

**Head size variation within broccoli (*Brassica oleracea* var.  
*italica*) plantings, causes and prediction  
for decision support**

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## Zusammenfassung

Eine termingenaue Erntereife der Kultur in geforderter Menge und Qualität ist Voraussetzung für einen gesicherten Absatz. Beim Anbau von Brokkoli (*Brassica oleracea* var. *italica*) bestehen dort zwei Hauptprobleme. Zum einem kommt es zu starken Abweichungen von geplanten Ernteterminen. Zum anderen wird in der Praxis eine starke Heterogenität von Einzelpflanzen beobachtet. Beides erschwert Prognosen über genaue Anlieferungsmengen zusätzlich. Die Folgen sind ein vorübergehendes Unter-oder Überangebot des Marktes mit starken Preisschwankungen. Entscheidungen über optimale Erntezeitpunkte sind stark von ökonomischen Aspekten und der gegenwärtigen Preissituation beeinflusst und können nur mit Hilfe einer genauen Erntevorhersage optimiert werden (Kapitel 1).

Die Ursachen für die auftretende Bestandesvariabilität soll ermittelt werden um ein besseres Verständnis der physiologischen Hintergründe und Pflanzenreaktionen auf Umweltbedingungen zu erhalten und diese in eine Prognose von Erntezeitpunkten und Bestandesstreuung einzubringen. Um die Hypothese zu prüfen, dass Unterschiede in der Dauer bis zur Kopfanlage von Einzelpflanzen Ursache für die Kopfgrößenstreuung sind und um die dynamische Entwicklung der Bestandesvariabilität zu untersuchen, wurde ein stochastischer Modellansatz in ein mechanistisches Modell integriert. Zunächst wurden die Effekte von streuender Jungpflanzengröße auf die Variation der Kopfgröße bei Einmalernte untersucht. Dabei wurde kein Zusammenhang zwischen der Jungpflanzenstreuung und der Bestandesvariabilität gefunden (Kapitel 2). Es wurde eine starke Variation in der Dauer bis zur Kopfanlage von Einzelpflanzen unter Feldbedingungen gemessen.

In der Folge wurde der Fokus auf Untersuchungen der Entwicklung der Pflanzen bis zur Kopfanlage in Klimakammerversuchen gelegt. Die Ergebnisse zeigten, dass die Jugendphase bereits bei Pflanzung abgeschlossen ist. Hier wurde ein Einfluss auf die sich im Feld etablierende Bestandesstreuung ausgeschlossen. Daten aus einem zweiten

Klimakammerversuch mit acht verschiedenen Temperatur Stufen zeigten eine hohe Variabilität in der Dauer bis zur Kopfanlage innerhalb der Versuchsvarianten. Diese Effekte wurden durch ein stückweise lineares Modell mit einer auf Variationskoeffizienten und Normalverteilungsfunktion basierenden stochastischen Subroutine beschrieben (Kapitel 3).

Es wurde dann geprüft, ob die Variation in der Vernalisation von Einzelpflanzen die Kopfgrößenvariabilität im Feld beschreiben kann (Kapitel 4). Zu diesem Zweck wurde das Vernalisationsmodell in ein auf Feldversuchsdaten basierendes Brokkoli Wachstumsmodell integriert. Als Modellbasis wurde eine Trockenmassesimulation und ein Verteilungsmodell für Blumenkohl genutzt. Das Modell wurde anhand von Felddaten neu parametrisiert und an das Wachstum von Brokkoli angepasst. Weitere Verbesserungen erfolgten durch die Implementierung von dynamischen *LUE*- und *SLA*-Parametern.

Eine Modellevaluierung an unabhängigen Felddaten zeigte, dass der Verlauf der Variation in der Kopfgröße über die Kulturdauer gut vorhergesagt wurde. Das Modell erklärt den Großteil der Variabilität der einzelnen Kopfgrößen bei Einmalernte. Unterschiede im Zeitpunkt der Kopfanlage sind sehr wahrscheinlich die Ursache für die auftretende Streuung in den Einzelkopfgrößen. Das Modell kann als Werkzeug zur Entscheidungsunterstützung genutzt werden. Simulationen können zu Prognose von exakten Erntemengen und zur Optimierung von Erntegängen in der Produktion eingesetzt werden. Eine weitere Einsatzmöglichkeit wäre eine Prognose des optimalen Erntezeitpunkts für eine nicht selektive Einzelernte.

*Schlagworte:* *Brassica oleracea* L. var. *italica*, Vorhersage der Zeit bis zur Ernte, Einzelpflanzenvariation.

## Summary

Two main problems in broccoli (*Brassica oleracea* var. *italica*) production had been reported. The first is a lack of predictability of time to harvest and strong deviations from planned harvest schedules. Consequences are temporary under- and oversupply of the market with strong price fluctuations. The second problem is a lack of uniformity of time to harvest on single plant basis which leads to increasing harvesting costs in practice (Chapter 1). Because of these problems predictions about exact delivery quantities are complicated.

The aim was to discover reasons for this plant to plant variability in order to obtain a better understanding of the physiological background and the plant response to environmental conditions to consider this in a harvest date prediction model. The outcome should be a functional tool for decision support. The effect of scattering in transplant size, different canopy structures (homogeny and alternating) and single plant growth on plant to plant variation was analysed. Transplant variation, inhomogeneity of the canopy structure and differences in individual plant growth rates were not correlated with the occurring variation in head size at final harvest (Chapter 2). Variation in time to head induction was discovered under normal field conditions. As a result of this the focus was placed on plant development until head induction and its variation. Results of cabinet experiments showed that the juvenile phase had been already passed at planting. Variation during the juvenile development phase of the crop was excluded as reason of the variation in the field. Data from a second cabinet experiment with eight different temperatures regimes showed a high variability in time to head induction of single plants. These effects were considered in a piecewise linear regression model to describe the facultative vernalisation response to temperature combined with one stochastic subroutine which describes the occurring variability in head induction (Chapter 3).

This model was implemented into a broccoli growth model to validate the hypothesis that this variation in vernalisation can explain the occurring variation in head size. (Chapter 4). For this task a dry matter production and portioning model for cauliflower was used and reparameterised for broccoli growth and improved through implementation of dynamic *LUE* and *SLA* parameters.

Evaluation of the model and the subroutine for development and variation was done using independent field data. Stochastic variation of time to head induction can predict head size variation of independent broccoli plantings. Variation in time to head induction is most likely the main cause of the observed head size variation. The model can find application in decision support to predict the optima dates for selective hand harvest to optimize harvest operations and to predict exact delivery quantities. Another application could be the prediction of one optima date for once over harvest. Furthermore the model can be used to make accurate forecasts of harvest dates for adjustments of marketing strategies.

*Keywords:* *Brassica oleracea* L. var. *italica*, prediction of time to harvest, plant to plant variability

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## List of abbreviations

<i>a</i>	Intercept dry matter fraction (exponential)	(-)
<i>a<sub>0</sub></i>	Conversion coefficient head fresh weight with stem portion of 0 cm from head dry weight	(g g <sup>-1</sup> )
<i>a<sub>5</sub></i>	Conversion coefficient head fresh weight with stem portion of 5 cm from head dry weight	(g g <sup>-1</sup> )
<i>a<sub>10</sub></i>	Conversion coefficient head fresh weight with stem portion of 10 cm from head dry weight	(g g <sup>-1</sup> )
<i>a<sub>LUE</sub></i>	Slope parameter of LUE (I)	(g DM MJ <sup>-2</sup> m <sup>-2</sup> d <sup>-1</sup> )
<i>b</i>	Relative increase dry matter fraction	(-)
<i>bias</i>	average difference between measured and simulated values	(Unit of measurement)
<i>c<sub>LAI</sub></i>	Productive part of LAI, limitation factor of LAI <sub>max</sub>	(-)
<i>c</i>	Increase dry matter fraction (linear)	(-)
<i>CMS</i>	Cytoplasmic male sterility	
<i>cv</i>	Coefficient of variation	%
<i>d</i>	Intercept dry matter fraction (linear)	(-)
<i>f<sub>max</sub></i>	Maximum fraction of dry matter growth allocated to the curd	(-)
<i>GA</i>	Gibberellin acid	
<i>h</i>	Constant allometric growth	(-)
<i>I</i>	Global radiation	(MJ m <sup>-2</sup> d <sup>-1</sup> )
<i>InitTotDM</i>	Initial dry weight	(g)
<i>k</i>	Light extinction coefficient	(-)
<i>LAI</i>	Leaf area index	(-)
<i>LAI<sub>0</sub></i>	Initial leaf area index of the parameterisation data set	(-)
<i>LAI<sub>max</sub></i>	Maximum Leaf area index of the	(-)

	parameterisation data set	
<i>LUE</i>	Light use efficiency	(g MJ <sup>-1</sup> )
<i>LUE<sub>0</sub></i>	Initial light use efficiency	(g MJ <sup>-1</sup> )
<i>MSD</i>	Mean squared deviation between measured and simulated values	(Unit of measurement)
<i>n</i>	Number of statistical replications	(-)
<i>N<sub>Le</sub></i>	number of visible leaves at the end of vernalisation	(-)
<i>N<sub>Li</sub></i>	number of visible leaves at flower induction	(-)
<i>N<sub>Lmax</sub></i>	maximum final leaf number	(-)
<i>p1...p10</i>	Percentile value of the normal distribution curve	(-)
<i>PAR</i>	Photosynthetic active radiation	(MJ m <sup>-2</sup> d <sup>-1</sup> )
<i>pd</i>	Plant density	(plants m <sup>-2</sup> )
<i>PI</i>	Switching point of exponential to linear increase of dry matter fraction	(°Cd)
<i>Q</i>	Amount of intercepted radiation	(MJ PAR m <sup>-2</sup> )
<i>Qd</i>	Daily amount of intercepted radiation	(MJ PAR m <sup>-2</sup> )
<i>QTL</i>	Quantitative trait locus	
<i>RMSD</i>	Root mean squared deviation of measured and simulated values	(Unit of measurement)
<i>RMAE</i>	Relative mean absolute error	(%)
<i>R<sup>2</sup></i>	Coefficient of determination	(-)
<i>S1</i>	Field trial variant small seedlings	
<i>S2</i>	Field trial variant big seedlings	
<i>S3</i>	Field trial variant median seedlings	
<i>S4</i>	Field trial variant alternating seedlings	
<i>S5</i>	Field trial variant unclassified seedlings	
<i>sd</i>	Standard deviation	(Unit of measurement)

$SLA$	Specific leaf area	$(\text{cm}^2 \text{g}^{-1})$
$SLA_0$	Initial specific leaf area	$(\text{cm}^2 \text{g}^{-1})$
$sla_a$	Slope specific leaf area $PAR_{14}$	$(\text{cm}^2 \text{g}^{-1} \text{MJ}^{-1})$
$sla_b$	Slope specific leaf area and time	$(\text{cm}^2 \text{g}^{-1} \text{d}^{-1})$
$t$	time	d
$T_{mean}$	Daily mean temperature	$^{\circ}\text{C}$
$Td_{max}$	Daily maximum temperature	$^{\circ}\text{C}$
$Td_{min}$	Daily minimum temperature	$^{\circ}\text{C}$
$T_s$	Temperature sum	$^{\circ}\text{Cd}$
$T$	Temperature treatment	$^{\circ}\text{C}$
$T1$	Cardinal temperature 1	$^{\circ}\text{C}$
$T2$	Cardinal temperature 2	$^{\circ}\text{C}$
$T3$	Cardinal temperature 3	$^{\circ}\text{C}$
$T4$	Cardinal temperature 4	$^{\circ}\text{C}$
$V_{max}$	Maximum Vernalisation rate	$\text{d}^{-1}$
$V_{min}$	Minimum Vernalisation rate	$\text{d}^{-1}$

Names and units of auxiliary variables are specified in the text.

## General introduction

### *1. Background*

Under today's market conditions producers must reach delivery commitments very closely. Harvest ability at fixed dates and defined product quantities and qualities are prerequisites for assured sales and for meeting market requirements and contracts. The production of broccoli throughout the growing season is characterized by its highly intensive use of production factors; this includes the use of transplants, net covering, use of plastic film or fleece, irrigation and a high input of chemical fertilizers and pesticides. Moreover, planting, fertilizing, weed control, plant protection and harvesting characterize a very labor intensive production. In comparison with other field grown vegetables the above-mentioned aspects make broccoli production expensive. Most growers aim for a continuous and stable market supply to avoid price fluctuations and keep the financial risk as low as possible. However, actual harvest times often diverge from planned schedules. The duration of the growing period between sets planted at the same calendar date during autumn in two different years can differ up to two weeks in temperate climate zones (Babik and Elkner, 1997). Reasons for this are variations in weather conditions and different responses to temperature during the different developmental phases (Grevsen and Olesen, 1999; Grevsen, 2000).

Harvest criterion for fresh market production is a head fresh weight of 500 g (approx. 16 cm head diameter). This defines the time to harvest as the time from planting until this size is reached. Broccoli is harvested when the head is in full growth, and since the period between optimal harvest size and loss of quality or marketability due to oversize is quite small, the harvest window of single heads is defined as the period between optimal harvest size and unmarketability. The harvest window of one planting set is defined as the period between the

first and the latest possible harvest of single heads within one planting set as limited by size and quality of the heads. Producers have the opportunity to adjust their total sales volume in times of high product supply in the market by cold storage of the produce for short periods to smoothen market supply fluctuations (Wurr et al., 1992). However, cold storage imposes additional costs and is useful only to overcome short phases of oversupply in the market, targeting to obtain a higher price during phases of undersupply of the market. The deviation from planned harvest schedules, the short harvest window, the short shelf life, and the limited suitability for storage of the product lead to a fluctuating market supply which is accompanied by high price fluctuations. For example, the price for fresh market broccoli in Germany in 2009 was 152 € (100 kg)<sup>-1</sup> on average. During weeks with a low market supply the price increased by 45 % to 220 € (100 kg)<sup>-1</sup> while during a period with high product availability on the market the price declined by 59 % to 90 € (100 kg)<sup>-1</sup> (AMI, 2010). The price fluctuations in 2008 and 2009 are shown in Figure I-1.

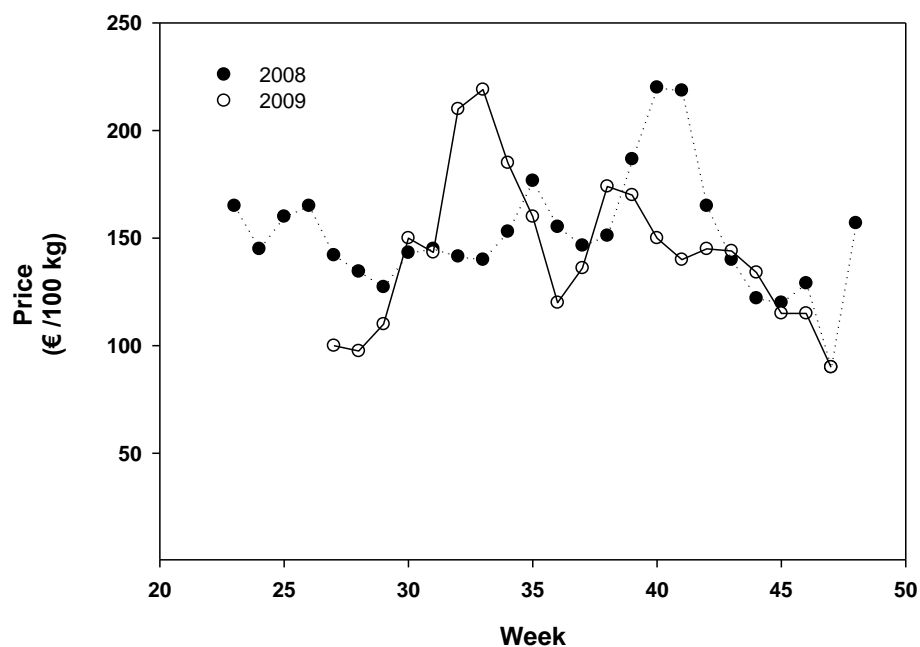


Fig.I-1. Market prices of broccoli on wholesale trading markets in Germany in the years 2008 and 2009 over calendar weeks 20-50 (data source: AMI, 2010).

Because of the uncertainty of weather conditions growers have to develop their strategies for planting, harvest and storage under economic considerations meaning that their annual profit depends on the possibility to compensate losses from weak sets. Generally, the grower is interested in selling a product of best quality and exactly on schedule to achieve the highest possible price. To mitigate price fluctuations, an increase in sales volumes by specific advertising and promotional activities by the retailers is possible. But for that exact estimations of delivery quantities are required before the expected delivery date. The amount of available product within one week has to be predicted as precise as possible. The predicted amount should match the real amount with an accuracy of at least 80 % (Behr AG, 2009 personal communication). In the end an accurate harvest prediction can support farmers to being able to optimize harvest schedules and pre planning marketing arrangements (Tan et al., 1997; 2000a).

In addition to the problems described above, a strong heterogeneity of broccoli head sizes within individual sets is observed in practice. Reasons for this variation are unknown and overall very little work has been done on this topic. The latest research has been done in Australia, where the main avenues to improve crop uniformity turned out to increase the plant density from 6 to 9 plants  $m^{-2}$  with direct sowing in single rows together with uniform irrigation and nitrogen application (Rogers, 2010). However, broccoli crops with plant densities higher than 4 plants  $m^{-2}$  are not suitable for fresh market production since the market required head sizes of 500 g fresh weight and such high planting densities will reduce the maximum single head size below this weight. Research in the past has shown that a large proportion of uniformity in time to harvest was attributed to genotypes and less to environmental conditions (Hulbert and Orton, 1984). Hulbert and Orton (1984) concluded that the development of a uniformly maturing hybrid is an issue of genotype selection. Although modern broccoli hybrid varieties and especially CMS cultivars showed less



variability than open pollinated cultivars in the past, this problem is still unsolved and it is still common in practice to harvest each crop a least three times by selective hand harvests. This makes predictions about exact delivery quantities additionally complicated. There is a high influence of the length of the cutting period and the numbers of selective hand harvests on harvest costs and the total production costs (Hulbert and Orton 1984; Olesen and Grevsen, 2000). Decisions about harvest operations must be taken under the aspect of price fluctuations and have to take economic aspects into account. If there is an oversupply in the market with severe price drops the growers may have to cancel an additional hand harvests and thus more than 30-40 % of the produce remain as waste or organic fertilizer in the field. This can lead to ecologically relevant problems due to the high nitrogen contents in the plant material and the high fertilization level during production. Additionally, this reduces the sales of growers and leads to economic losses.

Both problems, the deviation of planned harvest schedules and the plant to plant variation in time to harvest are still current and actual. The problem of unpredictability of harvest time in broccoli production and the sensitivity to high temperatures of the crop is addressed in current breeding projects and research programs (Farman and Björkman, 2011; Uptmoor et al., 2012). In addition to that and to address these problems, an advanced model-based prediction of exact harvest quantities and variation in time to harvest single plants could help to optimize the production and harvest operations with consideration of harvesting costs and the expected market prices.

## ***2. Harvest prediction models and physiological background***

The transition from the vegetative to the generative phase is essential for head formation in broccoli. It is crucial to understand the physiological background of head formation to be able to give reliable harvest date predictions. Generally, the development of the plant from planting to harvest can be divided into different growth phases: A juvenile phase defined as a non-sensitive phase to a cold stimulus, a head initiation phase and a head growth phase (Grevsen, 1998).

The duration of the juvenile phase was studied by several authors. Fontes et al. (1967) found that a cold stimulus accelerates curd induction for broccoli plants of varieties “Waltham” and “Green Mountain” only with a minimum age of 4 weeks. Fontes et al. (1967) concluded that a juvenile stage is existent in both broccoli cultivars. Comparable results were published 20 years later, the estimated juvenile phase of different broccoli cultivars ranged between 3-5 weeks (extremely early cultivars) to 4-6 weeks (early and intermediate cultivars) after seed germination (Fujime, 1988). Results of studies in Denmark indicate only a very short juvenile phase in transplanted Broccoli crops and the plants had probably already past the juvenile phase at transplanting (Grevsen and Olesen, 1999). Other studies considered the end of the juvenile phase when 4-5 leaves are visible (Wiebe, 1990; Mourão and Brito, 2000) or mean leaf number of 3.7 leaves exist (Grevsen, 1998). Since broccoli transplants were normally planted with visible leaf numbers of 3-5 leaves and 3-5 weeks after sowing this agrees with the above mentioned results. Other definition for the end of the juvenile phase is a fresh weight of 4-50 g per plant or a stem diameter of 5-8 mm (Miller et al., 1985; 1988). The description of a juvenile phase in broccoli prediction models is therefore not existent in most cases. Even when authors found hints for the existence of a juvenile phase they did not consider this in their prediction model. This is done because this stage is already completed at

planting, or the use of a phonological marker like leaf number seems to be not accurate enough (Wurr et al., 1995; Grevsen and Olesen, 1999) or they do not separate the crop development into different developmental phases (Marshall and Thomson, 1987 ab).

The development of broccoli from the vegetative to the generative stage and the response to different temperatures was analysed by several authors (Gauss and Taylor, 1969; Fujime, 1983; Wiebe, 1990; Wurr et al., 1991; 1992; 1995; Grevsen and Olesen, 1999). In general, broccoli has been considered to have a cold requirement for head formation (Fontes et al., 1967; Fontes and Ozbun, 1972). Gauss and Taylor (1969) observed that the reproductive differentiation of different broccoli sets grown under average temperatures of 18 °C and 30 °C showed a reproductive differentiation between 5-6 weeks after sowing. They concluded that broccoli does not have a qualitative vernalisation requirement and that temperature alone has no influence on the time to head induction. Wiebe (1990) concluded the same, according to his observations the development to head induction for early broccoli varieties was accelerated with increasing temperature while for later varieties vernalisation and decreasing temperature leads to an accelerated flower induction. Wiebe claimed that the cold requirement for curd induction was facultative for broccoli while it was obligatory in cauliflower. Data of 16 broccoli sets of 4 cultivars (Compacta, Comanche, Green Valient and Marathon) grown in Portugal over the whole growing season and with observations of two years showed that an obligatory cold requirement was not detected for any of these cultivars (Mourão and Brito, 2000). There are nevertheless high differences among different varieties. Some varieties require cool conditions (maximum temperature 23 °C) to induce and maintain vernalisation (Farnham and Björkman, 2011). Other authors found a limit of 17 °C for head initiation (Grevsen, 1998). Others studies in calabrese showed that after the apex diameter reached a value of 0.5 mm the production of secondary meristems was induced automatically and that low temperatures directly controlled the apex expansion rate (Wurr et al., 1995).

To predict the time to head induction some authors used thermal time models (Diputado and Nichols, 1989; Fyffe and Titley, 1989; Tan et al., 2000 ab). Existent thermal time models often simulate the development with three cardinal temperatures: a base temperature below which the plant does not develop, an optimum temperature at which the developmental rate is at its maximum, and a maximum temperature above which the developmental rates are zero (Tan et al., 2000). However, the authors of those studies were not always able to define a maximum temperature from their data sets (Tan et al., 2000). Values for reported cardinal temperatures range between 0 and 4.5°C for minimum temperatures and around 20- 21 °C for optimum temperatures (Fyffe and Titley, 1989; Diputado and Nichols, 1989). Fellows et al. (1997) simulated on the basis of data from cabinet experiments that the head induction not occurred at temperatures of 0, 30 and 35 °C. The time from planting to head induction was described through a linear relationship between the reciprocal of time to head induction and the mean air temperature, the thermal time required for head initiation was 680 °Cd and the base temperature was 0.7 °C (Mourão and Brito, 2000). In addition to temperature sum concepts some modeling approaches are very similar to the vernalisation descriptions in cauliflower (Wurr et al., 1995; Fellows et al., 1997; Grevsen, 2000). The optimum temperature for vernalisation marks a small plateau. Comparisons of the effect of cold temperatures on curd formation of cauliflower and broccoli were presented in the past (Wiebe, 1990). Several authors developed prediction models for production scheduling and prediction of time to harvest of broccoli and calculating mean time to harvest (Marshall and Thompson, 1987ab; Scaife et al. 1987; Pearson and Hardley, 1988, Wurr et al., 1991, 1992; Grevsen, 1998, 2000; Grevsen and Olesen, 1999; Tan et al. 2000 ab; Kläring et al. 2001). The model of Marshall and Thompson (1987) calculates the time from sowing to harvest depending on solar radiation and temperature. The influence of solar radiation was integrated

with a combined factor of temperature and radiation (Scaife et al. 1987), developmental stages of the crop were not considered. A later model describes head growth after vernalisation as a function of temperature sum with different base temperatures depending on cultivars (Pearson and Hardley, 1988). The model was extended to account for effects of plant density (Wurr et al., 1991). To describe the duration to head induction the effect of different temperatures was analysed and models for apex development were presented (Wurr et al. 1995 and Fellows et al. 1997; Grevsen and Olesen 1999). Researchers mainly attributed variations in time to harvest to the effects of different environmental factors on growth and development in different development phases of the crop (Grevsen, 2000). The juvenile is accelerated with increasing temperature while the head induction phase can be delayed. Functions with different temperature optima for different developmental phases can be found. These different developmental phases are considered in a model of Tan et al. (2000), analysing the accuracy of prediction with different model combinations. Additional work and investigations on broccoli growth was done in the following years. A significant effect of global radiation on head growth of broccoli was shown (Grevsen, 1998), indicating that prediction accuracy of simple temperature sum models is limited. An accurate prediction of plant growth and time to harvest should base on the mechanistic relationship between global radiation and plant growth. Beside calculation of dry matter production and the allocation of dry matter among the different plant organs is important for the simulation of plant growth and development (Marcelis, 1993). In 1999 a model for dry matter production and partitioning for cauliflower was presented (Kage and Stützel, 1999). For the vegetative and the curd growth phase, the model simulated dry matter production as the product of intercepted photosynthetically active radiation (PAR) and light use efficiency (LUE). Intercepted PAR was calculated according to Beer's law from leaf area index (LAI) and light extinction coefficient  $k$ . The loop to dry mass production was closed by multiplying the leaf dry mass with a value for the specific leaf area

(SLA) to obtain the LAI of the crop. During the vegetative phase an allometric function of dry matter partitioning between leaf and stem was used. After the vernalisation process was completed an empirical logistic function of temperature sum described the fraction of dry matter allocated to the curd. The model worked under the limitations of constant parameter values for LUE and SLA which limited the use of the model for growers and production scheduling since it was not possible to make accurate predictions without fitting the parameter LUE and SLA to the individual evaluation data sets (Kage and Stützel, 1999). In the following models improvements had been made by the implementation of a dynamic LUE and SLA depending on the radiation levels (Kage et al. 2001a; 2001b). Studies with broccoli showed comparable relationships between SLA, LUE and the radiation level. In summary, reduced radiation levels caused reduced dry matter production and an increase in SLA (Kläring, 1998; Mourão and Hadley, 1998; Francescangeli et al. 2007). Other studies had shown that light use efficiency of broccoli crops declined with increasing radiation level (Olesen and Grevsen, 1997). Based on these results a prediction model for broccoli should consider developmental phases as well as dry matter production and portioning to predict accurate harvest dates. Nearly all existing harvest prediction models for broccoli are empirical or deterministic; the output is a predicted mean harvest day for one canopy and a given simulation (i.e. Tan et al., 2000a). Also, most existing models of head induction and growth of broccoli (i.e. Tan et al. 2000a) calculate temperature dependency of organ formation and development. Prediction models which include dry matter production with dynamic changes in SLA and LUE due to environmental conditions or models which consider plant to plant variation are missing in literature as well as explanations for the occurring variability in broccoli.

### *3. Objectives and structure of experiments*

The objectives of this work is to identify reasons for head size variation as it is overserved in broccoli canopies in commercial production and to predict this variation by stochastic mathematical formulations and to integrate these equations in a mechanistic simulation model. The project aims to improve the prognosis of vernalisation and head growth. Comparison of the model simulation results to observed data should evaluate the source for variation in head size, to obtain a better understanding of the plant physiological background of head size variation and its formation. Another target is to understand and to predict the variability in growth and development of individual plants to optimize harvest operations and the prediction of exact delivery quantities. For hypothesis testing a combination of stochastic and mechanistic modelling is used. To study the transplant size variation in relation to head variability, field trials were conducted with different classes of transplants with defined differences in leaf area and leaf area variation (Chapter 2). Next to this the head induction and its variability in relation to temperature is examined in cabinet experiments with eight different temperature regimes and 10 replications per sampling date. The outcome should be a head induction model and a stochastic model to describe variability in development to head induction of single plants cohorts (Chapter 3). For prediction of time to harvest an empirical model of dry matter production and portioning in relation to global radiation and temperature will be derived from literature and field trials. The vernalisation and variation model is implemented as a subroutine, to test the hypothesis that differences in time to head induction are responsible for head size variation. Main task is to predict the variability of head size and its influence on the number of necessary hand harvests (Chapter 4).

Is the variation of head size of broccoli (*Brassica oleracea* var. *italica*) due to variability in seedling size?

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***Abstract***

A strong variability in head size of broccoli is usually observed in the field. The narrow harvest window (i.e. the period between optimal harvest size until beginning of quality decline and oversize) leads to the need for multiple hand harvests which increase production costs significantly. However, the reason for this variability is unknown. Variations in seedling leaf area of F<sub>1</sub> hybrids as large as 20 % can be observed. We tested the hypothesis that this variability is the cause of head size variation at harvest. In a second hypothesis we expect that crop heterogeneity increases over time due to interaction and competitive effects between plants. Total seedling leaf area was estimated based on the allometric relationship between leaf width and leaf area. Seedlings were sorted into three groups: small (below first quartile), median (around the second quartile, most homogeny) and big (above the third quartile). We created canopies with different compositions of plant size and variability.

Overall, five sets were studied over two years. In the first trial, a random sample of the whole seedling population was used as a standard and was compared to the plant classes median and alternating. In the latter, a small and a big plant were planted next to each other which allowed us to analyse the development of competitive effects. In all later experiments the two variants median and alternating were compared to seedling populations made up of the smallest or



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biggest seedlings. Vegetative and head growth pattern were analysed by measurements of leaf width and head diameter. In addition continuous sampling for fresh and dry weight was done. Total leaf area at final harvest was not correlated with seedling leaf area. The relative growth rates in leaf area of single plants across all plant classes were not different. No competitive effects were detected. After head induction the initial differences in leaf area and variability were statistically untraceable. No effect of seedling size on the variability in total plant size, relative head growth rates or head size variation was found. No differences in relative head growth rates but high differences in head induction of single plants were detected. These differences and not seedling size differences were responsible for the observed head size variation. Head induction occurred independently of plant size, and head size initially correlated poorly ( $R^2 = 0.08-0.33$ ) with total dry weight or plant size, at final harvest this correlation improved ( $R^2 = 0.26-0.70$ ). This is due to the source capacity (amount of intercepted radiation, produced assimilates) which becomes limiting in the later head growth phase. Plant with higher leaf area can intercept more light and can supply the head with a higher amount of assimilates. A negative correlation between final leaf number and head size of single plants was found. In conclusion our data suggest that differences in time to head induction were responsible for head size variation at harvest.

Keywords: Seedling, vernalisation, uniform canopy, variability, multiple harvests.

### ***1. Introduction***

A strong variability in time to harvest, defined as the duration from planting to harvest size (head fresh weight of ca. 500 g) between individual plants is usually observed in practical broccoli production. The harvest window of one planting set is defined as the time span between optimal harvest size and loss of marketability due to oversize or quality decline of single heads. The narrow harvest window and the short shelf life of the product necessitate several selective hand harvests in the same crop. In commercial production, up to three hand harvests are carried out to collect 80-90 % of the plants. Since harvest operations are responsible for a large fraction of the total production costs, the number of harvests has a great influence on the profitability of broccoli production. Harvest decisions are also influenced by market supply, market prices and delivery commitments. Under adverse market conditions picking percentages of only 50-60 % may occur in practice. The reasons for the variation in time to harvest are not explained in literature at present. In Australia, large investments have been made to develop a mechanical broccoli harvester (Dellacecca, 1996), but due to the temporal variability of crop maturity the harvest percentages obtained with the mechanical harvester did not exceed 50 %. Because of these problems a project aiming at developing agronomic strategies and using new broccoli varieties was carried out. The goal was a harvest percentage of not less than 90 % with once-over harvest (Rogers, 2010). One finding was the importance of the optimal planting date and period in relation to environmental conditions and optimal plant development. Main avenues to improve crop uniformity turned out to be to increase plant density from 6 to 9 plants m<sup>-2</sup> with direct sowing in single rows, and uniform irrigation and nitrogen application (Rogers and Rogers, 2010). It had been shown in other studies that the time of seedling emergence at direct seeding is as

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important as equidistant plant arrangements to produce homogeneous broccoli canopies (Peck and Clack, 1973). However, broccoli crops with plant densities above 4 plants m<sup>-2</sup> produce only small heads which are mainly suitable for the processing industry. For fresh market broccoli, studies regarding intra-crop plant variability in time to harvest are missing.

In cauliflower many attempts have been made to reduce the variability of head size, also aiming at narrowing the harvest window. In a study using six different cultivation treatments, slight improvements in homogeneity were achieved by grading for seedling size and root morphology (Salter, 1969b). With use of seedlings the variability in time to harvest compared to direct seeding was higher in two of three tested cultivars and increased with seedling age (Salter, 1969b). Other studies identified the variation in time of curd induction as reasons for the variability in time to harvest in cauliflower (Olesen and Grevsen, 2000; Booij, 1990a; Salter, 1969a). About 55 % of the variability in time to harvest could be explained by the variation in curd induction and temperature fluctuation during curd growth. Reasons for variation in curd initiation were plant to plant variation at the end of juvenility combined with variation in day temperature after the juvenile stages of the plant (Booij, 1990a). Therefore, attempts have been made to reduce the variation in time to harvest by cold treatments of seedlings before planting (Wurr et al., 1981b, 1982; Wiebe, 1975; Salter and Ward, 1974). The success of these treatments, however, was highly variable which may be explained by the timing of the treatments in relation to the plant developmental stage (Olesen and Grevsen 2000). Another explanation might be given by effects of differences in plant size. Size differences of seedlings might superimpose developmental homogeneity in the subsequent head growth phase in the field and thus partly offset the effects of the cold treatment before planting.

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Our own measurements on self-raised as well as on commercially produced seedlings in different raising systems showed coefficients of variation in leaf area (cm<sup>2</sup>) of around 20 % between plants of the same lot (identical F1 seed material and growing conditions). So far, there is no measurement-based study on the variation in time to harvest in relation to seedling size variation for broccoli. Since it is common in Europe to use seedlings in broccoli production for the fresh market, the question arises if it is possible to reduce the duration of the harvest period or the variation in time to head induction by selection of seedlings according to their size. If this was possible harvest operations and thus production costs could be reduced. In this case uniformity of seedlings could be an important quality parameter for seedlings used by growers.

The present state of knowledge leads us to two hypotheses which will be tested in this study: The first hypothesis postulates that for given growing conditions seedling leaf area is the main determinant of plant and head growth and thus time to harvest. According to this hypothesis we expect a shorter time to harvest for a canopy consisting of big plants in comparison with a canopy of small plants. In our second hypothesis we expect an increase of canopy heterogeneity over time due to interaction and competitive effects in heterogeneous compared to homogeneous canopies. Reduced variation in seedling size should reduce the variation of plant size at harvest accordingly.

## 2. *Material and Methods*

Starting with the hypotheses of this study the first goal is to determine the leaf area of individual plants non-destructively before planting to create canopies with different compositions of plant size and variability.

Seedlings of *Brassica oleracea* var. *italica* 'Ironman F1' were grown in the greenhouse (16/14 18 °C) with peat substrate (Potgrond P, Klaasman, Geeste, Germany). Calibrated seeds of 1.8-2 mm diameter were sown into plug trays of 60x40 mm (VP 96, Vefi, Larvik, Norway) directly. Since broccoli is sensitive to molybdenum deficiency, plants were fertilized with sodium molybdate ( $\text{Na}_2\text{MoO}_4$ ) in a concentration of 0.5 g L<sup>-1</sup> and a total amount of 2.2 g/1000 plants at the two-leaf stage. One week before planting, which occurred at the three leaf stage, the plants were transferred to outdoor conditions under netting for hardening. In early sets plants were covered by a fleece over night to avoid frost damage. To grade seedlings according to their leaf area a non-destructive method for leaf area measurement was developed and validated. Maximum leaf widths were measured using a ruler and a quadratic relationship between the maximum leaf width and the individual leaf area as measured with a leaf area meter (LI3100, Licor, Lincoln, NE, USA) was used (Fig.II-1). For establishment of the calibration function a sample of single leaves of 15 plants was used for each population of seedlings (set) at each planting date (Table. II-1).

The field trials were carried out on our experimental field in Hannover-Herrenhausen on a loamy sand soil. In our first planting set a randomised sample of the whole seedling population was used as a standard and compared to one group of seedlings selected around the population median. This seedling class showed the least variation in total leaf area and greatest homogeneity in canopy structure. A third variant consisted of an alternating canopy

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structure, created by planting a small plant next to a big plant. This was done in order to see whether competitive effects increase plant to plant. In all later sets the two variants median and alternating were compared with one seedling class consisting of a selection of the smallest seedlings of each sowing date, and with one group of the biggest seedlings selected from each sowing date. Before planting conventional soil tillage with plow and cultivator was used. Plants were irrigated by overhead precision irrigation to a level of 22 vol. % (85 % field capacity) controlled by TDR (0-30 cm) when the irrigation threshold level of 18 % by volume (70 % field capacity) was reached. The basic fertilization of the soil with 40 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 110 kg ha<sup>-1</sup> K<sub>2</sub>O and 10 kg/ha<sup>-1</sup> MgO was applied after soil analysis (CAL) before planting. Nitrogen fertilizer was applied as calcium ammonium nitrate (27 % N). The actual nitrogen state of the soil was determined by the N<sub>min</sub> method. The soil was fertilised to a target of 160 kg N ha<sup>-1</sup> in 0-30 cm depth at planting, and 360 kg N ha<sup>-1</sup> (0 - 60 cm sampling depth) in the 3<sup>rd</sup>-4<sup>th</sup> week after planting. A preventive treatment with 0.5 g / plant Nexion Neu (Scotts Celaflor GmbH, Mainz, Germany) against cabbage root fly was used for all plants at all plantings. Mechanical weed control was used to keep the plots free from weeds. Net cover against birds and insecticides were applied when needed. Overall, five sets of seedlings were grown at different planting dates during the growing seasons of 2011 and 2012. After classification, the seedlings were planted by hand at a density of 4 plants per m<sup>2</sup> (50 x 50 cm) in groups of different classes in size. In the field trials planting dates were arranged as main plots and plant size class as a subplot in a split-plot design with three replications as blocks. Random samples of four plants per replication were harvested from the field plots four times in 2011 and three times in 2012 for destructive measurements of leaf area (LI 3100, Licor, USA), fresh and dry weight of plant organs (stem, leaf, head). In addition, three plants per replicate were harvested for apex dissection. The plant apexes of all experiments in 2011 were

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analysed at least three times using a binocular (B061, Olympus, Tokyo, Japan). Head induction was considered as the switch from the vegetative to the generative phase and to have occurred when first flower buds were clearly visible on the apical meristem (Uptmoor et al. 2008). During the experiments in 2011 maximum leaf widths and head diameters of four plants per plot were measured non-destructively using a ruler and a B660 quicktest gauge (B660, Kroelin GmbH, Schlüchtern, Germany). For comparison of leaf areas, fresh and dry weights mean values of the four plants per replication were calculated and used for further statistical analyses. Thus, the standard deviation for treatments reflects the variation between experimental replications. For the examination of plant-to-plant variability, single plant values per replication were used to calculate the coefficient of variation for every replication separately. Statistical analyses were performed using R.2.15.2 (R Core Team, 2013). Multiple comparisons of the effects of seedling size and planting date on vegetative and generative growth parameters like leaf area and head sizes were conducted after analysis of variance (ANOVA), and functions of the R packages MVTNORM and MULTCOMP were used. To analyse the effects of seedling size and variation on the variation of leaf area and head size at final harvest, a likelihood ratio test was used to compare a mixed model with fixed effects and assumed variance homogeneity between the variants with one model assuming a seedling size dependent heterogeneity of variance. The data of the non-destructive measurements for the maximum leaf width and head diameter was analysed by regression after  $\ln$  transformation and by calculation of relative growth rates. Regression parameters and calculated parameters of single plants, namely the relative growth rate and the intercept points, were analysed by analysis of variance (ANOVA) and pairwise t-tests were conducted with Bonferroni adjusted p values. After linearisation by  $\ln$  transformation head diameter differences in relative growth

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rates (slope) and head induction (intercept) were also tested using the R package MULTCOMP function for generalised linear model hypothesis testing (glht) ( $p \leq 0.05$ ).

### 3. Results

The non-destructive leaf area determination method provided an accurate assessment of individual leaf area for each set of seedlings (Fig. II-1 and Table. II-1).

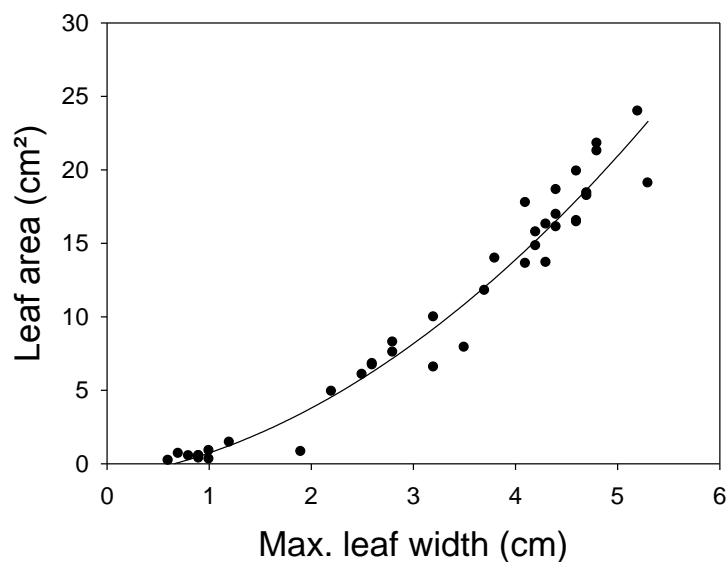


Fig. II-1. Example of the relationship between leaf area and leaf width. The solid line shows the regression line  $y = 0.78x^2 + 0.31x$ ,  $R^2 = 0.96$  and  $n = 39$ .



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Table II-1.

Regression parameters  $\pm$  standard deviation of the quadratic relationship  $y = ax^2 + bx$  between leaf area and leaf width, with coefficient of determination  $R^2$ , for the different experiments.

Experiment/Year	Parameter $a$	Parameter $b$	$R^2$
1 2011	$0.90 \pm 0.03$	$0.02 \pm 0.15$	0.99
2 2011	$0.73 \pm 0.27$	$0.68 \pm 0.06$	0.98
3 2011	$0.78 \pm 0.08$	$0.31 \pm 0.36$	0.96
4 2012	$0.84 \pm 0.10$	$0.26 \pm 0.39$	0.99
5 2012	$0.56 \pm 0.08$	$1.10 \pm 0.33$	0.97

Within sets leaf areas of individual seedlings were normally distributed as shown for the example of set 3, 2011 in Fig. II-2.

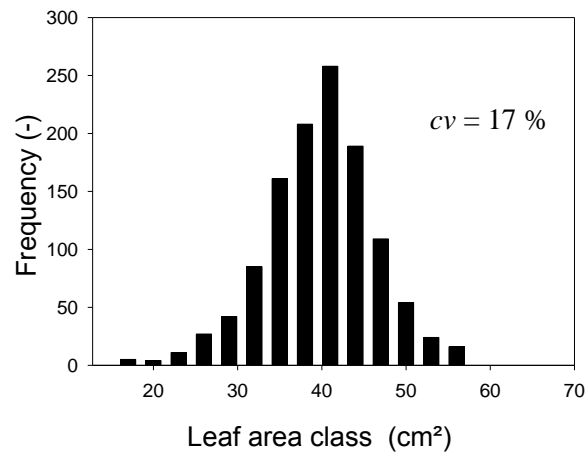


Fig II-2. Frequency distribution of plant leaf area (cm<sup>2</sup>) of 1440 seedlings with three leaves from set 3, 2011.

In the next step the seedling population was subdivided into defined leaf area classes. In all experiments, the plants of treatment S1 had significantly higher leaf areas than those of S3 and S2, the latter having significantly the smallest leaf area, directly after planting (Table. II-2).

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Table II-2.

Mean leaf area per plant (cm<sup>2</sup>) and coefficient of variation (%) of single plants at planting (destructive measurement using LI 3100).

Experiment Year	Variant				
	S1 Big	S2 Small	S3 Median	S4 Alternating	S5 Unclassified
1 2011			40.2 <i>b</i> 7.2 %	36.5 <i>a</i> 20.5 %	39.4 <i>b</i> 16.8 %
2 2011	53.8 <i>c</i> 15.9 %	37.1 <i>a</i> 9.5 %	45.7 <i>b</i> 6.2 %	48.5 <i>cb</i> 29.1 %	
3 2011	50.0 <i>c</i> 6.1 %	31.1 <i>a</i> 15.72 %	41.9 <i>b</i> 7.2 %	40.5 <i>b</i> 26.5 %	
4 2012	33.9 <i>c</i> 11.2 %	24.5 <i>a</i> 15.43 %	26.8 <i>ab</i> 6.8 %	29.2 <i>b</i> 17.6 %	
5 2012	36.8 <i>c</i> 10.7 %	24.3 <i>a</i> 14.31 %	30.9 <i>b</i> 7.4 %	31.3 <i>b</i> 22.4 %	

Different letters indicate significant differences between treatments within experiments (pairwise *t*-test,  $p \leq 0.05$ ,  $n = 3$  with 4 plants per replication).

The differences in leaf area at planting between small and big plants were 28 % on average. Mean leaf areas of S3 and S4 were on the same level at planting (except for experiment 1), but the coefficients of variation were 3-4 fold higher in S4 than in S3. All variants showed clear differences in variation of leaf area at planting as intended in our experimental approach. Influences of seedling size on leaf area of single plants later on were only found for harvest date one in experiment 2 in 2011 where the variant S2 (small transplants) showed the smallest leaf area (Table. II-3).

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Table II-3.

Mean leaf area per plant (cm<sup>2</sup>) of the second and third harvest (destructive measurement using LI 3100).

Experiment Year	Temp. Sum (°Cd)	Variant				
		S1 Big	S2 Small	S3 Median	S4 Alternating	S5 Unclassified
1 2011	470			659	626	664
	961			4999	4881	5483
2 2011	407	1354 <i>b</i>	1098 <i>a</i>	1114 <i>ab</i>	1305 <i>b</i>	
	882	7152	6694	6758	6702	
3 2011	613	915	797	762	893	
	1034	7736	7480	7531	7607	
4 2012	394	2364	2159	2380	2046	
5 2012	331	4179	3573	3663	3656	

Different letters indicate significant differences between treatments within experiments (pairwise *t*-test,  $p \leq 0.05$ ,  $n = 3$ ).

All other measurement dates showed no differences in leaf area between variants. Mean values of leaf areas of the different variants were close to each other and did not differ significantly, but the initial plant size differences tended to remain visible. Overall differences in leaf area showed differences between 3 % (harvest date 2 set 3) and 19 % (date 1 set 3). On average differences in mean leaf area between variants within one harvest date were less than 10 %. At final harvest the same trends reflecting the initial size differences in leaf area between the size classes were visible (Table. II-4).

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Table II-4.  
Mean leaf area (cm<sup>2</sup>) at final harvest (destructive measurement using LI 3100).

Experiment Year	Variant				
	S1	S2	S3	S4	S5
	Big	Small	Median	Alternating	Unclassified
1 2011			7106	7531	7637
2 2011	6055	5500	5921	5966	
	7496	6654	7343	6508	
4 2012	9040	8255	8976	8948	
5 2012	7098	6676	6696	6422	

No significant differences between treatments were detected.

Non-destructive measurements on single plants showed that their relative leaf area growth rates were not different between variants and decreased for all plant classes with increasing age. Tendencies of smaller relative growth rates for S1 (canopy established from big seedlings) compared to S2 (canopy established from small seedlings) were visible (Table. II-5).

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Table II-5.

Mean relative growth rates of leaf area ( $\text{cm}^2 \text{ cm}^{-2} \text{ }^\circ\text{Cd}^{-1}$ ) between measurement dates/Temperature sum ( $^\circ\text{Cd}$ ) and coefficient of variation (%) of single plants averaged over experimental replications (data from non-destructive measurements).

Experiment	Temperature sum after transplanting	Variant				
		S1 Big	S2 Small	S3 Median	S4 Alternating	S5 Unclassified
1 2011	129					
	227			0.0101 5.78	0.01 8.64	0.0105 4.24
	316			0.0118 3.94	0.012 6.21	0.0117 12.73
	466			0.0055 8.59	0.0057 8.03	0.0055 4.87
2 2011	142					
	260	0.0097 7.71	0.0106 10.52	0.0101 4.97	0.01 12.41	
	605	0.004 8.58	0.0042 13.52	0.0043 7.3	0.0042 9.47	
3 2011	176					
	268	0.0181 13.31	0.0193 18.65	0.0183 10.96	0.0198 14.1	
	395	0.0076 7.68	0.0078 7.13	0.0078 7.17	0.0077 9.97	
	511	0.0057 16.5	0.0059 10.25	0.0061 14.36	0.0059 16.92	

No significant differences between treatments were detected.

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A significant influence of seedling size on head size was found only for experiment 4 in 2012 where S2 (small transplants) resulted in significantly smaller heads in comparison with all other variants at final harvest (Table. II-6).

Table II-6.  
Mean head diameter (cm) at final harvest (destructive measurement).

Experiment Year	Variant				
	S1	S2	S3	S4	S5
	Big	Small	Median	Alternating	Unclassified
1 2011			16.1	13.7	15.9
2 2011	16.5	16.4	17.5	17.4	
3 2011	14.3	11.9	13.5	13.1	
4 2012	13.5 <i>b</i>	11.2 <i>a</i>	12.6 <i>b</i>	12.9 <i>b</i>	
5 2012	11.5	12.4	13.8	13.8	

Different letters show significant differences between variants for every single set (pairwise *t*-test,  $p \leq 0.05$ ,  $n = 3$ ).

In general, measured head size showed no differences between variants. The variation of single plant leaf area at final harvest was not correlated with transplant size variation in any of the experiments (Fig. II-3).

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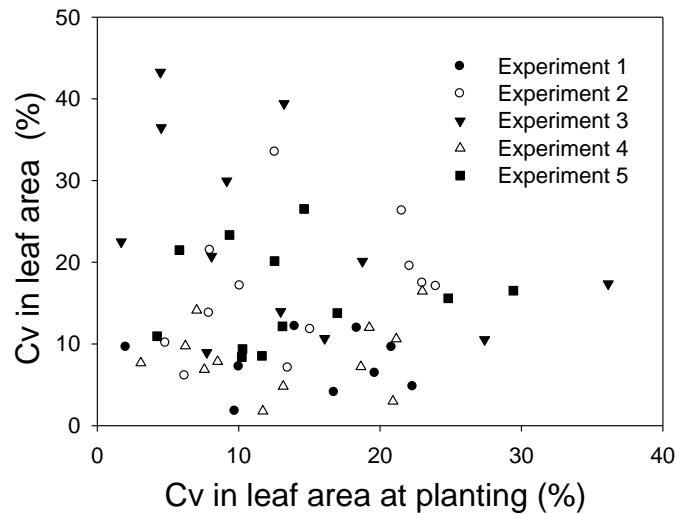


Fig.II-3 . Coefficients of variation (cv) in leaf area at planting of the different experiments calculated for each variant and experimental replication (destructive measurements) in relation to the coefficients of variation in leaf area at final harvest. No significant relationship was found by regression analysis.

The likelihood ratio test detected no effects of initial seedling size or variation on variance in leaf area at final harvest. We also found no effect of the alternating canopy structure in terms of differences in leaf area variation or head size variation at final harvest compared to the other variants. The variation of head size at final harvest was not correlated with the variation in leaf area at planting (Fig. II-4).

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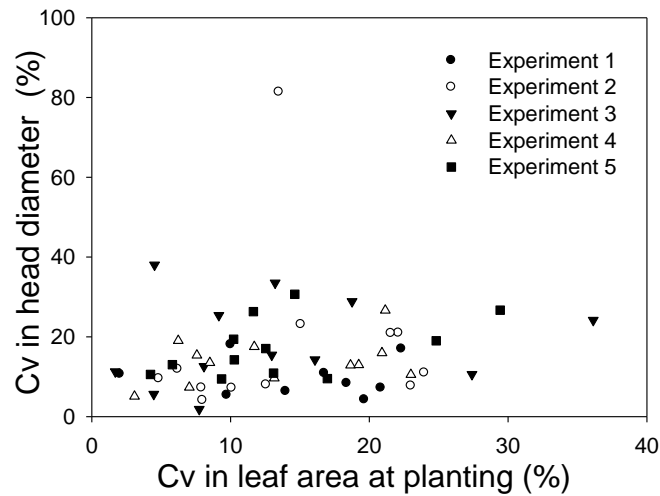


Fig.II-4 . Coefficients of variation in leaf area at planting (%) of the different experiments calculated for each variant and experimental replication (destructive measurement) in relation to the coefficients of variation in head diameter at final harvest. No significant relation was found by regression analysis. The result of the likelihood ratio test for differences in variance of head diameters between the variants showed also no significant effect of seedling variation on head size variation.

The likelihood ratio test showed no significant effects of seedling size variation on later head size variability indicating that head size variation is independent of seedling size variation.

Relative growth rates of head diameters did not differ between variants and the variation in relative head growth rates were not different for the different seedling classes (Table. II-7).



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Table II-7.

Mean relative growth rates of head diameter ( $\text{cm cm } ^\circ\text{Cd}^{-1}$ ) of the different variants (non-destructive measurements) and coefficient of variation in relative head growth rate.

Experiment Year	Variant				
	S1 Big	S2 Small	S3 Median	S4 Alternating	S5 Unclassified
1 2011			0.0064 9.10 %	0.0062 8.50 %	0.006 7.90 %
2 2011	0.0048 12.50 %	0.0056 12.10 %	0.0054 12.50 %	0.005 8.60 %	
3 2011	0.0059 18.30 %	0.006 9.60 %	0.0053 8.90 %	0.0059 12.60 %	

No significant differences were detected.

Intercepts of the exponential functions of head diameter showed no differences between variants, but high coefficients of variation for single plants within variants were measured (Table. II-8).

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Table II-8.

Mean y-intercepts of exponential regression of head diameter growth (cm) of the different variants (non-destructive measurements) and coefficients of variation in intercepts of single heads.

Experiment Year	Variant				
	S1 Big	S2 Small	S3 Median	S4 Alternating	S5 Unclassified
1 2011			0.022 76.90 %	0.026 73.50 %	0.031 72.70 %
2 2011	0.175 167.60 %	0.053 112.90 %	0.049 74.90 %	0.062 40.10 %	
3 2011	0.043 111.30%	0.014 61.10 %	0.044 78.00 %	0.046 84.50 %	

No significant differences were detected between treatments within experiments.

The coefficient of variation in intercept points of single heads ranged between 40.1 % and 167.6 % but was again not correlated with transplant characteristics. As for the destructive measurements we found no relationship between the coefficients of variation in leaf area at the beginning of the experiment and the coefficients of variation in head size at final harvest for any of our experiments by non-destructive measurements (data not shown). The transition from vegetative to generative stage, as observed by apex dissection, was not related to plant dry weight or plant class at planting (Fig. II-5).

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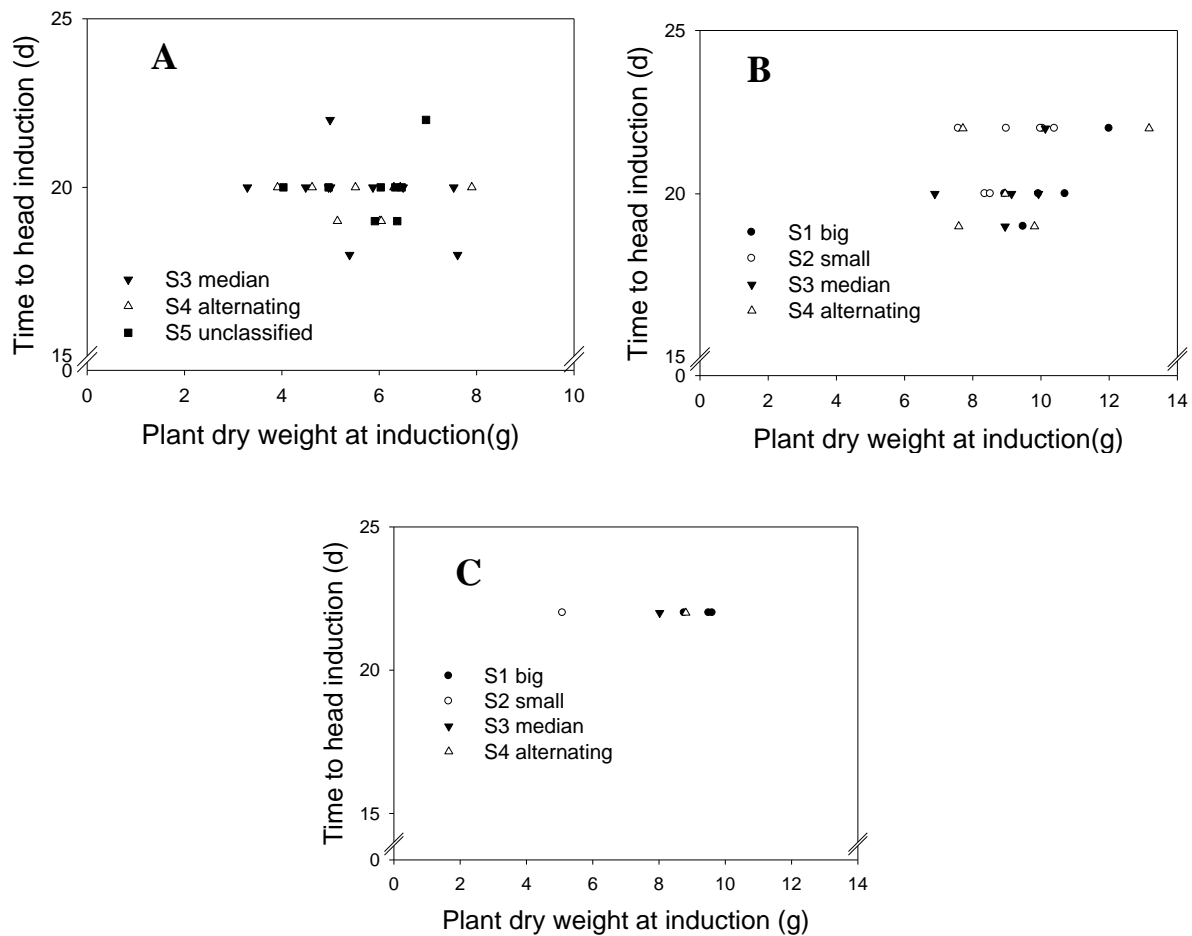


Fig. II-5. Time to head induction determined by apex dissection against total dry weight of single plants at head induction for the different classes of seedling sizes of experiment 1 2011 (A), experiment 2 2011 (B) and experiment 3 2011 (C). No significant relationship between time to head induction and plant dry weight was found by regression analysis; for the experiments in 2012 no apex dissection was conducted.

Head sizes were negatively correlated with the observed numbers of final leaves (Fig. II-6) indication that later transition to flower development results in smaller heads. We found no differences between variants and the variation in final number of leaves was less pronounced than the variation in head size.

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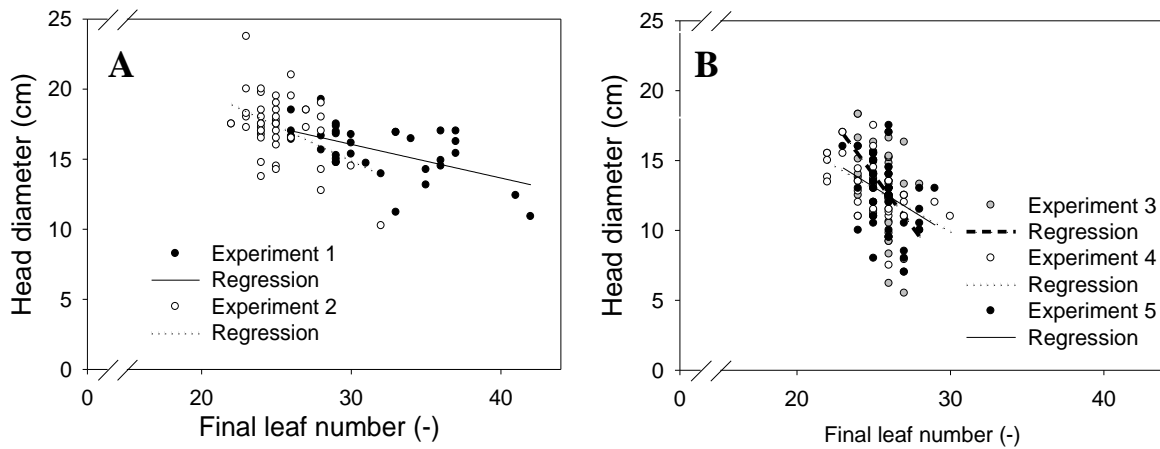


Fig. II-6. (A) Head diameter (cm) in relation to final leaf number of single plants observed in the experiments 1 and 2, lines show linear regression. For experiment 1 the regression equation (solid line) was  $y = -0.24x + 23.27$  and  $R^2 = 0.32$ , for experiment 2 the relationship was  $y = -0.50x + 29.94$  and  $R^2 = 0.20$  (dotted line). (B) Head diameter of the experiments 3, 4 and 5 in relation to final leaf number. Lines show linear regression. Regression equations were  $y = -1.47x + 50.81$ ,  $R^2 = 0.28$  (experiment 3, dashed line),  $y = -0.62x + 28.59$ ,  $R^2 = 0.25$  (experiment 4 dotted bold line) and  $y = -0.68x + 30.17$ ,  $R^2 = 0.12$  for experiment 5 (solid line).

The relationship between head dry weight and vegetative dry weight was weak for the early head growth stages but became stronger with increasing head size (Fig. II-7).

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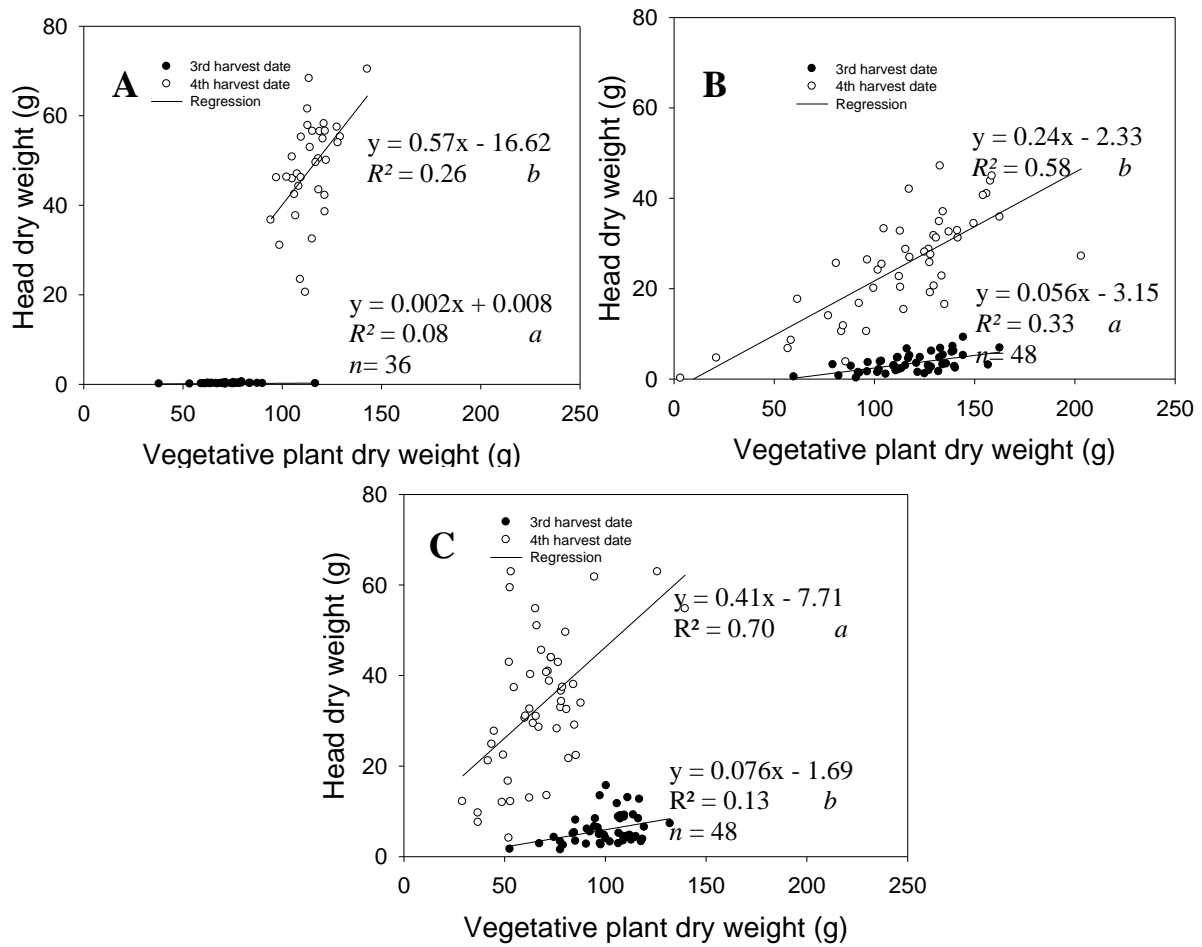


Fig. II-7. Head dry weight (g) in relation to vegetative plant dry weight (g) for set 1 (A), 2 (B) and 3 (C) 2011 of all variants the solid line was fitted by linear regression for each individual harvest date. Small letters show differences in slope.

#### 4. Discussion

To test the hypothesis that seedling size variability of broccoli “Ironman F1” is the reason for head size variation, field trials were carried out. Since the number of leaves is a widely used marker for the developmental stage of *Brassica* plants (Wiebe, 1990; Mourão and Brito, 2000; (Grevsen, 1998) in the beginning of the experiment seedlings with identical numbers of leaves were selected. This was done to ensure identical developmental stage and thus, differences in seedling leaf area size and leaf area variation were the main factors influencing head size variability independent of developmental differences before transplanting. In the next step seedlings were sorted into three groups: small (below first quartile), median (around the second quartile, highest homogeneity) and big (above the third quartile). Based on the sorting of seedlings, canopies with four different compositions of seedling size and seedling size variability were created. First measurements showed that different canopies with distinguishable pattern in leaf area and leaf area variation were created successfully (Table. II-2). The method of seedling classification provide suitable data to create canopies with different mean size in leaf area and variation in leaf area as it was intended.

The first hypothesis postulates that seedling leaf area is the main determinant of plant and head growth and thus time to harvest. This hypothesis could not be confirmed. Statistical analyses showed no differences in head size and relative head growth rates. This can particularly be explained by leaf area harmonization during growth. It was observed that differences in leaf area of different canopies at harvest dates were less than 10 % on average, contrasted by the initial differences in leaf area at planting of 28 % on average. One possible reason for level up of initial size differences was better establishment of small seedlings and faster recovery after the transplanting shock, indicated by tendencies of reduced relative

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growth rates especially in the beginning of the measurements (142-268 °Cd after planting) for plant stands established from big seedlings. Another reason could be a change in relative leaf area growth rate after reaching a specific size and thus plant size itself influenced its own growth pattern. One general assumption is that the relative growth rate for young or small plants is approximately constant because during early growth no limitations of growth factors occur. With increasing plant size the relative growth rate generally decrease (Rees et al. 2010). After a specific size had been reached the resources for growth become more and more limiting and the relative growth rate decreases size specifically. A small seedling remains for longer time in its exponential growth phase while for a big seedling the limitation of resources starts earlier and the relative growth rate declines at any earlier date. Anyhow this cannot be shown in the data because the temporal distances between measurements were long and the changing point from exponential to linear growth was not captured. However this hypothesis can explain observed harmonization of leaf area in the field. Another reason for observed harmonization of size in leaf area could be a change in relative leaf area growth rate after head induction. Differences in developmental stage cause differences in source sink relations, specifically a reduced vegetative growth rate after head induction in favor of a beginning and increasing dry matter allocation to the head. Due to this relationship a changed time to head induction had an impact not only on the duration of the vegetative development but also on the phase after head initiation. A plant with a long head induction phase forms more leaf area and intercepts a higher amount of PAR, which then influences its vegetative and head growth rate, this can be shown in the data were head size was correlated with total plant size at final harvest. Calculation of relative head growth rates showed constant relative head growth rates among all variants (Table. II-6) and very small differences between individuals even when the initial seedling size was highly variable. This suggest that light limitation or the amount of

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intercepted radiation by plants is not limiting for head growth during the early head growth phase. Leaf area and the amount of intercepted radiation seem to be sufficient to supply the head with sufficient assimilates in any case at this stage. At the same time regression analysis showed high differences in intercept points of single head growth curves across all variants.

In the regression analyses the intercept of the regression function was not fixed in order to estimate differences in head induction. Single head growth measured non-destructively showed a range of intercepts of 0.9-0.005 mm for single plants which is larger compared to the range 0.4 and 0.6 mm found by microscopic investigations of the apex of single calabrese plants at the point of head induction in a cabinet experiment (Wurr et al. 1995). Therefore the absolute values of the intercept points should be interpreted carefully. But the variability in intercept points found by regression analyses provides evidence for variation in head induction. Overall differences in relative head growth rates of single plants were small and in comparison to differences in intercept points interpenetrated as differences in head induction negligible. The results of the regression analyses agreed with results from apex dissection. The time of head induction did not differ between variants and was not related to the current plant size at measuring time (Fig. II-5). Since time to head induction occurred independently from plant or previous seedling size the data suggest that plants with earlier head induction showed a larger head at final harvest. Additionally measured head size was negatively correlated with numbers of final leaves (Fig. II-6). This provides additional indications that different times in head induction and not seedling size differences were responsible for the observed head size variation. This assumption is supported by results of other studies in cauliflower, where the authors concluded that variation in time of head induction plays a major role regarding variability in head size and in time to harvest variation (Olesen and Grevsen 2000; Booij 1990a; Salter 1969a). Moreover, we found only a weak correlation between head



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dry weight and vegetative plant dry weight in early head growth stages (Fig. II-7 A-C). Causes that lie behind it, were on the one hand that the switching from vegetative to generative phase occurred independent of plant size and on the other hand that the sink capacity of the head limited head growth especially during its early growth stage (Kage and Stützel, 1999). This means that head growth is not limited by plant size and assimilate supply in the beginning of head growth. During the later head growth stage the relationship between vegetative plant size and head size increased (Fig. II-7). During this growth stage it seems to be more important to supply the head with sufficient dry matter for maintaining the maximum head growth rate. A plant with a higher vegetative mass and a higher leaf area can intercept more light and supply the head with a higher amount of assimilates. Overall size differences in leaf area during later head growth stages, where bigger plants can intercept more radiation and supply the head with higher amounts of dry matter, cannot compensate differences in switching points which were independent from plant size or initial seedling size. Because of this, it is not possible to predict the head size from the initial seedling size.

The expectation of an increase in canopy heterogeneity due to interaction and competitive effects in heterogeneous and alternating canopies in comparison to a homogenous canopy could not be confirmed. Calculated relative growth rates showed no differences for small and big plants within the alternating canopy structure. After canopy closure competition for above ground resources, mainly light, would be theoretically size asymmetric, i.e. big plants would intercept over-proportional more light and thus suppress the growth of smaller individuals. Late destructive measurements ( $> 500$  °Cd after planting) in closed canopies (Table. II-3) did not support this assumption, since plant size differences do not increase. Measured leaf area data suggest no size asymmetric competition. No limitation or reduction in head growth was found due to competition in the alternating canopies where differences in variation of relative

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head growth rates were not distinguishable from those of other seedling sizes and canopy structures. It seems that competition for light has very little influence on broccoli plant size heterogeneity and that developmental differences have a stronger impact on vegetative and head growth pattern compared to initial size differences.

### **5. Conclusions**

Seedling size effects do not appear to be the causes for head size variation in broccoli. Differences in head induction independent of plant size or seedling size seem to be mainly responsible for the variability in head size and the occurring harvest windows in commercial production. Reasons for variability in head induction are unknown. Somatic or genetic differences in seedling material could be possible reasons for that.

## Prediction of plant to plant variability in head induction of broccoli

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### **Abstract**

Field experiments showed a strong variability in time of head induction within broccoli plantings. This appears to be the cause of the high variability in head sizes at harvest. To quantify head induction, variation in head induction and the juvenile development cabinet experiments were carried out. One cabinet trial quantified the end of the juvenile development stage by using plants of different ages and size. Every three days a new set of seedlings was raised (26 °C) and after youngest seedlings showed 1,5 leaves > 1 cm on average plants of different ages and with different number of visible leaves were transferred to cool conditions (10 °C).

Results suggest that the juvenile phase is already passed at planting. A second trial analysed head induction in relation to temperature. Head induction was determined by microscopical analysis of apical meristems and was considered to have occurred when first floral buds were clearly visible on the apical meristem. Additionally, final leaf number was determined non-destructively on a random sample of ten plants per treatment. With the results of the cabinet experiments we parameterized a dynamic model which considered the measured variation. The vernalisation phase is simulated as an optimum function of temperature by a piecewise linear regression. The variability in development is implemented by measured coefficients of variation combined with a normal distribution function. The model was evaluated using independent data of field crops of broccoli crops, grown in different regions, on different soil

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types, with different transplant raising systems and over a period of three years throughout the whole growing season. The model predicted the time to head induction well and was able to predict the variability in vernalisation occurring in the field. Furthermore we found a relationship between the final numbers of leaves and the time to head induction.

Keywords: *Brassica oleracea* L. var *italica*, juvenile phase, facultative vernalisation, variability, head initiation, prediction.

### ***1. Introduction***

In broccoli production a strong heterogeneity of head fresh weight of single plants within individual planting sets is observed. Although modern hybrid varieties (CMS cultivars) show less variability than traditional open pollinated cultivars, it is still common in practice to harvest single sets at least three times by selective hand harvests. Harvest percentages of around 85 – 90 % in total are common. The length of the cutting period directly influences harvest and production costs (Olesen and Grevsen, 2000; Wheeler and Salter, 1974). Harvest decisions must be taken by considering market supply, market prices and delivery commitments. Under adverse circumstances harvest percentages as low as 50-60 % occur resulting in economic losses (Personal communication, Dr. Carsten Bargmann AMG Agrarmanagement GmbH 2010). Broccoli (*Brassica oleracea* var. *italica*) and cauliflower (*Brassica oleracea* var. *botrytis* L.) are two varieties which can be freely crossed (Wurr et al., 1995). In cauliflower the phase of curd initiation can be seen as critical for variability in curd size (Booij, 1990a; Olesen and Grevsen, 2000). It was possible to predict the variability in curd size between individual plants by incorporating plant variability in the juvenile and curd induction phases into harvest prediction models (Olesen and Grevsen, 2000). In our field experiments, broccoli also showed a strong variability in head induction between single plants (unpublished data). This appears to be the cause of the high variability in head size at harvest which is commonly observed in practice. Knowledge of the time of floral initiation is essential to predict the time to harvest in broccoli (Tan et. al. 1998). Time to head induction was studied by various authors (Gauss and Taylor, 1969; Wiebe, 1990; Wurr et al., 1991; 1992; 1995; Grevsen and Olesen, 1999) but no studies with broccoli regarding plant-to-plant variability in different developmental stages have been carried out so far. Studies have shown

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that fluctuations in the production of broccoli are based on different temperature responses in the different development phases (Grevsen, 2000; Mourão and Brito, 2000). To analyse if the variation in head induction is related to variation in head size a precise description of the juvenile development and the vernalisation requirement of broccoli and their variability is necessary. The duration of the juvenile phase was studied by several authors. Fontes et al. (1967) found that a cold stimulus accelerates curd induction for broccoli plants of varieties “Waltham” and “Green Mountain” only in plants with a minimum age of four weeks and conclude that a juvenile phase existed in both broccoli cultivars. Similar results were published 20 years later, the estimated juvenile phase of different broccoli cultivars ranged between 3-5 weeks (extremely early cultivars) to 4-6 weeks (early and intermediate cultivars) after seed germination (Fujime, 1988). Results of studies in Denmark indicate only a very short juvenile phase in transplanted Broccoli crops and at transplanting time the plants were probably already past the juvenile phase (Grevsen and Olesen, 1999). Other studies considered the end of the juvenile phase when 4-5 leaves were visible (Wiebe, 1990; Mourão and Brito, 2000) or a mean number of 3.7 leaves was visible (Grevsen, 1998). Since broccoli transplants are normally planted with 3-5 visible leaves and 3-5 weeks after sowing this agrees with the above mentioned results. Another definition for the end of the juvenile phase is achievement of a fresh weight of 4-50 g per plant or a stem diameter 5-8 mm (Miller et al., 1985; 1988) this definition with such a wide range and is not precise enough for exact predictions and modelling purposes and will result in large errors (Pearson et al. 1994). The above mentioned studies explain why the juvenile phase is not considered in existent broccoli prediction models (Wurr et al. 1995; Grevsen and Olesen, 1999; Tan et al. 2000a;b). Even when hints for the existence of a juvenile phase were found they were not included in prediction models because this stage is already completed at planting, or the use of

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phenological markers like leaf number seemed to be not accurate enough (Wurr et al., 1995; Grevsen and Olesen, 1999). In another instance, crop development was not subdivided into different developmental phases (Marshall and Thomson, 1987 ab). The existence or absence of a juvenile phase is of particular importance for our understanding how *Brassica oleracea* plants develop (Wurr et al., 1995). Furthermore, the duration of the juvenile phase seems to be different among broccoli cultivars. The development of broccoli from the vegetative to the generative stage and the response to different temperatures was analysed before (Gauss and Taylor, 1969; Fujime, 1994; Wiebe, 1990; Wurr et al., 1991; 1992; 1995; Grevsen and Olesen, 1999). By some authors broccoli was considered to have a cold requirement for head formation (Fontes et al., 1967; Fontes and Ozbun, 1972). Gauss and Taylor (1969) observed that the time to head induction of different broccoli sets grown under average temperatures of 18 °C and 30 °C occurred 5-6 weeks after sowing. They concluded that broccoli does not have a vernalisation requirement and that temperature alone has no influence on the time to head induction. Wiebe (1990) c observed that head induction of early broccoli varieties was accelerated with increasing temperature while for later varieties vernalisation and decreasing temperature lead to an accelerated flower induction. The cold response for curd induction was facultative for broccoli while it was obligatory in cauliflower (Wiebe, 1990). Data of 16 broccoli sets of 4 cultivars (Compacta, Comanche, Green Valient and Marathon) grown in Portugal over the whole growing seasons of two years showed no obligatory vernalisation requirement (Mourão and Brito, 2000). There appear nevertheless to be high differences among different varieties. Some varieties seem to require cool conditions (maximum temperature 23 °C) to induce and maintain vernalisation (Farnham and Björkman, 2011). Grevsen (1998) found a limit of 17 °C for head initiation. Others studies in broccoli showed that after the apex had reached a diameter of 0.5 mm the production of secondary meristems

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was induced automatically, and that low temperatures directly controlled the apex expansion rate (Wurr et al., 1995). To predict the time to head induction thermal time models are used (Diputado and Nichols, 1989; Fyffe and Titley, 1989; Tan et al., 2000ab). Thermal time models often simulate the development with three cardinal temperatures: a base temperature below which no development takes place, an optimum temperature at which the developmental rate is at its maximum, and a maximum temperature above which the developmental rates are zero (Tan et al., 2000). However, maximum temperatures for head induction could not always be defined from the experimental data sets (Tan et al., 2000). For broccoli, base and optimal temperatures for head induction range between 0 and 4.5 °C and 20- 21 °C, respectively (Fyffe and Titley, 1989; Diputado and Nichols, 1989). Simulations have shown that head induction did not occur at temperatures of 0, 30 and 35 °C for some cultivars (Fellows et al., 1997)., the model assumed that head induction occurred when the apex had reached a diameter of 0.49 mm (Wurr et al.,1995). Increase in apex diameter was calculated as a function of temperature. Other studies used thermal time models. The thermal time required for head initiation was 680 °Cd and the base temperature was 0.7 °C (Mourão and Brito, 2000). In addition to temperature sum concepts some modeling approaches employed vernalisation descriptions similar to those used for cauliflower (Wurr et al., 1995; Fellows et al., 1997; Grevsen, 2000). Descriptions of the variability in head induction of broccoli are missing in literature. No modelling approach to predict this variation was published up to now. A prediction model of head induction and its variability can assist in evaluating the hypothesis that observed variation in head size is related to observed variability in head induction on field scale.

The goals of the present studies were (1) to quantify the length of the juvenile phase of the broccoli cultivar ironman F1, using the number of visible leaves as a morphological maker.



(2) To estimate the relationship between time to head induction and temperature and (3) to parametrise a piecewise linear model to predict time to head induction in the field from temperature data. (4) To analyze the variation in time to head induction in order to develop a stochastic model to predict variation in time to head induction on single plant level. (5) The non-destructively assessment of time to head induction from final leaf numbers.

## ***2. Material and Methods***

All experiments were conducted with broccoli plants (*Brassica oleracea* var. *italica* 'Ironman F1') which were raised at a daylength of 16 hours in growth cabinets equipped with high-pressure mercury lamps (HQI-BT 400W daylight, Osram, München, Germany). Plants were grown from calibrated seeds of 2.0-2.2 mm diameter in peat substrate (Potgrond P, Klaasman, Geeste, Germany). Since broccoli is sensitive to molybdenum deficiency, plants were fertilized with sodium molybdate ( $\text{Na}_2\text{MoO}_4$ ) in a concentration of  $0.5 \text{ g L}^{-1}$  and a total amount of 2.2 g/1000 plants before the four leaf stage. During the experiments the plants were fertilized with Scotts Universol orange (Universol Orange, Everris International B.V. Geldermalsen, Netherland) in a concentration of  $1 \text{ g L}^{-1}$  of irrigation water. Irrigation was applied on demand.

### ***2.1 Quantification of the length of the juvenile phase***

The first experiment was conducted to characterize the juvenile stage of the plants. The experimental set up comprised six plant age groups. Every three days a new set of seeds was sown in pots which were put in a growth cabinet with 16 hours of daylight and 26/24 °C (day/night). After emergence of the latest set all plants were transferred to two cabinets with

10/10 °C to accelerate flower induction. At this time the oldest plants had five visible leaves (> 1cm diameter), while the youngest plants had 1.5 leaves on average. The number of visible leaves of all variants at treatment start is shown in Table III-1.

Table III-1. Number of visible leaves at start of cold treatment in experiment 1.

Variant	Number of leaves > 1 cm
1	1.5
2	2
3	2.5
4	3
5	4
6	5

Head induction was determined on dissected plant apices using a binocular (B061, Olympus, Japan). Head induction was considered to have occurred when first flower buds were clearly visible on the apical meristem (Uptmoor et al. 2008) (Fig. III-1). Apex dissection was done at treatment days 10, 13, 15, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 40 and 42 on three randomly selected plants of each age group.

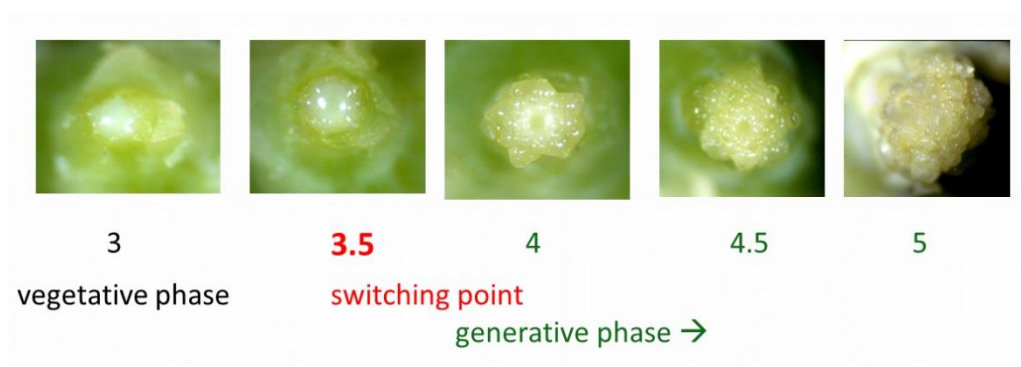


Fig. III-1. Scheme used to determine the level of development by apex dissection and binocular measurements (B061, Olympus) (based on Uptmoor et al., 2008). The time of head induction was considered as the point 3.5 when first flower buds were clearly visible on the apical meristem (Uptmoor, 2008).

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### *2.2 Estimation of the relationship between time to head induction and temperature*

In experiment 2 the heterogeneity of vernalisation in broccoli was examined. The experiment was divided into two cycles, and carried out in four cabinets with eight temperature regimes. In the beginning a set of 600 transplants was raised in one cabinet (16 hours of daylight, 26/24 °C). At the four-leaf stage 150 plants were transferred into each of four cabinets. At this time all plants had identical leaf numbers. Pots were placed with isometric plant distance on four tables of 90 cm height. The first experimental cycle consisted of temperature treatments 6, 12, 18 and 21 °C. The second pass consisted of temperature treatments 3, 9, 15 and 26 °C. Each chamber was equipped with temperature sensors (Tiny Tag view 2; PT1000 probe - PB-7002-1M5/3M, Gemini Data Loggers, West Sussex, UK) to check the accuracy of the cabinet temperature control. Target and measured temperatures during the experiment are shown in Table III-2.

Table. III-2. Temperature regimes during the cabinet experiment 2 for different growth stages and measured mean temperature and radiation at plant level with standard deviations ( $\pm$ ) within the cabinets.

Variant	Target	Set point Day	Set point Night	Measured Day	Measured Night	Measured Mean	Mean radiation
	°C	°C	°C	°C	°C	°C	$\mu\text{mol PAR m}^{-2}\text{s}^{-1}$
up to 4 leaves	26	24	26	25.9 $\pm$ 0.4	25.5 $\pm$ 0.4	25.7 $\pm$ 0.4	
1	3	1	3	3.7 $\pm$ 0.3	3.7 $\pm$ 0.3	3.7 $\pm$ 0.3	257.2 $\pm$ 17.08
2	6	4	6	6.2 $\pm$ 0.7	6.3 $\pm$ 0.3	6.3 $\pm$ 0.6	264.8 $\pm$ 31.1
3	9	7	9	9.5 $\pm$ 0.5	9.1 $\pm$ 0.1	9.4 $\pm$ 0.5	264.8 $\pm$ 13.94
4	12	10	12	13.1 $\pm$ 0.5	12.1 $\pm$ 0.2	12.8 $\pm$ 0.6	270 $\pm$ 17.6
5	15	13	15	15.9 $\pm$ 0.3	15.1 $\pm$ 0.1	15.6 $\pm$ 0.5	256.8 $\pm$ 20.51
6	18	16	18	18.1 $\pm$ 0.5	17.9 $\pm$ 0.2	18.0 $\pm$ 0.4	268 $\pm$ 17.3
7	21	19	21	21.1 $\pm$ 0.4	20.8 $\pm$ 0.1	20.9 $\pm$ 0.4	271 $\pm$ 14.1
8	26	24	26	25.9 $\pm$ 0.5	25.4 $\pm$ 0.4	25.7 $\pm$ 0.5	258.8 $\pm$ 24.1

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Before the temperature treatments started, the light conditions (PAR quantities) in every cabinet were measured with a LI-188B photometer (LI-188B, Licor, USA).

To exclude differences in light environments among experimental variants and between cabinets as a source of variation the height of the plant benches in the individual chambers was adjusted to ensure a uniform radiation level between the cabinets. To compensate for gradients within one cabinet plant benches were rotated in the growth chamber twice a week.

First measurements of the apex began 12 days after treatment start. Determination of head induction was done the same way as in experiment one.

The statistical analyses were performed using R.2.15.2 (R Core Team 2012). Multiple comparisons of the length of the development phases in experiment 1 and experiment 2 as well as on the data of final leaf numbers in experiment 2 were conducted after analysis of variance (ANOVA). Functions of the R.2.12.0 packages MVTNORM and MULTCOMP were used.

### ***2.3. Model development***

The model assumed 10 classes each reflecting 10 % of the plant individuals of one simulated population. An example for data normalisation (data set from cabinet with  $T = 6.3$  °C) and the allocation of the ten classes by the corresponding percentile values of the distribution curve is given in Fig. III-2.

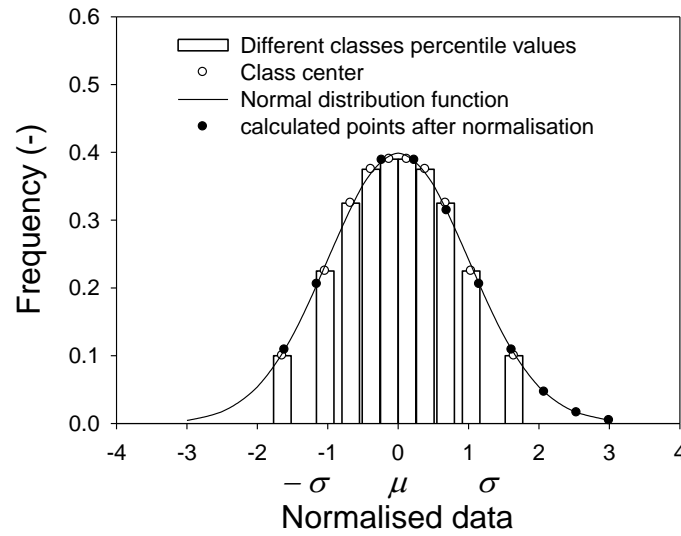


Fig. III-2: Example for data normalisation, class percentile values of the normal distribution curve after testing for normal distribution of the data set from cabinet 6 °C (Shapiro-Francia test  $p \leq 0.05$ ).

The simulation model calculates the mean vernalisation rate

$(dV/dt)_{50}$  depending on temperature using Eq.III-1.

$$\frac{dV}{dt_{50}} = \begin{cases} V_{\min} & T \leq T1 \\ \max \left[ \left( V_{\max} - \frac{V_{\max}}{T2 - T1} \cdot (T2 - T) \right); (V_{\min}) \right] & T1 < T < T2 \\ V_{\max} & T2 \leq T \leq T3 \\ \max \left[ \left( V_{\max} - \frac{V_{\max}}{T4 - T3} \cdot (T - T3) \right); (V_{\min}) \right] & T3 < T < T4 \\ V_{\min} & T \geq T4 \end{cases} \quad (\text{III-1})$$

In the model, the variation in vernalisation is considered by simulated standard deviation (sd)

(Eq.III-2.), calculated from a parameter for the coefficient of variation in vernalisation ( $\bar{cv}$ ).

$$sd = \left( \frac{dV}{dt_{50}} \right) * \bar{cv} / 100 \quad (\text{III-2})$$

In the next step calculated standard deviation values were normalised and assigned to the ten classes by multiplication of  $(dV/dt)_{50}$  and  $sd$  with given percentile values ( $p_i$ ) of the normal distribution curve (Table. III-3).

$$\left( \frac{dV}{dt} \right)_i = \left( \frac{dV}{dt} \right)_{50} + sd * p_i \quad (\text{III-3})$$

Table. III-3. Values for standardisation (percentile values of the normal distribution curve) of the different classes to describe the variability in development rates (Bohres, 1996, modified).

Class	Variable	Frequency	Percentile ( $p_i$ )
p1	-c_5	0.05	-1.64486
p2	-c_15	0.15	-1.03644
p3	-c_25	0.25	-0.67449
p4	-c_35	0.35	-0.38532
p5	-c_45	0.45	-0.12566
p6	c_45	0.55	0.12566
p7	c_35	0.65	0.38532
p8	c_25	0.75	0.67449
p9	c_15	0.85	1.03644
p10	c_5	0.95	1.64486

The concept of this model approach was taken from the cauliflower model Blukosim (Bohres 1996, Kage, 2010) which describes the variability in crop development by classes with assumed normal distributions and assumed coefficients of variation in the juvenile and vernalisation stage of the crop. The approach presented here only considers variation in vernalisation and is based on measured data from cabinet experiment 2.

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The model equation and the variation mapping approach as well as the model parameter fitting were realised by using ModelMaker4™ (ModelMaker4™, Modelkinetix, UK). Minimization of least square differences was achieved using the Marquardt algorithm in Modelmaker. The differential equations of the model were integrated numerically using the Runge-Kutta method with time steps of 1 day. Daily mean temperatures measured from local weather stations near to the field as well as from thermobutton (Thermobutton 22L, Progesplus, Willems, France) measurements within the plant stand were used as model input.

For each day the mean temperature  $T_{\text{mean}}$  was calculated from daily maximum and minimum temperature  $T_{\text{max}}$  and  $T_{\text{min}}$  (Eq. III-14).

$$T_{\text{mean}} = \frac{T_{d \text{ max}} + T_{d \text{ min}}}{2} \quad (\text{III-14})$$

### ***2.4 Model parametrisation***

For model parametrisation and to include the observed plant to plant variation into the prediction model for vernalisation the data sets from the different temperature regimes were analysed for normal distribution (Shapiro-Francia test) (Thode, 2002) using R packages from library NORTEST. In the next step the cumulative number of plants after head induction  $F(xt)_T$  (-) of temperature treatment T, were described by a logistic function (Eq. III-4).

$$F(xt)_T = \frac{a_T}{(1 + b_T e^{-k_T x t_T})} \quad (\text{III-4})$$

The Parameter  $a_T$  defines the maximum of each individual logistic function of treatment T;

this parameter becomes one when all plants of one temperature treatment T have induced the head (-).  $X_{t_T}$  is the time of the measurement after start of the cold treatment (d) in treatment T.  $k_T$  and  $b_T$  are parameters defining the point of inflection. The mean duration to complete vernalisation ( $\mu_T$ ) at each temperature treatment (T) can be calculated as the point of inflection of the logistic function:

$$\mu_T = -\frac{\ln(b_T)}{k_T} \quad (\text{III-5})$$

Figure III-3 illustrates the calculation procedure of the standard deviation and the coefficient of variation from the cumulative data (data set from cabinet with 6.3 °C).

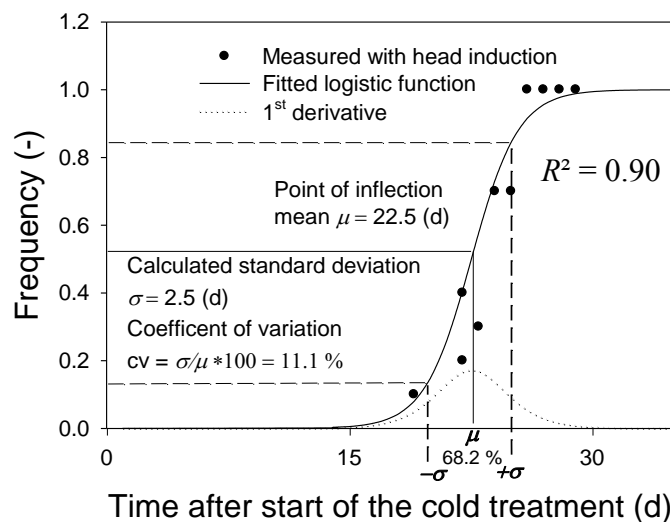


Fig.III-3. Frequency of plants after head induction in relation to time after start of the cold treatment for cabinet T = 6.3 °C, example for parameterization and fitting of the logistic function to estimate the mean and the coefficient of variation from the cumulative data.

Equation III-4 can be considered as the integral of the probability density function. Its first derivative describes the probability distribution of the data from each cabinet temperature T (Eq. III-6).



$$f(x)_T = F'(xt)_T = \frac{a_T b_T k_T e^{-k_T x T}}{(1 + b_T e^{-k_T x T})^2} \quad (\text{III-6})$$

The piecewise linear regression model (Eq. III-1) was fitted to one over  $\mu_T$  in relation to temperature of treatment T to model the vernalisation rate (Eq. III-7).

$$\frac{1}{\mu_T} = \frac{dV}{dt_{50}} = \begin{cases} V_{\min} & T \leq T1 \\ \max \left[ \left( V_{\max} - \frac{V_{\max}}{T2-T1} \cdot (T2-T) \right); (V_{\min}) \right] & T1 < T < T2 \\ V_{\max} & T2 \leq T \leq T3 \\ \max \left[ \left( V_{\max} - \frac{V_{\max}}{T4-T3} \cdot (T-T3) \right); (V_{\min}) \right] & T3 < T < T4 \\ V_{\min} & T \geq T4 \end{cases} \quad (\text{III-7})$$

$(dV/dt)_{50}$  reflects the mean vernalisation rate ( $d^{-1}$ ) at the 0.5 quintile (median) assuming a normal distribution as is the reciprocal value of the mean measured duration to head induction (d) for every temperature treatment T. Temperature dependent daily vernalisation rates  $(dV/dt)_{50}$  were simulated using a piecewise linear function with four cardinal temperatures ( $^{\circ}\text{C}$ ) for the minimum (T1), lower optimum (T2), upper optimum (T3), and maximum temperature (T4), a maximum vernalisation rate ( $V_{\max}, d^{-1}$ ) achieved at optimum temperatures and one minimum vernalisation rate  $V_{\min}$  to reflect the facultative vernalisation requirement of the crop (based on Wiebe, 1972, Wiebe 1990, modified). In the model the vernalisation process begins directly after planting (normally 3.5-4 leaf stage), and is completed when the sum of daily vernalisation rates reaches a value of 1. From the standard normal distribution table (Sachs und Hedderich, 2009) it can be seen that for normally distributed random variables 68.3 % of the values lie within one standard deviation around the mean ( $\mu \pm \sigma$ ). Thus, the standard deviation can be estimated from the logistic function (Eq. III-4) since the mean is

equal to the point of inflection of the logistic curve (Eq.III-5). For calculation of the standard deviation from the cumulative data of head induction, equation III-1 was rearranged (Eq. III-8), where  $xt_T$  corresponds to  $F(xt)_T$ . The value for the mean plus the positive standard deviation ( $x_{+T}$ ) can be derived from the curve by calculating the corresponding  $xt_T$  value to the given probability of 84.15 % ( $68.3/2 = 34.15 + 50$  %) (Eq.III-9). The value of the mean minus the negative deviation ( $x_{-T}$ ) can be calculated similarly from equation 10 to the given probability of 15.85 % ( $50$  % -  $34.15$  % =  $15.85$  %). This calculation was done for each temperature treatment T.

$$xt_T = \frac{1}{-k_T} \ln \left( \frac{1}{b_T} \left( 1 - \frac{a_T}{F(xt)_T} \right) \right) \quad (\text{III-8})$$

$$x_{+T} = \frac{1}{-k_T} \ln \left( \frac{1}{b_T} \left( 1 - \frac{a_T}{0.8415} \right) \right) \quad (\text{III-9})$$

$$x_{-T} = \frac{1}{-k_T} \ln \left( \frac{1}{b_T} \left( 1 - \frac{a_T}{0.1585} \right) \right) \quad (\text{III-10})$$

The average of the calculated positive standard deviation (Eq. III-9) and the negative standard deviation (Eq. III-10) was calculated from equation III-11 for every temperature treatment T:

$$\sigma_T = \frac{(x_{-T} + x_{+T})}{2} \quad (\text{III-11})$$

In the next step the coefficient of variation,  $cv_T$ , of temperature treatment T was determined (Eq.III-12):

$$cv_T = \frac{\sigma_T}{\mu_T} \cdot 100 \quad (\text{III-12})$$

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The mean coefficient of variation,  $\bar{cv}$ , was calculated over all temperature treatments T from all individual  $cv_T$  values (Eq.III-13) since no systematic differences were found between  $cv_T$  values of the different temperature treatments T.

$$\bar{cv} = \frac{1}{n} \sum_{i=1}^n cv_i \quad (\text{III-13})$$

One  $\bar{cv}$  value was used as input parameter for the simulation of the variance of developmental rates.

### ***2.5 Non-destructive estimation of time to head induction***

A random sample of ten plants per treatment in cabinet experiment two was chosen for counts of final leaf number. After all plants of one temperature treatment had switched into the generative phase, the ten plants were repotted into 3 L containers and further cultivated in the greenhouse to determine the final leaf number until the head was clearly visible. For the determination of final leaf number, all leaves up to the first inflorescence branch were counted.

In addition to the cabinet experiments field investigations of the development and leaf initiation of broccoli cultivar ('Ironman F1') grown on commercial farms in Mecklenburg-Western Pomerania and on the experimental farm in Hannover were conducted in 2010, 2011 and 2012. The data of leaf number counts was analysed by linear regression.

In order to allow a nondestructive determination of the end of vernalisation a relation between the number of visible leaves at the end of the vernalisation phase,  $N_{Le}$  (-) and the number of visible leaves at flower induction  $N_{Li}$  (-) was used. A non-linear regression with equation 15 (Uptmoor et al., 2008) was fitted using functions from the R package NLME in R.2.12.0 (R Development Core Team 2011).

$$N_{Li} = N_{Lmax} - (N_{Lmax} - c)e^{-kN_{Le}} \quad (\text{III-15})$$

where  $c$  is the intercept of the regression (-),  $k$  reflects a constant (-) and  $N_{Lmax}$  (-) is the maximum final leaf number (-) observed from the whole data set. This approach assumes a strong correlation between  $N_{Le}$  and the number of developed leaf primordia as well as that at the end of vernalisation all leaf primordia have been developed (Uptmoor et al., 2008). Rearranging equation (III-15) allows calculating the number of visible leaves,  $N_{Le}$ , at the end of vernalisation from final leaf numbers:

$$N_{Le} = \frac{\ln\left(\frac{N_{Lmax} - c}{N_{Lmax} - N_{Li}}\right)}{k} \quad (\text{III-16})$$

In order to estimate the time from transplanting to the end of vernalisation by non-destructive determination of final leaf numbers in the field a relationship between leaves > 1 cm and accumulated thermal time from planting was derived from field data using linear regressions in R. Thermal time was calculated from data of local weather stations near the field and from thermobutton measurements. Thermal time  $Tt$  was calculated from daily maximum temperature  $Td_{max}$ , daily minimum temperature  $Td_{min}$ , and with an assumed base temperature  $T_{base}$  of 0 °C (Tan et al., 2000ab):

$$Tt = \sum_{i=1}^n \left[ \frac{Td_{max} + Td_{min}}{2} \right] - T_{base} \quad (\text{III-16})$$

---

Leaf number,  $N_L$ , was calculated as:

$$N_L = l * T_t + N_{L0} \quad (\text{III-17})$$

Where  $l$  is the leaf initiation rate,  $T_t$  the thermal time after planting and  $N_{L0}$  the visible leaf number  $> 1$  cm at planting.

### *2.6 Calculation example to illustrate the stochastic model*

The following example illustrates how vernalisation and its variation are calculated in the model: Assuming that the plants had been just planted, the juvenile phase is passed, the plant begins its vernalisation phase with zero and that the average temperature for day one is 12 °C. According to equation 1 the daily vernalisation rate  $(dV/dt)_{50}$  is  $0.055 \text{ d}^{-1}$ . The output of equation one is in other words the mean vernalisation rate  $Vd/dt_{50}$  of the considered plant population consisting of ten classes with identical size in number of the simulated set. The standard deviation in vernalisation of this plant population is calculated from equation 2 and the parameter for the coefficient of variation  $cv = 11.69 \%$ .

$$sd = 0.055 \text{ d}^{-1} * 11.69 / 100 = 0.00643 \text{ d}^{-1}$$

In the next step the standard deviation is normalised and assigned to the ten classes (Eq.III-3) by multiplication of  $(dV/dt)$  (Eq. III-1.) and  $sd$  (Eq.III-2) with the percentile values derived from the normal distribution curve (Table. III-2) with intervals of 10 %, thus every class describes 10 % of the plant population.

The vernalisation of the individual classes were calculated from Equation III-3:

$$\frac{dV}{dt}_{0.05} = 0.055 + 0.00643 * (-1.644860) = 0.044$$

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$$\frac{dV}{dt}_{0.15} = 0.055 + 0.00643 * (-1.03644) = 0.048$$

....

$$\frac{dV}{dt}_{0.50} = 0.055$$

...

$$\frac{dV}{dt}_{0.80} = 0.055 + 0.00643 * (1.03644) = 0.062$$

$$\frac{dV}{dt}_{0.95} = 0.055 + 0.00643 * (1.644860) = 0.066$$

### ***2.7 Model evaluation***

The model was evaluated against independent field data from broccoli cultivar ('Ironman F1') grown on commercial farms in Mecklenburg-Western Pomerania and on the experimental farm of Leibniz Universität Hannover in the years 2010, 2011 and 2012. Evaluation sets include three different seedling production systems and seven different locations comprising three different soil types. For the evaluation of the vernalisation model, the leaf appearance model, calculation of the switching point from final leaf number measurements and for the description of the accuracy of our approach for variation mapping the quantities BIAS, MSD, RMSD, RMAE and  $r^2$  were calculated (Wallach et al. 2006; Kobayshi and Salam, 2000; Mayer and Butler, 1993).

The differences between model and measurement is formulated as:

$$D_v = y_v - y_s \quad (\text{III-18})$$

where  $y_v$  is the measured value for time  $v$  and  $y_s$  the value calculated by the model simulation for situation  $v$ .

The *bias* is the average difference between measured and calculated values:

$$bias = \frac{1}{N} \sum_{i=1}^N D_v \quad (\text{III-19})$$

MSD is the mean squared deviation between measured and calculated values:

$$MSD = \frac{1}{n} \sum_{i=1}^n (x_i - y_i)^2 \quad (\text{III-20})$$

The root mean squared deviation RMSD is the square root of MSD:

$$RMSD = \sqrt{MSD} = \sqrt{\frac{1}{n} \sum_{i=1}^n (x_i - y_i)^2} \quad (\text{III-21})$$

The coefficient of determination of the model is the percentage of the total variability explained by the model:

$$R^2 = \left( \left[ \frac{1}{n} \sum_{i=1}^n (x_i - \bar{x})(y_i - \bar{y}) \right] / (SD_m SD_s) \right)^2 \quad (\text{III-22})$$

RMAE is the relative mean absolute error, where each difference is divided by the corresponding observed value:

$$RMAE = \frac{1}{N} \sum_{i=1}^n \frac{|Y_i - \hat{Y}_i|}{|Y_i|} \quad (\text{III-23})$$

In addition to these calculations a graphical presentation of the agreement between measured (y-value) and calculated (x-value) values as 1:1 graph and graphed residuals was chosen (Wallach et al., 2006). Differences from a slope of 1 and an intercept of zero were analysed with the R package MULTCOMP and functions for generalised linear model hypothesis testing ( $p \leq 0.05$ ).

### 3. Results

#### 3.1 Quantification of the length of the juvenile phase

The time to head induction was strongly influenced by plant age and the number of visible leaves at the start of the cold treatment in experiment 1 (Fig. III-4).

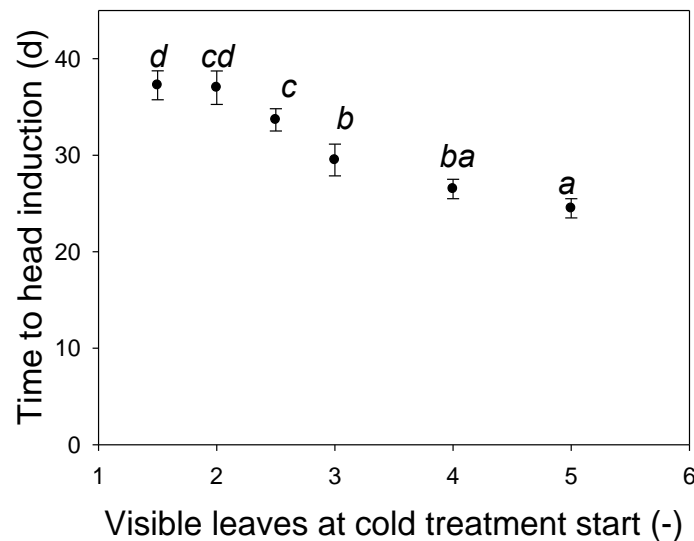


Fig. III-4. Mean time from start of cold treatment to head induction (days) with standard deviation (error bars) in experiment 1 in relation to the number of visible leaves at treatment start.

The duration from start of the cold treatment to head induction decreased with increasing plant age and the number of visible leaves at the start of the cool period. Differences between



plants with 1.5 or 2.5 visible leaf numbers at planting were small. From 2.5 visible leaves at the start of cold treatment, a significant drop in duration to head induction was visible and the shortest duration was observed for plants which had between 3 and 5 leaves at the start of cold treatment. An analysis of the thermal from start of the cold treatment to time to head induction showed a significant increase with increasing plant age and higher leaf number at the start of the cold treatment. A higher thermal time to head induction was found for plants which started the cold treatment with 2.5-5 leaves in comparison to plants which started the cold treatment with 1.5-2 leaves. A piecewise linear model fitted well to the data and yielded a breakpoint at 2.1 leaves. An analysis of the different phases of the experiment showed that thermal time to head induction of the cold treatment was reduced with increasing leaf numbers at the start of the cold treatment (Fig. III-5).

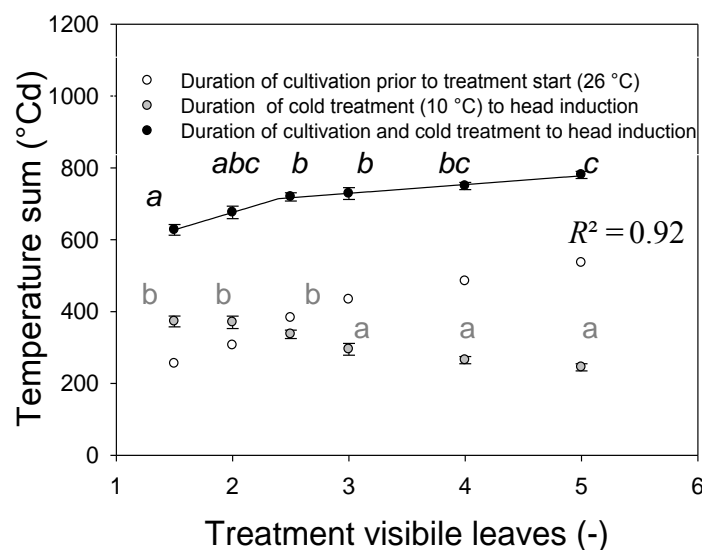


Fig. III-5. Mean temperature sum (white dots) of the cultivation period under 26 °C before start of the cold treatment in experiment one in relation to the number of visible leaves at start of the cold treatment. Mean temperature sum to head induction during cold treatment 10 °C (grey dots) with standard deviation (error bars) in relation to number of visible leaves at cold treatment start,. Mean temperature sum to head induction during cold treatment and the cultivation period (black dots) with standard deviation (error bars) in relation to number of visible leaves at cold treatment start (different letters indicate significant difference for pairwise t-test at  $p \leq 0.05$ ). Lines were fitted by linear and piecewise linear regression.

The duration of the pre-treatment cultivation period reflects the plant age at the beginning of the cold treatment and increased with increasing leaf number at the start of cold treatment. The final number of leaves when first flower buds were clearly visible on the apical meristem was calculated as the sum of visible leaves > 1 cm and leaf primordia. The results show that the plants which had a number of visible leaves of 1,5 and 2 at the start of the cold treatment had a smaller final leaf number in comparison with plants which started the cold treatment with a number of visible leaves between 2,5 -5 (Fig. III-6).

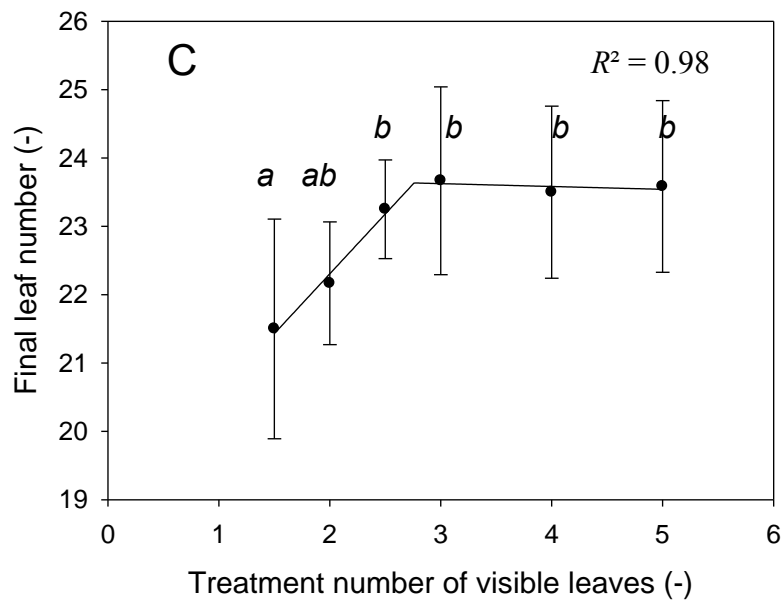


Fig. III-6. Mean final leaf number (dots) with standard deviation (error bars) at head induction in relation to the number of visible leaves at treatment start. Different letters indicate significant difference for pairwise t-test at  $p \leq 0.05$ .  $n = 12$  plants (4 dates  $\hat{=}$  3 plants per treatment).

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***3.2 Estimation of the relationship between time to head induction and temperature***

To ensure stable light environments among experimental variants and between cabinets in experiment 2 we measured the PAR light quantities. Overall we found just slight differences between our variants and cabinets. During cabinet experiment 2 the temperatures were monitored by temperature loggers. In all variants slight temperature fluctuations ranging within the measurement accuracy of the temperature sensor could be measured (Table.III-3). Overall temperatures were stable with only small deviations from the target values. In all subsequent calculations and data analyses we used the measured mean temperatures from the cabinet experiment. Measured thermal time from start of the temperature treatment to head induction significantly increased with increasing temperature, beginning with 96 °Cd for the coldest temperature treatment (Fig. III-7A). The required thermal time increased up to 1716 °Cd for the warmest treatment. The developmental rates to head induction increased with cabinet temperatures from 3.7 to 12.8 °C (Fig. III-7B). Within the range from 12.8 to 20.9 °C the rates decreased constantly. Between 20.9 °C and 25.7 °C we found only small differences between the developmental rates.

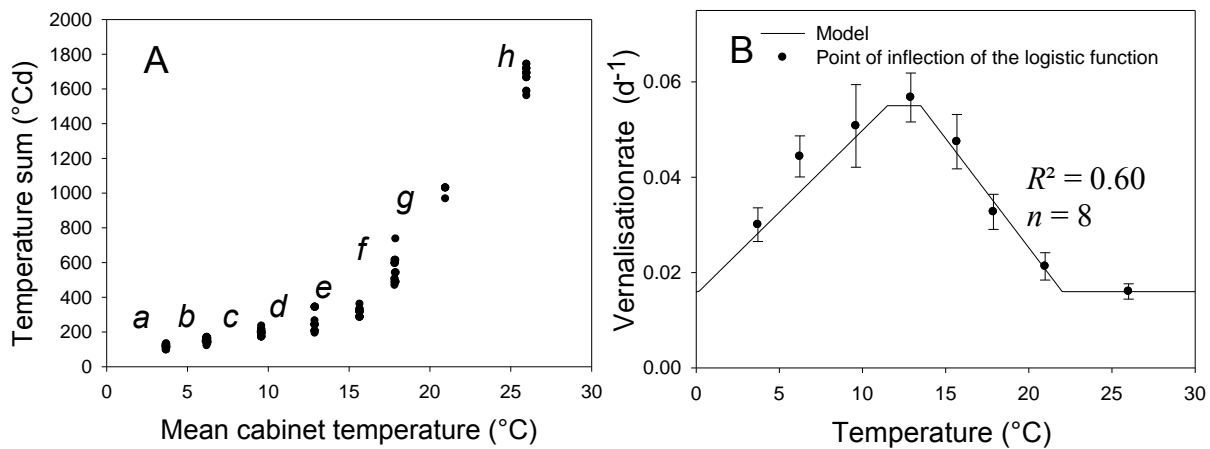


Fig. III-7. (A) Thermal time to head induction (°Cd) in experiment 2 in relation to the measured cabinet air temperature. Different letters indicate significant difference for pairwise t-test at  $p \leq 0.05$ . (B) Vernalisation response of broccoli shown as developmental rates (1/point of inflection of the logistic function of each cabinet); error bars represent the calculated standard deviation over measured cabinet temperatures. The solid line represents the fitted piecewise linear model for the prediction of vernalisation  $Vd/dt_{50}$ .

### 3.3 Model parametrisation

The calculated mean vernalisation rate  $VD/dt_{50}$  could be described well by the fitted piecewise linear function (Fig. III-7B). The function definition included a maximum and minimum development rate and four cardinal temperatures. The estimated model parameters are shown in Table III-4.

Table. III-4. Fitted model parameters based on the measured development rates in experiment 2 for the simulation of vernalisation depending on temperature with the piecewise linear regression model (Eq. III-1).

Parameter					
T1	T2	T3	T4	$V_{\max}$	$V_{\min}$
-4.5	11.5	13.5	25.5	0.055	0.016

For the parameterisation of the variation mapping approach we first calculated the coefficient of variation for each of our treatments according to Eq. III-12. The coefficient of variation over all temperature treatments was  $cv = 11.69\%$  and the graph suggested no relationship between the coefficient of variation and cabinet temperature (Fig. III-8).

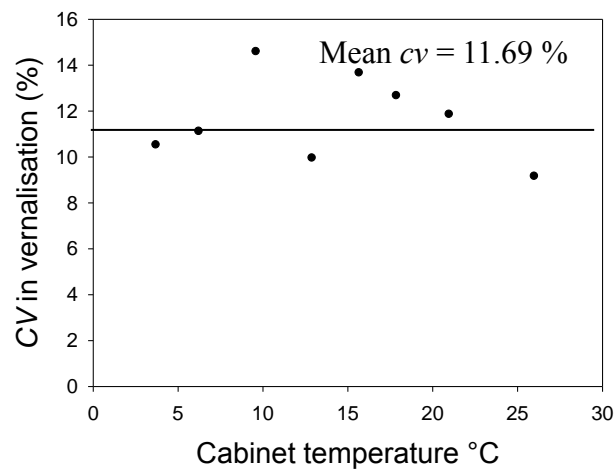


Fig. III-8. Coefficient of variation in development in relation to the measured cabinet temperatures.

Data sets from the different temperature regimes were analysed for normal distribution. The percentiles of each of the ten classes were derived from the normal distribution curve corresponding to the frequency of each class center (Table. III-2). The model was able to describe the cabinet data well with an  $R^2$  of 0.90 (Fig. III-6).

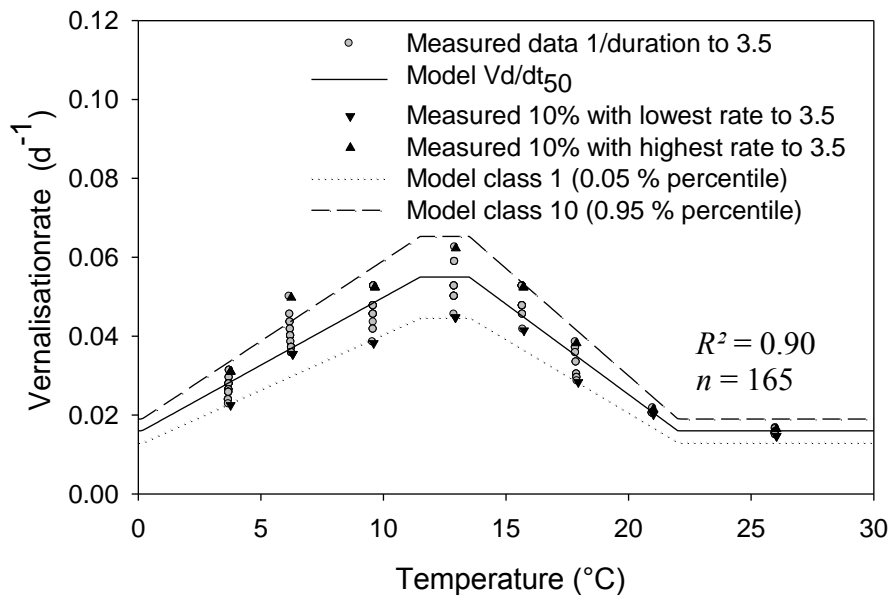


Fig.III-9. Measured vernalisation rate in cabinet experiment 2 (reciprocal of scored duration to head induction 3.5; Fig. III-1) over measured cabinet temperatures. The black solid line represents the fitted piecewise linear model for prediction of vernalisation ( $Vd/dt_{50}$ ) (Eq. 5; parameter Tab 3). The dotted black line shows the model output for class 1 (simulation of the 5 % percentile vernalisation rate). The dashed line showed the model output for class 10 (simulation of the 95 % percentile vernalisation rate).

### 3.4 Non-destructive estimation of time to head induction

In order to calculate the time to head induction from the final leaf numbers the relationship between temperature sum ( $^{\circ}\text{Cd}$ ) and the number of visible leavers was parameterized on the basis of field data, the calculated leaf initiation rate was 0.0206 leaves per growing degree day (Fig. III-10).

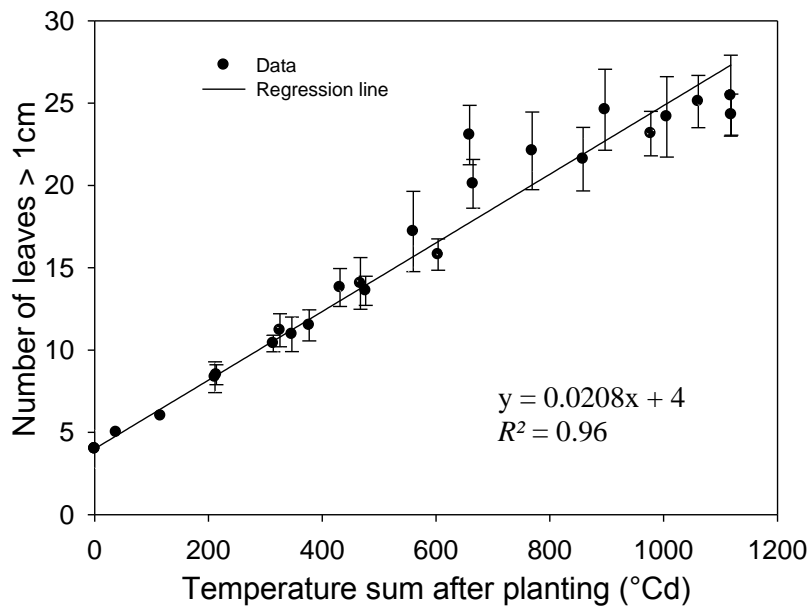


Fig. III-10. Number of leaves > 1 cm measured on commercial production fields in 2010 and 2011 in relation to temperature sum after planting. n = 26.

The final leaf number generally increased with increasing cabinet temperature but no significant differences between 6.3, 9.4, and 12.8 °C as well as between 15,6 and 18 °C could be identified (Fig. III-11A). Over all temperature treatments we found that final leaf numbers increased with thermal time to head induction (Fig. III-11B). For estimation of the duration of vernalisation from final leaf numbers in the field we used the relationship between final leaf number and the number of visible leaves > 1 cm at head induction (Figure III-11 C).

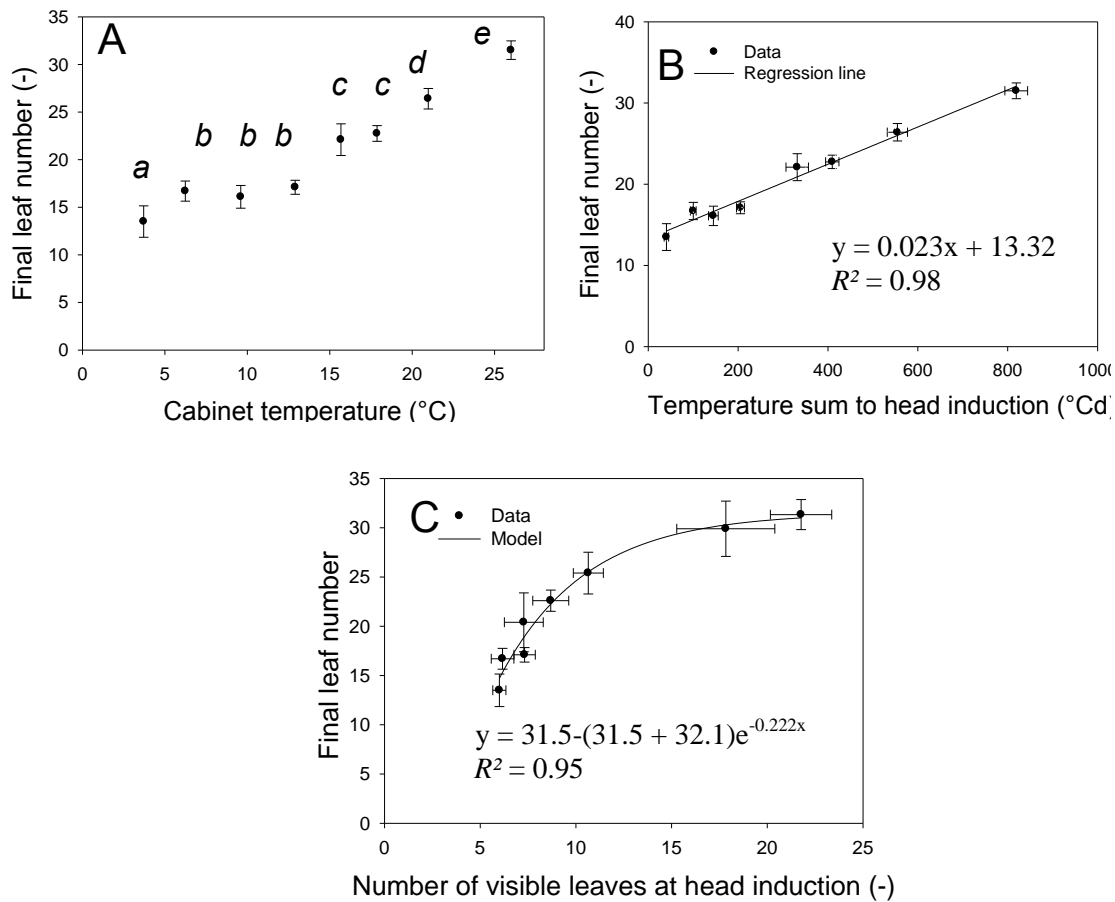


Fig.III-11. (A) Final leaf number of plants in experiment 2 scored after the head was clearly visible in relation to the mean measured cabinet temperature.

Different letters indicate significant difference for pairwise t-test at  $p \leq 0.05$ . (B) Final leaf number and in relation to the temperature sum to head initiation,  $n = 79$  plants (9-10 plants/treatment). Error bars show the standard deviation. The solid line was fitted by linear regression.

(C) Final leaf number in experiment 2 in relation to visible leaves at head induction for all temperature variants.  $n = 79$ . (B) Number of leaves  $> 1$  cm measured on commercial production fields in 2010 and 2011 in relation to temperature sum after planting.  $n = 26$ .



Using the leaf number at head induction and the measured leaf initiation rate of 0.0208 leaves per growing degree day (Fig. III-10) the time from planting to head induction was calculated (Fig III-12). Comparison of the calculated time to head induction based on final leaf number showed that the approach was able to predict the time to head induction from final leaf numbers with an relative model error RMAE of 21 % (Fig. III-12).

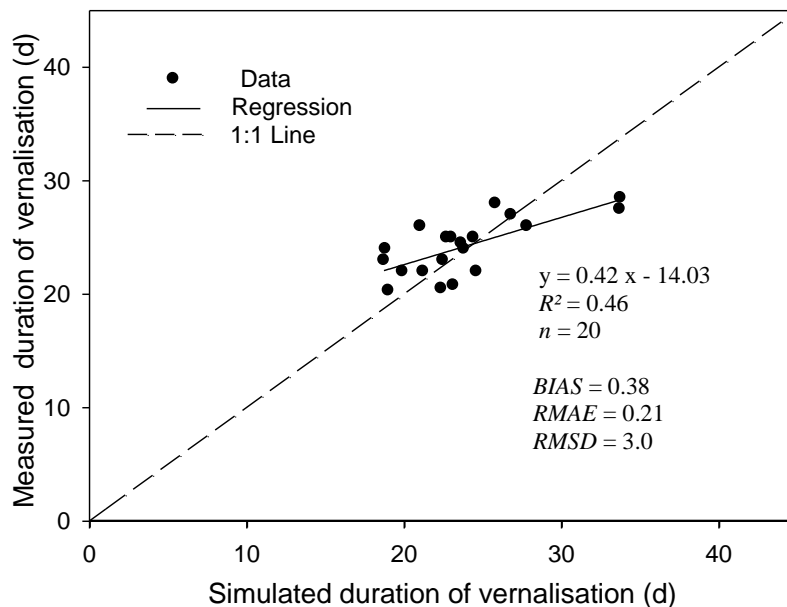


Fig. III-12. 1:1 Line for comparison between head induction calculated from final leaf numbers and observed duration (days) from planting to head induction for independent data from broccoli cultivar ('Ironman F1') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2010, 2011 and 2012.

**3.5 Model evaluation**

Evaluation of the vernalisation model against independent field data showed that the model was able to predict the mean duration of vernalisation under field conditions. The measured relative mean absolute error (RMAE) was 0.18 (Fig.III-13) A systematical model error and overestimation of the duration of vernalisation with increasing duration in head induction in the field was found. The vernalisation model (Eq III-7) provides comparable results and accuracy compared to the non-destructive method basing on counting of final leaf numbers in the field. Figure III-14 shows the 1:1 line between simulated and measured variation in vernalisation the model was able to predict the measured variability with an RMAE of 21 % . .

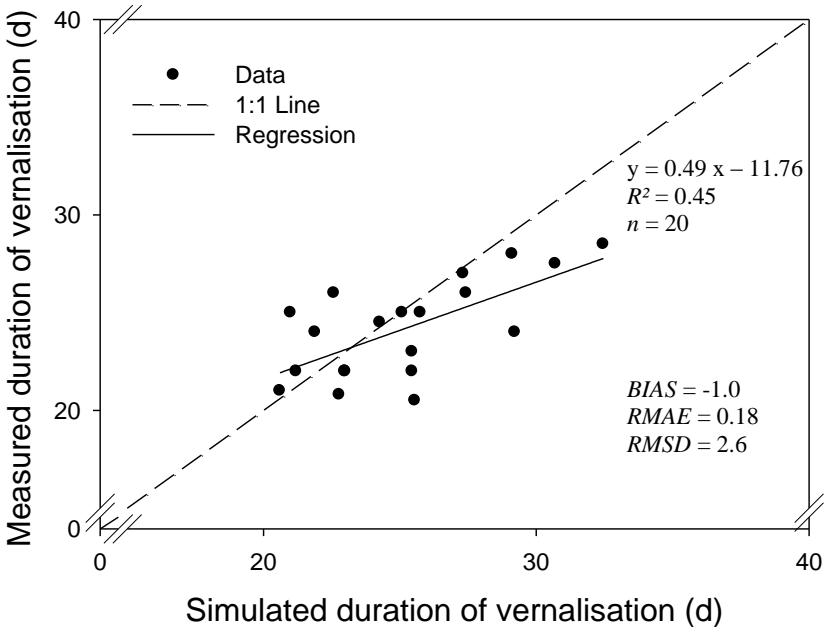


Fig. III-13. 1:1 Line for comparison between simulated and observed duration (days) from planting to head induction for independent data from broccoli cultivar ('Ironman F1') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2010, 2011 and 2012.

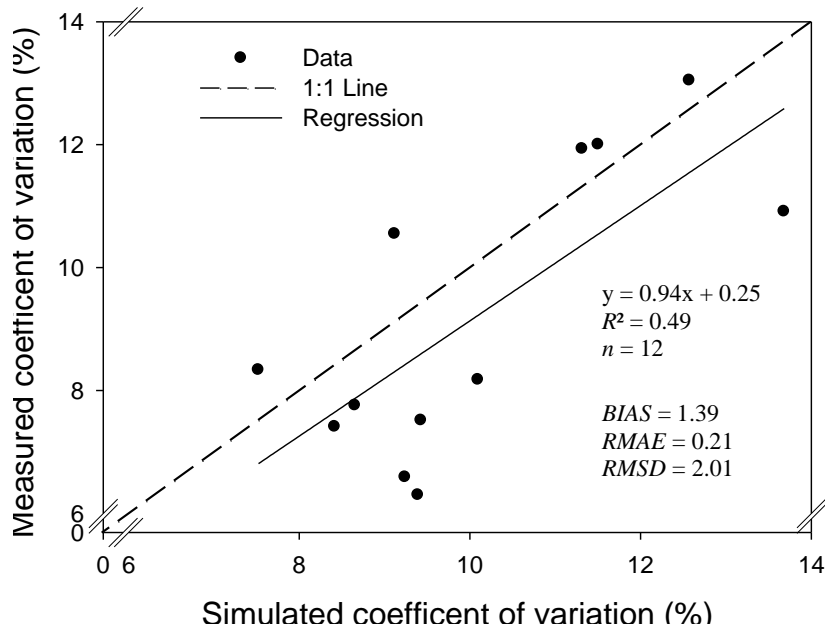


Fig.III-14. 1:1 Line for comparison between simulated and observed coefficient of variation in duration from planting to head induction for independent data from broccoli cultivar ('Ironman F1') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2010, 2011 and 2012.

#### 4. Discussion

We found evidence for the existence of a juvenile phase in broccoli, since final leaf numbers were significant lower for plants which started the cold treatment with smaller leaf numbers. The data suggest that the juvenile phase is already passed before planting and completed when around two leaves were visible. The determination of the duration of the juvenile phase is complicated in broccoli since the plants have a facultative vernalisation response and thus the cultivation period and plant age have an effect on the duration to head induction which is an artefact in our data set. The results of our study agree with results of other studies (Wurr et al., 1995; Fontes et al., 1967; Miller, 1988). Wurr et al. 1995 concluded from the exponential

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shape of their model of apex development of calabrese without implying a specific juvenile phase that the plant has to grow a certain time to produce a minimum dry weight, stem diameter or number of leaves before it significantly responds to vernalisation temperatures. This coincides with the results of our experiments. Some researchers used thermal time models to simulate the development with three cardinal temperatures: a base temperature below which the plant does not develop, an optimum temperature at which the development rate is at its maximum and a maximum temperature above which developmental rates approach zero (Tan et al., 2000). Our results show that broccoli required a significant shorter developmental time ( $^{\circ}\text{Cd}$ ) if the plant is exposed to cool conditions. Furthermore the relationship between thermal time to head induction and temperature was not linear. This relationship between cool temperatures and the developmental rate (Fig. III-7A, Fig III-9) showed that the concept of thermal time models which implies a linear relationship between developmental or growth rate and temperature (Bonhomme, 2000) is inapplicable to predict the time to head induction of the used broccoli cultivar.

Unlike cauliflower, broccoli is characterised by its facultative vernalisation requirement at least for the temperature range up to 25.7  $^{\circ}\text{C}$ . The results of the experiments showed that plants grown at 25.7  $^{\circ}\text{C}$  were still capable to induce the head. The shape of the vernalisation response curve and especially a comparison between 20.9 and 25.7  $^{\circ}\text{C}$  also suggest that the vernalisation response of the broccoli cultivar differs from a typical vernalisation response curve of an obligatory variety (i.e. cauliflower). Typical cauliflower cultivars showed a linear decrease of the vernalisation rate above the optimum temperature towards zero while this was not observed for broccoli “Ironman F1”. The model which implies a facultative vernalisation requirement was able to describe the observed independent field data well.

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Our results are supported by studies on the influence of high temperature on inflorescence development in broccoli at varying developmental stages. Daily temperatures of 35 °C for one week do not lead to a cessation of bud initiation at the apex (Björkman and Person, 1998). Tan et al. (2000) also reported that the definition of a maximum temperature corresponding to a developmental rate of zero was not always possible from experimental data for broccoli. Contrasting findings were shown by Wurr et al. (1995). In their cabinet experiments with calabrese (*Brassica oleracea* var. *italica*) plants under temperature regimes of 20.7 and 22.6 °C needed more than 70 days to induce the generative stage and the upper limit for effective vernalisation temperature was found to be 23.6 °C (Wurr et al., 1995). Wurr et al. (1993) pointed out that „the difference between perceived obligate and facultative (optional) cold requirements may simply be the level of ambient temperatures relative to the temperatures that permit vernalization. If the mean temperature is close to the upper limit for vernalization the requirement will appear to be obligate; if the mean temperature is close to the optimum temperature for vernalization the requirement will appear to be facultative.” This statement implies that researchers defining a facultative vernalisation response might have considered temperatures which were too close to the optimum or which were too far away from the upper limit for vernalisation. Our results show that the highest temperature regime (25.7 °C) was beyond optimal temperature conditions for head induction (Fig. III-9). Optimum vernalisation temperature is the temperature that lead to fastest induction of the generative phase (Wiebe, 1990) in our data 11.5-13.5 °C. The experimental data cannot say anything about the responses of the cultivar “Ironman F1” for temperatures above 25,7 °C. Anyhow existing contradiction regarding the obligatory or facultative vernalisation response of broccoli, could not fully resolved yet, the shape of the relationship between the developmental rate to head

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induction and temperature suggest that there is a bend of the negative slope above the optimum temperature and a flatten curve between 20.9 and 25.7 (Fig. III-9) which can be seen as an indicator for a facultative vernalisation response.

The vernalisation response curve of “Ironman F1” (Fig. III-9) showed a peculiarity compared to the reported vernalisation characteristics of broccoli. Wiebe (1990) gives a review of the vernalisation responses of different species and broccoli and reported that the optimum for vernalisation of broccoli is mostly 20 °C. While the optimum found in this study was 11.5-13.5 °C which is more characteristic for the response of head tolerant cauliflower varieties i.e. Fremont with an optima temperature range from 10-13 °C (Bohres, 1996).

A clear increase in final leaf number in relation to temperature for plants grown under temperatures higher than 18 °C was found (Fig. III-11A). This was accompanied with flatten of the vernalisation curve and a significant slowdown of developmental rates with increasing temperatures. The increase in leaf number was however not found for temperatures lower than 12.8 °C. Even the observed thermal time to head induction was different between these different temperature traits (Fig. III-7A). The final leaf number in relation to the temperature sum to head induction showed a slope of 0.02 (Fig. III-11B) which is identical with the leaf appearance rate found in the field observations (Fig. III-10). This relationship supports the hypothesis that the time to head induction can be predicted from final leaf numbers.

Published work modeled the physiological response of broccoli to temperature and predicted time to head induction (i.e. Wurr et al. 1995). The step forward of the presented work was to consider the occurring variability with the combination of the classically applied empirical relationship between temperature and developmental rate with a stochastic model part.

The mapping of variation in time to head induction under a constant temperature regime was one major goal of cabinet experiment two. A frequent rotation of plants was applied to avoid

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any effect of small scale differences in temperature or irradiance within the cabinets which might influence variation in time to head induction. A constant variation in time to head induction of 11.69 % over all temperatures treatments was detected. Data basis were measurements of 100 plants per temperature variant. The data showed that it was possible to capture the occurring variability in the experiments because it was possible to start the measurements when first plants (10% of a sample of 10 plants) induced their generative stage while other remained vegetative and end up when 90-100 % of the plants had induced the head (i.e. Fig. III-3).

The parameterisation approach of the mechanistic and the stochastic model parts by the use of logistic functions provided a precise description of the parameterisation data set (Fig. III-9). The application of this complicated parameterisation approach was necessary to overcome inaccuracies which were combined with the scheme used to determine the level of development by apex dissection (Fig. III-1). Especially the time between the different scheme points was not known and could not be defined. We had problems to capture the variability in the field because of a limited number of samples and the missing possibility for daily measurements in the field. Anyhow, our approach to model the occurring variability showed that we were able to describe the shape of the occurring variability in broccoli development in the field. Our vernalisation model was successfully tested against independent field data and under a broad range of environmental conditions and can be used to describe and predict vernalisation and occurring variation within the field. Furthermore we developed a non-destructive approach to estimate the time to the end of vernalisation in broccoli from counting final leaf numbers in the field.

### *5. Conclusions*

The results showed that the juvenile phase is already passed at planting, therefore this phase was not considered in the model to predict time to head induction and the occurring variation.

The model evaluation showed that time to head induction and the stochastic variation could be predicted with parameters derived from cabinet experiments and field weather data.

Scattering in time to head induction not differentiate between different cabinet temperatures and different field environments, this indicates that variation in time to head occurs independently from environmental conditions. The stochastic approach to predict the variation in time to head induction therefore could be used to test the hypothesis that variation in time to head induction is related to head size variation by integrating this model into a model for plant and head growth.



Modeling time to harvest and its variability of broccoli (*Brassica oleracea* var. *italica*): Model description, parameterisation and field evaluation

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**Abstract**

A strong variation of broccoli head sizes within individual plantings is observed in practice. Because of this problem it is common to harvest each crop a least three times by selective hand harvests. The length of the cutting period and the number of selective harvests influence harvest costs, so that plant to plant variation increases total production costs. Moreover, deviations from planned harvest schedules cause a fluctuating market supply which is accompanied by high price fluctuations. Decisions about harvest operations must be taken under the aspect of these price fluctuations and have to take economic aspects into account. Thus, harvest strategies and the optimal number for selective hand harvests influence the number of harvestable heads per date (biological properties) as well as by the current market supply and price situation (economic aspects). For planning of cutting and optimization of harvesting a forecast of the exact amount of harvestable heads per date is necessary. A model for growth, development and variability between plants in time to harvest of broccoli is presented here describing three linked processes: crop development to head induction and its variability, dry matter production and dry matter partitioning to the different vegetative organs and the head. The mean developmental time from planting to head induction is described as a function of temperature. To account for developmental variation we grouped the plants into 10 classes, each class representing 10 % of the plants of the whole crop. The developmental variation is based on the coefficient of variation in vernalisation determined

experimentally. After vernalisation is completed an expo-linear function of temperature sum describes the fraction of dry matter allocated to the head. Evaluation of the model against independent field data from the years 2011 and 2012 show that the model is able to predict total above-ground dry matter, dry matter of different plant organs, LAI, mean head size and variation in head size. The model explains on average 79 % (22 - 94 %) of the measured variability of individual plants in head size at final harvest by developmental variation. The coefficient of determination for simulated vs. observed standard deviations in head size for all measured data points is 67 %. For prediction of harvest dates a harvest criterion of 500 g fresh weight with a stem part of 0-10 cm is defined, assuming that no quality decline occurs in this range. The model calculates percentages of harvestable heads per date; this information is useful to maximise reaping percentages of individual harvests and can support producers to optimize their harvest operations for given market supply and price. Simulations showed that harvest percentages not less than 80% can be archived by 1,8 selective hand harvests on average.

Keywords: broccoli, vernalisation, variability, model, harvest time prediction, optimisation.

### **1. Introduction**

In broccoli (*Brassica oleracea* var. *italica*) production two main problems are frequently reported: One is a lack of predictability of time to harvest and strong deviations from planned harvest schedules (Hulbert and Orton, 1984). Harvest time delays are a well-known economic problem for growers and retailers. The market supply fluctuates during the growing season due to unpredictable climatic effects which affect head induction and head growth (Wurr et al. 1992). Consequences are temporary under- and oversupplies to the market. The fluctuation of the market supply is associated with strong price fluctuations (AMI, 2010). A major production goal is to ensure continuous market supply to avoid price fluctuations and keep the financial risk as low as possible. This problem is a task of current research and the development of a broccoli cultivar with a predictable time to harvest even under unfavourable weather conditions is still a major breeding goal (Farham and Björkman, 2011; Uptmoor et al., 2008 and 2012). However, all advances in breeding were not successful in overcoming this problem until today. The time to harvest maturity of individual sets is still erratic and hard to predict. The magnitude of price fluctuation may exceed 50 %: For example, the price for broccoli at wholesale trading markets in Germany in the years 2009 was 152 € (100 kg)<sup>-1</sup> on average. During weeks with a low market supply the price increased by 45 % to 220 € (100 kg)<sup>-1</sup> while during a period of high product availability on the market the price declined by 59 % to 90 € (100 kg)<sup>-1</sup> (AMI, 2010). The difference in the duration of the growing period of one planting set in two different years can be up to two weeks (Babik and Elkner, 1997). Researchers attributed variations in harvest time mainly to a variation in weather conditions and different responses to temperature during the different physiological developmental phases of the crop (Grevsen and Olesen, 1999; Grevsen, 2000). Producers have the

opportunity to adjust their economic strategies in relation to crop maturity and can smooth market supply fluctuations by cold storage of the product for short periods (Wurr et al., 1992). But this kind of storage means additional costs and can only be useful to overcome short phases of oversupply in the market in order to obtain higher prices during phases of undersupply of the market.

The second problem in broccoli production is a lack of uniformity of time to harvest which requires multiple harvests, causing high costs in practice. Research in the past showed that a large proportion of uniformity in maturity was attributed to genotypic variation and to a lesser degree to environmental conditions (Hulbert and Orton, 1984). Although modern broccoli hybrid varieties and especially CMS cultivars show less variability than cultivars in the past this problem is still unsolved. The reasons for the apparent variability in head development are largely unknown. In our own investigations on commercial farms coefficients of variation for head size between 25 and 35% were determined for the CMS variety “Ironman F1” in early as well as in late sets (unpublished data). On average, each set in practice requires at least three selective hand harvests that make up a significant proportion of the total production costs and also cause a high planning effort for the producer.

Therefore, predictions about exact delivery volumes are further complicated. There is a high influence of the length of the cutting period, the harvest costs and the total production costs (Hulbert and Orton 1984; Olesen and Grevsen, 2000). The determination of the optimal harvesting strategy is also influenced by the strongly fluctuating market prices. With oversupply of product in the market and strong price declinations the growers have to cancel an additional hand harvest so that more than 30-40 % of the product may remain as waste in the field. Because of these problems growers have to take their planting, harvest and storage decisions under economic considerations. To compensate for price fluctuations, the markets require exact estimations of delivery quantities as early as possible. For growers and

cooperatives it is useful to have an accurate forecast of harvest dates for adjustment of marketing strategies (Wurr et al. 1992; Tan et al., 1997; 2000a).

To address the problems described above model-based forecasts of harvest quantities and variation in time to harvest of single plants to predict the percentages of harvestable heads could help to optimise the production and harvest operations with consideration of harvesting costs and the expected market prices.

Several prediction models for production scheduling of broccoli and calculating mean time to harvest have been developed (Marshall and Thompson, 1987ab; Scaife et al. 1987; Pearson and Hardley, 1988; Wurr et al., 1991, 1992; Grevsen, 1998, 2000; Grevsen and Olesen, 1999; Tan et al. 2000 ab; Kläring et al. 2001). The model of Marshall and Thompson (1987) calculates the time from sowing to harvest depending on solar radiation and temperature. Influence of solar radiation was integrated with a combined factor of temperature and radiation (Scaife et al. 1987), but developmental stages of the crop had not been considered. A later model describes head growth after vernalisation as a function of temperature sum with different base temperatures depending on cultivars (Pearson and Hardley, 1988). The model was extended to account for effects of plant density (Wurr. et al., 1991). To describe the development phase between the vegetative and the generative stage, the effects of different temperatures were analysed and models for apex development were presented (Wurr et al. 1995; Fellows et al 1997; Grevsen and Olesen 1999). Effects of different growth phases are considered in a model of Tan et al. (2000). A significant effect of global radiation on head growth of broccoli was shown (Grevsen, 1998). Studies on light interception and light use efficiency (LUE) in broccoli were presented (Olesen and Grevsen, 1997). In cauliflower it was found that different radiation levels strongly affected the growth rates of the harvest organ (Rahman et al., 2007). This highlights that a reliable prediction of time to harvest should be based on the mechanistic relationship between global radiation and plant growth.

Effects of plant density on LUE of broccoli crops were presented (Francescangeli et al., 2006). Besides calculation of dry matter production the allocation of dry matter to the different plant organs is important for the simulation of plant growth and development (Marcelis, 1993). Dry matter allocation of broccoli in relation to different shading levels was analyzed by Francescangeli et al. (2007). A model for dry matter production and partitioning in cauliflower was presented by Kage and Stützel (1999). The model calculates dry matter production and partitioning using constant parameter values for LUE and specific leaf area (SLA). The model evaluation showed that prediction of dry matter and time to harvest was only possible by fitting SLA and LUE individually to every evaluation data set. This highlighted the importance of dynamic SLA and LUE parameter for prediction accuracy. Models improvements by implementation of dynamic LUE and SLA parameters depending on the radiation levels were carried out (Kage et al. 2001a; 2001b). In broccoli, reduced radiation levels also cause reduced dry matter production and an increase in SLA (Kläring, 1998; Mourão and Hadley, 1998; Francescangeli et al. 2007). Prediction models which include dry matter production with dynamic changes in SLA and LUE as functions of environmental conditions or models which consider plant to plant variation has been presented in literature . In cauliflower the time of curd initiation can be seen as the main factor influencing the variability in curd size and the resulting harvest window (Booij, 1990; Grevsen and Olesen, 2000). It was possible to describe the curd size variability of single plants by incorporating plant variability in the juvenile and curd induction phases in a harvest prediction model (Olesen and Grevsen, 2000). Here the question arises if this can also be shown for broccoli.

The goals of this study were (1) to test the hypothesis that differences in time to head induction are responsible for head size variation. To archive this one stochastic model subroutine predicting the variation in time to head induction will be implemented into a

simulation model for vegetative and head growth of broccoli. (2) Second aim is to estimate the relationship between the numbers of necessary selective hand harvests and variability of head size. Modell simulation should predict the optima numbers and dates of selective hand harvest to archive harvest amounts not less than 80 %.

## **2. Materials and Methods**

### **2.1 Field experiments**

For this study we used various plantings of broccoli (*Brassica oleracea* var. *italica*) of the variety 'Ironman F1'. On the one hand we used samples of 12 sets grown on commercial farms in North-East Germany in 2010, 2011 and 2012 planted at a density of 3.6 plants per m<sup>2</sup> (30 x 60 cm). The earliest planting date was on 17 March and latest planting date was on 20 June. Crops were grown on 8 different locations with varying soil type in 100 km radius of the village Gresse (53°26'528"N 10° 44'42" E). Randomized samples of 10 plants were collected one to two times per week in the field and divided into leaves including petioles, stems including side shoots and heads with defined stem parts of 0, 5 and 10 cm length. For measurements of plant dry weight whole plant parts were dried at a temperature of 105 °C until a constant weight was reached. The leaf areas of plants were measured using a leaf area meter (Li 3100, Licor, NE, USA). For parameterization of the model we used two data sets of 2010 collected on the commercial farms. Data from 2011 and 2012 was used for model evaluation. On the other hand we used experiments conducted on a loamy sand soil at the experimental farm of the Leibniz Universität Hannover (52° 23' 10" N, 9° 42' 28" E.) to complete our evaluation data set. Crops were planted in 2011 and 2012 with a total of five different planting dates arranged in two factorial split plot design, where effects of transplant

size and planting date were studied. Tillage, irrigation, fertilization and plant protection was conducted in accordance with the requirements of good agricultural practice. For the data analyses presented here we summarized the data of all different transplant sizes of each of the three replications, since no significant effect of transplant size on growth and time to harvest was found. Overall 5 sets of seedlings were grown at different planting dates during the growing season 2011 and 2012 with a plant density of 4 plants per m<sup>2</sup> (50 x 50 cm). Randomised samples of 16 plants per each of the three replications were harvested from the field plots four times in 2011 and three times in 2012 for destructive measurements of leaf area (Li 3100, Licor, NE, USA) and fresh and dry weight of plant organs (stem, leaf and head). For measurements of plant dry weight whole plant parts were dried at a temperature of 105 °C until a constant weight was reached.

## ***2.2 Model description***

The dry matter production and partitioning model by Kage and Stützel (1999) was used as mathematical basis for our model. The advantage of the LUE approach is its simple parameterisation in comparison to photosynthesis-respiration based modules (Kage and Stützel, 1999). However, it was shown that a constant LUE is not suitable to describe total dry matter production of cauliflower well whereas the use of a linearly decreasing LUE with increasing levels of daily global radiation along with a temperature correction factor for dry weight production showed comparable suitability for dry matter prediction as a photosynthesis based approach (Kage et al., 2001b). Therefore, improvements of the original model of Kage and Stützel (1999) through implementation of a dynamic LUE and a temperature factor for dry weight production were made. LUE in the model is described as:



$$LUE = LUE_{E0} - a_{LUE} I \quad (IV-1)$$

LUE depends on two parameters  $LUE_{E0}$  and  $a_{LUE}$  and the level of photosynthetic active radiation  $I$  (Kage et al. 2011b).

A temperature correction factor ( $f_{temp}$ ) was implemented.

$$\frac{dDM}{dt} = Q \cdot LUE \cdot f_{temp} \quad (IV-2)$$

where  $dDM/dt$  is the rate of increase of total dry matter,  $Q$  the amount of intercepted radiation and  $LUE$  the light use efficiency calculated using Eq. IV-1. The value of  $f_{temp}$  was set to 1 within the range from 10 to 25 °C mean daily temperature and decreased linearly to 0 from 10 to 0 °C and from 25 to 35 °C according to Kage et al. (2001). The value for the light extinction coefficient was set as 0.7.

Further improvements were done for a dynamic estimation of specific leaf area. This was based on a relationship between the intensity of incident photosynthetically active radiation (PAR) and the SLA of cauliflower (Alt, 1999) together with observations of a decreasing SLA with increasing plant age in broccoli (Uzen and Kar, 2004). The SLA is calculated as a function of average PAR during the last 14 days ( $PAR_{14}$ ) (Alt, 1999) and plant age (Leutscher and Vogelezang, 1990) in terms of days (d).

$$SLA = SLA_0 - (sla_a \cdot PAR_{14}) - (sla_b \cdot d) \quad (IV-3)$$

Other changes were done for calculation of dry matter partitioning to the head. In contrast to the cauliflower model of Kage and Stützel (1999) an expo-linear function describes the shape

of the growth fraction ( $f$ ) of the head in relation to the total growth rate ( $dDM/dt$ ). This shape reflects sink capacity limited growth (Kage and Stützel, 1999) based on the potential sink strength of the harvest organ (Marcelis et al. 1998) which was assumed to increase for broccoli during the whole head growth phase until its maximum fraction  $f_{max}$  of 0.84 is reached.

$$f = \begin{cases} ae^{bT_t} & T_t \leq PI \\ \min(cT_t + d; f_{max}) & T_t > PI \end{cases} \quad (IV-4)$$

Were  $a$  is the intercept of dry matter fraction, and  $b$  the relative increase in dry matter fraction (exponential phase).  $c$  is the increase dry matter fraction and  $d$  the intercept dry matter fraction (linear phase).  $PI$  describes the switching point of exponential to linear increase of dry matter fraction depending on head age in thermal time  $T_t$ .

Thermal time  $T_t$  was calculated based on Tan et al. (2000ab) according to equation III-16.

As harvest criteria a fresh weight of 500 g with a stem part from 0-10 cm has been defined, assuming that no quality decline occurred in this range.

The head fresh weight  $H_{FW}$  was calculated with an empirical regression equation based on the relationship between head dry weight ( $DM_H$ ) and head fresh weight with a defined stem part of 0, 5 and 10 cm ( $DM_{S0}$ ,  $DM_{S5}$ ,  $DM_{S10}$ ) (Eq. IV 6).

$$H_{FW} = a \left( \frac{(DM_H + DM_{S5})}{pd} \right) \quad (IV-6)$$

where  $pd$  reflects plant density (plants/m<sup>2</sup>).

The vegetative dry matter partitioning part of the original model was not changed but reparameterised.

Broccoli development to head induction was implemented on basis of results from cabinet studies (Chapter 3). The juvenile phase of the used cultivar had passed before transplanting so that the model does not require consideration of a juvenile phase. In the model the vernalisation process begins directly at planting and is completed when the sum of daily vernalisation rates reaches a value of 1. The piecewise linear regression model derived in Chapter 3 was used to describe the facultative vernalisation of the broccoli cultivar 'Ironman F1' (Eq. III-1).

The implementation of plant to plant variation in head induction (vernalisation) is from particular importance. We subdivided the developmental variation of individual plants into ten classes with equal size thus each class represents 10 % of the whole population of one planting set. The model calculates the mean development rate ( $\frac{dV}{dt_{50}}$ ) depending on temperature using Equation III-1. The variation is considered by standard deviation (Eq.III-2), calculated from one parameter for the coefficient of variation in vernalisation ( $cv$ )  $cv = 11.69$  % estimated in an cabinet experiment in 2011 (Chapter 3).

In the next step values were normalised and assigned to the ten classes by multiplication of mean  $\frac{dV}{dt_{50}}$  and  $sd$  with the percentile values of the normal distribution curve ( $p_i \dots p_n$ ) (Table. III-3) (Eq. III-3) corresponding to every class center for every time step.

The concept of this model approach was taken from the cauliflower model Blukosim (Bohres 1996, Kage, 2010) which described the variability in crop development by classes with assumed normal distributions and assumed coefficients of variation in the juvenile and

vernalisation stage of the crop. The stochastic model development, parameterization and evaluation is presented in Chapter 3.

### 2.3 Model parameterisation

The model equations and the variation mapping approach were implemented using ModelMaker4™ (ModelMaker4™, Modelkinetix, UK). Model parameter fitting was carried out using a function for multiple and nonlinear regressions in R. In R, packages of the library DAAG and the NLS procedure were used. In ModelMaker the differential equations of the model were integrated numerically using the Runge-Kutta method with time steps of 1 day. The intercepted radiation ( $Q$ ) was not measured directly in our experiments; therefore the  $LUE$  was also not calculated directly. Daily values for  $Q_d$  were calculated from daily radiation data and the  $LAI$  as a function of thermal time (Eq. IV-11) together with one assumed light extinction coefficient  $k = 0.7$  (-) in ModelMaker (Eq. IV-10).

$$Q_d = I(1 - e^{-k \cdot LAI}) \quad (\text{IV-10})$$

To determine  $Q_d$ , data from leaf area measurements of the two parameterisation data sets was used to estimate the  $LAI$  over thermal time  $LAI_{TS}$  using a logistic function and parameter estimations from R in the first step.

$$LAI_{TS} = \frac{LAI_{\max}}{\left(1 + \left(\frac{LAI_{\max}}{LAI_0} - 1\right) \cdot e^{(-cT_s)}\right)} \quad (\text{IV-11})$$

with  $LAI_{max}$  describing the maximum  $LAI$ ,  $LAI_0$  the initial  $LAI$  at planting,  $T_{sum}$  the thermal time after planting and  $c$  the productive part of the  $LAI$  which limits the maximum  $LAI_{max}$ .

The calculated amount of daily intercepted radiation (Eq. IV-10) was summed up over time to calculate  $Q$  and parameter values for  $LUE_{EO}$  and  $a_{LUE}$  were estimated with data of the measured amount of above ground dry mass ( $g\ m^{-2}$ ) ( $DM_t$ ) and from the sum of the calculated amount of daily interception radiation  $Q$ , using functions for minimisation of last square differences and the Marquardt algorithm in ModelMaker4™. In addition to that a parameter for  $LUE$  was estimated by linear regression between the manually calculated amount of  $Q$  and the measured above-ground dry mass in R which provides identical results.

$$LUE_{EO} = \frac{dDM_t}{dt} \frac{1}{I(1-e^{-kLAI})} \quad (IV-12)$$

All estimated and used model parameters for the simulations of time to harvest are shown in table IV-1.

## ***2.4 Model based hypothesis testing and model simulations***

For the hypothesis testing the commercial software product ModelMaker® (ModelMaker4™, Modelkinetix, UK) was chosen because it allows formulation of individual processes independently (modularity), the software can be used to test a single hypothesis or a single hypothetical cause of plant to plant variation, and provides tools for parameter estimation and statistical model evaluation. Thus for small scale problems like head size variation the ModelMaker® software seemed to be a suitable tool for model based testing of hypotheses

(Kage, 2000). To test the hypothesis that variation in time to head induction is the main reason for head size variability the stochastic part describing variation in time to head induction of single plant cohorts was implemented as the only source of variation in the model. The model output for head size variation was then compared to measured head size variability. To analyse the relationship between head size variation and the number of selective hand harvests a fresh weight of 500 g was defined as harvest criteria. In commercial broccoli production this fresh weight is achieved by cutting of heads (inflorescences branches) with varying stem portion. In the model a varying stem portion from 0-10 cm was implemented, assuming that no quality decline occurred in this range. The model derived a harvest window on the basis of this varying stem portion and calculated the time from the beginning of harvest ability (500 g fresh weight with 10 cm stem portion) and the end of harvest ability (500 g fresh weight with 0 cm stem portion). The head fresh weight  $H_{FW}$  was calculated with an empirical regression equation based on the relationship between head dry weight ( $DM_H$ ) and head fresh weight with a defined stem part of 0, 5 and 10 cm ( $DM_{S0}$ ,  $DM_{S5}$ ,  $DM_{S10}$ ).

Plotting the simulated proportion of harvestable heads against time after planting provides a graphical tool to find the optimal number and the optimum dates for selective hand harvests (Fig. IV-1).

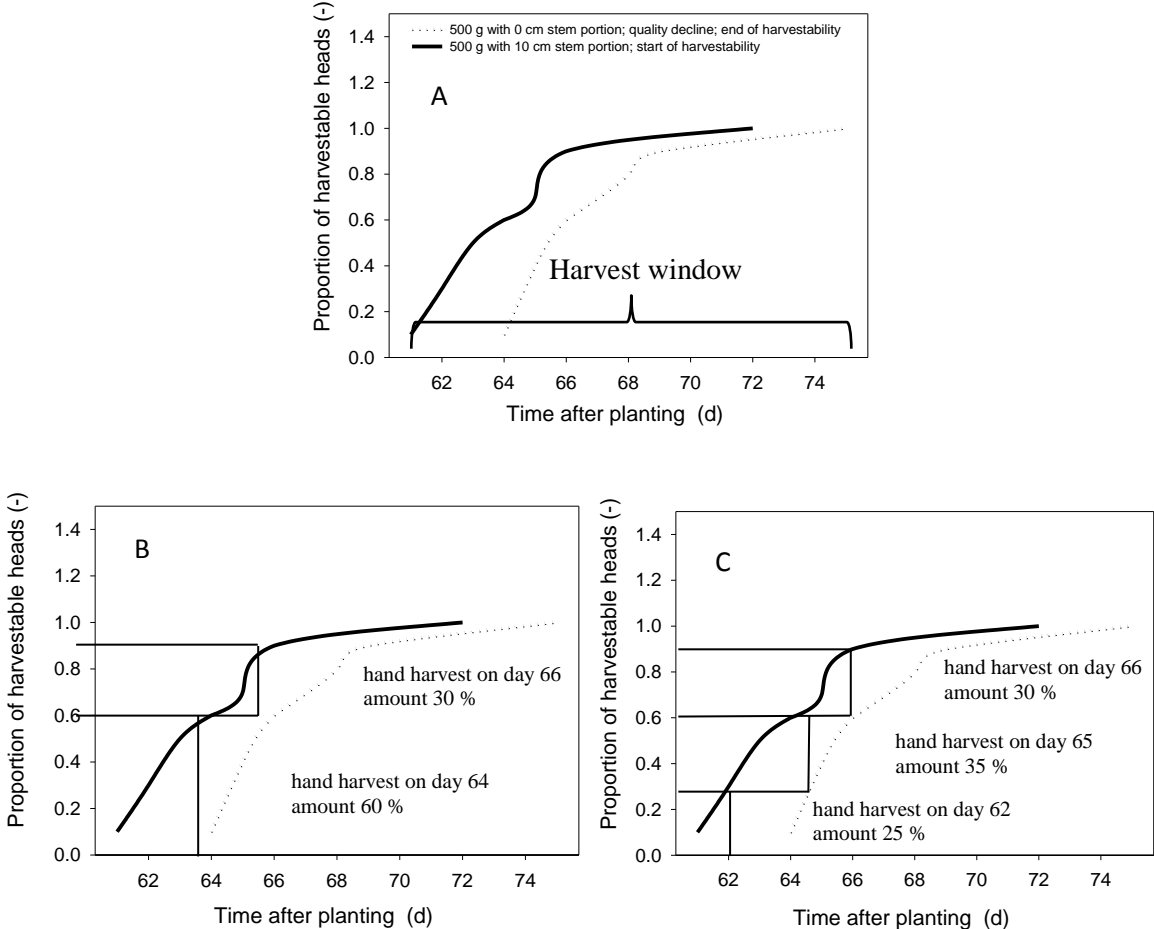


Fig. IV- 1. Example of simulated proportion of harvestable heads against time after planting for weather data Herrenhausen 2011 plating date (125 DOY), deviation of the harvest window (A), (B) example for scenario analyses with two harvest dates, the vertical solid lines marking a date for one selective hand harvest corresponding to a certain amount of harvested heads (solid horizontal lines), in this example one selective hand harvest is carried out on day 64 after planting and another on day 66 after planting. (C) Example for scenario analyses with three selective hand harvests on day 62, 65 and 66 after planting.

The vertical solid lines marked a date for one selective hand harvest corresponding to a certain amount of harvested heads (solid horizontal lines). A vertical line of maximum length represents a date for a single selective hand harvest with maximal harvest percentage by single harvest. As an example for optimization of harvest operations a scenario with different planting sets is simulated. For the scenario analyses several sets (planting dates DOY (A 80, B 90, C 100, D 110, E 120, F 130, G140, H 150, I 160) were simulated using weather data from

Herrenhausen 2011. The model output was graphically analyzed to find the minimum number of hand harvest to archive harvest percentages not less than 80 %.

For model run, model inputs were global radiation ( $\text{MJ m}^{-2}$ ) and temperature ( $^{\circ}\text{C}$ ) measured 2 m above ground. Daily weather data for 2010-2012 were provided by the Institute of Meteorology and Climatology, Leibniz Universität Hannover, for Herrenhausen and by the Behr AG ([www.behr-ag.com](http://www.behr-ag.com)) for commercial farms in North-East Germany. For commercial crops weather data were averaged from a total of four weather stations in the vicinity of the fields.

The values of global radiation  $I$  ( $\text{MJ m}^{-2}$ ) were converted to PAR by multiplication with the factor 0.5 (Szeicz, 1974).

### ***2.5 Model evaluation***

The model was evaluated against independent field data from broccoli cultivar 'Ironman' grown on the experimental farm of the Leibniz Universität of Hannover (5 sets) and on commercial farms in North-East Germany (10 sets) in the years 2011 and 2012. Evaluation sets include two different seedling production systems, three different soil types and seven different locations. For the evaluation of the model, the quantities *BIAS* Eq. III-19, *RMSD* Eq. III-21 and *RMAE* Eq. III-23 (relative to the observed mean value) and  $R^2$  Eq. III-23 were calculated according to Kobayashi and Salam (2000) and Mayer and Butler (1993). In addition, a graphical representation of the agreement between measured (y-value) and calculated (x-value) values as 1:1 graph and graphed residuals was chosen (Wallach et al., 2006). Differences in slope from 1 and differences in intercept points from zero were analysed



with R package MULTCOMP function for generalised linear model hypothesis testing (*glht*) ( $p \leq 0.05$ ). The statistical analyses were performed using R.2.15.2 (R Core Team 2012).

### **3 Results**

#### **3.1 Model parameterisation**

The vernalisation model, the model of developmental variation and used parameter values of those models were acquired and validated by Zutz et al. (2015). Used percentile values of the normal distribution curve are shown in table III-3, for details see Chapter 3. A high variability of SLA in the parameterisation data set was observed. We were able to describe this variability by a multiple linear regression model with influences of average PAR during the last 14 days ( $PAR_{14}$ ) and plant age with a goodness of parameter fit of  $R^2 = 0.70$  and  $p \leq 0.01$ . We took the allometric dry matter portioning approach from the cauliflower model of Kage and Stützel (1999) and re-parameterised this approach for the broccoli cultivar used. In both of our parameterisation data sets a linear relationship between the logarithms of leaf and stem plus side shoot dry matter could be found (Fig. IV-2). Differences in slope and intercept between both parameterisation data sets were small and we fitted one regression to both data sets. Result showed a coefficient of determination of  $R^2 = 98 \%$ .

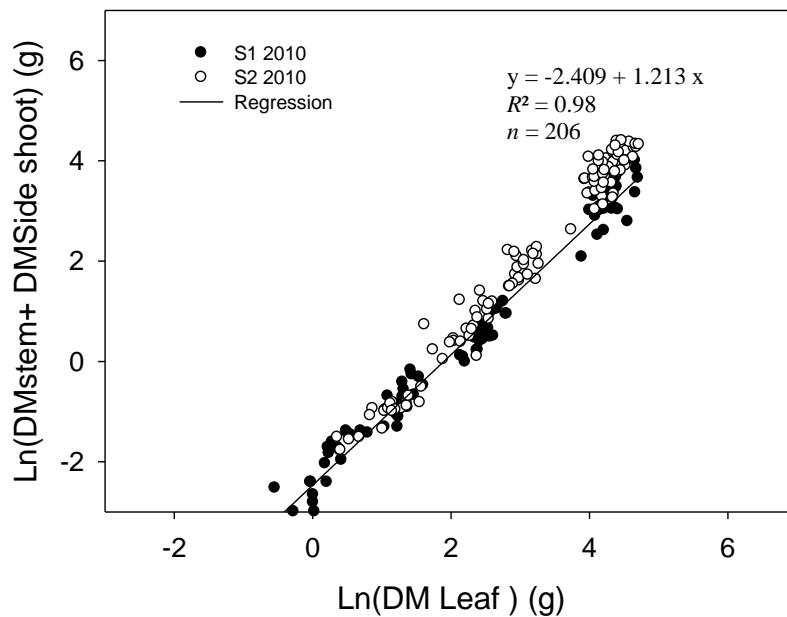


Fig. IV-2. Relationship between the logarithms of leaf and stem dry mass for two sets grown on commercial farms in north-east Germany in 2010 (parameterisation data set), the black circles represents one set planted in spring (planting date DOY 138), the white circles show data of one summer set (planting date DOY 182).

An expo-linear function of temperature sum after head induction describes the fraction of dry matter allocated to the head in proportion to the total dry matter growth rate. The function described the observed fractions well with a coefficient of determination of 0.96. The point where the relationship became linear was found at 322 °Cd. The maximum dry matter fraction allocated to the head was fixed to 0.84 (maximum measured value from the parameterisation data set) (Fig. IV-3).

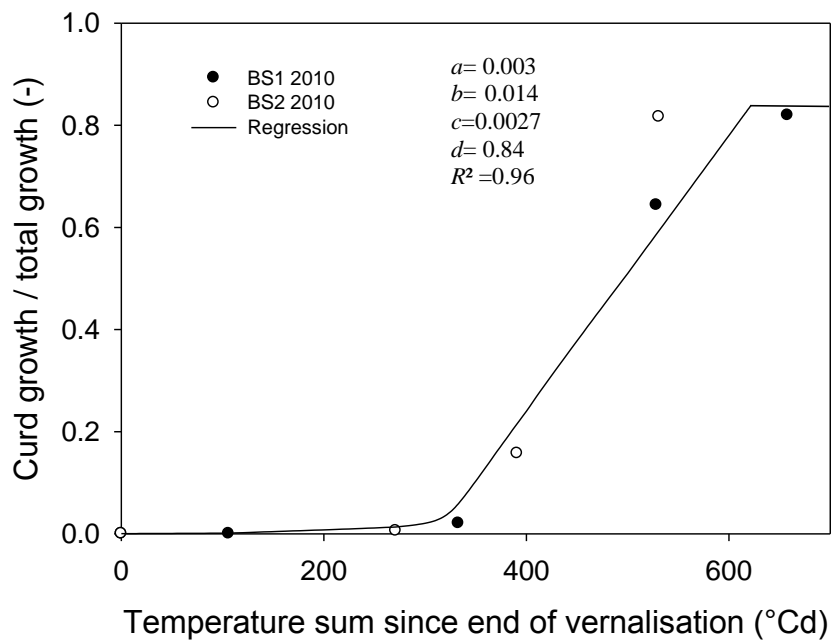


Fig. IV-3. Fraction of the head growth rate on total growth rate as a function of temperature Sum since end of vernalisation for two sets grown on commercial farms in north-east Germany in 2010 (parameterisation data set), the black circles represents one set planted in spring (planting date DOY 138), the white circles show data of one summer set (planting date DOY 182). The line was fitted using an expo-linear function; fitted parameters and  $R^2$  are shown.

The head fresh weight  $H_{FW}$  was calculated with an empirical regression equation based on the relationship between head dry weight ( $DM_H$ ) and head fresh weight with a defined stem part of 0, 5 and 10 cm ( $DM_{S0}$ ,  $DM_{S5}$ ,  $DM_{S10}$ ), the  $R^2$  of this relationship was 0.95 for stem portion of 0 and 5 cm and  $R^2 = 95$  for 10 cm stem portion (Fig. IV-4) .

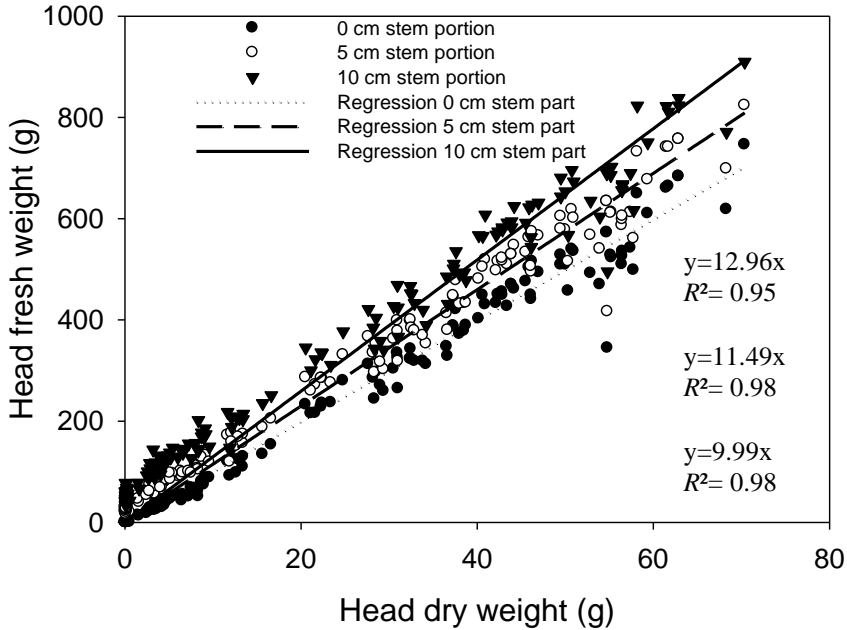


Fig. IV-4. Relationship between head dry weight and fresh part with different stem portion used to simulate the fresh weight of heads with different stem parts at target weight of 500 g; the lines were fitted by linear regression, fitting parameters and  $R^2$  are shown

Chapter 4: Modeling time to harvest and its variability of broccoli (*Brassica oleracea* var. *italica*) model description, parameterisation and field evaluation

Table IV-1. Used and estimated model parameter to simulate the crop growth and development.

Maximum Vernalisation rate	$V_{\max}$	0.055	$d^{-1}$
Minimum Vernalisation rate	$V_{\min}$	0.016	$d^{-1}$
Cardinal temperatures 1	T1	-4,5	$^{\circ}C$
Cardinal temperatures 2	T2	11.5	$^{\circ}C$
Cardinal temperatures 3	T3	13.5	$^{\circ}C$
Cardinal temperatures 4	T4	25.5	$^{\circ}C$
Coefficient of developmental variation	cv	11.69	%
Percentiles of normal distribution curve	p1...p10	Table 2	(-)
Maximum LAI of the parameterisation data set	$LAI_{\max}$	3	(-)
Initial LAI of the parameterisation data set	$LAI_0$	0.01	(-)
Productive part of LAI, limitation factor of $LAI_{\max}$	c	0.01	(-)
light extinction coefficient	k	0.7	(-)
Initial light use efficiency	$LUE_0$	5.4	( $g\ MJ^{-1}$ )
Slope parameter of LUE (I)	$a_{LUE}$	0.31	( $g\ DM\ MJ^{-2}\ m^{-2}\ d^{-1}$ )
Initial specific leaf area	$SLA_0$	189.86	( $cm^2\ g^{-1}$ )
Slope specific leaf area $PAR_{14}$	$sla_a$	6.00	( $cm^2\ g^{-1}\ MJ^{-1}$ )
Slope specific leaf area time	$sla_b$	1.03	( $cm^2\ g^{-1}\ d^{-1}$ )
initial dry weight	InitTotDM	0.75	(g)
Intercept dry matter fraction (exponential)	a	0.0003	(-)
Relative increase dry matter fraction	b	0.014	(-)
Increase dry matter fraction (linear)	c	0.0027	(-)
Intercept dry matter fraction (linear)	d	0.84	(-)
Switching point of exponential to linear increase of dry matter fraction	PI	322	( $^{\circ}Cd$ )
maximum fraction of dry matter growth allocated to the curd	$f_{\max}$	0.84	(-)
constant allometric growth	g	1.213	(-)
constant allometric growth	h	-2.409	(-)
plant density	pd	3.6-4	(plants $m^{-2}$ )
Conversion coefficient head fresh weight with stem part of 5 cm from head dry weight	$a_5$	11.49	( $g\ g^{-1}$ )
Conversion coefficient head fresh weight with stem part of 10 cm from head dry weight	$a_{10}$	9.99	( $g\ g^{-1}$ )
Conversion coefficient head fresh weight with stem part of 0 cm from head dry weight	$a_0$	12.96	( $g\ g^{-1}$ )

### 3.2 Model evaluation and hypothesis testing

Measured and simulated dry matter partitioning to the different plant organs, leaves, stem + side shoot and head (%) showed a good agreement between simulated and measured data points (Fig.IV-5).

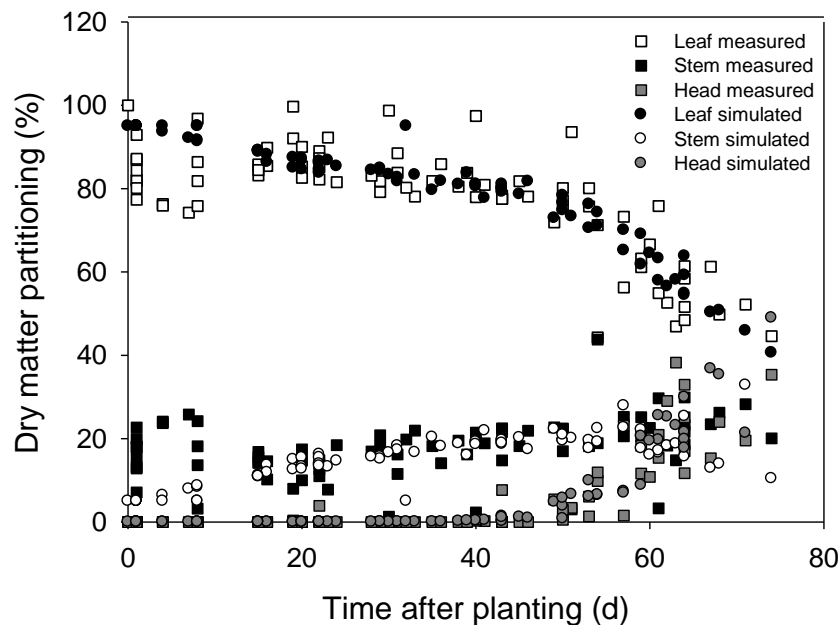


Fig. IV.5. Measured and simulated dry matter partitioning to the different plant organs vs. time after planting for sets grown on commercial farms in north-east Germany and on the experimental farm in Hannover in 2011 and 2012; squares are measured values, circles are simulated values. The white square and the black circles represents leaves, the black squares and the white circles show stem portions and the grey circles and grey squares showed the portion of dry weight allocated to the head.

The dry matter production as well as the portioning of the model showed an appropriate performance. The application of the model on recorded weather data of independent data sets in 2011 and 2012 resulted in a coefficient of determination of 0.59 between observed and simulated SLA. On average the model overestimated the observed SLA with  $9.01 \text{ cm g}^{-1}$  (BIAS). The statistical analyses showed a slope not significantly different from one and an

intercept not significantly different from zero (Fig IV-6). The overall performance of the SLA sub model showed a, RMSD of 28.27 ( $\text{cm g}^{-1}$ ) with a relative mean absolute error of around 14 % of the observed mean SLA (RMAE= 0.14).

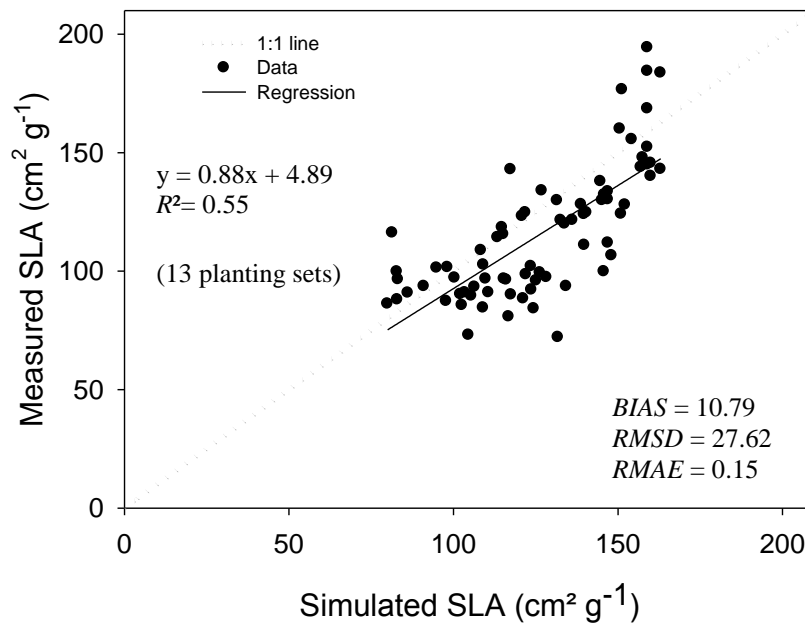


Fig. IV-6. Comparison between simulated and observed specific leaf area (SLA) for independent data from broccoli cultivar ('Ironman') grown on commercial farms in north-east Germany and on the experimental farm in Hannover in 2011 and 2012. Line was fitted by linear regression where the slope was not significantly different from one and the intercept was not significantly different from zero.

A comparison of the observed and simulated LAI shows a slight overestimation by the model for LAIs above 2.2 (Fig. IV-7). The model was able to predict the observed LAI well, the correlation coefficient between observed and simulated was 0.95, the slope of the linear regression was significantly different from one and the intercept was not significantly different from zero. On average the model underestimates observed LAI values with 0.14 (BIAS) and the RMSD of the model was 0.22 (-) with an RMAE of 0.22 %.

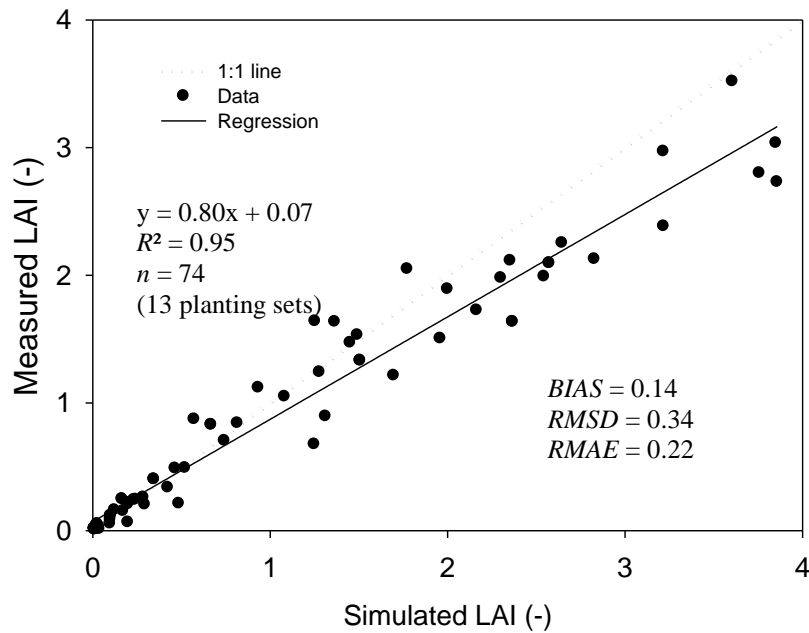


Fig. IV-7 1:1 Line for comparison between simulated and observed LAI for independent data from broccoli cultivar ('Ironman') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2011 and 2012. Lines were fitted by linear regression where the slope was significantly different from one and the intercept was not significantly different from zero.

The dry matter production over time is described well by the model. A 1:1 comparison showed good model performance. The slope was close to one; and the intercept was close to zero (Fig. IV-8). We observed no systematic model error or trend in the model residuals against the measured above ground dry mass.



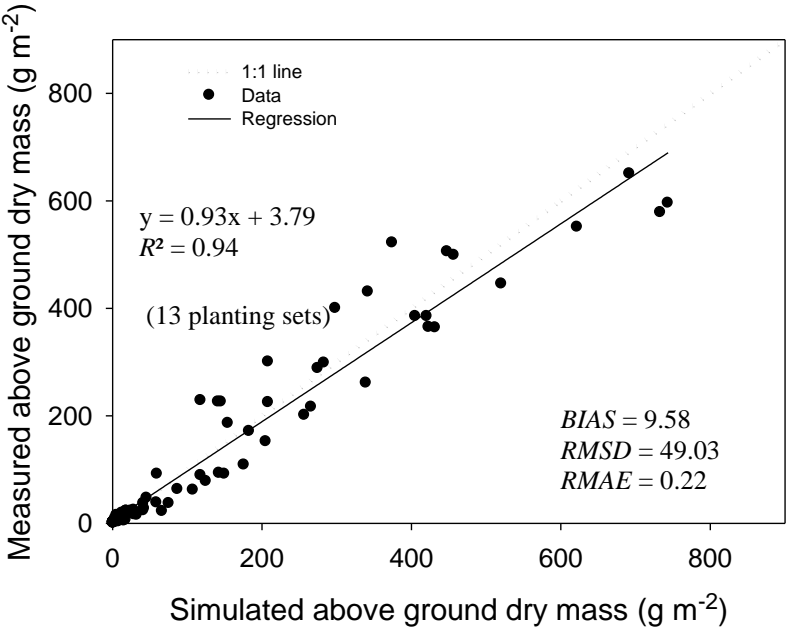


Fig. IV-8. 1:1 Line for comparison between simulated and observed above ground dry matter for independent data from broccoli cultivar ('Ironman') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2011 and 2012. Lines were fitted by linear regression where the slope was not significantly different from one and the intercept was not significantly different from zero.

On average the model was able to predict the observed head fresh weight well, the slope was significantly different from one and we found an intercept of 22.79 g, on average the model overestimates the observed head fresh weight by 28.04 g (BIAS) and has an accuracy of  $\pm 71.08$  g (RMSD) (Fig.IV-9). We found no systematic trend in model residuals.

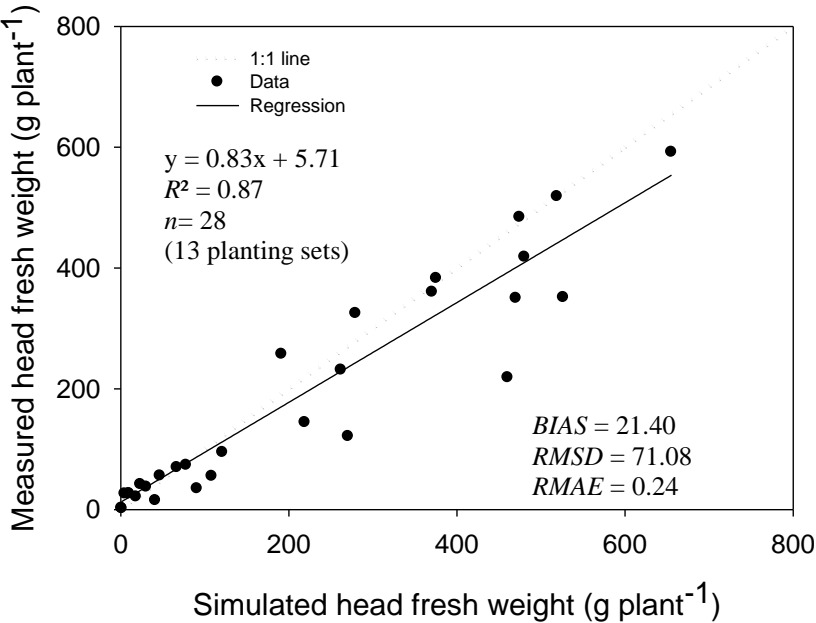


Fig. IV-9 1:1 Line for comparison between simulated and observed head fresh weight for independent data from broccoli cultivar ('Ironman') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2011 and 2012. Lines were fitted by linear regression where the slope (A) was not significantly different from one and the intercept was significantly different from zero.

A 1:1 line comparison between measured and simulated standard deviation of head fresh weight showed a slope of 0.67. The model explains a high percentage of the measured variability of individual plants in head size at final harvest by differences in time to head induction and was able to predict the development of head size variation for independent data sets (Fig. IV-10).

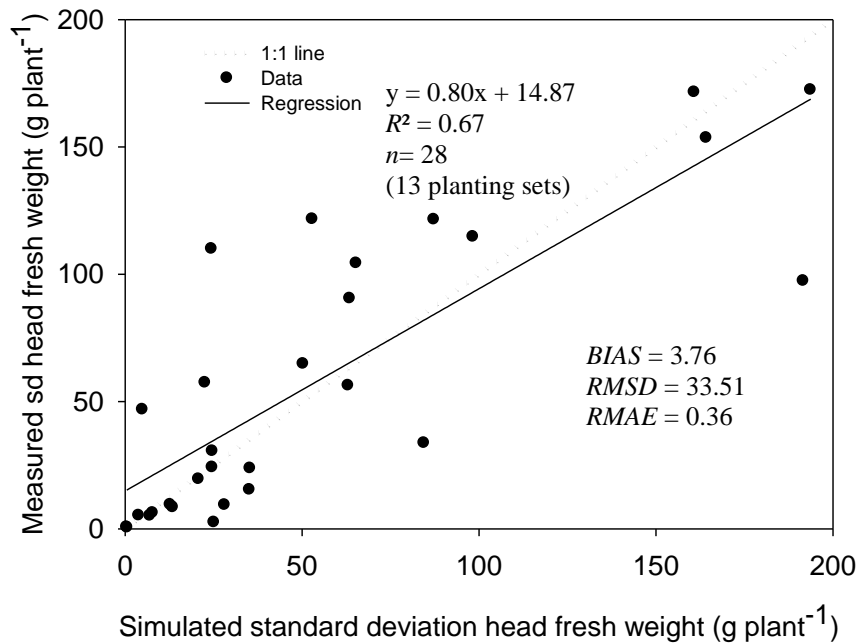


Fig. IV-10. 1:1 Line for comparison between simulated and observed standard deviation of head fresh weight for independent data from broccoli cultivar ('Ironman') grown on commercial farms in North-East Germany and on the experimental farm in Hannover in 2011 and 2012. Lines were fitted by linear regression were the slope was significantly different from one and the intercept was significantly different from zero.

Simulated and measured distribution of head sizes of sets grown at Herrenhausen in 2011 showed a comparable distribution of measured and simulated head weights, even though the model overestimates head fresh weight by 14 % (Fig.IV-11).

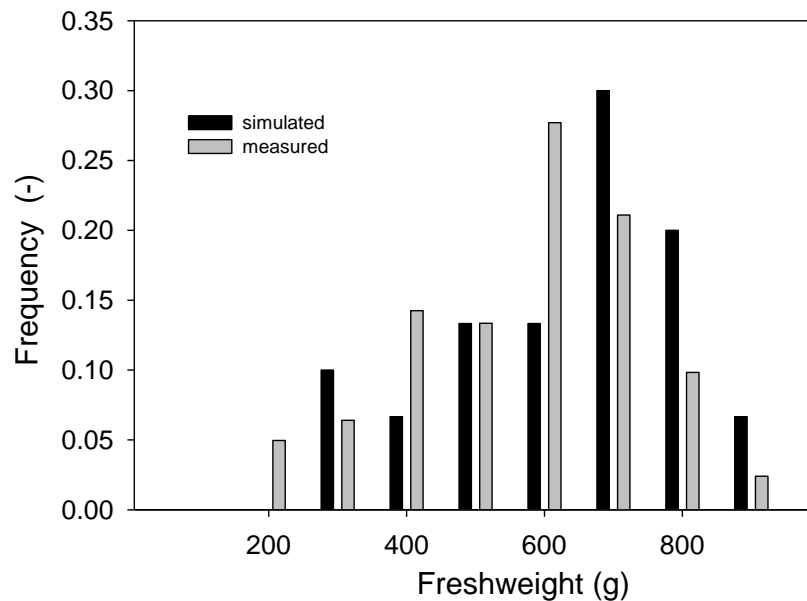


Fig. IV-11. Simulated and observed frequency distribution of head fresh weight for independent data from broccoli cultivar ('Ironman') grown on experimental farm in Hannover in 2011; data from three sets at final harvest, total number of measured plants 132 (mean measured head fresh weight 487 g, mean simulated fresh weight 566 g).

### ***3.3 Model simulations and optimised harvest dates***

The model output of the scenario analyses showed different shapes of the individual curves defining the harvest ability of the different simulated sets (Fig. IV-12 A-I). The search for optimum harvest dates with the aim to minimize the total number of hand harvests with given harvest amount not less than 80 % was strongly influenced by the different shapes of the curves and the simulated head size variability. Fig. IV-12 C showed one set with small head size variation and a narrow harvest window of six days. The graph of this set suggest that a harvest percentage of 80 % can archived by one single harvest on day 63 after planting. Another extreme regarding the simulated head size variation was shown by one set with simulated planting date DOY 140 (Fig. IV-12G). The harvest window of this sets started on day 58 after planting and end on day 70 after planting, in this set the harvest amount of 80 %

could be archived by two selective hand harvests on day 61 and day 66 after planting. Overall sets the average number of hand harvests archiving harvest amounts not less than 80 % was  $1.8 \pm 0.4$ .

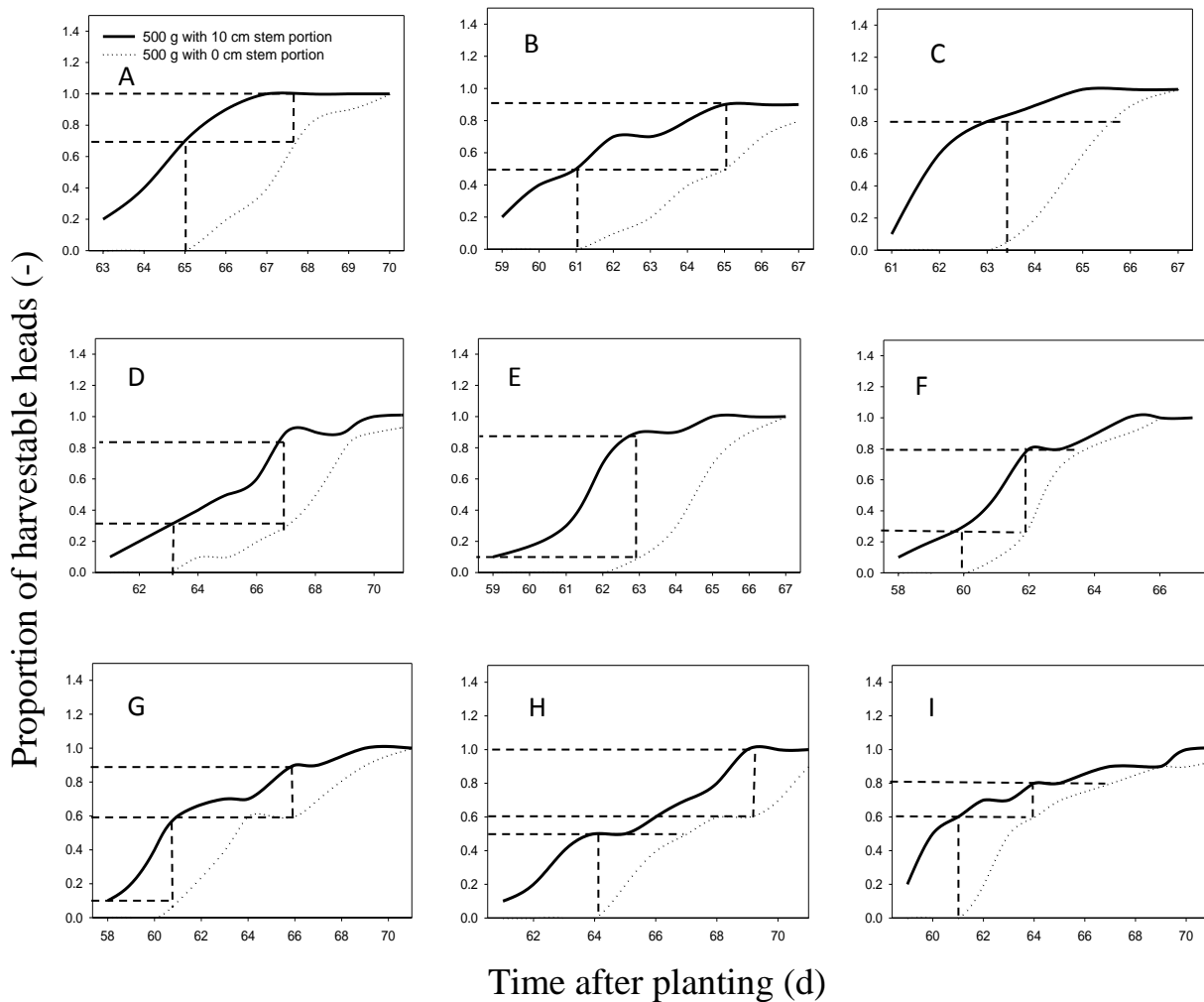


Fig. IV-12. Example for model optimized harvest schedule. Simulated proportion of harvestable heads against time after planting for weather data Herrenhausen 2011 plating date DOY (A 80, B 90, C 100, D 110, E 120, F 130, G140, H 150, I 160 ). Dashed vertical lines show optimal dates for hand harvest to minimize the number of selective hand harvests, horizontal dashed lines are the corresponding harvest amount, the average number of hand harvests archiving harvest amounts not less than 80 % was  $1.8 \pm 0.4$ .

#### ***4. Discussion***

The aim of this chapter was to test the hypothesis that differences in time to head induction are responsible for head size variation. To archive this one stochastic model subroutine predicting the variation in time to head induction was implemented into a simulation model for vegetative and head growth of broccoli. The model consists of three linked processes: crop development and its variability to head induction, dry matter production based on intercepted radiation and dry matter partitioning to the different vegetative organs and the head. Results in the past had shown that constant *LUE* and *SLA* values only poorly able to predict total dry matter production of brassica crops (Stützel and Kage, 1999; Kage et al., 2001), it had been shown for cauliflower that improvements in prediction of total above ground dry matter can be archived by implementation of dynamic *LUE* and *SLA* sub models. Models with dynamic *LUEs* and *SLAs* for cauliflower had shown comparable performance and goodness of prediction of total dry matter than more complex photosynthesis/respiration based approaches (Kage et al. 2001). Therefore suggestions of Kage et al. (2001) were taken into account and a dynamic *SLA* depending on the level of global radiation and plant age was implemented. The *SLA* sub model predicts the measured *SLA* of our crops with a coefficient of determination of  $R^2 = 0.59$ . Results showed that the *LAI* calculated from dry matter allocated to the leaves and the *SLA* was predicted well for independent data sets. We further implemented a dynamic *LUE*. The model showed a good accuracy of prediction of total dry matter of independent data sets. The model is intended primarily for use as a scientific tool to test the hypothesis that variation in head induction is responsible for head size variation. We evaluated the model against data from different sets with varying locations with different planting date's throw-out the whole growing season, with different plant rising systems and mainly with data input from local weather stations. All planting sets were irrigated and optimally supplied with nutrients

but anyhow the verification of crop growth is affected by local differences in soil and weather conditions and as well from errors of the sub model for vernalisation. Especially the global radiation data was not determined near to the plant stands. Even though some single sets deviate from the model prediction the model showed a good estimation for the different quantities on average. The model showed a good accuracy and was able to predict the dry matter partitioning to the different organs (Fig. IV-5), the LAI (Fig. IV-7), the total above ground matter (Fig- IV-8), and the head fresh weight (Fig. IV-9) for independent data sets well. The model performance was comparable to the accuracy of a model for time to harvest of broccoli from Tan et al. (2000 ab). The model showed as good predictions as dry matter production based models of cauliflower (Stützel and Kage 1999, Olesen and Grevsen). While the model Kage and Stützel (1999) works with the disadvantage that exact prediction was only possible if the SLA and LUE was estimated for every individual planting set. The model of Olesen and Grevsen (2000) gave although comparable accuracy but the authors adjusted the length of the time to curd induction to match the observed end of vernalisation, which was not done in model evaluation here. One aim of the presented study was to predict the occurring variability of single heads within one plant stand. The plant variability was simulated by implementation of variability in the vernalisation phase with class formation and calculation of the deviation from the mean development rate using a normal distributed deviation and a delay procedure (Bohris, 1996). The variation model was parameterised with data from a cabinet experiment and evaluated against independent field data (Chapter 3). The growth of the different head classes after head induction was simulated by dry matter allocation to the head, simulated as a fraction of total growth as a exponential function of temperature sum (Fig. IV-3). No additional source of variability was included but due to the relationships of dry matter partitioning in the model the enlarged vernalisation duration of one class had impact not only on the duration of the vegetative development but also on the phase

after head initiation. A plant with a long vernalisation phase formed more leaf area and intercepted a higher amount of PAR, which then influenced its vegetative and later head growth rates of this class. The variation description in the model was comparable and quiet similar to one approach for simulating the variability in the juvenile and vernalisation phase in cauliflower based on an erlang distribution and curd cohort building (Olesen and Grevesen, 2000). The use of this distributed delay procedures gives a good way of including plant to variation in maturity into an otherwise deterministic model (Olesen and Grevsen, 2000) and was although used to include variability in other existing growth models .i.e. for cotton (Sequeira et al., 1993). The presented model parameter for variation was a coefficient of variation  $c_v$  determined from a cabinet experiment with an mean observed standard deviation of vernlisation of  $\pm 3.5$  days (Chapter 3). This is comparable to the parameter value used from Olesen and Grevesen (2000) witch based although on cabinet data and a measured standard deviation in time to curd induction of 2.59 days. The simulated standard deviation matched the shape of the measured standard deviation. Which was although found in the published cauliflower model (Olesen and Grevsen, 2000). The proportion of standard deviation and head fresh weight fits the observation at the final harvest with an accuracy of 67 % on average. The distribution of measured head size showed the same shape compared with the distribution of head size simulation (Fig.IV-11). This suggests that variability in vernalisation and head induction is causal for a large part for the apparent heterogeneity of individual head sizes. It was assumed that the majority of the variation in time to harvest in cauliflower crops could be explained by combined effects of variation in time to curd induction and by temperature variation during curd growth (Booij, 1990a). The agreement between simulated and measured variation of head fresh weight for sets where the simulated time of head induction meets the measured time of head induction confirms the assumption that this is although true for broccoli crops. From the model simulation, the cumulative size distribution



of heads at any time point and the distribution of time to harvest with the resulting harvest window can be estimated (Fig. 12 A-I). This can be used to optimize harvest operations by improved predictions of time to harvest of individual plant cohorts. Model scenario analyses had shown that there is a relationship between the numbers of necessary selective hand harvests and variability of head size (Fig. 12 A-I). Prediction of the optimal numbers and optimal dates of selective hand harvest to archive harvest amounts not less than 80 % showed that the average number for selective hand harvests which is three in commercial practice can theoretically be reduced to less than two harvests on average. Here, however, labor- and storage capacities, contracts and the respective market prices should be considered.

### ***5. Conclusions***

Stochastic variation of time to head induction can predict head size variation of independent broccoli plantings. Variation in time to head induction is most likely the main cause of head size variation. The simulation of head size variation provides the basis to estimate numbers of selective hand harvests required to archive harvest amounts not less than 80 %. Simulation results suggest that the number of necessary selective hand harvests can be reduced. Compared with today's practical standard (three selective hand harvest on average with 80 % harvested heads) the model simulation showed that with less than two selective hand harvests on average, harvest percentages not less than 80 % can be archived. The model can find application in decision support to predict the optima dates for selective hand harvest to optimize harvest operations and to predict exact delivery quantities. Another application could be the prediction of one optima date for once over harvest. Furthermore the model can be used to make accurate forecasts of harvest dates for adjustments of marketing strategies.

### General discussion

A strong variability in head size of broccoli is usually observed in the field. The variability in head size leads to variation in time to harvest which requires multiple harvests and causing high costs in practice. Thus the objectives of this work were the identification of reasons for head size variation and to predict this variation by stochastic mathematical functions and to integrate these equations in a mechanistic simulation model. The project also aimed to improve the prognosis of vernalisation and head growth. The comparison of the model simulation results to observed data should evaluate the source for variation in head size, to obtain a better understanding of the plant physiological background of head size variation and its formation. Simulation modeling allows the quantitative testing of hypothesis (Kage, 2000) and can help to understand dynamic processes such as genesis of head size variation during field growth which cannot easily be directly analyzed. Additionally the model formulations summarize the main results to a decision support tool for production scheduling and model based optimization of harvest operations.

To provide the basis for a suitable mechanistic plant growth model, published work predicting plant development, growth and time to harvest of broccoli and cauliflower was analyzed and summarized (Chapter 1). The literature survey highlighted the importance of global radiation for head growth of broccoli (Grevsen, 1998).

This indicates that prediction accuracy of simple temperature sum models is limited. Thus an accurate prediction of plant growth and time to harvest has to base on the mechanistic relationship between global radiation and plant growth. Additionally an appropriate sub model for dry matter partitioning is necessary. Evaluation of existing models for dry matter production and partitioning showed that accurate predictions require dynamic SLA and LUE

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parameters (Kage and Stützel, 1999; Kage et al. 2001ab). Prediction models of broccoli which include dry matter production using dynamic SLA and LUE parameters have not been published yet. Models which consider plant to plant variation were missing in literature as well as explanations for the occurring head size variability.

In a first step the impact of seedling size variation and canopy heterogeneity directly after transplanting on head size variation was analyzed in field trials (Chapter 2). The field trials showed that relative growth rates in leaf area of single plants across all plant classes (small, median, and big) were not different. No competitive effects were detected and no effect of seedling size on the variability in total plant size, relative head growth rates or head size variation were found. The variation of initial seedling leaf area was not correlated with later head size variability. However, huge differences in head induction of single plants independent from seedling size or dry weight at measuring time were detected. In line with this we found a negative correlation between final leaf number and head size of single plants. Both suggested that differences in head induction and not seedling size differences are responsible for the observed head size variation. Compatible with findings of size independent time to head induction, measurements of head growth showed that head size in the early head growth phase correlated poorly ( $R^2 = 0.08-0.33$ ) with total dry weight at this time. At final harvest this correlation improved ( $R^2 = 0.26-0.70$ ). Reasons that lie behind are size depending advantages in competition of plants for light resources where bigger plants can intercept more light and supply the head with a higher amount of assimilates. The relationship between plant size and head size underlines that simple temperature sum models are not able to predict head growth sufficiently and that the model structure should include the functional relationship between leaf area, light interception and plant growth (Chapter 1,4).

Since the data from the field trials (Chapter 2) suggested that differences in time to head induction of single plants contribute significantly to the observed head size variation the focus of further studies was set to plant development and its variability. In broccoli transition from the vegetative to the generative phase is essential for head formation. At this stage it was crucial to gain a functional relationship between temperature and developmental time to head induction of the studied cultivar “Ironman F1”. The experimental set up was designed in order to quantify the functional relationship between temperature and time to head induction and to acquire data of variability in time to head induction for the parameterization of a stochastic model to predict the variability of plant development (Chapter 3). The results of one first cabinet trial showed that the juvenile phase of the cultivar is already passed at transplanting, since the number of leaves, a widely used marker for the developmental stage of *Brassica* plants (Wiebe, 1990; Mourão and Brito, 2000; Grevsen, 1998) was above the 2.5 leaves and was not different in all analyzed plantings in commercial productions and in the field experiments in Hannover. The juvenile development stage was not considered to contribute to the formation of head size variation.

A second trial analyzed head induction in relation to temperature and described the variation. The results of this cabinet experiment were summarized and transferred to field conditions by a dynamic model which considered the measured variation by a stochastic part. The next step was the implementation of the vernalisation and of the variation model into a growth model for broccoli to evaluate if the simulation of the variability in vernalisation can predict the variability of head size within independent broccoli sets (Chapter 4). Based on literature and of findings from field trials (Chapter 2) a mechanistic model structure was chosen to consider the functional relationship between size in leaf area, light interception and growth dynamically and to link this to dynamic changes in the developmental stage of the

plant. The model consists of three linked processes: crop development to head induction and its variability (stochastic submodel, Chapter 3), dry matter production and dry matter partitioning to the different vegetative organs and the head (mechanistic model) (Fig V1). The current implementation established a link between stochastic and normal distributed plant development to head induction and plant growth which is not realized by other broccoli models. This allowed a quantitative testing of the hypothesis that differences in head induction was one main reason for head size variation. The plant growth model considers dynamic changes in SLA and LUE parameters, to take account of published model evaluations which showed that accurate predictions require dynamic changes of SLA and LUE parameters (Kage and Stützel, 1999; Kage et al., 2001ab). Evaluation of the model against independent field data from the years 2011 and 2012 showed that the model was able to predict observed above ground dry matter, dry matter portioning to the different plant organs, the LAI, mean head size and variation in head size. The implementation of dynamic SLA and LUE parameters provides clear improvements regarding prediction quality compared to published models with static SLA and LUE parameters (i.e. Kage and Stützel, 1999).

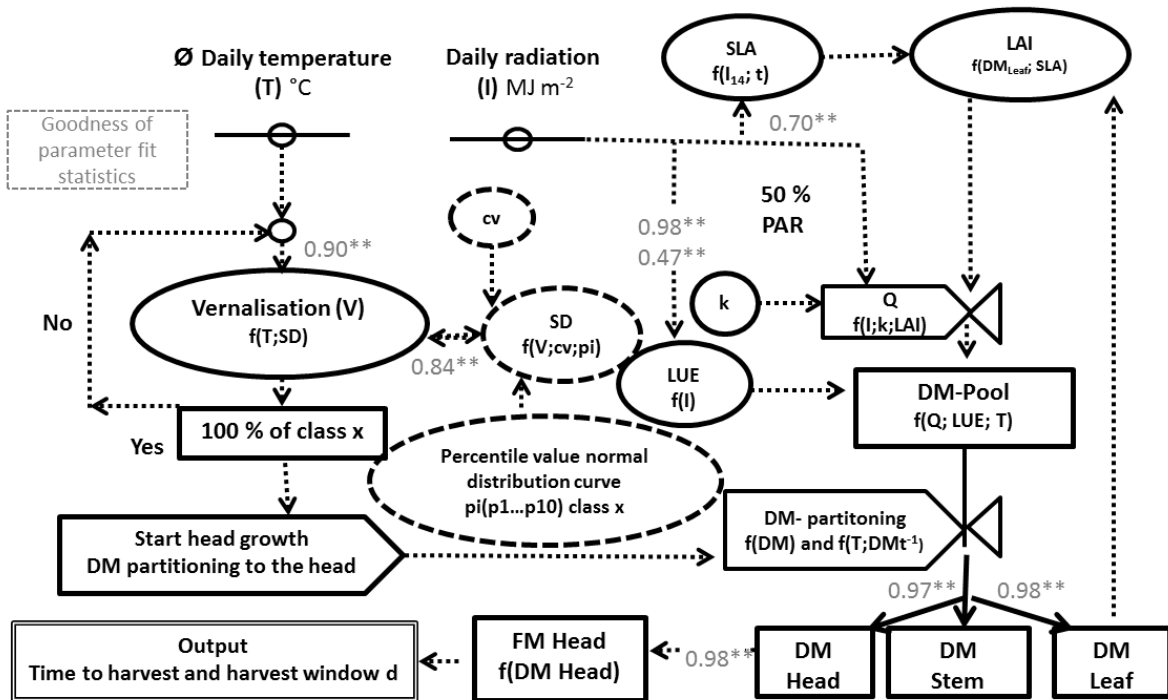


Fig. V-1. Relational diagram for the presented growth and development model of broccoli and goodness of parameter fit statistics of the parameterisation data set. Dashed line show stochastic model parts, solid boxes visualize mechanistic model parts. Boxes are compartments representing the state variables  $f(t)$  of the model, cycles are parameters, ovals are variables and valves are rate variables, solid lines are representing mass flow and dotted lines represents influences ( $DM$  = Dry matter,  $FM$  = fresh matter,  $LAI$  = Leaf area index,  $k$  = light extinction coefficient,  $Q$  = intercepted radiation,  $LUE$  = Light use efficiency,  $SLA$  = specific leaf area,  $cv$  = coefficient of variation,  $pi$  percentile values of the normal distribution curve corresponding to each individual class  $x$ ,  $SD$  = calculated standard deviation). Signif. Codes: ‘\*\*\*’ 0.01, ‘\*\*’ 0.05, ‘\*’ 0.1.

The model explains 22 - 94 % in the mean 79 % of the measured variability of individual plants in head size at final harvest by developmental variation. The coefficient of determination between simulated and observed standard deviations in head size for all measured data points was 67 %. The hypothesis that size independent differences in time to head induction are the main reasons for head size variation can be confirmed. The stochastic model part was comparable and quite similar to one approach for simulating the variability in the juvenile and vernalisation phase in cauliflower based on an erlang distribution and curd cohort building (Olesen and Grevsen, 2000). The use of the “distributed delay” approach had

proven its suitability to include stochastic variation into deterministic models of cauliflower (Olesen and Grevsen, 2000) and other crops, i.e. for cotton (Guitierrez et al., 1984). The simulated standard deviation matched the shape of the measured standard deviation. The same was found in the published cauliflower model of Olesen and Grevsen (2000). The authors concluded that differences in the duration of the juvenile phase and the duration of the vernalisation phase were responsible for observed curd size variation. The model of Olesen and Grevsen (2000) gives comparable accuracy but the authors adjusted the length of the time to curd induction to match the observed end of vernalisation. This was not done in the model evaluation presented here. Thus our broccoli model is a step forward regarding the prediction of head size variation of independent data sets. The model predictions with respect to the apparent variability in the crop shows an accuracy which is not realized by any other published prediction model for *Brassica*. Anyhow, the model formulation assumed no other sources of variation for head size variation than differences in time to head induction, this have to be discussed critically. Many other sources for head size variation, for example local variation in soil conditions, would be conceivable. Many studies have shown that yield variation can be attributed to differences in soil conditions (Hakojärvi et al., 2013, Cassel et al., 2010). But local differences in soil conditions were not considered here because of two reasons. The first reason was the result of measurements in commercial practice which have shown that head size and head size growth rates were randomly distributed across the field and there was no systematic increase in variability with lag distance. (i.e. Fig. V2).

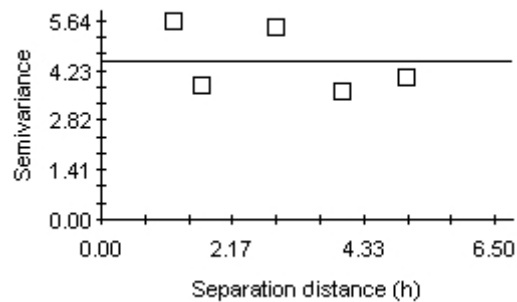


Fig. V-2. Typical variogram showing broccoli head weight across a commercial field (i.e. lag Distance of one set from 2010 grown in North-East Germany).

This result does not exclude differences in soil texture, soil water content or bulk density due to compaction. But the results showed that these differences, if they are existent and related to head size, were randomly distributed. The second reason was, that spatial crop growth patterns and spatial differences in soil conditions were generally very site specific. To consider those differences in a general model for the testing of hypothesis seemed not to be reasonable. Furthermore our data from filed experiments (Chapter 2) showed, that there was a weak correlation between head dry weight and total plant dry weight in the beginning of head growth. Plant size and differences in total plant growth were no decisive factors regarding head size according to our results. Differences in soil conditions may although provide abiotic stress (local water or salt stress) which may influence the hormonal balance of plants. Especially it is known that exposure to several stresses, including cold, salt and osmotic stress reduces the level of gibberellins (GA) in many plant species which contribute to plant growth restrictions (Colebrook et al., 2014). Results of experiments with cauliflower suggest, that the application of gibberellins advances curd initiation in cauliflower under suboptimal conditions for curd induction (Fernandez et al., 1997; Booij, 1990b). Similar effects can be excluded as reasons for differences in time to head induction in our observations, since differences in time to head induction were not correlated with a small plant size which would be an indicator for



a stress based reduction in GA synthesis. The reasons for the observed differences in head induction are still unknown. The stochastic part of the model was purely descriptive. Possible causes and tasks for future research will be discussed in the outlook part of this work. Despite these open questions this work and the model summarized the results and main findings in an applicable tool for decision support and research. The presented method included plant variability into a mechanistic model. Especially the presented parametrization method of the stochastic part of the model from the cumulative data of plants which induced the head (Chapter 2) can find applications in other models and modelling work with respect to variation in plant development. The prediction of the phenotypic behavior and the stochastic variation in plant development under a broad range of environmental conditions also provides potential applications for plant breeding, if genetic based parameters could be linked to the stochastic variable. Furthermore the model can be applied in commercial broccoli production in order to optimize harvest operation and to predict the market supply. For decision support in commercial production and prediction of harvest dates, a harvest criteria of 500 g fresh weight with a stem part from 0-10 cm is defined, assuming that no quality decline occurs in this range. The model calculates percentages of harvestable heads per date; thus harvest strategies and the optimal number for selective hand harvests can be calculated. Together with information about the current market supply and price (economic aspects) the simulation results can be used for planning of pricking, optimization of harvesting, and for maximization of pricking percentages of individual harvests. Simulations showed that harvest percentages not less than 80 % can be archived by 1.8 selective hand harvests on average. This showed that there is a potential for optimization of hand harvest in commercial production where three and more hand harvests on average are standard.

*Future research needs*

The objective of this work was to identify the main sources that are responsible for head size variation of broccoli. For this reason a large sample size and many single plant measurements with a high number of replicates were used. Based on this, it was impossible to consider different varieties and genotypes in this study. Hence, in the present work the commonly used cultivar “Ironman F1” was chosen. Thus future studies should comprise a set of genotypes, since the time to head induction in relation to environmental conditions in broccoli is strongly controlled by genetic factors (Uptmoor et al., 2008). Research in the past has shown that a large proportion of uniformity in time to harvest was attributed to genotypes and less to environmental conditions (Hulbert and Orton, 1984). Hulbert and Orton (1984) concluded that the development of a uniformly maturing hybrid is an issue of genotype selection. Although modern broccoli hybrid varieties, and especially CMS cultivars, showed less variability than open pollinated cultivars in the past, the problem of head size variation is still unsolved. Environmental effects like variation in temperature were excluded in the cabinet experiments and observed variation in time to head induction was on the same level compared with variation in time to head induction within the field. The reason for this is unknown. Normally all F1 hybrids were almost completely genetically identical. For this reason genetic differences cannot be the cause of differences in time to head induction in the first viewing. But it is well known that even in vitro plant cell and tissue culture propagated plants induce phenotypic variation at several levels. In large scale clonal propagation there has been a concern using molecular markers for evaluating stability, these marker analyses mostly reports moderate frequencies of genetic variation, but variation in DNA methylation patterns seems to be much more frequent and in some cases this has been directly implicated in

phenotypic variation (Miguel and Marum, 2011). Because of these observations epigenetic effects may be one source for the observed variation in time to head induction of the used F1 hybrid. To figure this out and to quantify epigenetic variation and its influence on variation in time to head induction would be a promising task for future research. For further research it would be also from major interest to use a mapping population to detect the genetic variation regarding the variation in time to head induction. The model predicts the phenotypic variation of single plant cohorts stochastically with one single parameter, namely the coefficient of variation in time to head induction. As in this study the variation in time to head induction of one single cultivar could be predict from one single parameter, it seems to be promising to link the phenotypic model and the stochastic parameter to a QTL model to solve genotype by environment interactions. The results of those studies may generate information which will allow the development of a stable genotype in the future.

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Lindemann-Zutz, K., Fricke, A. and Stützel H. (2015, submitted to *Scientia Horticulturae*): Prediction of time to harvest and its variability of broccoli (*Brassica oleracea* var. *italica*) Part I. Plant developmental variation and forecast of time to head induction.

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## Unreviewed magazines

Stützel, H. und, Zutz, K. (2013): Terminproduktion von Gemüse im Freiland. ZVG Report, FGG Förderungsgesellschaft Gartenbau mbH.

## Conference paper

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