

Comminution properties of biomass in forage harvester and beater mill and its particle size characterization

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Abstract. It was found that the differences in the distribution of particle length were the species feature of energetic plants. The length distribution of comminuted willow particles showed the highest mean value and good regularity, while the topinambour mixture was the shortest and most irregular in the mixture of energetic plants. Connectivity between mean values of particle length plants broken up in the forage harvester and in the beater mill, calculated by geometric mean method and according to Rosin-Rammler-Sperling-Bennett model, was very high, as testified by square correlation values, which amounted to 79.4 and 97.8% respectively. The research allows proving the practical possibility of using the RRSB model to determine the amount of separated material meeting dimension requirements for production of formed fuels.

Key words: energetic plants, sieve separator, chopping, milling, particle length

INTRODUCTION

Particle size is one of the most essential properties of comminuted material. For a spherical particle, its size is directly defined as its diameters (the distance between two points on the particle) or calculated from the two-dimensional surface section or three-dimensional volume (Bitra et al., 2008). All three of these estimates are consistent. For some nonspherical regular-shaped particles, such as a cone or a cuboid, more than one particle size dimension must be specified, while irregularly shaped particles require derived diameters to define their particle sizes.

Among all the different particle sizes, sieve diameter is the factor most often used to characterize plant comminuted material.

Among many different types of size distribution functions, either in cumulative or relative percentage frequency form, the Rosin-Rammler-Sperling-Bennett (RRSB) function is useful for monitoring grinding or breaking-up operations for highly skewed distributions (Ahmed & Drzymala, 2005; Domoradzki et al., 2005; Bitra et al., 2008).

The purpose of the work was to compare size distributions for comminuted particles of seven energetic plant species in the flywheel cutting unit of a forage harvester and in a beater mill, and to present possibilities of using the Rosin-Rammler-Sperling-Bennett model to predict separation of the amount of cut and milled material meeting requirements set for the size of particles used in production of pellets or briquettes.

MATERIAL AND METHODS

The chopped plant particles of willow (*Salix viminalis*), rose (*Rosa multiflora*), giant miscanthus (*Miscanthus sinensis gigantean*), polygonaceous (*Polygonum sachalinense*), spartina (*Spartina pectinata*), Virginia mallow (*Sida hermaphrodita*) and topinambour (*Helianthus tuberosus*) were taken as research material. These plants are cultivated on the plots of the Experimental Station in Skieriewice belonging to Faculty of Agriculture and Biology WULS in Warsaw.

Harvesting of energetic plants was carried out directly from the field in December 2007 with the use of a tractor-trailed forage harvester Z374. The chopping unit of the forage harvester was equipped with 5 knives and straight thrower blades with a sharp tool rake and also with a smooth-surface bottom plate. The cutting disc rotational speed amounted to 1000 rpm. The set working parameters allowed for cutting frequency 83 Hz and theoretical length of chopped plant material particles 8.8 mm.

In field conditions the plants' moisture content during harvest varied from 22.6 to 52.2% (wet basis, Table 1). The obtained chips and chaffs of mean dimension from 9 to 11 mm were dried in natural conditions to moisture content within the narrow range from 5.6 to 7.3% (Table 1). The dried plant material was milled in a beater mill equipped with a sieve with diameter openings of 10 mm.

Table 1. Plant material moisture contents during harvesting and milling and for broken-up and milled plant mixture during investigation of particle size distribution.

Faze	Plant material moisture contents, %						
	Willow	Giant miscanthus	Spartina	Polygonaceous	Rosa	Virginia mallow	Topinambour
Harvesting	51.0±0.9	46.4±0.6	40.4±0.5	52.2±0.8	43.7±0.6	22.6±0.3	23.3±0.3
Distribution of broken-up material	49.0±0.8	46.1±0.6	39.9±0.5	51.7±0.7	43.4±0.5	22.3±0.3	23.1±0.3
Milling	6.2±0.2	5.6±0.1	6.0±0.1	6.5±0.1	7.3±0.2	5.6±0.1	6.1±0.1
Distribution of milled material	6.0±0.1	5.5±0.1	6.0±0.1	6.5±0.1	7.0±0.2	5.5±0.1	6.0±0.1

Methodology of investigations included the following:

- Determination of particle length distribution on a sieve separator,
- Calculation of geometric mean and standard deviation of particle length,
- Calculation of hypothetical dimension of sieve opening diagonal, at which 50% of comminuted material would be screened (according to the Rosin-Rammler-Sperling-Bennett model),
- Approximation of particle length cumulated real distribution by the Rosin-Rammler-Sperling-Bennett model.

For the evaluation of comminuted material length distribution a sieve separator designed by Lisowski and Sar (Lisowski et al., 2008) was used. The procedure of investigation was carried out according to the ASAE S424.1 Standard (2003).

Geometric mean length and geometric standard deviation were calculated based on mass fraction using the following equations (ASAE Standards, 2003):

$$x_{gm} = \log^{-1} \left[\frac{\sum(m_i \log x_{si})}{\sum m_i} \right] \quad (1)$$

$$s_{gm} = \log^{-1} \left[\frac{\sum(m_i (\log x_{si} - \log x_{gm})^2)}{\sum m_i} \right]^{\frac{1}{2}} \quad (2)$$

where, x_{gm} is geometrical mean length, mm; s_{gm} is geometrical standard deviation (dimensionless); x_i is diagonal of sieve openings of i th sieves, mm; $x_{(i-1)}$ is diagonal of sieves openings in next larger than i th sieves, mm; x_{si} is the geometric mean length of particles on i th sieve or $x_{si} = \sqrt{x_i x_{(i-1)}}$, mm; m_i is mass on i th sieve, g (ASAE Standards, 2003).

Cumulative undersize mass percentage data obtained through ASAE sieve analysis was regressed using RRSB distribution equation (Ahmed & Drzymala, 2005):

$$Y = 100 \left[1 - 2 \left[\frac{x}{x_{50}} \right]^n \right] \quad (3)$$

where, Y is cumulative undersize mass, %; x is particle size assumed equivalent to diagonal sieve opening, mm; x_{50} means a sieve opening size, at which 50% of the comminuted material sample would be sifted out, mm; n – is the distribution parameter or Rosin-Rammler skewness parameter (dimensionless). The SPSS v.14 statistical packet was used to obtain n and x_{50} unknown value coefficients.

The investigations of the distribution of plant particle length were carried out at the Department of Agricultural and Forest Machinery, WULS in Warsaw. The measurements were executed directly after harvest, therefore moisture content of the chopped material was only slightly lower (Table 1).

RESULTS AND DISCUSSION

By comparing the particle length distributions (Fig. 1) and coefficients of these distributions, at forms of kurtosis and skewness indexes (Table 2), it was found that the distributions of particle lengths obtained after cutting of energetic plants were characterized by a stronger right-hand skewness and they were more slender than distributions of particle lengths obtained after milled material.

The statistically significant differences in the amount of plant material were found for the sieves. The highly statistically significant difference was in the share of broken-up plant mass, because the value of the F-test was equal to $F_{v1=5, v2=270} = 1351.3$, at $\alpha < 0.0001$, and for milled material $F_{v1=5, v2=84} = 3214.2$, at $\alpha < 0.0001$.

It was found that there was an interaction for plant species and sieve opening dimensions, $F_{v1=30, v2=270} = 8.3$ and $F_{v1=30, v2=84} = 39.0$ respectively (in both cases at $\alpha < 0.0001$). A detailed analysis proved that the shares of the biggest and smallest mass particles were comparable. Greater differences in the mass share occurred for the smallest particles of the rose and topinambour, which contained almost twice as many of these particles in relation to the remaining plant species. Similarly, the share of milled material mass particles at the biggest dimension openings was comparable and

formed a homogenous group. The greatest differences in distributions occurred among the finest particles, found on the bottom. The share of the smallest particles of topinambour and spartina was 47%, and for the remaining plants 28% (Fig. 1).

A statistically significant difference was found among geometric mean values and standard deviation of broken-up material particle length for species of energetic plants. The Fisher-Snedecor statistics value for the geometric particle length amounted to $F_{v1=6, v2=45} = 49.3$, while for standard deviation $F_{v1=6, v2=45} = 109.2$ (in both cases at critical significance level $\alpha < 0.0001$). Similarly, geometric mean and standard deviation values of milled material particle length were statistically significantly different, because Fisher-Snedecor statistics values amounted to $F_{v1=6, v2=14} = 65.8$ and $F_{v1=6, v2=14} = 13.5$ (in both cases at critical significance level $\alpha < 0.0001$).

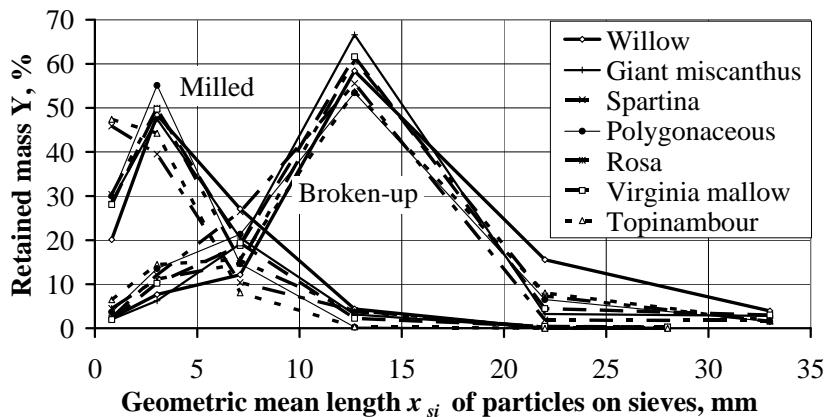


Figure 1. Particle size distribution between separate sieves by geometric mean length x_{si} of particles on each sieve of broken-up and milled plant material.

Table 2. Statistic parameters of particle size distribution of plant broken-up and milled material.

Parameter	Willow	Giant miscanthus	Spartina	Polygonaceous	Rosa	Virginia mallow	Topinambour
Broken-up plant material							
Skewness	3.34	5.53	3.26	4.21	3.45	4.16	4.35
Kurtosis	1.30	1.54	0.28	0.68	1.41	1.20	1.28
Milled plant material							
Skewness	1.45	1.13	1.21	1.71	1.47	1.37	1.27
Kurtosis	-0.33	-0.85	-1.29	-0.36	-0.63	-0.62	-1.34

Basing on the Duncan test, homogeneous groups of broken-up material particle length geometric means were distinguished (Table 3). The homogeneous group means that values of x_{gm} belong to the same group, e.g. for milled plant material of miscanthus and Virginia mallow and rose. Practically, this means that suppleness of these energetic plants to milling is the same. The homogeneous group of the lowest value was found for topinambour and the highest, for willow. Similarly, the particle length of milled material from these plants forms homogeneous groups of extreme values. For the remaining plants no close links exist but cohesion between rankings for broken-up and

milled material length geometric means is found in almost all cases. Such cohesion was not found for spartina and polygonaceous only. The distribution of broken-up willow particle lengths were characterized by the highest geometric mean and was well equalized, while the topinambour mixture was shortest and the most irregular. Standard deviations of particle lengths of milled material were better equalized than in the broken-up material, but their absolute values were high. Therefore, geometric mean length values affected the relative spread of distribution. The mixture of the milled willow material, similarly to the broken-up material, was the longest and the most equalized, while for topinambour it was the shortest, but the most irregular.

Table 3. Geometric mean length x_{gm} and geometric standard deviation s_{gm} and homogeneous group for principle length of broken-up and milled plant material.

Broken-up plant material			Milled plant material		
Plant	x_{gm} , mm (s_{gm})	Homogeneous group	Plant	x_{gm} , mm (s_{gm})	Homogeneous group
Willow	11.31 (1.95)	x	Willow	3.13 (2.20)	x
Giant miscanthus	10.47 (1.86)	x	Giant miscanthus	2.60 (2.29)	x
Virginia mallow	9.87 (1.88)	x	Virginia mallow	2.57 (2.25)	x
Rosa	9.30 (2.11)	x	Rosa	2.46 (2.27)	x x
Spartina	8.96 (1.94)	x x	Polygonaceous	2.33 (2.11)	x
Polygonaceous	8.79 (2.06)	x	Spartina	1.93 (2.37)	x
Topinambour	8.40 (2.31)	x	Topinambour	1.76 (2.14)	x

The parameters of the RRSB model for particle length distributions were calculated (Table 4). The F Fisher-Snedecor statistics and R^2 determinations are also presented in Table 4. The high values of evaluation statistics prove that approximations of particle length distributions by the RRSB model are correct.

Table 4. Coefficients of the Rosin-Rammler-Sperling-Bennett model.

	Broken-up plant material				Milled plant material			
	n	x_{50} , mm	F	R^2	n	x_{50} , mm	F	R^2
Willow	1.61	8.57	595.3	96.4	1.20	2.00	478	97.4
Giant miscanthus	1.54	8.88	1426	95.3	1.03	1.55	1892	99.3
Spartina	1.42	7.69	716.3	94.7	0.74	0.88	577	97.8
Polygonaceous	1.52	6.52	2624	97.8	1.05	1.27	259	95.2
Rosa	1.46	6.74	498	95.8	0.96	1.47	1826	99.3
Virginia mallow	1.67	7.73	1851	97.9	1.00	1.57	1078	98.8
Topinambour	1.35	5.95	1018	95.7	0.86	0.76	335	96.3

By comparing size values x_g with x_{50} , a high correlation was found between geometric mean values x_g and median values x_{50} of broken-up ($R^2 = 79.4\%$) and milled material ($R^2 = 97.8\%$) particle length. Hence it can be assumed that the RRSB model closely approximates the particle length distribution both of broken-up and milled material.

Using the RRSB model we can calculate the amount of comminute material meeting dimension requirements for production of pellets or briquettes. The

dimensions amount to 3.2 and 8.0 mm respectively. It is possible to separate 82.8–92.9% of the mixture obtained after milled material by using a sieve with 3.2 mm openings size. It is possible to separate 44.6–64.5% of the mixture obtained after broken-up material by using a sieve with 8.0 mm openings size.

CONCLUSIONS

Since during the harvesting of energetic plants by a forage harvester and during the milling of plant material in a beater mill the same technical parameters of working units were applied, one can conclude that physical properties of plants significantly influenced the mean values of particle lengths of plant material. The harvesting of plants was carried out at different moistures (Table 1), which could affect the breaking up of material. The milling of plant material was performed at comparable moistures, therefore the final effect of this process depended on the physical properties of plants only. In working processes complex phenomena connected with grinding, breaking-up, compressing and crushing of plant material take place. The deciding factors are the following: coefficients of external and internal friction, moisture content of plants and their dimensions, hardness, firmness, rigidity as well as the plant constitution connected with its external and internal structure. Lack of soft parts (e.g. leaves) in willow and its greater hardness are the basic factors resulting in a higher share of longer particles in the mixture. The plants most susceptible to comminution are the topinambour and the spartina. These properties can affect working parameters of briquetting or pellet machines and the firmness and durability of briquettes or pellets or the necessity of diversification of the beater mill working parameters. The particle length distributions of broken-up and milled plant material are well approximated by the RRSB model. The research allows proving the practical possibility to use the RRSB model to determine the amount of separated material meeting dimension requirements for production of formed fuels.

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Effects of biopesticide Neem EC on the *Mamestra brassicae* L. (*Lepidoptera, Noctuidae*)

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Abstract. Third instars larvae of the cabbage moth, *Mamestra brassicae* L. were exposed to Neem EC (M/S RYM Exports, The Indian Neem Tree Company) by a leaf-dip experiment (concentration of azadirachtin – 100 ppm). The after-treatment parameters studied the duration of the larval stage, mortality, and weight of pupae. Neem EC has shown significant prolongation in the development of larvae of *M. brassicae*: 18.5 days in the test variant, versus 14.8 days in the control. Neem EC also caused lethal failure of larval-larval and larval-pupal ecdysis, which is typical for insecticides possessing morphogenetic activity commonly referred to as IGR-activity. The mortality of larvae, prepupae and pupae in the test variant was significantly higher than in the control. The experiment in laboratory revealed that Neem EC inhibited larval development, greatly increased mortality, had antifeedant/deterrent effects and acted as a growth regulator for *M. brassicae* larvae and pupae.

Key words: Neem EC, *Mamestra brassicae*, larval stage, mortality, pupal mass

INTRODUCTION

The cabbage moth (*Mamestra brassicae* L.) is among the key caterpillar pests of cabbage in most European countries. Depending on the climate, *M. brassicae* develops one to ten generations per year; in Estonia this pest is univoltine or partially bivoltine. The polyphagous larvae are mainly recognized as cabbage pests, but have also been reported from a wide range of other plant species. Insecticide application against the larval stage of the *M. brassicae* is the primary method of control, but environmental concerns may jeopardize their continued use. The use of natural compounds in place of conventional insecticides can reduce environmental pollution, and avert insecticide-induced pest recurrence. Extracts from the neem tree (*Azadirachta indica* Juss.) have emerged as an excellent alternative to synthetic insecticides for the management of insect pests and neem extracts have been developed as commercial insecticides in several countries.

The compounds from the neem tree have a number of properties useful for insect pest management. These include toxicity, repellence, feeding and oviposition deterrence, insect growth regulator activity, etc. (Ascher & Meisner, 1989; Schmutterer, 1990; Mordue (Luntz) & Blackwell, 1993; Mordue (Luntz), 2004) and more than 140 active principals have been identified in the neem tree (Koul, 2004). The key insecticidal ingredient is azadirachtin, a steroid-like tetraneortriterpenoid, responsible for both antifeedant and toxic effects in insects (Govindachari, et al., 2000; Koul, 2004). Furthermore, azadirachtin blocks the synthesis and release of moulting

hormones from the prothoracic glands, leading to incomplete ecdysis in immature insects (Isman, 2006). Different insect species show varying degrees of sensitivity to various extracts and components. Naumann & Isman (1995) used three concentrations of an oil-free neem seed extract to deter oviposition in noctuid moths and found that these neem-based products were not effective. According to Turcani (2001), the neem seed kernel suspension reduced the damage by *Pieris brassicae* L. larvae but failed to give any significant protection to castor, *Ricinus communis* L. leaves against the red hairy caterpillars, *Amsacta albistriga* Walker. Neem preparation has a positive effect in controlling the turnip root fly, *Delia floralis* (Fall.) and the cabbage moth, *M. brassicae* (Karelina et al., 1992; Meadow & Seljåsen, 2000; Seljåsen & Meadow, 2006), but Päts & Isman (1998) found no effect on hatching or larval development for the cabbage root fly, *D. radicum* (L.) and Neemix, which kills and repels a variety of insects, had no effect on populations of the beet armyworm, *Spodoptera exigua* (Hübner) larvae. These controversial results suggest that the efficacy of any neem-based insecticides on pests should be investigated before widespread use in the field. This research addresses the need for finding effective options for managing the insect pest *M. brassicae* in Estonia in the face of the declining availability and popularity of conventional chemical insecticides. In this study, we have been interested in the effects of Neem EC, widely used in the warmer climate areas against different pest species, on the cabbage moth, *M. brassicae*. Furthermore, there have been few experiments for biological preparations against cabbage moth.

MATERIALS AND METHODS

The egg clutches of *M. brassicae* were collected from the cabbage fields near Tartu, Estonia and incubated in Petri dishes. As the cabbage moth is a long-day insect, and a winter diapause is induced by short day lengths, the larvae were reared in insectaria at photophase 12:12, 20 ± 2 °C, and $75 \pm 10\%$ relative humidity. The larvae were fed on cut leaves of the cabbage (*Brassica oleracea* var. *capitata* L.), which were replaced as appropriate. Larvae pupate in the soil at a depth of about 3–5 cm; therefore ca 10 cm thick layer of peat was placed on the bottom of the rearing vessels at the end of the last instar.

We obtained the preparation for experiments from India (M/S RYM Exports – The Indian Neem Tree Company). The larvae hatched from one egg clutch were divided into 2 groups, control and test. Eight replicates were used ($n = 240$). Third instar larvae of the *M. brassicae* were exposed to the Neem EC (1% azadirachtin) by a leaf-dip experiment (concentration of the active ingredient was 100 ppm). The leaves of 6 cm diameter cut from middle leaves of cabbage were dipped for 10 seconds into the treatment (test) or distilled water (control) and left to dry for 30 minutes on filter paper. Larvae were forced to feed on neem-treated leaves for 48 h and thereafter fed with untreated leaves. The after-treatment parameters studied the duration of the larval stage, mortality, and weight of pupae. The larvae were checked every day and the leaves replaced. The pupae were weighed one month after pupation with an analytical balance to 0.1 mg.

Data were analysed by means of ANOVA procedures, and means were compared by the Student's t-test, at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Neem EC causes significant prolongation in the development of test larvae of *M. brassicae*. The time needed for completion of the larval stages by individuals fed on treated cabbage increased significantly (Student's t-test; $t = 2.21$, $df = 30$, $p = 0.001$, Fig. 1), 18.5 ± 6.17 days in the test variant, versus 14.8 ± 1.57 days in the control.

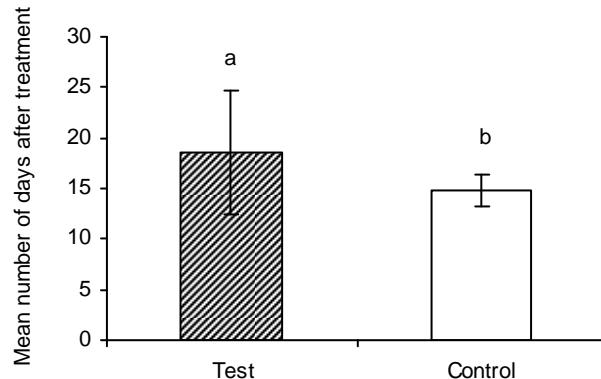


Figure 1. Mean ($\pm SD$) number of days of the larval stage of *Mamestra brassicae* after treatment with Neem EC. Different letters indicate statistically significant differences ($P < 0.05$, Student's t-test).

The mortality of larvae (Student's t-test; $t = -9.57$, $df = 14$, $p < 0.05$, Fig. 2), prepupae and pupae (Student's t-test; $t = -8.74$, $df = 14$, $p < 0.05$, Fig. 2) in the test variant was significantly higher than in the control. Neem caused lethal failure of larval-larval and larval-pupal ecdysis, which was typical for insecticides possessing morphogenetic activity commonly referred to as IGR-activity.

Results showed that pupae whose larvae had been feeding on the control cabbage were significantly heavier than the larvae fed on the treated plants (Student's t-test; $t = 2.153$, $df = 24$, $p = 0.04$, Fig. 3). The mean $\pm SE$ body mass was 394.9 ± 68.8 mg in the control and 332.1 ± 79.6 mg in the test.

The experiment revealed that Neem EC increased mortality, inhibited larval development, and acted as a growth regulator for *M. brassicae* larvae and pupae. Neem EC deterred feeding by cabbage moth larvae. Sample pieces were taken from the treated cabbage leaves only on the second day; they had been eaten considerably less than the control. Yoshida & Toscano (1994) found that the relative consumption rate of *Heliothis viridescens* (Fabricius) larvae treated with neem extract was 25% of the control. Greenberg et al. (2006) found that neem-based insecticides deterred feeding by beet armyworm (*S. exigua*). The neem tree contains different substances in its tissues, many of which are biologically active against insects as antifeedants (Jacobson, 1987; Mordue (Luntz), 2004). The primary active ingredient of neem-based pesticides is azadirachtin. Inhibition of feeding behaviour by azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells or

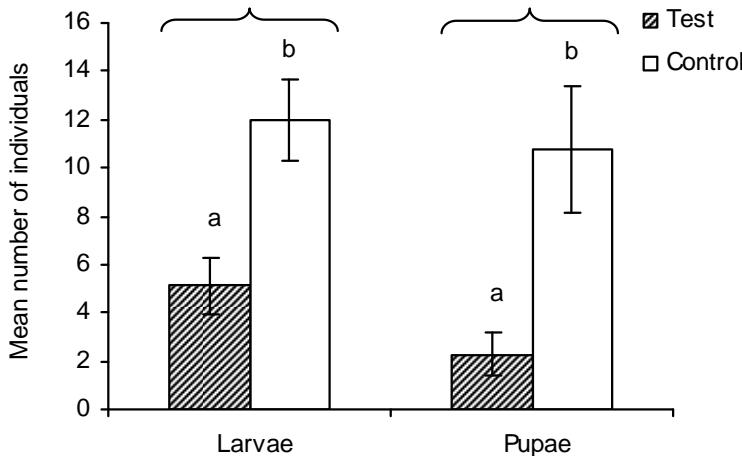


Figure 2. Survival of larvae and pupae of *Mamestra brassicae* on plants treated with Neem EC (test) or distilled water (control). Means followed by the different letters above bars (\pm SD, standard deviation) indicate statistically significant differences ($p < 0.05$; Student's t-test). The horizontal clippers indicate that statistical comparison is validated by grouping the measurement results of the same groups (larvae or pupae).

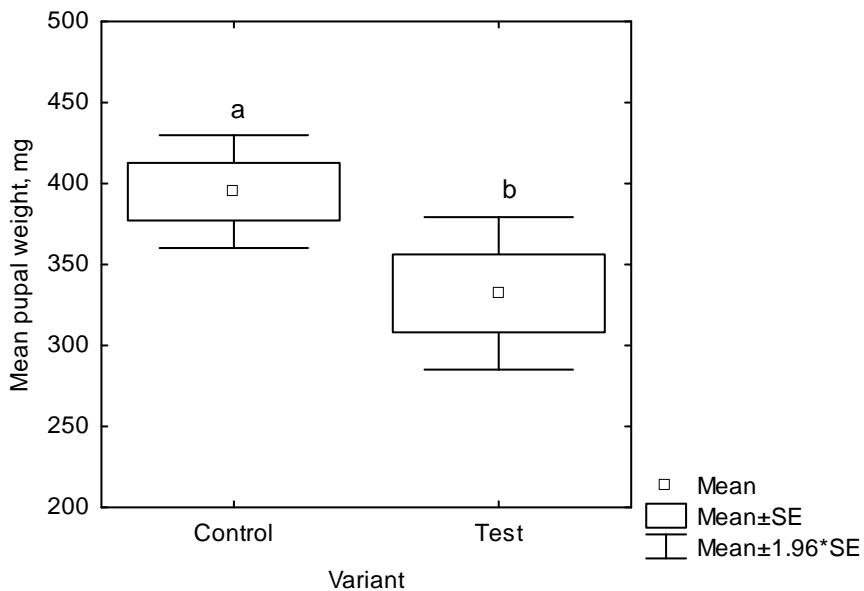


Figure 3. Mean body mass standard error (\pm SE) and 95% confidence intervals ($\pm 1.96 \times$ SE) of *Mamestra brassicae* pupae in the test and control variants. Bars with different letters are significantly different ($p < 0.05$; Student's t-test).

both (Mordue (Luntz) & Blackwell, 1993). The Neem EC did not produce a rapid mortality. No larvae perished within the first days of the experiment; mortality began on the 4–5th days, when moulting to the 4th growth stage began. Insects treated

with neem extracts rarely show immediate symptoms; death may be delayed a week or longer (Pavela & Holy, 2004). However, experiments by Luik & Viidalepp (2001) showed that when caterpillars of *P. brassicae* were fed with cabbage treated with Neem-Azal T/S, all caterpillars perished within 7 days. Coudriet et al. (1985) detected that neem seed extract prolonged larval development, and induced larval mortality of *Bemisia tabaci* (Gennadius) on cotton foliage. In our experiments caterpillars of the treated variant were delayed both in their growth and development in comparison with the control; test larvae perished directly before moulting or in the process of moulting, as they were unable to shed the old integument. The mortality observed during the moulting process, with production of larval-pupal intermediates; support the proposition that the action of neem ingredients is connected with endocrine events in the insects. To be successful, metamorphosis requires a careful synchrony of many hormones and other physiological changes. Metamorphosis occurs when the *corpora allata* stop secreting juvenile hormone (Gilbert et al., 1980). It can occur only when the insect has reached a certain minimum mass characteristic of the growth stage. Neem EC caused lethal failure of larval-larval and larval-pupal ecdysis, which is typical for insecticides possessing morphogenetic activity, commonly referred to as IGR-activity. It is possible that azadirachtin, because of its similarity to ecdysone, which controls the process of metamorphosis as the insects pass from larva to pupa, will block off these vital hormones and interrupt their life cycle (Sridharan, 2007). Another characteristic was that defects occurred both in moulting into the 4th instar and the following 5th instar and during pupation. Therefore it may be concluded that a higher than normal juvenile hormone level persisted in the organism, allowing growth but inhibiting metamorphosis. It is known that neem may act as an exogenous juvenile hormone or its analogue (Schmutterer, 1987). However, it is also possible that, as a result of intoxication, histopathological changes may occur in the *corpora cardiaca* of the insect, causing disorders in the secretion of hormones (Mordue (Luntz), 2004). A significant reduction in the average pupal mass was also observed, presumably due to reduced feeding on neem-treated cabbage. The results are promising in developing effective approaches to control *M. brassicae*, but more studies are needed to evaluate the field efficacy of Neem EC.

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Occurrence and distribution mating types A1 and A2 of *Phytophthora infestans* in eastern Estonia

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Abstract. *Phytophthora infestans* (Mont.) de Bary is one of the most serious and economically important pathogens in potato fields worldwide, including Estonia. Under favourable conditions it can destroy the whole potato haulm and cause a considerable yield loss. In Estonia, the average yield loss due to late blight can reach 20–25% and in untreated fields, even more. Without control of potato late blight it is not possible to achieve high-quality crop yield. In Estonia, fungicides are used routinely in conventional potato production, but under favourable conditions for the disease, with heavy pressure of the pathogen, protecting large areas is complicated. *P. infestans* isolated from potato leaves and tubers were collected from a region of Eastern Estonia during 2001–07. In total, 133 isolates were assessed for mating type. The average frequencies of mating types A1 and A2 were 59% and 38% respectively. The data indicate that in the Estonian population of *P. Infestans*, both mating types occur simultaneously within fields, with a potential for sexual recombination.

Keywords: mating type, *Phytophthora infestans*, sexual reproduction

INTRODUCTION

Late blight is caused by the fungal-like oomycete *P. infestans*, which can reproduce both sexually and asexually. For sexual reproduction, *P. infestans* requires both A1 and A2 mating types to produce gametangia (Fry & Goodwin, 1997). The centre of diversity of this oomycete is located in the highlands of Mexico (Fry & Goodwin, 1997), where both mating types originate. At least two different migration events from Mexico have occurred. The first is postulated to have occurred before 1845, after which *P. infestans* swept through Europe and Ireland resulting in the death of over one million people due to starvation, and emigration of 1.5 million people to other parts of Europe or North America (Drenth *et al.*, 1994). The second migration occurred in the 1970s to 1990s, bringing the A2 mating type out of Mexico and also containing genetically diverse and aggressive strains (Fry *et al.*, 1993). In Estonia, the A2 mating type was detected for the first time in 1987 by researchers of The All Union Institute of Plant Pathology (Vorobyeva *et al.*, 1991). In the present study, phenotypic characteristics of *P. infestans* isolates, collected from potato plants from 2001 to 2007

in eastern Estonia, are reported. The main aim was to detect the temporal changes in population parameters throughout the seven years within a distinct location. It was also observed if the mating type ratio in this area suggests the occurrence of sexual reproduction. The results are compared with the findings of similar studies performed in other European countries in recent years.

MATERIALS AND METHODS

In total, 133 isolates of *Phytophthora infestans* were collected from Estonia from 2001 to 2007. Most of the isolates originated from leaves and a minority, from tubers. The isolates were sampled randomly from a location in eastern Estonia; the procedure was repeated for seven years. All sites used in the seven years were experimental field trial plots at the Jõgeva Plant Breeding Institute (one experimental field per year, labeled here as Jõgeva 2001, Jõgeva 2002, Jõgeva 2003, Jõgeva 2004, Jõgeva 2005, Jõgeva 2006 and Jõgeva 2007). The study area is characterized by high genetic diversity of the host plants including several genotypes that have race-specific genes (Runno-Paurson *et al.*, 2009). No fungicides were used for late blight control.

Blighted leaves (one per plant) were collected in the period from the emergence of disease until the end of the growing season in all years. Fourteen to forty leaflets, each with single lesions, were collected from individual plants. Isolations were carried out as described in Runno-Paurson *et al.* (2009).

For mating type determination a method described by Runno-Paurson *et al.* (2009) was used. In 2002 and 2003, the determinations were conducted at Cornell University by using a tester strain (US970001 for the A1 mating type and US940480 for the A2 mating type). In all other years, the tester isolates described by Lehtinen *et al.* (2007) were used.

Statistical analyses were performed with the SAS/STAT version 9.1 (SAS Institute Inc., Cary, NC, USA). Differences in the prevalence of the two mating types among *P. infestans* isolates between years were tested using a logistic analysis (GENMOD procedure in SAS) with a multinomial response variable (A1, A2, or both).

RESULTS AND DISCUSSION

In total, 133 isolates were assessed for mating type. The frequencies of A1 and A2 were 59% and 38% respectively. Self-fertile isolates were also found (3%). There were considerable differences ($\chi^2 = 45.74$, df = 12, p < 0.001) in the proportion of A1 and A2 between years: A2 mating type was found in all years except in 2002 (Figure 1). The frequency of A2 mating type was significantly higher in 2001, 2003, 2004, 2005 and 2007 (41–71%).

The average percentage of A2 mating type in the current study was 38%, which is similar to the results of previous studies from 2002 to 2003 (Runno-Paurson *et al.*, 2009). There have been reports of a lower A1:A2 ratio from several European countries (Hermansen *et al.*, 2000; Bakonyi *et al.*, 2002a; *et al.*, 2002b; Cooke *et al.*, 2006; Lehtinen, *et al.*, 2007). A higher proportion of A2 mating type has been found in Austria, The Netherlands and Poland (Avendaño Córcoles, 2007; Zwankhuizen *et al.*, 2000; Śliwka *et al.*, 2006).

We found no consistent directional change in the proportions of mating types throughout the study years. However, the prevalence of A2 mating type fluctuated strongly, being almost absent in some years and making up over half of the population in others (figure 1). More precisely, in 2001, 52% of the isolates were A2 mating type, whereas in 2002, A2 mating type was not found. Since then the proportion of A2 mating type increased abruptly from 0% to 44% in 2003 and 61% in 2004. Even though the proportion fell to 3% in 2006, it increased once again to 71% by 2007.

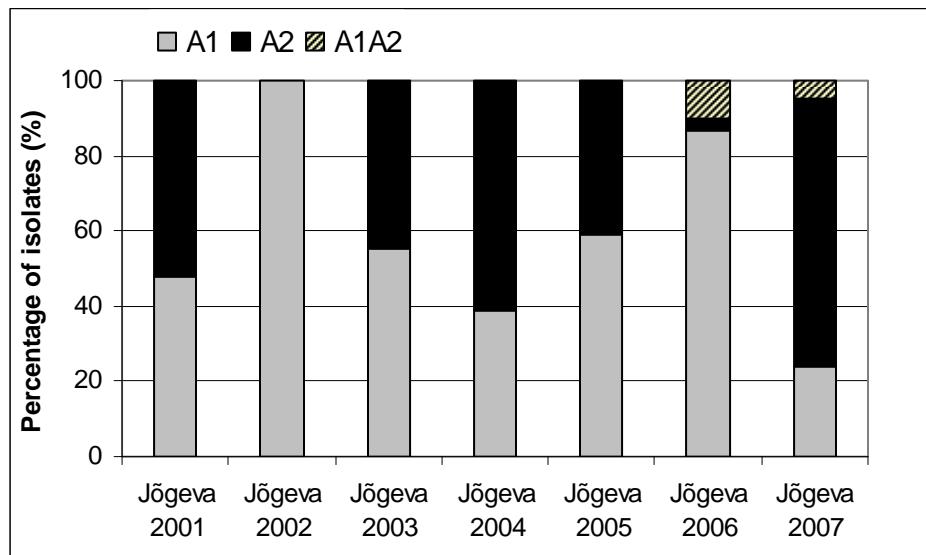


Fig. 2. Percentages of mating types among isolates of *Phytophthora infestans* from eastern Estonia during 2001–2007.

In general, it can be noted that in many years the ratio of the two mating types appeared to be close to 50:50, which is the most effective ratio for sexual reproduction. Both mating types were detected in most (six out of seven) of the studied plots. The presence of both mating types in the same field indicates that oospores would be produced in potato foliage (Turkensteen *et al.*, 2000). This changes pathogen epidemiology substantially. Sexual reproduction increases genetic diversity and leads to soil contamination with oospores and associated early late-blight infection. Previous results by Runno-Paurson *et al.* (2009) indicated that blight epidemics could be severe both in large conventional productions and in organic fields. The results of this study confirm the same situation in experimental fields. Therefore, organic farm managers who do not use fungicides are recommended to apply other preventive methods such as crop rotation. This can be important because continuous potato cropping in small scale conventional fields further increases the risk of oospore-derived infections and may cause earlier attacks and consecutive yield loss (Runno-Paurson *et al.*, 2009).

CONCLUSIONS

The results of this study indicate that the ratio of *P. infestans* A1:A2 mating types is suitable for sexual reproduction. In most of the studied years, both mating types were

found in the same field plots. The probable occurrence of oospore formation makes it important to apply crop rotation as a preventive measure.

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The incidence of fungal diseases in oat leaves and yields as affected by fertilizer and chemical inputs in Estonia

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Abstract. Field experiments were conducted for three years and were designed to study the effects of fertilizers on fungal disease infections and yield of two oat genotypes. The impact of the different levels of fertilization has been noticed at the level of crown rust (induced by *Puccinia coronata*) and oat leaf spot (induced by *Pyrenophora avenae*). Four fertilizer doses (N_0 = untreated control $N_0P_0K_0 \text{ kg ha}^{-1}$; $N_1 = N_{60}P_{13}K_{23}$; $N_2 = N_{100}P_{22}K_{39}$; $N_3 = N_{140}P_{31}K_{54}$) and two variants of chemical treatments (variant 1 – without chemicals; variant 2 – with chemicals as growth regulator, fungicide and thrice with foliar fertilizer) were used. The significant differences in levels of disease infection and grain yields between inputs and varieties were observed. The infection level of both oat diseases was mostly influenced by the yearly weather conditions. By using variant 2, including fungicide, the infection of *Puccinia coronata* decreased considerably. The fertilizer input increased the grain yield of the oat varieties. Oat grain yields were higher in treated plots in variant 1 than in variant 2, due to weather conditions.

Keywords: oat, fertilization, fungicides, diseases, yield

INTRODUCTION

Nutrients from the rhizosphere may aid foliar pathogen survival in soil and influence disease epidemiology. Balanced and adequate fertility for any crop reduces plant stress, improves physiological resistance and decreases disease risk (Krupinsky et al., 2002). The effect of disease control on yield is of economic significance (White et al., 2003). Oat leaf spot and crown rust are diseases that have often been found in Estonia. Oat leaf spot or seedling blotch, caused by *Pyrenophora avenae* (Ito & Kurib., conidial stage: *Drechslera avenae* syn. *Helminthosporium avenae*) is a seed-borne pathogen; the fungus also survives on host debris (Clifford, 1995). Oat crown rust, caused by *Puccinia coronata* f. sp. *avenae*, is a long-cycled rust fungus that has a species of buckthorn (*Rhamnus* sp.) as an alternate host (Šebesta et al., 1997).

There is comparatively little published on the influence of the basic and foliar fertilization on the diseases' intensity and the degree of attack on oat plants; traditionally, oat has been viewed as a minor crop and has received little research attention. If chemicals are not used against crop lodge and fungal diseases, it is not worth fertilizing cereals with large nitrogen rates because cereal productivity does not increase significantly (Lisova et al., 1996). In general it is suggested that oat requires less nitrogen fertilizer and chemicals to optimize yield than other spring cereals.

The present experiments were designed to study foliar disease management options in basic fertilization and chemical inputs in oat. The objective was to determine the yield responses of oat to basic fertilization applied to the soil and to determine if the oat crown rust and leaf spot incidence and severity would be reduced with chemical inputs.

MATERIALS AND METHODS

The trials were made on Calcaric (Eutric) Cambisol (FAO classification) soil (clay loam; pH_{KCl} 5.8; P 170–190 mg kg⁻¹; K 153–206 mg kg⁻¹; Ca 1440–1600 mg kg⁻¹; Mg 57–71 mg kg⁻¹; Cu 1.2–1.3 mg kg⁻¹; Mn 36–46 mg kg⁻¹; B 0.48–0.92 mg kg⁻¹). Two oat varieties Villu (Estonia) and Flämingsprofi (Germany) were included in the trial. Non-treated seeds were sown in 9 m² plots at the rate of 600 germinating seeds per 1m² in three replicates using a randomized block design, in early May. Four levels of fertilization (N0 = untreated control N₀P₀K₀ kg ha⁻¹; N1 = N₆₀P₁₃K₂₃; N2 = N₁₀₀P₂₂K₃₉; N3 = N₁₄₀P₃₁K₅₄) were applied to the soil before sowing using a complex fertilizer Kemira Power (N₁₈P₄K₇). Additionally, for fertilization, two different variants were utilized (1 – only soil fertilizer doses and non treatment; 2 – soil fertilizer doses and chemical treatment). In variant 2 the full dose of 1.0 l ha⁻¹ (GS 32) foliar spraying with plant growth regulator CCC (a.i. 750 g l⁻¹ chlormequat chloride) and leaf fertilizer Folicare 8 kg ha⁻¹ (N₁₂P₂₀K₇ g kg⁻¹ at GS 21–22; N₁₈P₈K₁₅ at GS 51–52; N₁₀P₂K₃₃ at GS 71–72) were applied. Fungicides Tilt 250 EC (a.i. g l⁻¹ 250 propiconazole) 0.5 l ha⁻¹ (GS 29–30) in 2006 and, in 2007 and 2008, Folicur EW 250 (a.i. g l⁻¹ 125 tebuconazole) 1.0 l ha⁻¹ (GS 50–51) were used against foliar diseases in variant 2.

Disease observations were made using a modified septoria and rust disease assessment keys (James, 1971). The assessments of the diseases' severity were carried out on ten randomly chosen tillers per plot in four replications. Visual scoring was made on the 1–9 point scale (1 – no infection, 9 – highly infected). The infection level was expressed as an average of the infection score at milk ripening stage (GS 75) on the top three leaves of the plant. Phenological growth stages were determined according to Zadoks scale of cereals (Zadoks et al., 1974) when > 50% of the plants reached the target growth stage. Grain yield (kg ha⁻¹) was measured on dried and cleaned seeds and expressed on the basis of 14% moisture content. Data were analyzed by factorial analysis of variance using the Agrobase statistics software (Agrobase™, 1999).

Field meteorological station Metos Compact recorded the weather data during the trial period. The average temperature of 15.4°C during the trial period in 2006 and 2007 was higher than the 14.2°C long-term average; 13.9°C for the same period in 2008 was lower, respectively (Table 1). There was little precipitation in 2006 and 2007. A monthly period of drought was observed during the tillers in 2006 and 2007 and during stem elongation in 2007. The grain harvest period was wet and rainy in 2008; precipitation exceeded the long-term average period by 96 mm.

RESULTS AND DISCUSSION

The relations between the plant and the pathogen are of a nutritional nature. The influence of the diseases on the plants depended on a particular genotype, fertilizer input and climatic conditions, which varied yearly (Krupinsky et al., 2007). The

influence of the year, variety, fertilizer input and their joint effect on the infection severity are shown in Table 2. By focusing on pathogen data, it was confirmed that in the basic fertilization conditions crown rust correlated highly with yearly climatic conditions, variety, fertilizer input and their interactions ($R^2 = 0.933^{***}$). In both variants, the year had the most impact on the infection of crown rust: 0.704 and 0.449 respectively. By using the chemical treatment the effect of the fertilizer input on crown rust distribution was non-significant. In variant 2, the influence of variety on trial results was more considerable than in variant 1.

Table 1. Mean temperature (°C) and precipitation (mm) for growing seasons 2006–2008 and long-term average (1922–2007) at Jõgeva PBI.

Month	Air temperature °C			85-years average	Precipitation mm			85-years average
	2006	2007	2008		2006	2007	2008	
May	10.5	11.5	10.4	10.2	36	62	22	50
June	16.0	15.7	14.2	14.4	40	42	107	66
July	18.1	16.8	15.6	16.7	11	86	56	81
August	16.8	17.7	15.4	15.3	74	64	195	87
May-August	15.4	15.4	13.9	14.2	161	254	380	284

Table 2. Infection of oat varieties by *Puccinia coronata*, *Pyrenophora avenae* and grain yield at different variation sources (ANOVA).

Source of variation	<i>P. coronata</i>	SED(df)	<i>P. avenae</i>	SED(df)	Yield	SED(df)
Variant 1						
Year	0.704***	0.1866 (2)	0.610***	0.1741 (2)	0.139***	124.2 (2)
Variety	0.054***	0.1523 (1)	0.007 ns	0.1421 (1)	0.037***	101.4 (1)
Fertilizer input	0.025***	0.2154 (3)	0.085***	0.2010 (3)	0.552***	143.4 (3)
Variety by year	0.090***	0.2638 (2)	0.063***	0.2462 (2)	0.046***	175.6 (2)
Year by fertilizer input	0.058***	0.3731 (6)	0.067**	0.3482 (6)	0.134***	248.3 (6)
R ²	0.933***		0.838***		0.914***	
Variant 2						
Year	0.449***	0.1445 (2)	0.196***	0.1132 (2)	0.712***	107.0 (2)
Variety	0.142***	0.1180 (1)	0.157***	0.0924 (1)	0.004*	87.4 (1)
Fertilizer input	0.012 ns	0.1668 (3)	0.155***	0.1307 (3)	0.158***	123.6 (3)
Variety by year	0.087***	0.2043 (2)	0.064**	0.1601 (2)	0.007*	151.4 (2)
Year by fertilizer input	0.069*	0.2890 (6)	0.142***	0.2264 (6)	0.074***	214.1 (6)
R ²	0.779***		0.739***		0.958***	

* significance at $p < 0.05$; ** significance at $p < 0.01$; *** significance at $p < 0.001$; ns – non-significant

Results revealed that the oat leaf spot correlated highly with year, fertilizer input, the interactions of variety x year and year x fertilizer input ($R^2 = 0.838^{***}$). This conclusion was also reported by Krupinsky et al. (2002). The impact of the variety on oat leaf spot was insignificant in the untreated variant and this adverted to the fact that in natural conditions the leaf spot resistance of varieties was quite similar. The leaf

spot infection depended on several factors, such as genotype, year and the interaction of year x fertilizer input in variant 2, whereas crown rust infection was primarily associated with weather conditions. The grain yield of oat was influenced mostly by fertilizer input (0.552***) in variant 1 and by year (0.712***) in variant 2. The variation of average oat yields of the two variants was determined by both factors, 0.369*** (year) and 0.291*** (fertiliser input) respectively. The influence of other factors turned out to be much less.

The results demonstrated that the increased level of fundamental fertilization in variant 1 increased the intensity of *P. coronata* and *P. avenae* (Table 3). A trend of crown rust infection decreased in variant 2; this was apparent at higher N-doses. This indicates that, to some degree, fungicides help to prevent the increase in the disease infection level normally associated with fertilizer use.

Table 3. Average infection of foliar diseases of oat varieties at different input levels during the years 2006–2008.

Variety	Variant	<i>P. coronata</i> (1–9 scale)				<i>P. avenae</i> (1–9 scale)			
		2006	2007	2008	average	2006	2007	2008	average
Villu	1+N 0	1	4	6	3.7	3	3	4	3.3
	1+N 1	1	5	6	4.0	4	4	5	4.3
	1+N 2	1	6	5	4.0	3	4	6	4.3
	1+N 3	1	6	7	4.7	4	5	5	4.7
	2+N 0	1	2	3	2.0	3	3	3	3.0
	2+N 1	1	2	3	2.0	3	2	3	2.7
	2+N 2	1	3	3	2.3	3	3	3	3.0
	2+N 3	1	4	2	2.3	3	4	4	3.7
Flämingsprofi	1+N 0	1	3	4	2.7	3	3	5	3.7
	1+N 1	1	5	3	3.0	3	4	5	4.0
	1+N 2	1	6	3	3.3	2	4	6	4.0
	1+N 3	1	6	3	3.3	2	4	7	4.3
	2+N 0	1	2	2	1.7	2	2	3	2.3
	2+N 1	1	2	2	1.7	2	2	3	2.3
	2+N 2	1	2	1	1.3	2	3	3	2.7
	2+N 3	1	2	1	1.3	2	3	4	3.0
<i>LSD 0.5</i>		0.0	0.8	0.6	0.4	0.5	0.4	0.6	0.3
Average of Villu		1.0	4.0	4.4	3.1	3.3	3.5	4.1	3.6
Average of Flämingsprofi		1.0	3.5	2.4	2.3	2.3	3.1	4.5	3.3
<i>LSD 0.5</i>		0.0	0.4	0.3	0.1	0.2	0.2	0.3	0.1

As to the adjusted average for the experimental years, more severe crown rust infection was recorded in trials with the Estonian variety Villu. The infection level of oat leaf spot was more severe and exceeded the average of both varieties in variant 1 in 2008. Oat crown rust occurs worldwide, infecting cultivated oat (Šebesta et al., 1997) and has often been found in Estonia (Sooväli & Koppel, 2003). In our trials, oat crown rust occurred in two years (2007, 2008) out of three. The incidence and severity of the degree of crown rust attack in the untreated variants demonstrated that weather conditions for disease development in natural conditions were favourable in 2008. In

variant 2, fungicide treatment had a significant protective effect against diseases in both varieties. The decrease in disease severity accomplished with chemical treatments was consistent in the study. In the case of fungicide treatment, N fertilization levels apparently had no significant effect on disease severities.

The grain yields of oat varieties based on different inputs are shown in Table 4. Some earlier studies had suggested that the amount of N fertilizer needed to maximize yield has varied between years (May et al., 2004; Mohr et al., 2007). The trial yield level average varied significantly by years from 3988 kg ha⁻¹ in 2006 to 6064 kg ha⁻¹ in 2008. The average yield level of 2007 was 4540 kg ha⁻¹. The weather conditions during the experimental years were diverse, causing differentiation of yields. In 2006 and 2007 the grain yields in variant 2 were lower compared to the yields in variant 1, which may have resulted from warm and dry growing conditions causing crop losses due to environmental stresses. In the 2008 trial the opposite effect was observed. As the results obtained indicate, the response of grain yield to soil fertilization depends greatly on moisture (Peltonen-Sainio, 1997). The results of our study support previous research (Mohr et al., 2007) indicating that the average grain yields at the N1–N3 fertilizer levels were quite similar in both variants but were considerably higher compared to yields at the N0. The intensity of fertilizer input caused significant differentiation in yields of the tested varieties, where the variety Flämingsprofi had higher yield over the average of the tested years.

Table 4. Dependency of grain yield of oat on different inputs at the Jõgeva PBI in 2006–2008.

Variety	Variant	Grain yield, kg ha ⁻¹			
		2006	2007	2008	average
Villu	1+N 0	2726	3077	4214	3339
	1+N 1	4556	5200	6411	5389
	1+N 2	4274	6251	5458	5328
	1+N 3	5102	6594	4447	5381
	2+N 0	2397	3198	4587	3574
	2+N 1	3153	4028	6897	4693
	2+N 2	3299	4041	7215	4852
	2+N 3	3675	4745	6476	4965
Flämingsprofi	1+N 0	3354	2907	4550	3603
	1+N 1	4985	5063	6949	5666
	1+N 2	5195	5969	6948	6037
	1+N 3	5792	6185	6278	6085
	2+N 0	3252	2914	4615	3594
	2+N 1	3302	3669	7300	4757
	2+N 2	3963	4063	7636	5219
	2+N 3	4241	4733	7041	5338
LSD 0.5		430	294	418	275
Average of Villu		3715	4,642	5713	4690
Average of Flämingsprofi		4260	4438	6414	5037
LSD 0.5		215	147	209	138

CONCLUSIONS

We conclude that the basic fertilization applied to the soil in moderate and higher NPK-doses determines the increase of the disease attack degree as compared to the non-fertilized variants. As expected, the fungicide application demonstrated a reduction of crown rust and oat leaf spot attack in all fertilizer levels. In this study there was absolutely no infection of crown rust in the case of the variety Flämingsprofi in N100 and N140. The oat leaf spot attack in variety Villu decreased to very low when fungicides were used in years of moderate infection severity, 2006 and 2007, when the basic fertilization applied to the soil brought about a higher yield increase for both varieties. The yield increase resulting from intensive fertilizers with fungicide and application of growth regulator was significantly higher only in 2008 than that from soil fertilization.

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Preliminary results of nitrogen uptake with mown grass in an apple orchard under influence of mulch and irrigation

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Abstract. Nitrogen leaching from intensive agriculture systems is one of the major contributors responsible for nitrate concentration increasing in groundwater. The aim of the study was to determine the content of total nitrogen in the orchard lawn and use this parameter as a correction factor for reduction of nitrogen fertilizers application and to include the nitrogen from mown grass into the N balance and turnover calculation. The investigation was conducted at the Latvia State Institute of Fruit-Growing, Dobele in 2009, on the basis of an existing field experiment planted in 1997 with cultivar `Melba` (rootstock B 9). The influence of soil moisture management treatments were compared near the alleyway: control, sawdust mulch and fertigation. The alleyway was covered by grass vegetation (*Lolium perenne* L. and *Poa pratensis* L.). Total nitrogen was determined using the Kjeldahl method. The preliminary results show that the mulch used in tree strips in an apple orchard had a significantly negative influence on the concentration of nitrogen in the dry matter of mown grass of the alleyway compared with control and fertigation. Near the mulching treatment the concentration of nitrogen in dry matter of mown grass in the alleyway was 2.23%, but near the control and fertigation treatments it was 8% higher. Nitrogen concentration in the grass of the alleyway was significantly influenced by the time of grass mowing. Nitrogen uptake with mown grass biomass in the alleyway near the control in tree strips was 33.36 kg ha⁻¹, near the mulch treatment it was higher by 6%, but in fertigation treatment - by 20%.

Key words: *Malus domestica* Mill., mineral nutrition, nutrient uptake

INTRODUCTION

Nitrogen is one of the most important nutrients necessary for many functions of plants, e.g. shoot growth, fruit and flower bud set, and fruit size. If apple trees lack nitrogen, the shoot growth is weak, leaves become light green to yellowish-green which in turn has a negative effect on photosynthetic intensity (Cmelik et al., 2006). Nitrogen, mainly in organic matter, is also one of the preconditions for soil fertility. Lack or excess of nitrogen in the soil greatly depends on the type of farming and technologies used. It has been found that at farms where post-harvest residue remains on the field and is ploughed down, the total loss of nitrogen is significantly lower, because nitrogen returns to the soil as organic matter and will take part in the natural process of the soil cycle (Līpenīte & Kārkliņš, 2007). If the mown grass is left in the

orchard, the plant nutrients will be recycled and humus content in the soil will also be increased (Hoagland et al., 2008). This will provide several positive consequences because the buffer capacity of the soil will increase. It will reduce the possibility for nutrient leaching, especially nitrogen, improve soil aeration, and reduce the evapotranspiration, to form the more favourable physical properties. As a result it will positively influence not only the growth of apple tree roots, but also the soil microbiological processes and finally the soil fertility. This is important for many aspects of agriculture, but especially concerning sustainable farming practice and for development of organic and integrated fruit growing technologies, where use of mineral fertilizers is restricted or only minimally acceptable. The environmental and human health concerns take priority.

Grass mown in orchards can be considered as a green manure contributing to the total plant nutrient turnover. Nitrogen accumulated in the grass biomass due to the high competitive ability of cereals is not lost from the root zone. After mowing it will become the nitrogen source for the successive plants including apple trees, especially if the grass is moved to the tree strips. Therefore to plan fertilization, this amount of nitrogen might be included in calculations as an input value.

The aim of the investigation was to clarify the amount of nitrogen in grass grown in an apple orchard alleyway influenced by treatment of soil moisture management in the tree strips.

MATERIALS AND METHODS

The investigation was carried out at the Latvia State Institute of Fruit-Growing, Dobele, in 2009 on the basis of an existing field experiment planted in 1997 with cultivar `Melba` on the rootstock B 9 (Rubauskis et.al., 2004). The planting distance of the trees was 5 × 4 m. The canopies of trees were trained as slender spindles.

The climate was as follows in 2008: the period of vegetation, when air temperatures are 5°C or higher was 204 days (average of long-term 135 – 145 days); the average air temperature was 8.1°C (long-term 5.5 °C) and amount of precipitation 531 mm (similar to long-term 560 mm), however the amount of precipitation in the vegetation period was 312 mm). Soil of the experimental plot was Haplic Luvisol (Hypereutric), sandy loam, organic matter content – 25 g kg⁻¹ (Tyurin method), pH – 6.5 (in 1 M KCl). Plant available P₂O₅ was 300 mg kg⁻¹ and K₂O – 190 mg kg⁻¹, MgO – 162 mg kg⁻¹ (DL).

The following treatments of soil moisture in the tree strips (1 m wide) were compared: control – no regulation methods; sawdust mulch and fertigation. In the mulch treatment the soil surface was covered with a 10 – 20 cm layer of sawdust, which was renewed three times every three years. In the irrigation treatment 'Den' type pipelines with built-in drippers spaced 0.38 cm apart were used. The irrigation provided effective moistening of a 1 m wide zone in sandy loam soil or about 25% of the orchard area. In 2009 irrigation for the trees provided an additional 353 litres of water.

In the alleyways (3 m wide) grass *Lolium perenne* L. and *Poa pratensis* L. were sown in proportion 1:3. In time, after planting, of the orchard, 'weeds' such as white clover (*Trifolium repens*) and dandelion (*Taraxacum officinale*) spread in the grass lawn. During the growing season, the tree strips were maintained free of the

grasses using herbicides such as basta and glifosate. The trees were provided with 9 g N and 12 g K₂O using ammonium and potassium nitrate. The grass in the alleyway was not fertilised. Grass samples were collected as the grass was mowed – 3 times during 2009, May 20, June 21 and August 11, and left on the lawn. From the beginning of the growing season up to May 19 (first grass mowing) the average air temperature was 13.6 °C, precipitation, 9.3 mm, till June 21 – 14.8 °C and 93 mm, but till August 11 – 18 °C and 96 mm correspondingly. The total nitrogen was determined using the Kjeldahl method. The nutrient uptake was calculated as kilograms per hectare area.

The results of the investigation were analyzed using dispersion analysis ANOVA, as well as descriptive statistics (*Descriptive statistic*).

RESULTS AND DISCUSSION

Results of the investigation showed that the concentration of nitrogen in the grass of the alleyways in the apple orchard was influenced by the soil moisture regulation treatments that were placed near the tree strips – sawdust mulch or fertigation ($n = 54$, $P < 0.05$).

The highest concentration of nitrogen was in the grass samples when the fertigation treatment was applied to the tree strips, but it did not significantly differ from the concentration in the control treatment (Fig. 1).

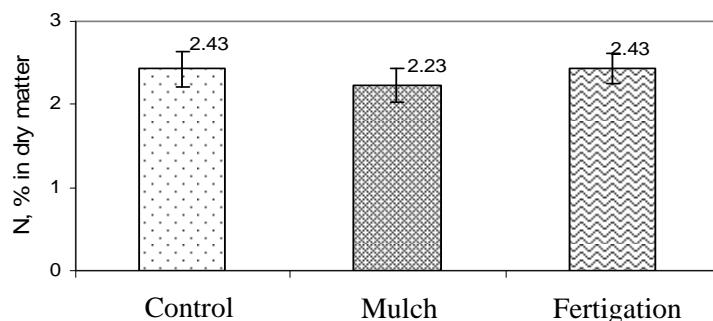


Figure 1. Nitrogen concentration in grass of alleyway depending on the treatment in the tree strips.

However, when using mulch treatment in the tree strips, the nitrogen concentration in the grass of the alleyways was only 8% lower than in other places that were treated. The difference is statistically significant ($P < 0.05$). The significantly lower nitrogen content may be explained by the fact that the nitrogen which is used by micro-organisms during decomposition of sawdust for their life functions has not yet been fully released and the immobilization process continues. When apple cultivar ‘Melba’ on dwarf rootstock B 9 root distribution was investigated (Surikova et. al., 2008), it was noticed that roots of the grass lawn of the alleyway reached the tree strips; depending on the treatment in strips, that may explain some results of the grass investigation. It is known also that if the organic matter at the beginning of decomposition has a proportion of C/N up to 20, then mineralization exceeds immobilization, but if this proportion is more than 30, then immobilization dominates

over mineralization (Wickramasinghe et al., 1985). In sawdust, depending on its origin (deciduous or coniferous tree species), the C/N proportion may reach as much as 400 (Shengzuo et al., 2008), so it is possible that the decaying process of the sawdust has not finished.

Nitrogen concentration in the grass of the alleyways was the lowest during the first mowing time (Fig. 2). At the second mowing tendencies were observed for the nitrogen concentration in the grass of the alleyways to increase, but no significant differences were found between the first and second time ($P > 0.05$). At the third grass mowing the nitrogen concentration in the grass of the alleyways of the control treatment increased by 19%, near the mulch treatment by 23%, but near the fertigation treatment by 25%; the differences were significant ($P < 0.05$).

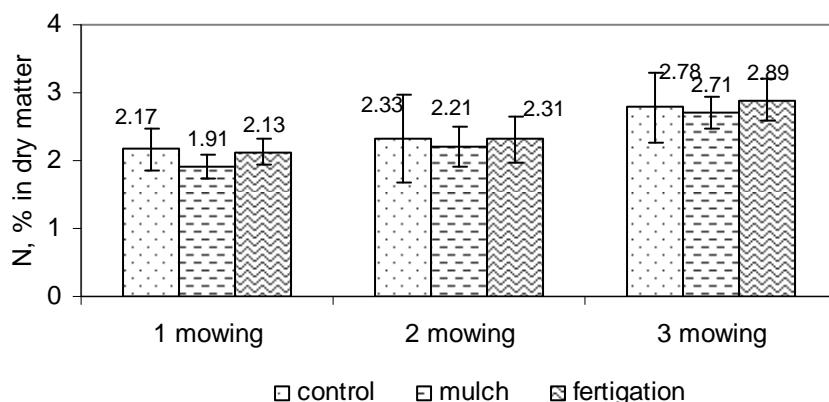


Figure 2. Nitrogen concentration in grass of alleyway depending on the treatment in strips and mowing time.

Yet there is not clear evidence that the nitrogen concentration in grass of the alleyways was influenced only by the mowing time. Theoretically, nitrogen concentration in plants should decrease during the growth season (Nurzinski et al., 1990), but in this study the concentration increased. The contradiction may be explained by the fact that the grass was not in the same stage of development at each mowing time. In the lawn cut on May 20 the cereal grasses were already at the beginning of bloom, while on August 11 the lawn was at a much earlier stage of development. According to several studies (Burke & Morris, 1993), the concentration of nitrogen is higher in young annual plants and plant parts. Nitrogen in plants has a high reutilisation capacity. Depending on the age of the annual plant, as much as 70–80% of N in the plant is repeatedly utilised (Adamec, 2002). It is possible that in the control treatment (resulting from a periodic lack of moisture) the changes of N concentration and the reutilisation process were slower. This may explain the relatively high dispersion of data in the control treatment. The nitrogen concentration could also be affected by air temperature and precipitation during the growth of grass, the proportion of legumes in the grass of the alleyway, along with other factors, but at all mowing times a tendency is noted for a reducing influence of mulch on nitrogen concentration in the grass.

Although nitrogen concentration in the grass of the alleyway near the control and fertigation treatments was significantly higher than near the mulch treatment (Table 1),

the total uptake of nitrogen with mowed grass in the control treatment remained the lowest and was significantly different from nitrogen uptake near the mulch and fertigation treatments in the tree strips, where uptake was higher by 6 and 20% ($P < 0.05$). Such differences were observed because the biomass of mowed grass significantly differed among treatments near the alleyway and mowing times. Precipitation from the beginning of the growth till first mowing on May 20 was only 9.3 mm, so, in the fertigation and mulch treatments with better soil moisture, the grass alleyways biomass was bigger. Still, these results contradict findings of other researchers, that plant biomass significantly increases with the use of fertigation, not with mulch (Nicholas, 2004). The contradiction may be explained by the fact that the effect of fertigation may become evident at a later stage of the growth period. This is witnessed also by the results of the present investigation, because the effect of fertigation on grass biomass was proven only for the 2nd and 3rd mowing time. Grass mown on August 11 no longer showed significant differences among treatments. ($P > 0.05$), because the amount of precipitation increased.

Table 1. Grass biomass and nitrogen uptake.

Cut	Treatment in tree strips near the alleyway					
	Control		Mulch		Fertigation	
	Biomass, kg ha ⁻¹	N uptake, kg ha ⁻¹	Biomass, kg ha ⁻¹	N uptake, kg ha ⁻¹	Biomass, kg ha ⁻¹	N uptake, kg ha ⁻¹
1	282.40 ^a	6.69 ^a	365.50 ^{b*}	7.68 ^{b*}	359.33 ^{b*}	8.42 ^{b*}
2	443.54 ^b	11.47 ^{c*}	503.39 ^{c*}	12.32 ^{d*}	542.12 ^{c**}	13.93 ^{d*}
3	494.27 ^c	15.20 ^{e*}	503.48 ^c	15.40 ^e	567.69 ^c	18.58 ^{f*}
Per season,	1220.21	33.36	1372.38*	35.40	1469.14*	40.93*

a, b, c, d, e, f, – significantly different within columns ($P < 0.05$)

*– significantly different within rows ($P < 0.05$)

The investigations of the grass of the alleyway in orchards have not been undertaken before in Latvia, so there are no data about the rate of decomposition of mown grass and the return of nitrogen into the turnover, but researchers in other countries (Shengzuo et al., 2007; Tagliavini et al., 2007) have found that nitrogen recycles even 1–2 years after the grass has been cut. In addition, a study (Cazzato et al., 2004) shows that by throwing the mown grass into the tree strips the soil organic matter is significantly supplemented, which has a positive influence on nitrogen turnover and its availability for plants. It could be influenced also by climate differences. However results are only preliminary. They can explain only some tendencies that could be influenced by climate and other uncontrolled situations. The results of this investigation provide a basis for additional research, the results of which might make it possible to establish fertilising plans in Latvia.

CONCLUSIONS

The preliminary results show that the mulch used in tree strips in an apple orchard had a significantly negative influence on the concentration of nitrogen in the dry matter of mown grass of the alleyway as compared with control and fertigation.

Near the mulching treatment the concentration of nitrogen in dry matter of mown grass in alleyway was 2.23%, but was 8% higher near the control and fertigation treatments.

Nitrogen concentration in the grass of the alleyway was significantly influenced by the time of grass mowing.

Nitrogen uptake with mown grass biomass in the alleyway near the control in the tree strips was 33.36 kg ha^{-1} : near the mulch treatment it was higher by 6%, but in the fertigation treatment – by 20%.

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Amounts of nitrogen and carbon returned to soil depending on green manure and the effect on winter wheat yield

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Abstract. The trials were carried out during the 2006–08 growing seasons at the Department of Field Crop Husbandry in the Estonian University of Life Sciences. A field experiment was conducted to investigate the effect of green manure treatments on the yield and yield quality of winter wheat. The total phytomass of leguminous green manures ploughed into soil in 2007 varied from 10.3 Mg ha⁻¹ with the bird's foot trefoil to 13.9 Mg ha⁻¹ with the white sweet clover. The root mass of legumes comprised 37–54% of the total biomass. The amount of carbon applied into the soil with the green material and roots of legumes varied from 4.43 Mg ha⁻¹ to 5.98 Mg ha⁻¹. The amounts of nitrogen were up to 274 kg of N ha⁻¹. The highest wheat yields were attained in treatments with lucerne and red clover as preceding crops. Compared to the N₀ treatment, the extra yield reached 3.26 Mg ha⁻¹ with green manures. Both green manures and mineral fertilizers enhanced the quality of the winter wheat yield, but the results did not vary among different green manures.

Key words: green manure, nitrogen, carbon, grain yield, protein, gluten index, volume weight

INTRODUCTION

One of the key factors in increasing the yield and quality of crops is appropriate manuring. In the present economical situation the sales prices of crops have decreased considerably compared to previous years, whereas the prices of pesticides and fertilizers have risen, leaving the farmers with fewer financial resources. Given the increased prices of mineral fertilizers, leguminous green manure crops have become important organic fertilizers both in organic as well as traditional production due to their ability to bind air nitrogen and carry nutrients (P, K) to deeper plough layers. Biological N fixation is one of the primary sources of N in organic farming (Berry et al., 2002). Some mineral fertilizers used in agriculture can be replaced by green manuring, which reduces the cost of production (Poutala et al., 1994). A high soil N fertility, e.g. from incorporated green manure crops, imply a risk of N leaching (Askegaard et al., 2005). Organic matter content is generally regarded as one of the main indicators of soil quality (Schjønning et al., 2004). Organic matter helps to improve the humus status of soil, thus also improving the soil structure, and physical as well as hydrophysical properties. Abundant application of organic matter into soil has a positive effect on soil biota and the soil's biological activity. Also, the nutrients released from organic matter increase the yield of succeeding crops. Beneficial effects of the preceding crop on water use efficiency and reduction in crop diseases can in some cases account for up to 50% of the yield response of the succeeding crop (Harper et al., 1995).

Green manure crops are most effective in organic farming where the main issues concern the application of nutrients into the soil and growing grains with high-quality properties. One of the major benefits of increasing the soil organic N levels through green-manure crops is an increase in the mid-growing season N mineralisation, which in most cases translates into a higher grain N content (Olesen et al., 2009). In the production of high-quality milling wheat, late manuring with nitrogen is especially important. Under good humidity conditions, the optimum period for late manuring is the heading phase. Manuring stimulates growth in protein content during this period. Nitrogen applied at a later period primarily enhances gluten content in wheat (Järvan et al., 2007). Applying the total amount of nitrogen fertilizer at the beginning of the growing period could result in superfluous vegetative growth, lodging and decrease in the yield and quality of wheat (Brown et al., 2007). With green manure, large amounts of nitrogen are applied into the soil, but nitrogen is released gradually because organic matter is decomposed over a long period of time. With green manure, crops are provided with nitrogen throughout their growing period. Research has shown that 82–84% of the red clover's effect is realized in the first year, and 16–18% in the second year as an after-effect (Vil & Võsa, 2005).

MATERIALS AND METHODS

The trials were carried out during the 2006–08 growing seasons in the Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences (58°23'N, 26°44'E). The size of each test plot was 30 m², with 4 replications. The soil was sandy loam Stagnic Luvisol in the WRB 1998 classification. The mean characteristics of the humus horizon were as follows: C_{org} 1.3–1.4 %, N_{tot} 0.10–0.11%, P 3.3–3.5 mg 100g⁻¹, K 15–17 mg 100g⁻¹.

Plant analyses were conducted at both the Department of Soil Science and Agro-Chemistry of EMU and the Estonian Agricultural Research Centre laboratories. Acid digestion by sulphuric acid solution was used to determine N, P, and K content in plant material. The Dumas Combustion method was used to determine the content of carbon in the plant biomass. The crude protein (CP) concentration in feed was determined using the Kjeldahl procedure. Wet gluten content (WGC) and gluten index (GI) were determined by ISO 21415-2:2006. Yield (Y), 1000 kernel weight (TKW) and volume weight (VW) was calculated as the average of 8 replications (2 from each plot).

The preceding crop in 2005 was spring barley. The field experiment was established in 2006 using the following variants of green manure crops and fertilisation:

Variant A) spring barley (*Hordeum distichon* L.) with undersowings of (i) red clover (*Trifolium pratense*), (ii) lucerne (*Medicago sativa*), (iii) hybrid lucerne (*Medicago media*), (iv) bird's-foot trefoil (*Lotus corniculatus*), (v) white sweet clover (*Melilotus albus*)

Variant B) spring barley with mineral fertiliser rates (i) N₀ – the control variant (ii) N₁₀₀, (with cereal sowing); the same, for 2007.

The succeeding crop winter wheat (*Triticum aestivum*) "Ramiro" was sown at the beginning of September 2007. The seed rate of germinating grains of cereals was 500 m⁻² every year. Green manure crops were sown according to the following norms: red

clover 7.5 kg ha⁻¹, lucerne 6.5 kg ha⁻¹, hybrid lucerne 10 kg ha⁻¹, bird's-foot trefoil 6 kg ha⁻¹ and white sweet clover 18 kg ha⁻¹. In 2006 barley straw was removed. In the beginning of August 2007 the biomass of legumes and barley straw were ploughed into the soil. Samples of the aboveground biomass (0.25 m² from each plot) were taken before harvesting the cereals. The root mass was taken from 0–30 cm in depth (by 10*20 cm frame from each plot), washed, dried and weighed. Biomass from the undersowing samples was separated into leguminous and cereals.

In variants with undersowings (A) the aboveground biomass and the root mass of leguminous crops were measured before ploughing.

The vegetation period of 2006 had a high temperature regime and low precipitation. The first half of the vegetation period (up to 31 July) was very dry, with only half of the average precipitation in Estonia. In 2007, the average temperature was higher whereas the average precipitation was lower than in previous years. The drought reached its peak in August. The average temperature of the 2008 vegetation period was lower than in many previous years. Drought in spring and high average precipitation in August had an influence on the yield and quality of wheat.

The analysis of variance (ANOVA) was used to evaluate the impact of the experimental variants on the yield and yield quality.

The relationship between the C/N ratio (y) and the nitrogen content (x, %) of the organic matter is reflected in the following regression equation:

$$y \text{ (C/N)} = 42.977x^{-1.0035}, R^2 = 0.99; p < 0.000.$$

The objectives of the trial include examining the capacity of the second vegetation year leguminous green manures to form biomass; analyzing the amount of nitrogen and carbon returned to soil, and determining the effect of these factors on the yield and quality of the succeeding crop.

RESULTS AND DISCUSSION

In 2007 barley pure sowings, the amounts of nitrogen returned to the soil with straw and roots were 39 kg ha⁻¹ and 57 kg ha⁻¹ on the background of N₀ and N₁₀₀ respectively. The respective amounts for carbon were 1.83 and 2.62 Mg of C ha⁻¹. The phytomass returned to the soil in barley sowings was 4.26 and 6.10 Mg of dry matter ha⁻¹ on the background of N₀ and N₁₀₀ respectively.

The total phytomass of leguminous green manures ploughed into soil in 2007 varied from 10.3 Mg ha⁻¹ with the bird's foot trefoil to 13.9 Mg ha⁻¹ with the white sweet clover. The phytomass of hybrid lucerne was 12.5 Mg ha⁻¹. The formation of legume mass is influenced by various factors. The trials have shown that red clover is more stable and resistant to unfavourable conditions than other legumes (Talgre, et al., 2009a, 2009b). White sweet clover and lucerne are more sensitive to climatic and agrotechnical factors.

The root mass of legumes comprised 37–54% of the total biomass. The amount of carbon applied to the soil with the green material and roots of legumes varied from 4.43 Mg ha⁻¹ (bird's foot trefoil) to 5.98 Mg ha⁻¹ (white sweet clover). The amount of nitrogen returned to the soil was dependent on the leguminous crop; up to 274 kg of N ha⁻¹ were applied into the soil (Fig. 1) based on the treatment. Earlier research has also

proved that leguminous crops can bind 200–300 kg of N ha^{-1} per year (Viil & Võsa, 2005; Talgre, et al., 2009b). The biological production of green manures, as well as the amounts of nitrogen they bind and the C/N ratio of organic matter vary according to the crop species, soil and farming techniques. The decomposition of organic matter in soil is largely determined by its C/N ratio. The smaller the C/N ratio of organic matter and the greater its nitrogen content, the more nitrogen is released into soil from green manure mineralisation (Kumar & Goh, 2002). The C/N ratio of the applied organic matter varied significantly. The C/N ratio of barley straw and the aboveground biomass of leguminous crops were 65–69 and 20–23 respectively.

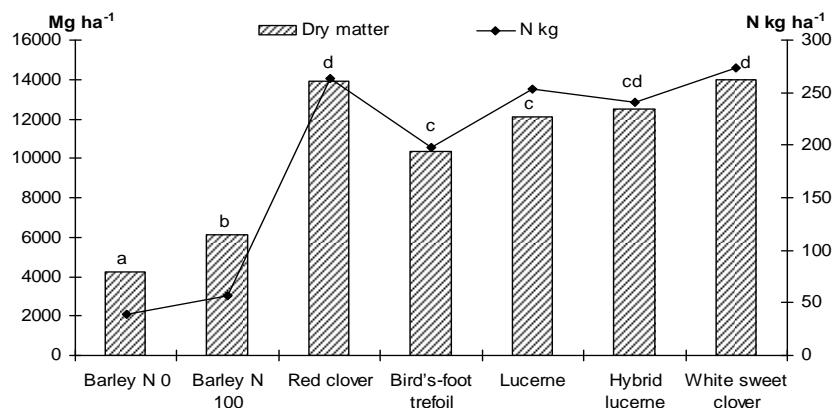


Figure 1. Quantities of dry matter (Mg ha^{-1}) and N (kg ha^{-1}) applied into soil in 2007.

Means followed by the same letter are not significantly different ($P < 0.05$).

In the 2007 trial, winter wheat was sown as a succeeding crop. Despite the drought in spring, the conditions were favourable for the yield formation of winter crops, though the quality of the yield was influenced by the rainy harvesting period. Aside from weather, other factors that may influence the yield of winter wheat are crop variety and nutrient supplies. Traditionally, winter wheat is known by its higher yield potential and spring wheat by better baking quality (Swenson, 2006).

The highest wheat yields were attained in treatments with lucerne and red clover as preceding crops. Compared to the N_0 treatment, the extra yield reached 3.26 Mg ha^{-1} with green manures. After the use of bird's foot trefoil, the yield was equal to the treatment in which 100 kg of mineral nitrogen had been applied (Fig. 1). Also Maiksteniene and Arlauskiene (2004) show that the highest wheat yield is attained when wheat is grown after lucerne as a preceding crop, the yield being 18.5% higher than after clover. Higher grain yields are usually associated with lower protein concentration (Blackman & Payne, 1987). Protein is a primary quality component of cereal grains. Protein content can be increased with higher nitrogen fertilizer norms (Peterson, 1976). In the present trial, protein content increased compared to the N_0 treatment, but remained lower than the protein content of wheat (13–15%). The protein content of wheat grains was 11.7–12.8% on the background of green manures, and had a lower level in the treatment where hybrid lucerne and bird's foot trefoil had been grown as preceding crops. Protein content is positively correlated with wet gluten content (Fredericson et al., 1998) which is strongly influenced by the growing

environment (Grausgruber et al., 2000). The trial also showed that the wet gluten index increased according to a rise in protein content. One of the most used criteria of wheat quality is volume weight, which is an indication of the density and soundness of the wheat. Volume weight is influenced by many factors, including fungal infection, insect damage, kernel shape and density, agronomic practice and the climatic and weather conditions (Gaines et al., 1997). In the trial, the volume weight remained low. After the application of green manures, the volume weight of all treatments was higher than the volume weight of the N₀ treatment (Table 1).

Table 1. Yield and yield quality of winter wheat *Ramiro* depending on preceding crop.

Preceding crop	Y (Mg ha ⁻¹)	TKW (g)	VW (gl ⁻¹)	CP (%)	WGC (%)	GI (%)
Barley N0	2.89	29.8	753	9.4	x	x
Barley N ₁₀₀	5.78	40.0	796	12.9	30.8	55
Red clover	5.95	40.3	801	12.2	23.3	88
Lucerne	6.15	40.8	802	11.9	23.0	87
H. lucerne	5.98	40.6	800	1.7	22.8	92
Sweet clover	5.39	39.8	800	12.2	24.6	98
Bird's foot trefoil	5.78	40.4	799	11.8	22.5	91
<i>LSD</i> _{0.05}	582	1.4	5.3			

x – non-washable

Kernel weight, usually expressed in grams per 1000 kernels, is a function of kernel size and density. Kernel weight increased in all treatments compared to the N₀ treatment, but there was no plausible difference between green manure treatments.

Wet gluten, obtained by mixing flour and water, increases the volume of bread. Grains should contain at least 26% of wet gluten, with the best wet gluten content being 28–29%. Wet gluten content in grains was increased both with mineral fertilizers as well as on the background of the after-effect of green manures, but remained below the norm in all treatments. Kangor et al. (2007) have also shown that wet gluten content increases both with root as well as foliar fertilization.

Gluten index is used to measure the quality of wet gluten: the optimum index is 60–90 and the satisfactory index, 41–59. In treatments where green manures were grown as preceding crops the gluten index rose by 33–43 units as compared to the N₁₀₀ treatment. The increased gluten index is an indication of the higher quality of wet gluten and enhanced baking properties.

CONCLUSION

The biological production of green manures, as well as the amounts of nitrogen they bind and the C/N ratio of organic matter, vary according to the crop species. The highest phytomass and amount of nitrogen returned to soil was obtained by growing white sweet clover and red clover; the lowest respective results were obtained with bird's foot trefoil. The winter wheat yield was lower after white sweet clover than after other leguminous preceding crops despite the highest amount of nitrogen applied into the soil. This is probably due to the slower decomposition of white sweet clover. The highest wheat yields were attained in treatments with lucerne and red clover as

preceding crops. Both green manures and mineral fertilizers enhanced the quality of winter wheat yield, but the results did not vary among different green manures.

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Control possibilities of *Apera spica-venti* (L.) P.Beauv. in winter wheat with autumn and spring applications of herbicides in Latvia

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Abstract. This paper presents results on weed control and yield responses in winter wheat grown after winter oilseed rape and after winter wheat, using data from field trials with a range of herbicides registered for use in Latvia that were applied either in the autumn or in the spring. *Apera spica-venti* was the dominant weed in these trials, accounting for 70–80% of the total weed biomass. Spring application of herbicides did not provide good control of *Apera spica-venti* up to harvest time: the infestation at application time was more than 140 plants per m². Autumn application of appropriate herbicides gave satisfactory control of *Apera spica-venti* up to harvest time in the following year. All herbicide treatments significantly increased crop yield but the autumn applications gave significantly greater increases than nearly all spring applications.

Key words: *Apera spica-venti*, winter wheat, yield, herbicide application in autumn and in spring

INTRODUCTION

Economic pressures have led many arable farmers in Latvia and other countries of northern Europe to adopt crop rotations based on the sequence of winter oilseed rape followed by winter wheat or successive crops of winter wheat. From 2000–2008 the areas of winter wheat and oilseed rape in Latvia increased annually: winter wheat, from 117.4 to 170.4 thousand ha; oilseed rape from 6.9 to 82.6 thousand ha. On the larger farms the main crop rotation is now based on winter wheat and winter oilseed rape.

These rotations have brought about changes in the weed flora such that *Apera spica-venti* is now an important target for control and serious yield losses have occurred in winter wheat crops where the *Apera spica-venti* has not been controlled effectively. For example, Bartels (2004) found a grain yield loss of 3 t ha⁻¹ in untreated plots which were infested with 200 *Apera spica-venti* plants m⁻² compared to treatments providing successful grass weed control. Increased infestations in winter cereals have also been reported by Danish researchers: Andreasen & Stryhn (2008) and Melander et al. (2008). In a ranking of the 15 most important weed species found in winter cereal crop systems in 26 European countries, *Apera spica-venti* was ranked fifth among all weeds and first among grasses (Schroeder et al., 1993). Weed population in treatments without herbicide application reveal the efficiency of weed management in previous years. Weed populations were more influenced by the preceding crop and by the timing of herbicide application than by the tillage system

(Streit et al., 2003). To improve the efficiency of weed control and to reduce reliance on herbicides in cropping systems with reduced tillage intensity, Streit and his co-workers stated that further research was needed to determine the beneficial effects and the optimization of crop rotation and appropriate weed control.

In recent years the average air temperatures in Latvia, during both the autumn and the spring-summer growing periods, have generally been higher than the long-term averages. These warmer temperatures have provided improved conditions for the germination and development of weeds in autumn-sown crops.

Cereals are most sensitive to competition from weeds in their early stages of growth and, especially in autumn sown crops, grass weeds can be extremely competitive in the early stages, particularly in cereals established with reduced cultivations (Tottman et al., 1982). Autumn application of herbicides will control weeds that could survive during winter to affect winter wheat growth and will provide better conditions for competition by the crop when vegetative growth begins in spring (Pilipavičius et al., 2010). Competition from autumn germinated broad-leaved weeds is generally less severe than that from grass weeds (Tottman et al., 1982). The most favourable application timing for grass weed control is between the 3-leaf stage and the beginning of stem elongation (Hacker et al., 1999). The importance of herbicides that can control grass weeds and dicotyledonous weeds by autumn application is described by Brink & Zöllkau (2004).

To control the yield-reducing weeds, wheat growers in Latvia have used a variety of herbicides applied either in autumn or in spring. During the past five years the range of new herbicides intended for autumn application has increased. This could improve the possibilities to control the weeds in the autumn and so avoid the greater influence on grain yield.

MATERIALS AND METHODS

Field experiments were established in the Jelgava municipality in 2006–07 and 2007–08 in winter wheat. The evaluations of herbicide efficacy in the cereals followed EPPO Guideline PP 93 (2). Field trials were arranged in randomized blocks with 4 replicates. Plot size: 30 m² (3 m x 10 m).

In 2007 the sod-calcareous clay soil in the field had pH_{KCl} 7.2, an organic content of 2.3%; winter wheat cultivar ‘Zentos’ was sown on 05.09.06. The previous crop was winter oilseed rape. In 2008 the sod-calcareous loamy sand soil in the field had pH_{KCl} 6.1, an organic content of 1.8%; winter wheat cultivar ‘Tarso’ was sown on 09.09.07. The previous crop was winter wheat. In both fields minimal soil tillage was employed and a fertiliser top-dressing of 120–170 kg ha⁻¹ was applied.

The herbicides used in the trials were: Alister Grande OD 217.5 (iodosulfuron-methyl-sodium 4.5 g L⁻¹ + mesosulfuron-methyl 6 g L⁻¹ + diflufenican, 180 g L⁻¹, Bayer CropScience); Hussar Activ OD 417 (iodosulfuron-methyl-sodium 10 g L⁻¹ + 2,4-D 377 g L⁻¹ as 2-ethyl-heyl ester, Bayer CropScience); Monitor (sulfosulfuron 750 g L⁻¹, Monsanto); Arrat (tritosulfuron 25% + dicamba 50%, BASF). The surfactant Kemiwett Plus (alcohol ethoxylate) was added to the tank mix of Monitor and Arrat.

Herbicide treatments were applied using a knapsack sprayer “Gloria” with flat-fan nozzles XR TEEJET 8003VS, delivering a spray volume of 300 L ha⁻¹ at a pressure of

300 kPa. Other plant protection measures were applied to the whole trial as necessary.

For the assessment for *Apera spica-venti*, the flowering panicles numbers were recorded at three random places within each plot with the aid of a 0.25 m² frame: when the growth stage of the crop was 86–87 BBCH on 25 July 2007 and 81–83 BBCH on 15 July 2008 (Tables 1, 2).

The total yield of grain from each plot was harvested by trial combine “Sampo 500”, respectively on 8 August 2007 and 30 July 2008, recalculated to t ha⁻¹ and given at 100% purity and 15% moisture content (Tables 1, 2).

To determine the relationship between the yields of winter wheat and the numbers of *Apera spica-venti*, the flowering panicles, the data from the untreated plots and the plots treated with herbicides were subject to analyses of variance and regression analysis. For statistical analysis the data were subjected to single factor analysis of variance using GenStat for Windows version 12. The treatment means were separated at the 95% probability level (LSD) using Student's *t*-test. Significant differences are stated next to the relevant figures in the tables: treatments marked with the same letter are not significantly different.

Weather conditions in 2006–2007 and 2007–2008. In autumn 2006 the herbicide treatments were applied on 26 September. During the third 10-day period of September the weather was unusually warm: the air temperature was more than 3 °C above the long-term norm and without precipitation. The weather continued to be warmer than average throughout October and there was sufficient precipitation to provide very good conditions for weed growth after the autumn herbicide application (Figs 1, 2).

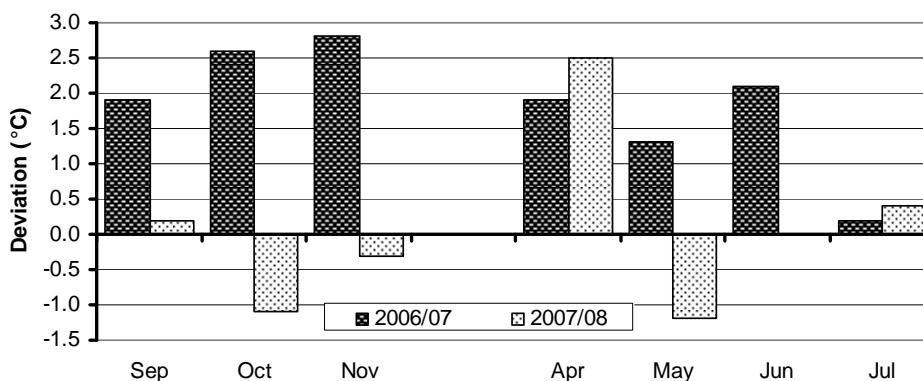


Figure 1. Deviations of monthly mean temperatures from long-term monthly means during growing seasons 2006–07 and 2007–08; data from Jelgava HMS.

In spring 2007 the weather around application time in the third 10-day period of April was unusually warm: the air temperature was more than 4 °C above the norm, but precipitation was only 36% of the norm for that period. Two rainy days occurred before herbicide application (on 21 and 23 April) and that provided moist soil conditions. During the remainder of the vegetative period, up to the end of July, the weather was warmer than the long term-averages with sufficient precipitation in almost all months for good growth (Figs 1, 2).

The autumn 2007 herbicide treatments were applied on 28 September. The weather during the first and second 10-day periods of September was cooler than the

long-term averages and some very rainy days occurred during the second 10-day period. Overall the daily mean temperature for the month was slightly above the long-term average and the precipitation was only 56.5 % of long-term average (Figs 1, 2). Around application time in spring 2008, during the second 10-day period of April, the mean daily temperature was higher than the long-term average and there was sufficient precipitation for plant growth. Overall, the weather during the vegetative period was drier than average which affected crop and weed emergence and development unfavourably.

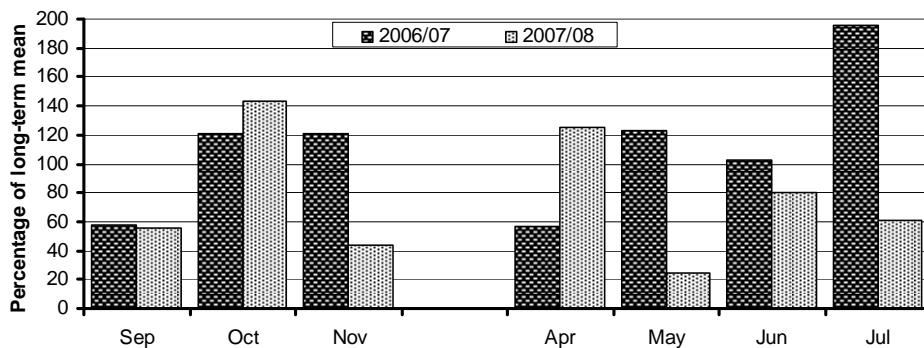


Figure 2. Total monthly precipitation as percentage of long-term monthly mean during growing seasons 2006–2007 and 2007–2008; data from Jelgava HMS.

RESULTS AND DISCUSSION

In autumn 2006 the emergence of the wheat plants was even and the plants were well developed, at 14–15 BBCH stage, when the autumn herbicide treatment was applied. The infestation of *Apera spica-venti* within the trial was 32 plants m⁻², up to 2 leaf stage (3–4 cm). In spring 2007 the winter wheat was at the end of the tillering stage (28–29 BBCH) when the spring herbicide treatments were applied and the density of *Apera spica-venti* in the untreated plots was 142 plants m⁻², mostly at 8 cm in height and well developed.

In autumn 2007 the winter wheat was at the 13–14 BBCH stage when the autumn herbicide treatment was applied. The growth stage of *Apera spica-venti* was up to 2 leaf stage and the infestation was very high: more than 430 plants m⁻². The spring herbicide treatments were applied on 16 April 2008 when the *Apera spica-venti* plants were at the tillering stage (12 cm in height) and the infestation was still very high: more than 424 plants m⁻².

In both of these winter wheat experiments dicot weed species were also recorded; the main species were: *Viola arvensis*, *Thlaspi arvense* and *Centaurea cyanus* as well as volunteer oilseed rape (*Brassica napus*). The biomass of the dicot weeds was only 20–30% of the total weed biomass. Most of the weed biomass was accounted for by *Apera spica-venti* and the high infestation of this grass species suppressed the growth of the dicot weeds.

The efficacy of the herbicide treatments in controlling *Apera spica-venti* was evaluated by counts of flowering panicles close to harvest time. All the herbicide treatments gave significant reductions in *A. spica-venti* panicle numbers compared with

untreated (Tables 1, 2). Control of *Apera spica-venti* was satisfactory (95–96%) only in the plots where the herbicides had been applied in the autumn. The performance of the herbicides on *A. spica-venti* in reducing the numbers of flowering panicles was the same in both years.

Table 1. Numbers of *Apera spica-venti* and winter wheat yields, 2006–2007.

Treatments, dose per ha	Numbers of <i>Apera spica-venti</i> flowered panicles		Grain yield of winter wheat	
	number m ⁻²	decrease, %	kg ha ⁻¹	± kg ha ⁻¹
1. Untreated	190.0 a	-	1858 a	-
2. Alister Grande, 0.8 L*	8.3 c	96	5596 c	3738
3. Hussar Activ, 0.8 L **	79.3 b	58	3259 b	1401
4. Hussar Activ, 1.0 L **	68.0 b	64	2587 ab	729
5. Monitor, 0.018 kg + Arrat, 0.2 kg**	46.7 bc	75	3324 b	1466
LSD 5%	58.06	-	1031.4	-

* in autumn 2006 ** in spring 2007

The average yield in the control plots was very low especially in 2007 (only 1858 kg ha⁻¹), because of the very high infestation of *Apera spica-venti*. Despite the moderate weed control in the herbicide treatments applied in spring, all treatments increased grain yield statistically significantly (Tables 1, 2). The increases given in the treatments where the herbicide had been applied in autumn were significantly higher than all but one of the increases where the herbicides had been applied in spring. The application with herbicides in spring could reduce the infestation of *Apera spica-venti*, but the crop stands were not as dense as in treatments where the herbicide application was made in the autumn. In weather conditions that were favourable for weed germination and development during the vegetative period, new weeds emerged and competed with the winter wheat and gave rise to problems with green weed material at harvest.

Table 2. Numbers of *Apera spica-venti* and winter wheat yields, 2007–2008.

Treatments, dose per ha	Numbers of <i>Apera spica-venti</i> flowered panicles		Grain yield of winter wheat	
	number m ⁻²	decrease, %	kg ha ⁻¹	± kg ha ⁻¹
1. Untreated	204.0 a	-	2606 a	-
2. Alister Grande, 0.8 L*	10.3 c	95	4732 c	2126
3. Hussar Activ, 0.8 L **	88.3 b	57	3576 b	971
4. Hussar Activ, 1.0 L **	84.0 b	59	3858 bc	1252
5. Monitor, 0.018 kg + Arrat, 0.2 kg**	62.0 bc	70	3403 ab	797
LSD 5%	57.90	-	961.2	-

* in autumn 2007; ** in spring 2008

In both experiments there was a strong, significant, negative linear relationship between winter wheat yield and the numbers of *Apera spica-venti* flowered panicles (respectively $r_{yx} = -0.73$, $P < 0.001$; $r_{yx} = -0.64$, $P < 0.01$). The determination coefficients showed that 54% of the variations in the grain yield in 2007 and 41% in 2008 could be explained by the infestation of *Apera spica-venti* in the trial plots.

CONCLUSIONS

1. The application of herbicides in spring could not provide good control of *Apera spica-venti* up to harvest time when the infestation of this weed species at the application time was very high (more than 140 plants per m²).
2. Application of appropriate herbicides in autumn for *Apera spica-venti* control provided satisfactory control up to harvest time in the following year.
3. The winter wheat crop in plots where herbicides were applied in the autumn was more even, denser and better developed than in plots where herbicides were applied in the spring. In the spring-treated plots the crops became thin and in open places, weed plants that were not controlled by the herbicides could regrow and develop well during the growing season up to harvest time.
4. For the best yield results herbicides should be applied in the autumn, especially when the weather is favourable for prolonged development of weeds and the infestations of competitive weed species like *Apera spica-venti* are very high.

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The impact of a farm's annual cattle slurry yield on the options for moving the slurry from stable to plot: a simulation study

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Abstract. The economical efficacy is substantial on both occasions for feeding plants with nutrients and moving the manure from stables to the plots. The aim of the present research is to explain the limit values for the annual amount of slurry and average plot distance on a farm as conditions to decide in favour of a personal eco-friendly slurry distributor or custom equipment. In their previous researches, the authors have composed models to calculate slurry management costs for different technologies depending on plot distance, taking into account ammonia emissions. In the present study, simulations were made using the composed calculation models to compare slurry distribution costs for four slurry application technologies.

Calculations show that if the annual amount of slurry exceeds 4000 m³, then for plot distance over 2 km, custom slurry distribution is cheaper than using the farm's own equipment. However, if the annual quantity of slurry exceeds 16,000 m³, then the limit value for distance is 5 km.

If the annual amount of slurry is 4000 m³, then full custom service is cheaper than the technology in which the farm's own slurry distributor and custom transportation is used. In the case of the annual amount of 16,000 m³, it is less expensive to use the farm's own slurry distributor and custom transportation. In order to benefit from the use of the farm's own distributor the minimum value for annual slurry amount is 5600 m³.

Key words: ammonia emissions, slurry application technology, plot distance, performance, operation costs, custom machines, annual slurry amount

INTRODUCTION

On the basis of environmental impacts in agricultural production, the following pollution subdivisions can be distinguished: point pollutants (animal farming, manure storages, etc) and diffuse pollution (e.g., pollution from manure distribution in the fields) (Dämmgen *et al.*, 2007). Leakage of farmyard stores and runoff following slurry application to the land can lead directly to losses of organic matter, nutrients and pathogenic micro-organisms, with potential consequences for both stream ecology and human health (Naden *et al.*, 2009). These diffuse losses have mainly been characterised in terms of nutrients (Vadas *et al.*, 2007).

Ammonia volatilisation can be a major source of N losses from applied slurry (Lewis *et al.*, 2003). Ammonia emission has been studied in several countries. The emission is magnified by higher air temperature during the spreading, wind (Misselbrook *et al.*, 2005), higher pH, content of solid matter and ammonium nitrogen of the slurry (Mattila, 2006), as well as by high soil pH and temperature (Sommer *et al.*, 2003; Misselbrook *et al.*, 2005) and low soil moisture (Jokela & Meisinger, 2008).

Although gas emission, leaching of nutrients and odour have undesirable effects on the environment, the contribution of manure to plant nutrition and build-up of soil organic matter is considered to have a positive effect.

To utilise the nutrients contained in manure and minimise air pollution, it is essential to apply technology suppressing the gas emission from slurry distributed on the field. In the Defra (2006) project, the impact of different spreading devices on the ammonia emission was compared in the UK, Germany, Denmark and Finland. The average values of reduction of ammonia emission compared to technology where slurry was broadcast-spread and not incorporated from that research are as follows: trailing hose 32%, trailing shoe 60%, open slot injection 67%, closed slot injection 82% and deep injection 86%. By IPCC (2007) the ammonia emission factors for different application technologies are the following: 70% for broadcast spreading, without incorporation, 20% for spreading with a trailing hose, 10% for spreading with an open slot shallow injector and 1% for spreading with a closed slot. The effect of the use of slurry depends also on the time-lag between spreading and incorporation. The time-lag depends *inter alia* on the distance to the manure storage if incorporation is consecutive (one-man system) (Huijsmans and de Mol, 1999). Paudel *et al.* (2009) determined by a GIS-based model a least-cost dairy manure application distance for Louisiana's major dairy production area. A comparison between the dairy manure and commercial fertilizer application under three consistent rules – N, P₂O₅, and K₂O – revealed that the use of dairy manure is not economical after 30 km for N and 15 km each for P₂O₅ and K₂O.

Plant nutrient overloads can result from several forms of mismanagement, including over-fertilisation of crops (Gerber *et al.*, 2005). The objective should be to apply slurry to match the needs of the crop both in terms of amount and timing, attempting to minimise nutrient losses while maintaining adequate yields. Nutrient absorption by soil and plants is a complex of factors including soil, climate conditions, season and plant species (Lewis *et al.*, 2003).

In order to decrease excessive application of nutrients, it is not advisable to use more manure than the soil and yield properties allow. The herd size determines proportionally the area needed for distributing the manure produced by animals. However, the larger the areas, the longer are the average manure transportation distances (Tamm, 2009). The farm's annual slurry quantity and transportation distance as the selection criteria of slurry application technology should be explained. Schindler (2009) has published data for choosing the machines for the slurry delivery chain depending on those criteria in average production conditions of Germany with labour cost 16 €ha⁻¹ and fuel price 1.45 €l⁻¹. In Estonia these values are 3.8 €ha⁻¹ and 0.58 €l⁻¹, respectively. Thus, the German data are not applicable to Estonia and no literature is available with similar data for Estonia. The equipment for slurry application can be the farm's own or rented from a service provider. There are no data published about a farm's annual slurry quantity as a decision criterion to choose one's own or custom machinery.

Therefore, the present paper compares slurry distribution costs considering a farm's annual slurry quantity and average transportation distance in the case of four technological approaches for average Estonian production conditions:

- 1) incorporating disc device – the slurry is simultaneously distributed and mixed with soil;
- 2) incorporating disc device as in variant no. 1, but the slurry is transported to the

- 3) slurry spreading by trailing hose spreader plus a separate operation to incorporate the slurry to the soil; and
- 4) custom slurry distribution: slurry is transported by tank trucks to the plot and distributed with a self-propelling and incorporating slurry distributor.

The results from this study are considered to be targeted for slurry producers, to enhance their knowledge of the impact of the farm's annual slurry quantity and plot distance on the technological options.

MATERIALS AND METHODS

In calculations, it was presumed that manure comes from the farm's own production and the only costs arise from transportation and distribution. The calculation model is composed by the authors and has been previously published (Tamm & Vettik, 2008). The model contains components from the method, applied to evaluate options for exploitation of a plot considering costs depending on plot distance (Tamm, 2009). The prices of fuel and custom works used in calculations are from summer 2009. The prices of machines are collected from KTBL (2008).

Four simulated cases for slurry handling have been studied. A description of the technological sequence for slurry handling is as follows:

- 1) mixing – pumping from storage into the distributor tank – transporting with distributor to the plot – distribution and mixing with soil simultaneously;
- 2) mixing – pumping from storage into the custom truck tank – transporting with truck to the plot – pumping from the truck tank into the distributor tank – distribution and mixing with soil simultaneously;
- 3) mixing – pumping from storage into the distributor tank – transporting with distributor to the plot – distribution onto the soil with trailing hoses – separate operation to incorporate the slurry to the soil; and
- 4) mixing – pumping from storage into the custom truck tank – transporting with truck to the plot – pumping from truck tank into the custom distributor tank – the custom distributor tank distributes and mixes slurry with soil simultaneously.

Before slurry transportation and its distribution for slurry mixing and pumping 15 kW electrical device with performance $4.5 \text{ m}^3 \text{ min}^{-1}$ (price is 4605 €) is applied. From the observations of ERIA researchers, the slurry should be mixed the entire time the distribution lasts. On the plot, the distributor's own pump is used for over-pumping.

In all technological variants the distributor has a tank with 15 m^3 volume, fuel price is 0.58 €l^{-1} and labour cost is 3.8 €h^{-1} . The distributor used in variants 1 and 2 is equipped with a 4.5 m wide disc device (price of distributor is 52,560 €); tractor power is 158 kW (price is 102,560 €). The distributor used in variant 3 is equipped with a 12 m wide trailing hose spreader (price for whole system is 42,200 €) and the tractor engine power is 102 kW (price is 76,730 €). In variant 4, a custom self-propelled distributor equipped with a 4.5 m wide disc device is used with the engine power of 246 kW. The price of custom work with this distributor is 2.2 €m^{-3} .

If custom work is used only for transportation of the slurry to the field (variants 2 and 4), then the tanker lorry with initial cost 1.3 €m^{-3} is rented. If the distance exceeds 7 km, then 0.07 €m^{-3} per every extra km must be added to the initial cost.

In the 3rd technological variant a field-operation-unit containing a 158 kW tractor and a 4 m wide disk harrow (price is 31,950 €) to mix slurry with soil is used. The time span between slurry distribution and mixing with soil may not exceed 4 h.

Ammonia emission factors used for technologies are as follows: 20% for spreading with a trailing hose (variant 3) and 5% for incorporating the disc device (as the average value between values for spreading with an open slot shallow injector and for spreading with a closed slot) (variants 1, 2 and 4) (IPPC, 2007).

The annual work capacity for the spreader is 4000 m³ and 16,000 m³. The slurry rate was 40 m³ ha⁻¹ and the plot area was 20 ha for all technological variants. The operations are considered to be performed before the cereal is sown.

RESULTS AND DISCUSSION

Simulations were made using composed calculation models to compare slurry distribution costs for four slurry application technologies considering the farm's annual slurry quantity and distance to the plot. The results for technological variant 1 (farm's own soil mixing disc device) and 4 (custom slurry distributor) are shown in Fig. 1.

Fig. 1 indicates that if the annual quantity of slurry exceeds 4000 m³, then for a plot distance over 2 km, custom slurry distribution is cheaper than the use of the farm's own equipment. Slurry management costs for 2 km and 4000 m³ is 3.5 €m⁻³ both in the case of variant 1 and 4. However, if the annual quantity of slurry exceeds 16,000 m³, then the limit value for distance is 5 km. For variant 1, slurry management costs for 5 km distance are 4.7 €m⁻³ and 3.5 €m⁻³ for annual slurry amounts 4000 m³ and 16,000 m³, correspondingly. The greater the annual amount of slurry, the cheaper is management of the slurry per m³; Huijsmans *et al.* (2004) got similar results. However, a greater amount of slurry needs a larger distribution area, which requires a longer distance and a greater cost for slurry transportation.

Dr. Schindler (2009) has published data for a slurry distributor with a 16 m³ tank and a 6 m wide slot injector. If the distance to the plot is 2 km, the plot area is 10 ha, and the farm's annual quantity of slurry is 4,800 m³, then the slurry distribution cost is 4.85 €m⁻³. For 5 km, this cost is 6.43 €m⁻³. The higher costs brought out by Schindler compared to our figures are probably induced by a more expensive distributor (it is wider and has a somewhat bigger tank, requiring a more powerful tractor), higher labour cost and fuel price.

The calculations show that distribution is cheaper (ca 0.64 €m⁻³) in the case of the trailing hose spreader (variant No. 3), because of the greater work width and cheaper machine price; Huijsmans *et al.* (2004) and Schindler (2009) had analogous results. Considering the impact of the art of distribution of slurry on the loss of nitrogen by ammonia emission it is essential to incorporate slurry into the soil on arable land. The slurry incorporation performed for diminishing the ammonia emission is a separate operation with a cost of ca 25.6 €ha⁻¹. This result is the same as by using an incorporating spreader and, therefore, the results are not presented separately in the figure.

If the slurry distributor is used for slurry distribution only, then the custom tank lorry is used for transporting the slurry to the plot (variants 2 and 4); results are presented in Fig. 2. The eco-friendly slurry application equipment is expensive; therefore, it is most effective to use these machines for distribution, rather than for the

transportation of slurry (Tamm, 2009). Thus, the separate vehicles with slurry tanks should be used to transport the slurry to the plot especially for longer distances. In Estonian conditions the maximum distance for transporting the slurry by distributor itself to the plot is about 4 km (Tamm & Vettik, 2008).

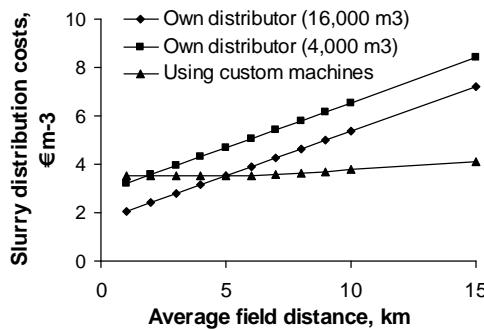


Figure 1. Slurry distribution costs in the case of farm's own distributor and using custom machines.

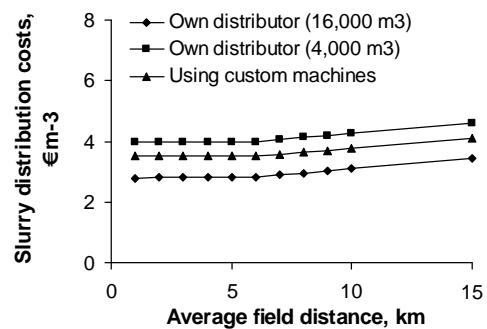


Figure 2. Slurry distribution costs if custom tank lorry is used for transportation and spreading is performed by farm's own distributor or custom distributor.

Figure 2 demonstrates that full custom service (variant 4) will be cheaper than the farms' own slurry distributor and custom transportation (variant 2), if the annual amount of slurry is 4000 m³. If the annual amount is 16,000 m³, then it will be less expensive to use the farm's own slurry distributor and custom transportation. For variant 2, slurry management costs for 5 km distance are 4.0 €m⁻³ and 2.8 €m⁻³ for annual slurry amounts of 4000 m³ and 16,000 m³, correspondingly. In order to benefit from the use of the farm's own distributor the minimum value for annual slurry amount is 5600 m³ by our calculations. Sørensen *et al.* (2003) report that use of distributors with a large tank volume is rational when the annual slurry amount exceeds 9000 t. If that amount remains under 3000 t, it is not at all profitable to own a distributor; the custom distribution is cheaper.

CONCLUSIONS

Before investing in eco-friendly but expensive slurry distribution technology, the farmer has to calculate whether his farm has enough slurry to ensure a lower work price than custom service. The calculations show that, in the conditions used in our simulations, the minimum value for annual slurry amount is 5600 m³ to own a distributor. We also found that the distribution cost in the case of a trailing hose spreader with an extra operation for soil mixing is equal to the distribution cost of incorporating a disc distributor. In the first case the additional time and labour should be taken into account for the soil-mixing operation. The ammonium emission is also somewhat higher than for other technologies compared in the present study. For longer distances to the plot, the farmer should consider hiring a custom tank lorry for slurry transportation, and the farm's own distributor should be used only for distribution on the plot.

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Example:

A study of synergistic effect of greater wax moth *Galleria mellonella* (Lepidoptera: Pyralidae)

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²Institute of ...

Abstract. In laboratory pupal...

Key words: *Galleria mellonella*, respirometry, synergistic interaction

INTRODUCTION

In many countries rural inhabitants for insect control use various local plants (Smith & Jones, 1996; Brown et al., 1997; Adams, 1998).

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In case of **two** authors use **&**. In case of more than two authors, reduce to first author "et al."

Smith & Jones (1996); (Smith & Jones, 1996)

Brown et al. (1997); (Brown et al., 1997)

Adams (1998); (Adams, 1998)

When referring to more than one publication, arrange them using the following keys: 1. year of publication (ascending), 2. alphabetical order for the same year of publication:

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Shiyatov, S. G. 1986. *Dendrochronology of the Upper Timberline in the Urals*. Nauka, Moskva, 350 pp. (in Russian).

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Use ‘.’ (not ‘,’) : 0.6 ± 0.2

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Without space: 5°C, 5% (not 5 °C, 5 %)

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Spaces: 5 h, 5 kg, 5 m, C : D = 0.6 ± 0.2

Use ‘kg ha⁻¹’, (not ‘kg/ha’)

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