Predicting Malting Barley Protein Concentration

Based on Canopy Reflectance and Site Characteristics

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Abstract

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The preferred grain protein concentration (CP) of malting barley is 10.5-11.0%, but 9.5-11.5% is acceptable. It is a challenge for farmers to achieve this target with crops grown in heterogeneous fields and exposed to fluctuating weather conditions. There are also economic and environmental reasons to balance the supply of nutrients to plant requirements. This forms the basis for precision agriculture, where barley has received limited attention. The key factor for precision agriculture in malting barley is the ability to predict CP from early observations of the crop so as to control a second fertiliser application.

This thesis investigates the possibility of predicting malting barley grain CP at an early stage of development and of using a second fertilisation application during growth for total nitrogen (N) adjustment. Three experiments were conducted. The first consisted of eleven field trials (1992-1994) and was used to compare broadcasting/harrowing and combi-drilling for applying full-rate fertiliser at sowing using two types of fertilisers; pure N and one also containing phosphorus (NP). The second experiment consisted of sixteen fertiliser field trials (2001-2003) and was used to examine the possibility of postponing the decision on total N. The third consisted of three evenly fertilised fields (2002-2004). In experiments 2 and 3, canopy reflectance was measured at developmental stages BBCH 32, 45 and 69. Soil macronutrients, organic matter and mechanical composition were analysed in all experiments.

Malting barley yield was higher when fertiliser was combi-drilled into the soil and when NP fertiliser was used. Grain CP was predicted in the field ($R^2_{adj} = 0.73$) from soil electrical conductivity (SEC_a), the canopy reflection-based vegetation index (VI) TCARI/OSAVI estimated at BBCH 32 and the sum of daily maximum temperatures during anthesis and grain filling (STS). In the fertilisation trials, CP was predicted ($R^2_{adj} = 0.83$) by sowing day number and the VI TCARI evaluated together with solar angle at measurement. Grain yield was independent, and grain CP almost independent, of whether all fertiliser was applied at sowing or divided between sowing and BBCH 32.

Keywords: canopy reflectance, grain crude protein, *Hordeum distichum*, sowing day number, soil electrical conductivity, temperature sum, vegetation index

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Prognoser av proteinhalten i maltkorn baserat på fjärranalys och miljöfaktorer

Proteinhalten i maltkorn bör ligga i spannet 10.5-11.0% för att fungera optimalt i mälteriet, men 9.5-11-5 accepteras. Det är svårt att uppnå detta för odlaren, eftersom maltkornet odlas på heterogena jordar och också är utsatt för varierande väder. Det finns också både ekonomiska och miljömässiga skäl att anpassa gödselmängden så att den lagom täcker växternas behov. Detta är grundtanken inom precisionsodling, där korn hittills fått begränsad uppmärksamhet. Nyckelfaktorn för precisionsodling i maltkorn är att kunna prediktera proteinhalten tidigt, för att därigenom kunna anpassa gödslingen.

Denna avhandling undersöker möjligheterna att förutsäga proteinhalten i maltkorn tidigt i grödutvecklingen och att använda en andra gödselgiva under grödutvecklingen för att justera totalmängden gödselkväve. Tre experiment genomfördes. Det första bestod av elva gödslingsförsök i maltkorn åren 1992-1994, vilka jämförde kombisådd med gödseln nedharvad i såbädden med två olika gödselmedel; rent N samt ett samgranulerat kväve och fosfor (NP) medel. Det andra bestod av sexton gödslingsförsök i maltkorn åren 2001-2003, avsedda att undersöka möjligheten att skjuta upp beslutet om den totala kvävenivån. Det tredje bestod av tre maltkornsfält på samma gård, vilka följdes åren 2002-2004. I experiment 2 och 3 mättes beståndets reflektans vid tre stadier BBCH 32, 45 och 69. Växtnäring i marken, organiskt material och mekanisk analys analyserades i alla experiment.

Skörden av maltkorn blev högre när kombisådd tillämpades och NP gav högre skörd än rent N, även utanför de områden som tillämbar kombisådd. Proteinhalten kunde predikteras i fälten (R^2_{adj} = 0.73) från jordens elektriska konduktivitet (SECa), vegetationsindexet TCARI/OSAVI mätt vid BBCH 32 och en temperatursumma från dagliga maxtemperaturer under blomning och kärnfyllning (STS). I gödslingsförsöken kunde proteinhalten predikteras (R^2_{adj} = 0.83) från dag-nummer vid sådd och vegetationsindexet TCARI från BBCH 32, tillsammans med solvinkel vid mätningen. Kärnskörden var opåverkad och proteinhalten nästan opåverkad av om all gödsel gavs vid sådd eller om den delades upp mellan sådd och begynnande stråskjutning.

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Abbreviations

α	Solar angle
AL	Soil analysis, “Plant available nutrients” (K-AL, P-AL etc.)
BBCH	Crop growth stage (Lancashire *et al.*, 1991)
C	Carbon
Ca	Calcium
CAN	Calcium ammonium nitrate
CP	Crude protein
d.m.	Dry matter
DM	Dry matter
Dnr_{sow}	Day number at sowing
Dnr_{harv}	Day number at harvest
g	gram
GPS	Global positioning system
GS	Crop growth stage (Tottman & Broad, 1987)
HCl	Soil analysis, “Plant unavailable soil reserves” (K-HCl etc.)
K	Potassium
kg	Kilogram
Ks	Kalksalpeter (calcium nitrate)
kt	kiloton, thousand metric ton
LAI	Leaf area index
m.c.	Moisture content
MC	Moisture content
mg	Milligramme
Mg	Magnesium
Mt	Megaton, million metric tons
N	Nitrogen
nm	Nanometre
P	Phosphorus
S	Sulphur
SOM	Soil organic matter
VI	Vegetation index
VIs	Vegetation indices

Appendix

Papers I – IV

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Pettersson, C.G. 2007. Reappraisal of methods of application of nutrients at sowing on the yield, grain protein content and nitrogen economy of malting barley in Sweden. *Submitted manuscript*.

II. Pettersson, C.G., Söderström, M. & Eckersten, H. 2006. Canopy reflectance, thermal stress, and apparent soil electrical conductivity as predictors for within-field variability in grain yield and grain protein of malting barley. *Precision Agriculture 7*, 343-359.

III. Pettersson, C.G. & Eckersten, H. 2007. Prediction of grain protein in spring malting barley grown in northern Europe. Accepted for publication in *European Journal of Agronomy 2007-04-12*.

IV. Pettersson, C.G., Eckersten, H. & Ritz, C. 2007. Prediction of malting barley N concentration from reflectance-based Vegetation Indices. *Manuscript*.

Responsibilities

In Paper I, Pettersson used data from Yara, and set up the aim of the analyses. Pettersson was responsible for the analysis, guided by Ritz, and the writing, guided by Frankow-Lindberg and Hay.

In Paper II, Pettersson set up the aim for the experiment, organised it and was responsible for the practical work. Pettersson was responsible for the analysis, guided by Söderström who did the interpolating. Pettersson was responsible for the writing, guided by Frankow-Lindberg, Eckersten and Hay.

In Paper III, Pettersson set up the aim for the experiment together with a group from the field trial coordination of middle Sweden, which also carried out the trials. Pettersson was responsible for all canopy sampling. Pettersson was responsible for the analysis guided by Ritz, and the writing guided by Eckersten and Hay.

In Paper IV, the same dataset as in Paper III was used. Pettersson was responsible for the analysis together with Ritz, the writing was done by Pettersson guided by Eckersten and Hay.

Introduction

The malting barley crop

Barley was grown when agriculture was first developed in Mesopotamia, was essential in Ancient Egypt and was the most important bread cereal of both the Greeks and the Romans. At present, barley is the fourth most cultivated cereal in the world, after wheat, maize and rice. The species is divided into three subgroups, six-row (*Hordeum vulgare*), two-row (*Hordeum distichum*) and intermediate (*Hordeum irregulare*), and both spring- and autumn-sown types are grown. The major use of barley is for animal feed, brewing malt and human food. Both two-row and six-row barley are used for malting, but the best malt quality for beer is produced from spring-sown two-row varieties. Barley is a short season, early maturing crop mainly adapted to a cool to temperate climate, but it can be found at the outer edges of agriculture, such as desert oases or the slopes of the Himalayas. Barley is not cultivated in the warm-humid climate of the tropics (Harlan, 1986).

Production, trade and use

Global barley production for 2005 has been estimated at 140 million metric tonnes (Mt). Most barley is consumed locally and only 17.5 Mt goes into international trade on a global level. The main barley importers are Saudi Arabia (feed), China (malt) and Japan (food & malt); the main exporters are Australia, Ukraine, EU and Canada. The global malting barley demand in 2005 was 23 Mt and the main production areas were EU (10.6 Mt), Australia (2.5 Mt), USA (2.3 Mt) and Canada (1.9 Mt). The main exporters were Australia (1.7 Mt), EU (1.0 Mt) and Canada (0.9 Mt). (Barley Australia, 2007; Saskatchewan I&R, 2007; Euromalt, 2007). Sweden exports both malt and malting barley. Swedish industrial malting barley consumption is 250 thousand metric tonnes (kt) per year and the Swedish malting barley crop amounts to 350 kt, out of a total barley crop of 1500 kt.

Malting and malt

Malting consists of *steeping*, where the moisture content (MC) of the grain is increased from 13% to 45% MC; *germination*, where the grain germinates under aeration and shuffling for 4-5 days; and *kilning*, where the germination process is stopped by heating. The *malt* is then dried, *roasted* to the preferred colour and stored at 4.0-4.5% MC. Malt is the most important raw material for beer and the only permitted source of fermentable carbon in countries observing the *Reinheitsgebot*, a German purity law from 1516 that is the oldest consumer protection regulation still in force. Malt is a source of carbon, such as starch and malt sugars, and of enzymes that produce fermentable sugars from starch sources other than malt ‘adjuncts’.

During the last two centuries malting has become an industrialised process and during recent decades the size of malting plants has grown dramatically. Because of this, the required characteristics of the barley grain have changed and have become more stringent in terms of appropriate mean levels and supply of large lots with consistent quality. For the best malting process all grain has to germinate simultaneously, which is the reason why only the size-stratified grain fraction over 2.5 mm is used in the process.

High germination rates, good grading and a suitable grain protein concentration (CP) are the most important factors for malting barley at farm level. The acceptable protein range for European malting barley is 9.5-11.5% CP on a dry matter (DM) basis, but the industry prefers a narrower range (10.5-11.0%). Too much protein lowers the extract yield, clouds the beer and slows down the start of germination, while too little protein results in lower enzyme activity and slow growth of the yeast in the brewery. As simultaneous germination is a key factor in the malting industry, any factor causing variation in the germination rate reduces the malt quality. For this reason, damage to the husk or uneven grain MC or grain CP all result in lower quality. Uneven protein levels as such can also cause common quality problems in malt that are not usually described as protein-related (Palmer, 2000). To produce malting barley with high vitality, good grading and undamaged husk, it is necessary to keep the crop free from fungal infection and to use appropriate techniques during harvest, drying and storage. To improve the production of appropriate and uniform grain CP in the field, new knowledge is needed.

Grain protein establishment

Malting barley growers all over the world are challenged by the difficulty in producing barley with appropriate grain CP (*e.g.* Correll *et al.*, 1994; Birch, Fukai & Broad, 1997; Wang, Zhang & Chen, 2001; McKenzie, Middleton & Bremer, 2005; Mengel, Hütsch & Kane, 2006). One reason for difficulties in establishing acceptable grain CP concentration is that it depends on both the nitrogen (N) supply from fertiliser and soil and the carbon (C) supply from the atmosphere. This renders N and C dynamics in the soil/-plant system sensitive to environmental changes.

Temperature effects

In classical field experiments in England, the proportion of grain C translocated from stem storage during grain filling was found to vary between 60% in a hot and dry year to 15% under favourable grain filling conditions (Austin *et al*., 1980), resulting in large differences in both yield and grain CP. As the translocation of stem N is not as sensitive as the translocation of stem C, disturbance from high temperature during grain filling tends to result in higher protein levels (Tester *et al*. 1991; Savin & Nicolas, 1996; Savin, Stone & Nicolas, 1996; Passarella, Savin & Slafer, 2002). A general rule of thumb is that environmental stress to the crop during grain filling increases grain CP.

Several mechanisms have been suggested for the reduced carbon loading in cereal grains exposed to high temperatures. In phenological growth models (Jamieson *et al.*, 1998), elevated temperatures result mainly in a shorter grain filling period and thus a lower carbon yield. High temperatures (> 25 ºC) at anthesis might also limit carbon yield by reductions in the number of starch cells in the grain, possibly causing a sink limitation to yield (May & Buttrose, 1958; Brooks *et al*., 1982; McDonald *et al*. 1991; Tester *et al*., 1991). High temperatures at anthesis may also reduce the capacity of UDPsucrose synthase (EC 2.4.1.13), an enzyme that splits sucrose into glucose units during grain filling (MacLeod & Duffus, 1988). As carbon is preferably transported in the phloem as sucrose and as starch synthesis starts with the formation of glucose, lack of this enzyme might create a situation, occurring mainly above 25-30 °C, where grain filling is source-limited even when C from photosynthesis is abundant.

When water is available, plant biomass is related to cumulative temperature, thermal time. The common way to estimate this is to calculate growing degree-days (GDD) as the cumulative sum of daily average temperatures above a base temperature defined as the temperature below which no development of the studied process occurs. By optimising the base temperature with statistical methods, the explanatory power of GDD and the linearity between GDD and the studied variable can be maximised (Kirby, Appleyard & Fellowes, 1982, 1985).

During grain filling, high daily GDD increments accelerate the development rate and shorten the duration. Tashiro and Wardlaw (1989) reported a similar reduction in grain filling duration for both wheat and rice with increasing daily mean temperatures (T_{MEAN}) in the interval 18 to 33 ºC, and that rice, but not wheat, could compensate for this with increased grain filling rate. The resulting grain weight was reduced by 5% for each 1 ºC over 18 ºC for wheat, while the grain weight in rice remained constant up to 27 ºC, above which the weight fell to a level similar to wheat. Wardlaw and Wrigley (1994) distinguished between 'moderate temperatures', with T_{MEAN} of 15-25 ºC and daily maximum (T_{MAX}) < 32 ºC, and 'heat shock conditions', with $T_{MAX} > 32$ ºC.

In the present work, an alternative temperature sum, accumulating T_{MAX} instead of T_{MEAN}, was used to maximise the sensitivity to short high temperature events during grain filling. The 'Stress Temperature Sum' (STS) was calculated, starting at growth stage 45 (BBCH 45, late boot stage; Lancashire *et al.*, 1991) accumulating daily T_{MAX} above a threshold temperature (T_b) for three weeks, to cover both anthesis and grain filling:

$$STS(T_b) = \sum_{t=BBCH\,45}^{BBCH\,45+3\,weeks} T_{MAX}(t) - T_b \qquad T_{MAX} > T_b \qquad (1)$$

The impact of daylength

The reaction of the crop to temperature is dynamic. Cultivars used at high latitudes are day-length sensitive, which means that each step in their phenological development requires a lower GDD amount when the plants are exposed to long-day conditions (*e.g.* Paynter, Juskiw & Helm, 2004). At high latitudes, this results in a lower maximum leaf area index (LAI_{MAX}) during grain filling, as both number and expansion of leaves are related to cumulative GDD during canopy development (*e.g.* Kirby, Appleyard & Fellowes, 1982; Juskiw, Jame & Kryzanowski, 2001). The cultivar thus starts grain filling at high latitudes at a lower LAI than at low latitudes. In this way it has enough time to mature during the remaining growing period, but has a lower yield potential.

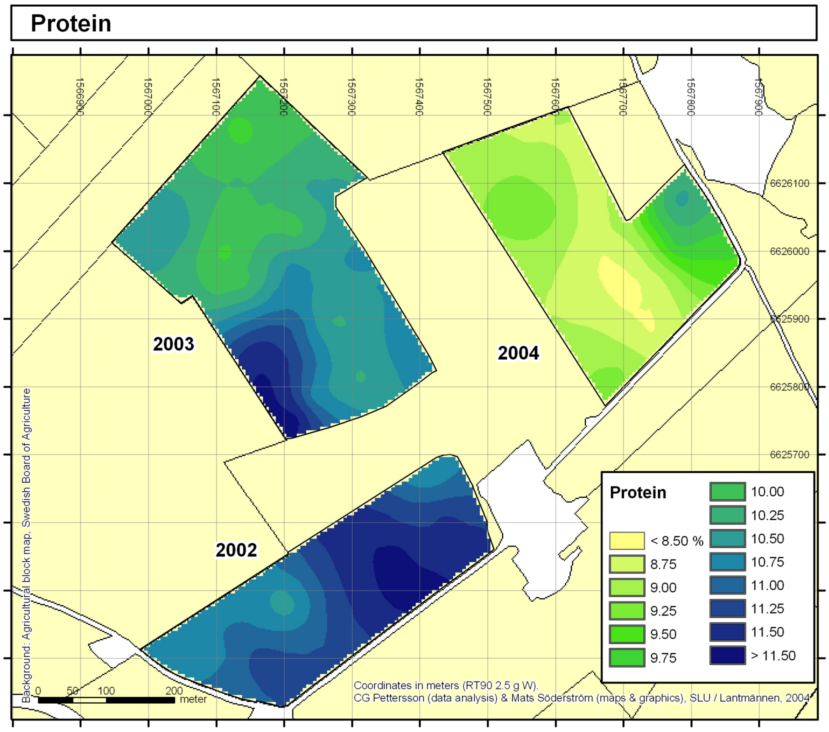

Fig. 1. Grain CP levels in three fields of malting barley (cv. Astoria) in Sweden 2002-2004 (Experiment 3). The map represents data from 219 GPS-positioned plots, harvested with a plot-combine in three consecutive years. The management of the crops was similar for the three seasons.

Precision Agriculture

The first yield loggers using the global positioning system (GPS) were mounted in combine harvesters in the early 1990s and became common some ten years later. The yield maps produced from this equipment led to a general awareness that the yield and quality of crops vary dramatically across fields, and also motivated systematic research on these variations (*e.g.* Stafford, 1999). For a long time, the main direction of work was that yield maps from harvesters would provide enough information to control site-specific fertilisation. Functioning management zones can be constructed with yield maps as the main input, if massive amounts of high quality data are available and are evaluated with advanced methods (Blackmore, 2000). However, with the data quality possible to obtain using yield logging harvesters on practical farms, management zones appear to be out of reach (*e.g.* Joernsgaard & Halmoe, 2003). A more successful approach has been to use measured soil properties to create management zones according to the expected soil N mineralisation and crop potential, but the patterns can change between years, and grain CP has proven difficult to predict in this way (Delin, Linden & Berglund, 2005). Apparent soil electrical conductivity (SECa) has been used as a cheap and fast method for soil mapping. However, the generality of the method has been questioned, as changes in SECa values could depend on SOM, clay content, salinity and soil porosity (Sudduth, Drummond & Kitchen, 2001). These factors influence the crop differently along a SECa gradient. However, when combined with soil topography data SECa has been useful for maximising yield, but not grain CP in response to N fertilisation (Delin, 2005). Another way of getting information about crop status is to make direct measurements on the crop itself. Canopy reflectance has the potential to link the status of the developing crop to grain CP at harvest, and thus the potential to act as a control variable for fertilisation (*e.g*. Börjesson & Söderström, 2003; Lark & Wheeler, 2003).

Protein varies both within fields and between years. Figure 1 shows the distribution of grain protein in malting barley grown on similar Swedish clay loam soils, with the same fertilisation regime, during three seasons (2002-2004) (**Paper II**). The mean grain CP in 2002 was nearly ideal, at 10.8% ±1.5 %-units. The other two crops had a similar variability, but different means. To control grain CP level within fields and between years, the main tool available to the farmer is the fertilisation rate, which should be adjusted in relation to the differing potential crop demand in different parts of the field. This would in most cases call for two-step fertilisation, enabling observations to be made on the established crop before the decision on final N-rate is taken.

The main problem with two-step fertilisation of a short-season crop, and especially malting barley, is the risk of causing systematically higher grain CP and lower yields, instead of using the crop potential efficiently. To avoid this, early canopy development must not be nutrient-limited. Swedish barley trials from the 1990s (**Paper I**) showed that combi-drilling in which the solid fertiliser was drilled between every second seed row and 40 mm below the base of the seedbed was the most efficient application method (Huhtapalo, 1982). Other trials from the 1990s showed that grain protein levels were less dependent on the total N application when NPKS was combi-drilled compared with pure N, and that 60 kg N ha^{-1} combi-drilled as NPKS produced as many tillers as 100 kg N ha^{-1} combi-drilled as NS (Pettersson, 2007). That study concluded that a moderate amount of combi-drilled NPKS would be the best application at sowing, if a second application was planned. Calcium nitrate (Kalksalpeter, Ks, Yara), the most water-soluble fertiliser on the market and the most readily available for root uptake in draughts (McTaggart & Smith, 1995), was chosen for the second N application. Applying calcium nitrate to barley without generating systematically higher protein levels is possible at BBCH 32 (two nodes) but not later (Anderson, 1990).

Canopy reflectance

The use of canopy reflectance for monitoring crop status is based on the ability of chlorophyll and canopy dry matter to absorb and reflect radiation. In the visible range, 400-700 nanometres (nm), chlorophyll absorbs most of the radiation. In the near infrared range (NIR; 750 nm and above) chlorophyll does not absorb radiation and most incoming radiation is reflected instead. The degree of reflection increases with increasing canopy biomass (Fig. 2). Canopy reflectance is expressed as the reflected fraction of incident radiation, where 0 denotes total absorption and 1 denotes total reflectance.

Both crops and natural vegetation have been systematically monitored by satellite since the early 1970s (Short, 2006). Satellite-based remote sensing measurements of crop canopy reflectance are continuously produced and today individual fields and even parts of fields can be monitored (Begiebing *et al*., 2005; Blondlot, Gate & Poilvé, 2005). Remote sensing is also performed from aeroplanes (*e.g*. Galvao *et al.*, 2004). Crop sensors based on canopy reflectance can be used to adjust the fertilisation rate continuously (Reusch, Link & Lammel, 2002; Scotford & Miller, 2005), and such systems have proven to work well in wheat (Link & Jasper, 2003). A crop sensor can either be passive, measuring the incoming sunlight and the crop reflectance simultaneously, or active, itself emitting the radiation and then measuring its reflection. A problem with passive sensors for fertilisation control purposes is that the use is limited to daytime and the outcome is potentially influenced by both solar angle and radiation intensity. This problem has been addressed by optimising the parts of the reflectance spectrum used, actively using the problematic measurement details as covariates in the prediction models and utilising measurements from several directions for each data point (Reusch, 2003). The other option is to use active sensors, which can be operated at any time of the day (Reusch, 2005; Schwab *et al*., 2005).

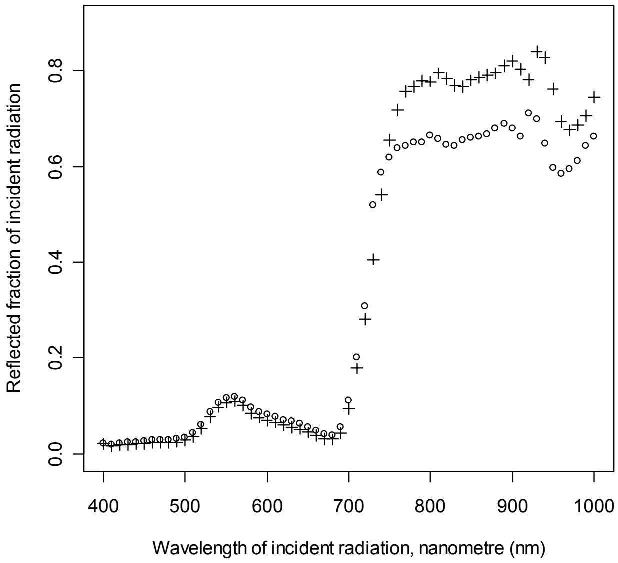

Fig. 2. Malting barley canopy reflectance at BBCH 69 (anthesis complete), fertiliser treatment A (o, No fertilisation), and D (+, 130 kg N ha^{-1}). Mean values of four field trials 2003 in Experiment 2.

Objectives

The overall objectives of this thesis were to monitor the variation in grain CP in malting barley under different environmental conditions, and to examine the ability of dominant factors to predict the variation in grain CP. As the ambition was to make the predictions useful as a basis for fertilisation systems permitting an adjustment of the final N level, factors observable at an early stage of crop development were preferred in the predictions. The specific questions addressed were:

How does grain yield relate to fertiliser application method? Two methods were examined: i) Uniform broadcasting on the soil surface followed by harrowing into the seedbed; and ii) combi-drilling to 40 mm below the base of the seedbed between every second seed row.

Are the established recommendations regarding combi-drilling in Swedish barley valid or do they need to be altered?

Is it possible to use a two-step fertilisation regime for malting barley during the short development period available in Sweden without producing systematically higher grain CP and lower grain yield?

With which factors, and how early, could the within-field variations in grain CP be predicted in uniformly fertilised fields of malting barley?

With which factors, and how early, could the variation in average grain CP in malting barley among fields be predicted? Does varying fertilisation rate or choice of cultivar influence the predictions?

What is the ability of different types of vegetation index (VI) to predict grain CP and grain yield of malting barley?

To what extent do measurement details such as solar angle and radiation intensity influence VI predictions?

Materials and Methods

The papers in the thesis are based on data from eleven field trials in 1992-1994 (**Paper I**), sixteen field trials in 2001-2003 (**Papers III and IV**) and three years of uniformly fertilised fields on one farm in 2002-2004 (**Paper II**). All experiments were performed in southern Sweden and selected malting barley varieties were used (Fig. 3).

Experiment 1

Eleven field trials were performed to compare the two commonly used methods of applying fertiliser to barley in Sweden. The trials were located in southern Sweden (55º55’ - 59º36’N, 12º21’ – 16º 39’E) and carried out during the period 1992-1994 using a randomised block design with three replicates. The cultivar used was Golf (Nickerson, England), at that time the major malting barley variety in Sweden. Two fertiliser application methods were compared: i) Uniform broadcasting of the fertiliser on the soil surface followed by harrowing to produce a uniform distribution in the seedbed before sowing; and ii) combi-drilling of fertiliser between every second seed row and 40 mm below the base of the seedbed. Two types of fertiliser (calcium ammonium nitrate (CAN) and compound calcium ammonium nitrate with phosphorus (NP)) were investigated in combination with application methods (i) and (ii), resulting in four treatments. The soils were analysed for plant nutrients and daily precipitation was recorded for each trial (for details see **Paper I**).

Experiment 2

Sixteen fertiliser field trials were performed to evaluate the ability of different observable factors to predict a wide grain CP range and also to test the possibility

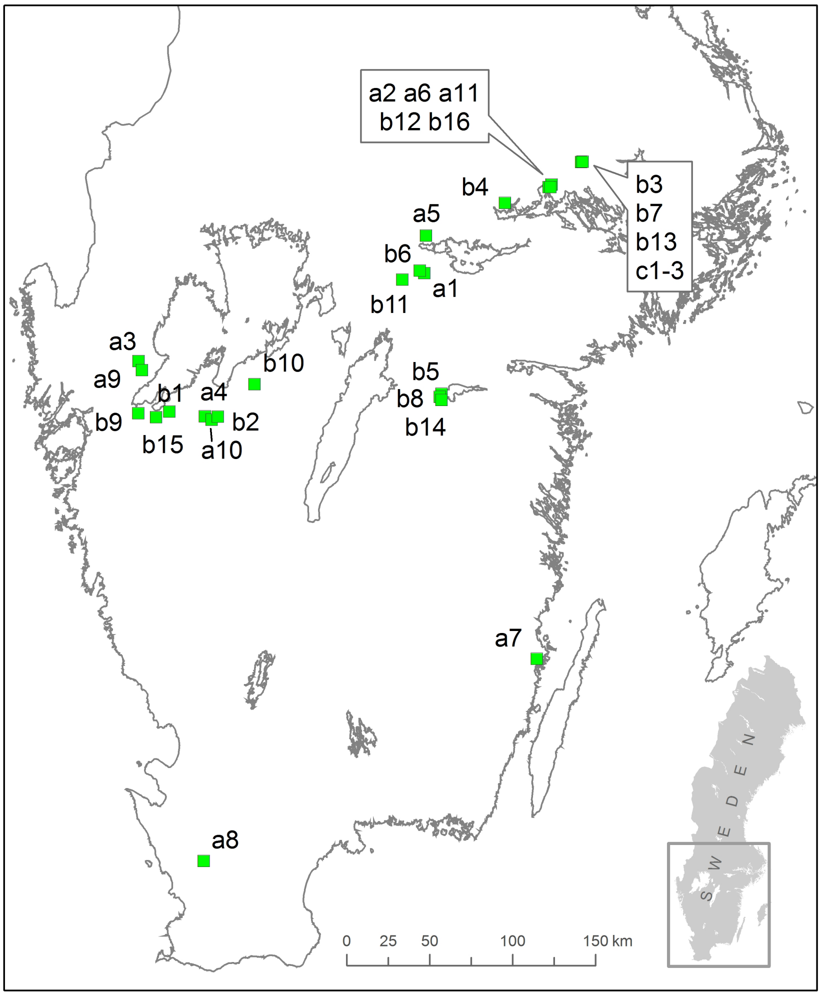

Fig. 3. The positions of the field trials in the thesis. Experiment 1 = 'a', Experiment 2 = 'b' and Experiment 3 = 'c', numbering according to **Paper I-IV**.

of fertilising malting barley in two steps. These trials were located in southern Sweden (58º19' - 59º44'N) and carried out in the period 2001-2003. The objective was to evaluate the possibility of using canopy reflectance at BBCH 32 to predict grain CP in two malting barley cultivars, cv. Astoria (Secobra, France) and cv. Wikingett (Svalöf-Weibulls, Sweden). Two different application strategies were compared: i) Application of the full N rate at sowing (combi-drilled; standard practices); and (ii) a combi-drilled starter fertilisation at sowing and a supplementary fertilisation at BBCH 32. The soil was analysed at sowing for mineral N in the 0-60 cm layer and for phosphorus (P), potassium (K), magnesium (Mg), and soil organic matter (SOM) in the 0-30 cm layer. Canopy reflectance was measured at BBCH 32, 45 and 69. Crop samples of 0.25 m^2 were cut at BBCH 77 (late milk) within the fertilised plots, but outside the net harvesting plots. Plots of 24 m^2 were harvested at BBCH 89, and grain samples were analysed for grain CP and grading. Rainfall and temperature were recorded for each trial on a daily basis (for details see **Papers III and IV**).

Experiment 3

Three uniformly fertilised fields on the same farm were studied during the period 2002-2004 to test the ability of observable factors to predict the within-field variation in CP in malting barley grain (Fig. 1). Precipitation and temperature were recorded on a daily basis. The cultivar used was Astoria (Secobra, France) and soil type was clay loam. A total of 81 fix points were established and used as positions for soil sampling, canopy reflectance measurements and canopy sampling (plot size 0.25 m^2) during grain filling and at harvest. Canopy reflectance was measured at BBCH 32 and BBCH 69 (anthesis finished), and nine VIs were estimated for each developmental stage. The soil at sowing was analysed for mineral N in the 0-60 cm layer and for phosphorus (P), potassium (K), magnesium (Mg), and soil organic matter (SOM) in the 0-30 cm layer. The fields were also surveyed with an EM38 device (Vlotman, 2000) measuring soil apparent electrical conductivity (SECa). The 81 fix points were sampled by hand (0.25 m^2) at BBCH 87 (hard dough), and machine harvesting was performed for 24 m^2 plots at BBCH 89 (fully ripe) in the corners of a systematic grid with 36 m edges, resulting in a total of 219 harvested and analysed plots over the three-year period. To make the 24 m^2 plot data represent the same locations within the fields as the fix point samples, the former were interpolated by ordinary block kriging using GS+ (Anon, 2004) to the positions of the fix points (for details see **Paper II**).

Results and Discussion

Fertilising malting barley

Our observations confirmed that malting barley needs to be fertilised at sowing. When no fertilisation was applied, early canopy development was nutrient-limited and the grain yield low. There was less influence on grain CP, which was roughly similar for unfertilised and plots with 70 kg N ha^{-1} at sowing (Fig. 4) (**Papers III and IV**). Combi-drilling the fertiliser between every second seed row at 40 mm below the base of the seedbed resulted in higher yields than when the fertiliser was harrowed into the seedbed (**Paper I**). The accepted guiding principle in practical agriculture, *i.e.* to combi-drill when the amount of rainfall during crop establishment is expected to become a limiting factor for canopy development (Mattsson, 1974; Hartman & Nyborg, 1989), was not confirmed. Combi-drilling was always a better practice than harrowing the fertiliser into the seedbed and its superiority was not dependent on limited rainfall after sowing. Instead, a response was found to the abundance of nutrients such as K and Mg (cations) in the soil. Combi-drilling was correlated to higher yields when K and Mg were abundant. This indicates that it might be of interest to consider the adhesion of ammonium ions to soil particles

(Yadvinder-Singh *et al.*, 1994; Tong *et al.*, 2004) when decisions are being made about fertiliser application method. Combi-drilling both N and P to barley also resulted in a higher harvest/application ratio than when the fertiliser was harrowed into the seedbed, indicating both an economic and a potential environmental advantage of combi-drilling (**Paper I**).

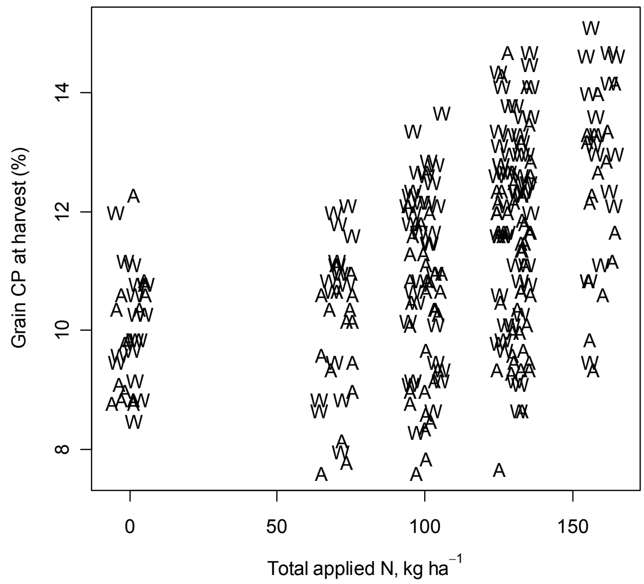

Fig. 4. The relationship between malting barley grain CP at harvest and total applied N. The dataset from Experiment 2 was used, and the N levels were jittered to make the figure read clearer. The plotting symbols indicate the cultivars Astoria (A) and Wikingett (W)

Two-step fertilisation of malting barley, where the first instalment was applied at sowing and the second at BBCH 32-37, resulted in the same grain yield and grain CP as when all fertiliser was applied at sowing. However, when 60 kg N ha^{-1} was applied in the second instalment, the grain CP was slightly higher, while the percentage of grain over 2.5 mm was lower than for corresponding fertiliser treatments with the same total N (Table 1). However, the magnitude of the grain CP change was insignificant compared with the expected natural variability (Fig. 4), indicating that a two-step application of fertiliser might be a practical alternative to full fertiliser rate at sowing, allowing for an adjustment of total N level until BBCH 32 (**Papers III and Paper IV**).

Predicting spatial patterns

Vegetation indices, soil and temperature

When estimated at BBCH 69, all VIs tested gave significant correlations to grain CP, whereas at BBCH 32 only VIs utilising the green (550 nm) waveband gave strong correlations to grain CP. The within-field variability in grain CP was of similar magnitude in all years, but the mean grain CP differed substantially between years (Fig. 1). Using the TCARI/OSAVI vegetation index (Haboudane *et al.*, 2002) estimated at BBCH 32 together with apparent soil electrical conductivity (SECa) measured during autumn in the first year of the study, it was possible to capture most spatial variability over the fields, but not the variations in yearly means among fields and years. However, when the exposure of the crop to high temperatures during grain filling, expressed as the cumulative daily maximum temperature (Eq.1; with T_b = 20 ºC), was included in the prediction, it was possible to predict both the within- and between-field variations in grain CP (R^2_{adj} = 0.73) and grain yield (R^2_{adj} = 0.90). The VIs in more widespread use, such as NDVI and REIP (Reusch, 1997), correlated well with grain yield and grain CP when estimated at BBCH 69 but not at BBCH 32 (**Paper II**), confirming the results of Börjesson & Söderström (2003).

Predicting factors for grain CP

Canopy N concentration

There was a high correlation between grain CP at BBCH 89 and canopy N concentration at BBCH 77 (R^2 = 0.63) (Fig. 5). Cv. Wikingett associated a higher grain CP to a particular canopy N concentration compared with cv. Astoria, but the two cultivars showed the same relationship to changes in canopy N concentration (**Paper IV**). This suggests a potential to use canopy N concentration for grain CP predictions (Molina-Cano, Gracia & Ciudad, 2001), but it would be impractical and costly for within-field variation, as many analyses would be needed from each field (Fig. 1). However, the results indicate that VIs correlated to canopy N concentration, rather than canopy N amount, might be better for predictions of grain CP.

Soil electrical conductivity

Predicting grain CP in fertilisation trials (Experiment 2) is somewhat different from predicting it in uniformly fertilised fields (Experiment 3), since SECa could be used as a descriptor of soil changes within Experiment 3, and was useful in the regression for grain CP. In the field trials in Experiment 2, however, SECa could not be used, as the EM38 measuring device gives the conductivity values on a local scale that is reset for every new measurement.

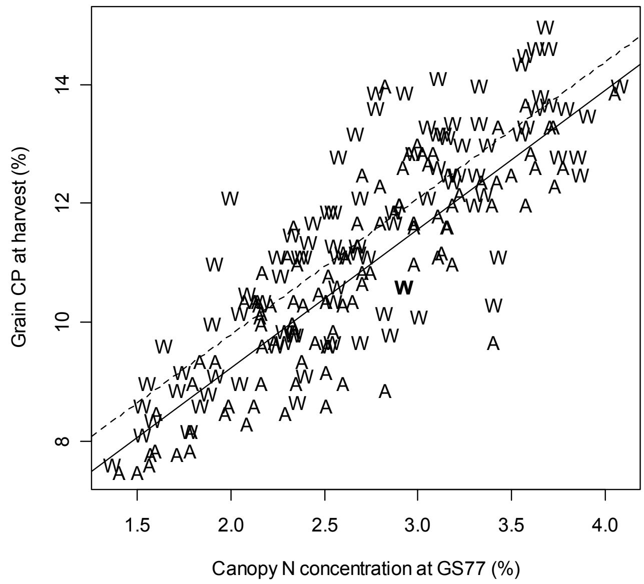

Fig. 5. The relationship between canopy N concentration at BBCH 77 (GS77), and grain CP at BBCH 99, for malting barley fertilised with 0 – 160 kg N ha^{-1}. The cultivars Astoria (A, ——) and Wikingett (W, ------) got the same regression slopes, but Wikingett produced a higher grain CP from the same N concentration in the canopy. The relations were independent of fertilisation. $R^2 = 0.63$

Day of sowing

It was possible to evaluate the effect of day number of sowing (Dnr_{sow}) on grain CP from Experiment 2, as the data covered a large range of sowing day numbers from 92 (2 April) to 142 (22 May). Day number at sowing correlated significantly to grain CP. Late sowing resulted in late harvest but also in a shorter growing period. For every three days of delay, the crop lost one growing day (Fig. 6), which is in line with the results of *e.g.* Juskiw, Jame & Kryzanowski (2001). Day number at sowing and the STS temperature sum were not statistically independent, and day number successfully replaced STS in the regressions when sixteen different sowing days were used (**Paper III**).

Tissue temperature and water balance

The crop reacts to the tissue temperature and not to meteorological standard air temperature, which was used in this study. The energy balance of the crop determines tissue temperature; low water availability increases the risk of high temperatures, and increases the protein level of the crop. Many researchers have found that water availability is a key factor for protein content in small grains, see for example Hector, Fukai & Goyne (1996), Dalal *et al.* (1997), Broner, Thompson & Dillon (1997), Bertholdsson (1998) and Delin (2005). It is conceivable that the temperature factor would contribute even more strongly to grain CP predictions with a water factor included in the model, but this was not done in the current study.

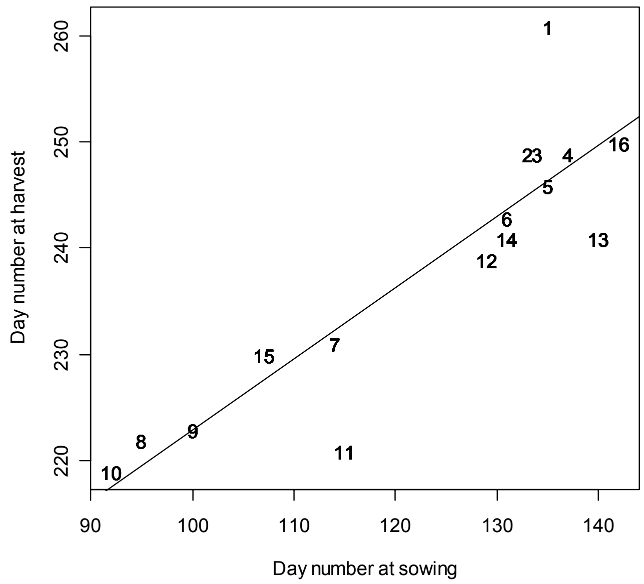

Fig. 6. The relationship between day number (from January 1st) at sowing (Dnr_{sow}) and day number at harvest (Dnr_{harv}). ($Dnr_{harv} = 156 + 0.67\ Dnr_{sow}$). All field trials in Experiment 2, with the numbering from **Paper III and IV**.

Minimal regression input

The final grain CP regressions based on data from Experiment 2 only used sowing day number and the Transformed Chlorophyll in Absorption Index (TCARI) vegetation index (Haboudane *et al.*, 2002) estimated at BBCH 32 to predict grain CP ($R^2_{adj} = 0.78$). The regressions were not sensitive to fertilisation, but to cultivar. The same VI estimates at BBCH 32 (TCARI and TCARI/OSAVI) correlated best to grain CP in both experiments 2 and 3, although in Experiment 2, TCARI was marginally better (**Paper III**). The ratio was used as the VI part of grain CP regressions in **Paper II**, but this was based more on the recommendations from Haboudane *et al.* (2002) than from a real need. The TCARI/OSAVI ratio was suggested to solve a problem at TCARI levels below 0.1. As no TCARI scores this low appeared in fertilised barley, there was no problem to solve and TCARI could be used in the basic form (**Paper IV**). The TCARI VI was estimated using the following equation:

$$TCARI = 3\ [(R_{700} - R_{670}) - 0.2\ (R_{700} - R_{550})\ (R_{700} / R_{670})] \quad (2)$$

where R_j denotes the fraction of incident radiation reflected from the canopy at j nm.

The use of different VIs and measuring details

The Red Edge Inflexion Point (REIP) index was the most universally usable VI, as it correlated significantly to all dependent variables tested in Experiment 2, estimated at all sampling stages (**Paper IV**). However, to control variable rate fertilisation, it is important to make the strongest possible predictions of grain CP at developmental stage BBCH 32. At this stage TCARI(32) produced the strongest correlations for grain CP among all VIs in a simple regression only using the VI, and the correlation was improved if solar angle and radiation intensity also were used; $R^2_{adj} = 0.53$ compared to $R^2_{adj} = 0.41$. This should be contrasted with $R^2_{adj} = 0.23$ for the second best VI, REIP(32), which was insensitive to all covariates (**Paper IV**).

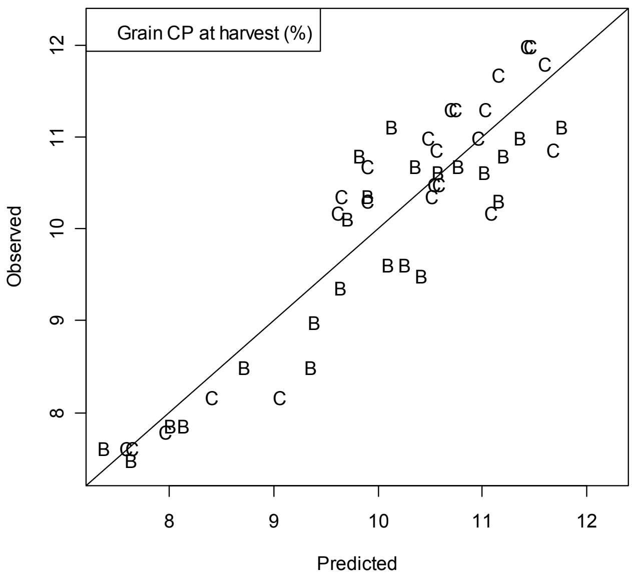

Fig. 7. The relationship between observed grain CP in cv. Astoria fertilised 70-100 kg N ha-1, and a prediction using sowing day number, TCARI(32) and solar angle (α) at canopy reflection measuring (Eq.3). $R^2_{adj} = 0.83$

The best prediction was achieved for cv. Astoria fertilised with 70-100 kg N ha^{-1} at sowing. The prediction of grain CP ($R^2_{adj} = 0.83$; Fig. 7) was based on data available at BBCH 32 in experiment 2 and used sowing day number (Dnr_{sow}) and TCARI(32) together with solar angle (α) at measurement, according to the following equation:

$$\text{Grain CP} = 7.7 + 0.06\ Dnr_{sow} - 21.4\ \text{TCARI(32)} - 0.05\ \alpha \quad (3)$$

Conclusions

The variation in grain CP within uniformly fertilised fields could be predicted using VI, the STS temperature sum accumulated during grain filling and SECa (R^2_{adj} = 0.73). Using a dataset with a wide range of sowing dates, information on sowing day number (Dnr_{sow}) and solar angle at canopy reflection measurements (α) improved the correlation (R^2_{adj} = 0.83). The best single VI was TCARI, but Dnr_{sow} was also a successful single predictor.

A dose of fertiliser at sowing was necessary for good crop establishment and best use of applied N was achieved when a fertiliser product containing N and P was applied by combi-drilling. The total fertilisation effect was similar when all N was applied at sowing or when a two-step strategy was used. This implies that spring-sown malting barley grown in Sweden can be successfully fertilised with a two-step strategy.

The highest correlation of canopy reflectance at BBCH 32 to harvested grain CP, as well as to canopy N concentration, was achieved with VIs that utilised the green reflectance band at 550 nm in their algorithms.

Future work

Sensor-controlled fertilisation

The relationship of sowing day and TCARI(32) to grain CP suggests that there is a possibility to control and adjust N application rate at BBCH 32 to achieve specific grain CP at harvest. It was beyond the scope of this project to evaluate such a fertilisation regime, but such an evaluation is necessary. Moreover, the role of sowing day in the grain CP algorithm could theoretically be expected to be general, but the exact formula derived is only valid for the latitudes for which it was derived. To use the approach further south than Lat. 58° N, the impact of sowing date has to be investigated together with the influence of latitude, asking for datasets with a larger north-south coverage than in the present study.

Adjustment of start fertilisation

The relationship between sowing day and grain CP was found to be influential, with two weeks of sowing delay related to an increase in grain CP of 1%-unit, corresponding to a fertilisation difference of 40 kg N ha^{-1}. However, it cannot be claimed from this that two weeks of delay automatically motivate 40 kg N ha^{-1} less fertilisation. Fertilisation for optimal grain yield might prove to correspond to higher grain CP for a late sowing date than for an early date, and identifying the matching best choice of fertilisation might be more complex than only aiming for a safe grain CP, but this question needs more research.

Regional prognoses

This study evaluated variation between years and within fields and also investigated the possibility of carrying out adjustments at farm level. Accurate regional protein prognoses early in the season would be valuable as a planning instrument. A new Swedish project within this area (Rymdstyrelsen, dnr 163/06) is starting in 2007, using the results from the present study. The purpose is to utilise satellite images, together with regional weather data, N-sensor data and samples from a limited number of fields, to produce regional grain CP prognoses for malting barley.

The water factor

To get a better understanding of tissue temperature, the water factor could be included using the dataset from Experiment 2. A soil water model for each field would be needed so that the crop water situation during grain filling could be described and evaluated together with temperatures and irradiance during grain filling. Another approach to get a measure of the water factor could be to use remote sensing. There are possibilities to calculate VIs that compare the biomass (NIR) with a sensor band sensitive to the water in the crop, but this was not possible from the sensor wavelengths available in the current dataset.

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As the project has been done part-time, with some responsibilities for me as project manager at Lantmännen, and both jobs have periodically been challenged by my part time farm. I want to thank, and apologise, everybody who has been disappointed by me because something else was more urging, but who still have some hope left. I have worn out a number of bosses at Lantmännen during the project, but to the remaining Lars: I will be more pushing in the projects from now on!

What are friends for, if not to take help from? So thank you Anders F, who has been both ploughing and harrowing at home when I have been writing – that's the right spirit for a Journalist! And thank you Johan and Bodil, who did the sowing this spring and also will help with the party.

The PhD student group at EVP, which turned into VPE (with some losses and some gains), have been worth a fortune to keep up the spirit in the work. We have been in somewhat different phases in life during our PhD periods, with me being a father to teenagers while several of you have been on maternal (or paternal) leave, it has never felt as a problem (at least not for me). Some of you have left the department, but anyhow: Thank you Hanna and Johan for your really high spirits and Eva who seems to have any amount of energy to cheer the rest of us up. Erik, who is on paternal leave from the disturbed insects but beats me in the finish anyhow, Anuschka, who is actually interested in nitrogen, and beside of that is prepared to take on any discussion, in any language. Sandra and Liv, who struggle with the weeds in between the kids. Maria, who counted half a million nematodes – compared to that my trip was easy, Johanna who counted nearly as many (but spores instead). Tomas, my roommate for two years, who probably had trouble with me talking too much, your relaxed humour, can really make the day! And slightly outside the PhD student group but right outside my door, Nilla thanks for the chats. And all the rest of you, who just don't turn up in my mind right now – Thank you for the nice atmosphere you created at VPE.

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