received May 1982

Jouko Sippola
Agricultural Research Centre
Institute of Soil Science
SF-31600 Jokioinen, Finland

SELOSTUS

Vertailu maan orgaanisen hiilen määrittämisestä kuiva- ja märkäpolttomenetelmällä

JOUKO SIPPOLA

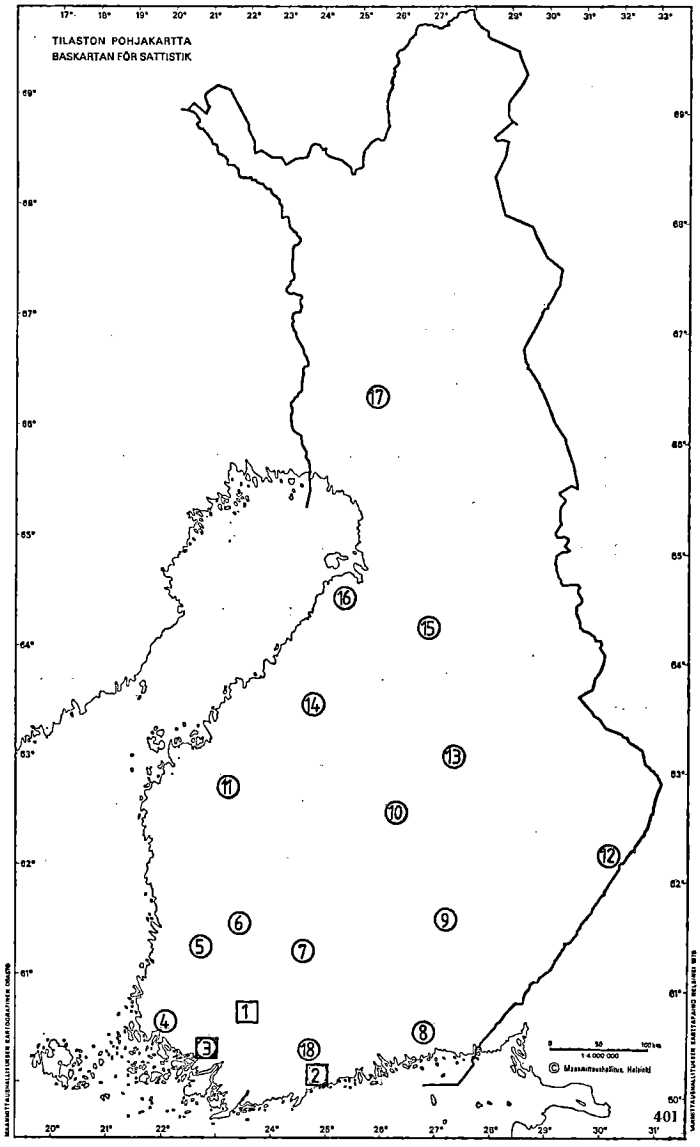
Maatalouden tutkimuskeskus

Maan orgaanisen aineksen määrittämisessä ovat märkäpolttomenetelmät olleet suosittuja nopeutensa ja yksinkertaisuutensa takia. Nopeilla märkäpolttomenetelmillä voidaan kuitenkin määrittää vain helposti hapettuva orgaaninen aines, eikä siten kaikki maan orgaaninen hiili tule mukaan analyysissä. Kuivapolttomenetelmässä näyte poltetaan happivirrassa ja vapautunut hiilidioksidi mitataan. Menetelmän suurimpana virhelähteenä pidetään sitä, että käytetyissä lämpötiloissa karbonaatit hajoavat vapauttaen hiilidioksidia.

Kuivapolttomenetelmällä saatiin kivennäismaista keskimäärin 6—7 % korkeampia orgaanisen hiilen pitoisuuksia kuin märkäpolttomenetelmällä (taulukko 1). Eloperäisten maiden ryhmässä ero oli 12 %. Tämä johtui todennäköisesti orgaanisen aineksen epätäydellisestä palamisesta märkäpolttomenetelmässä. Ero olisi ollut ilmeisesti pienempi, mikäli kullakin maalajiryhmällä olisi käytetty humuspitoisuuttaan vastaavia standardimaita. Kalkitusaineiden karbonaatin sisältämän hiilen aiheuttama virhe kuivapolttomenetelmän tuloksiin on alle 1 %, silloin kun käytetään kalkkia 5—10 tn/ha.

Kaksoismääritysten perusteella laskettu määritysmenetelmien toistettavuutta kuvaava variaatiokerroin oli kuivapolttomenetelmällä pienempi kuin märkäpolttomenetelmällä. Kuivapoltto antoi siis tarkempia tuloksia. Koska kuivapoltto on lisäksi märkäpolttoa nopeampi suorittaa ja siinä tarvitaan vain vähän kemikaaleja, voidaan sitä pitää märkäpolttomenetelmää soveliaampana maan orgaanisen hiilen määrittämiseen.

CONTENTS



INSTITUTES, EXPERIMENTAL STATIONS AND BUREAUS OF THE AGRICULTURAL RESEARCH CENTRE

1. Administrative Bureau, Information, Library, Institutes of Soil Science, Agricultural Chemistry and Physics, Plant Husbandry, Plant Breeding, Animal Husbandry, Animal Breeding; Bureau for Local Experiments, Central Laboratory, Computing Service (JOKIOINEN) — 2. Institutes of Plant Pathology and Pest Investigation; Pesticide Regulation Unit (VANTAA) — 3. Institute of Horticulture (PIIKKIÖ) — 4. South-West Exp. Sta. (MIETOINEN) — 5. Satakunta Exp. Sta. (KOKEMÄKI) — 6. Sata-Häme Exp. Sta. (MOUHIJÄRVI) — 7. Häme Exp. Sta. (PÄLKÄNE) — 8. Kymenlaakso Exp. Sta. (ANJALA) — 9. South Savo Exp. Sta. (MIKKELI) — 10. Central Finland Exp. Sta. (LAUKAA) — 11. South-Pohjanmaa Exp. Sta. (YLISTARO) — 12. Karelia Exp. Sta. (TOHMAJÄRVI) — 13. North Savo Exp. Sta. (MAANINKA) — 14. Central Pohjanmaa Exp. Sta. (TOHOLAMPI) — 15. Kainuu Exp. Sta. (VAALA) — 16. North Pohjanmaa Exp. Sta. (RUUKKI) — 17. Lapland Exp. Sta. (ROVANIEMI) — 18. Swine Research Exp. Sta. (HYVINKÄÄ).

SISÄLLYS — CONTENTS

Helsinki 1982. Government Printing Centre

ISSN 0570-1538