On seed physiology, biomechanics and plant phenology in *Eragrostis tef*

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On seed physiology, biomechanics and plant phenology in *Eragrostis tef*

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General Abstract

• ***Background*** Teff (*Eragrostis tef* (Zuccagni) Trotter) is a C_4 annual grass species (*Poaceae*) originating from Ethiopia. Teff cultivation in the Netherlands is thought to be economically feasible because teff grains and flour do not contain gluten and are rich in iron. These two characteristics make teff a desirable ingredient in health products, particularly for celiac disease patients. At the start of this project Dutch teff yields were modest (1.0 - 1.5 $Mg{\cdot}ha^{-1}$). The sowing and harvest dates were (too) late in the season and the crop was sensitive to lodging. Here, lodging is defined as the permanent displacement of shoots from their vertical due to root or shoot failure.

• ***The objective*** of this research is to detail some processes that underlie the sensitivity to lodging and the late harvest. Therefore we studied seed germination, lodging resistance, day length response, pace of leaf appearance.

• ***Germination*** of teff can be described by assuming a normally distributed rate of germination within the seed population. Minimal and maximal temperatures required for germination depend on water availability (water potential). Conversely, the minimal required water potential for germination depends on temperature.

• ***Lodging*** was inevitable for teff grown on a Dutch sandy soil. We identified that not only the shoots of teff are prone to lodging, but that the roots are also a major factor in the lodging process. Furthermore, water adhering to the shoots alone, without wind action, could induce lodging in the studied cultivars.

• ***Flowering*** in teff is significantly delayed by exposure to long days. Teff is therefore a short day plant; not only panicle initiation, but also development and outgrowth of the panicle were influenced by photoperiod.

• ***Phyllochron***, defined as the time required between the appearance of two successive teff leaves, increased abruptly for the last few leaves on the main stem of teff. After re-evaluation of literature data this abrupt increase in phyllochron seemed to be also present in both wheat and rice. The delay is most likely independent of temperature, but might be related to the moment of panicle initiation.

• ***In conclusion***, the study on teff identified clear targets for breeding towards a high-yielding cultivar in the Netherlands.

Key words: Teff (*Eragrostis tef* (Zuccagni) Trotter), germination, temperature, model, leaf appearance, phyllochron, development rate, lodging, biomechanics, safety factor, flowering, heading, day length, photoperiod.

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1

General Introduction

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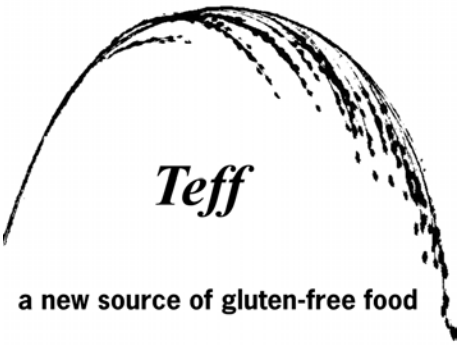

General introduction

This general introduction'sets the scene' for this thesis and ultimately converges to the general objective of this study. As starting point, compendious information on the cereal teff (*Eragrostis tef* (Zuccagni) Trotter) (Fig.1.1) is provided, the main reasons for cultivation in the Netherlands are presented, and teff's global growing areas are identified. Next, the history and meaning of the scientific name '*Eragrostis tef* (Zuccagni) Trotter' are explained. To draw a clear picture of the plant itself the physical appearance (morphology) of teff is described (Fig.1.1). The introduction of a new plant species, such as teff, into the Netherlands could cause biological hazards, therefore certain possible threats to the Dutch environment are discussed. This is followed by a discussion on the main yield-reducing pests and diseases of teff, and by information on teff production and consumption in its natural habitat, Ethiopia. The work towards the general objective of this thesis is subdivided into four research topics. Each of the four research topics is addressed in an independent chapter. The four topics follow from a brief analysis of preliminary field trials, from currently known Ethiopian yield constraints, and from differences in environmental conditions between Ethiopia and the Netherlands.

What is teff and where does it grow?

Eragrostis tef is an annual grass species (*Poaceae*) that most likely originates from Ethiopia (Vavilov, 1951). Teff is believed to be an ancient crop, the cultivation dating back to at least 3.000 B.P. (Mengesha, 1966; Costanza *et al.*, 1979). This C_4 cereal crop species (Kebede *et al.*, 1989) can be cultivated both for its grains, suitable for human consumption, and for its straw, usable as fodder, building material and various other domestic purposes.

Teff was recently introduced to the Netherlands. This project was funded by the Dutch Technology Foundation STW with the objective to facilitate a successful introduction of teff to Dutch agriculture. The main reason that teff production is thought to be economically feasible in the Netherlands is that teff grains and flour are rich in certain minerals, especially iron (Mengesha, 1966; Abebe *et al.*, 2007; Verdonschot *et al.*, 2008) and more importantly do not contain gluten (Spaenij- Dekking *et al.*, 2005). Gluten is a multi-protein complex in seeds that can cause coeliac disease in genetically predisposed humans. The absence of gluten makes teff a desirable ingredient in health products, particularly for celiac disease patients. Teff can replace gluten-containing cereals in products such as pasta, bread, beer, cookies and pancakes.

Before the introduction to the Netherlands, the crop was already introduced to Australia, India and South Africa as forage in the late 19th century by military and political agents of the British Empire (Costanza *et al.*, 1979). In several other countries teff is or was grown mainly, but not exclusively, as fodder: Argentina (Nicora, 1939), Ukraine (Krasnokutskii & Konstanc, 1939), Malawi, Zaire, Sri Lanka, New Zealand, Mozambique, Uganda, Tanzania, Palestine, Kenya, Canada (Ketema, 1997), USA (Castellani, 1948) and parts of Asia (Castellani, 1948).

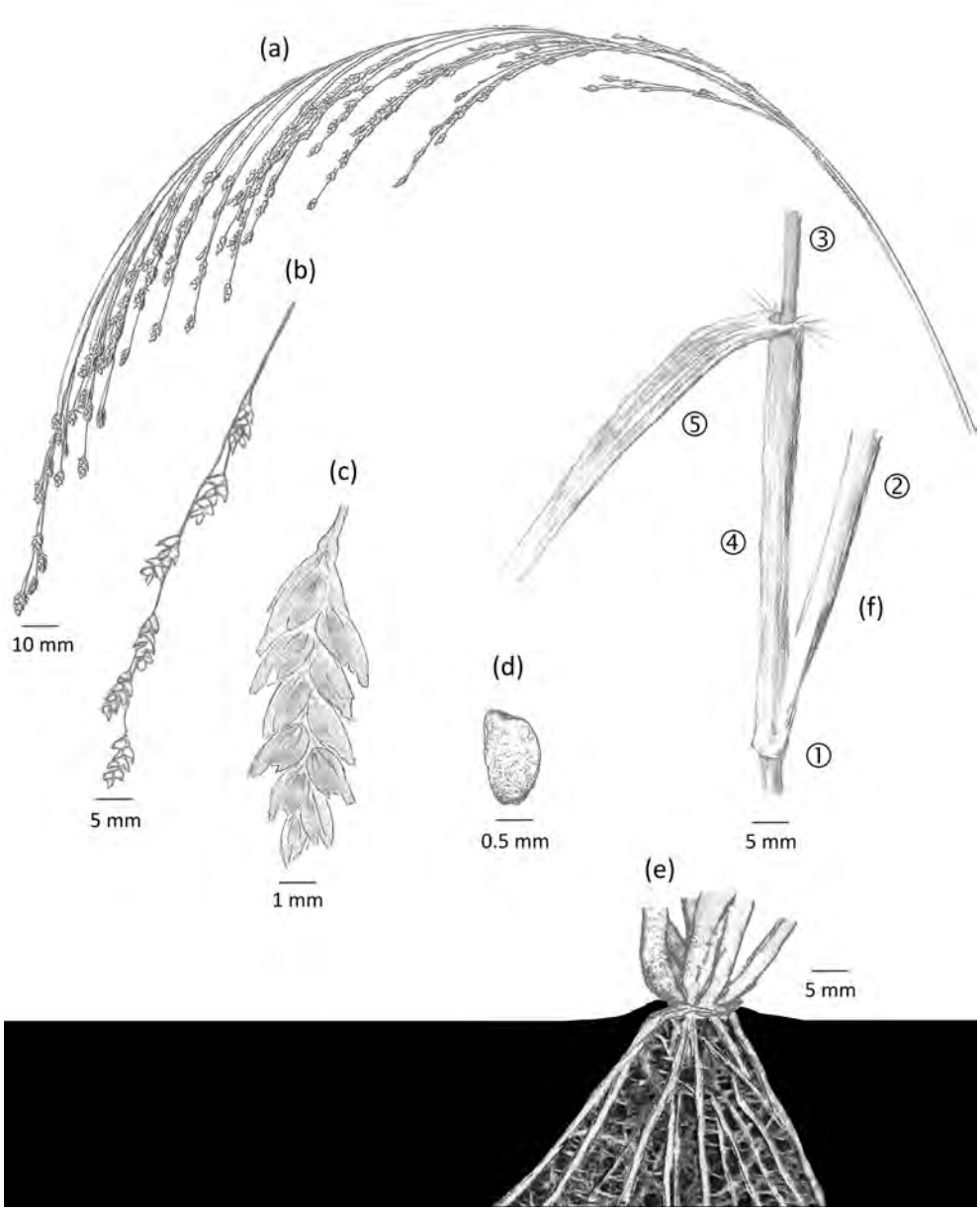

Fig. 1.1: Drawing of (a) ripening inflorescence of teff (drooping panicle); (b) branche of the panicle, containing spikelets; (c) individual spikelet, containing teff grains; (d) individual teff grain; (e) teff stubble and root system; (f) teff phytomer: (1) node, (2) axillary branche, (3) internode, (4) leaf sheath and (5) leaf blade.

How teff received its common and scientific name

The common name of *Eragrostis tef* (Zuccagni) Trotter, is often spelled "teff" or "t'ef" and is considered by many to originate from the Amharic word teffa, which means 'lost', since grains spilled on the ground are so small that they are lost.

According to Costanza *et al.* (1979), Attilio Zuccagni was the first to publish a botanical description of teff as a species and named it *Eragrostis tef* in 1775. In 1851, however, Jacquin described this same species as *Poa abyssinica* (Costanza *et al.*, 1979) in the genus *Poa*; *abyssinica* is referring to the Empire of Ethiopia, historically known as Abyssinia. Later teff was thought to belong to the love grass genus, *Eragrostis* and accordingly named *Eragrostis abyssinica*. In 1918, Trotter rediscovered the original description of Zuccagni, hence the current name featuring two authors: *Eragrostis tef* (Zuccagni) Trotter.

Plant morphology

Most of the observations on the diverse appearance of teff described here, I did during my research at Wageningen University and during visits to Dutch teff breeders. These breeders showed me fields with mixed teff landraces, i.e. local teff varieties originating from Ethiopia. I found that the length of full-grown plants ranged between approximately one and two meters depending on the variety. Some teff varieties can produce numerous tillers and each tiller can produce axillary branches. Axillary branches are defined as branches that arise from buds in the axils of leaves higher up the culms, where stem internodes have expanded (Doust, 2007). Teff shoots are often thin, 1-4 mm in diameter, and the shoots of most varieties easily bend. Teff possesses small (10 - 100 mm) and long (40 - 700 mm) leaf blades, a culm can produce 1 to 13 leaf ranks, the ligule of teff is very short (0.5-1 mm) and ciliated (Fig.1.1). The inflorescence of teff plants is a panicle (Fig.1.1). Among teff varieties differences in panicle shape can be enormous: from long to short, compact to open and from erect to drooping. In general a panicle can contain over 1000 spikelets and each spikelet has 2 to 20 florets. These florets are bisexual and teff is self-pollinating, but chasmogamous, i.e. its flowers open, making cross pollination possible (Berhe, 1976; Ketema, 1983). Yet, the degree of outcrossing in teff is very low, i.e. 0.2-l.0% (Ketema, 1997). Heading, defined as emergence of the tip of the inflorescence from the sheath of the flag leaf, and pollination almost coincide. From here on we will use the term heading instead of flowering. Each floret has a lemma, a palea, three stamens, an ovary and mostly two, in some cases three, feathery stigmas (Ketema, 1983). Teff has 40 chromosomes (2n = 4x = 40) with a relatively small genome size of about 730 Mbp (Jones *et al.*, 1978; Ayele *et al.*, 1996). Grains are approximately 0.5-1.7 mm long, and 0.2-1.0 mm in diameter, and have a l000-kernel weight of 0.25 - 0.5 grams. Grain colour varies between varieties, from plain white through dark yellow and bright red to dark brown. The rooting system of teff is fairly shallow with most of the roots in the top 20 cm of the soil, however, during field observations we did observe some thin roots at more than 1 m depth.

Teff as a pest?

The introduction of a new plant species into the Netherlands could cause biological hazards. The more so because in the 20th century pilosa (*Eragrostis pilosa*), closely related to teff, became rapidly a common species in the Netherlands. As indicated by the Dutch vegetation data base (Schaminée *et al.*, 2007), pilosa is mostly growing along road sides and in urban areas. According to W. Ozinga (personal communication, 2010) pilosa does not cause a great threat to the Dutch vegetation, neither does it cause great inconvenience to Dutch citizens. In the Netherlands seeds must possess some form of dormancy in order to remain viable until the next growing season. Whereas pilosa seeds can be dormant (Tenner, 2004), teff seeds are free of dormancy and therefore most of the teff seeds that are shed on the soil will germinate that same season. Therefore it is unlikely for teff to become invasive. In accordance with this, no spontaneous teff re-growth was observed on the fallow fields the year after agronomic trials in Wageningen, dating from 2005 to 2008. These observations together with the absence of dormancy, and absence of invasive problems in other countries, indicate that current teff varieties are unlikely to become troublesome invasive plants. There is, however, still the potential threat of teff being a host for plant diseases that are new to Dutch natural vegetation and agriculture.

Pests and diseases in teff

Although teff is known for its high pathogenic resistance, more than 24 fungal pathogens and several nematodes have been reported to cause some harm to teff in Ethiopia (Amogne *et al.*, 2000). Impact of the majority of these diseases is 'minor', only teff rust (*Uromyces eragrostidis* Tracy), head smudge (*Helminthosporium miyakei* Nisikado) and damping-off caused by *Drechslera* spp. and *Epicoccum nigrum* are important diseases in Ethiopia (Amogne *et al.*, 2000). Welo bush-cricket (Decticoides brevipennis Ragge) (Ragge, 1977; Stretch *et al.*, 1980) and caterpillars of noctuid moths (in particular species belonging to the genus Spodoptera, i.e. army worms) are the cause of most of the insect damage in Ethiopia (Ketema, 1997). Reports on viral or bacterial diseases in relation to teff yield are scarce. It has however been reported that teff can be a host of both dwarf mosaic virus and sugarcane mosaic virus (Bekele *et al.*, 1995).

In the Netherlands we found that both thrips (Family: *Thripidea* in particular the species: *Limothrips cerealium*; *Thrips angusticeps*; *Frankliniella tritici*) and aphids (Family: Aphidoidea in particular the species: Aphis frangulae and *Aphis nasturtii*) can substantially reduce yield, if no management action is taken. Teff in the Netherlands is also susceptible to several pathogenic fungi: *Fusarium graminiarum*; *Fusarium culmorum* and several *Pythium* species. Some nematodes can cause damage at the early stages of development *viz*: *Meloidogyne chitwoodi* and *Pratylenchus penetrans*. Although we did not observe any teff-related plant diseases that are new to the Netherlands since the introduction of teff in 2001, we should not yet exclude such a possibility.

Teff production and consumption in Ethiopia

The Ethiopian national average teff grain yield is as low as 1.1 Mg·ha^{-1} (CSA Ethiopia, survey 2005-2010). For teff to be economically feasible in the Netherlands yield have to be in the order of 2.5 - 3.0 Mg·ha^{-1}. Thus, Dutch yields have to be two to three times as high as the average Ethiopian yield. It is therefore interesting to identify the main yield constraints that underlie this low national average of Ethiopia.

The low national average is partly associated with constraints such as water logging, drought and nutrient limitation (Tulema *et al.*, 2005). Although farmers can use teff as a drought-tolerant crop, water remains a major yield constraint (40-50% yield reduction) in rain-fed teff cultivation (Yizengaw & Verheye, 1994).

Another cause for the currently low teff grain yields is lodging. Lodging can be defined as the permanent displacement of a plant from the vertical (Berry *et al.*, 2004). Solving teff's lodging problems would dramatically increase actual yield and therefore lodging resistance is the main focus in several breeding programmes (Assefa *et al.*, 2011; Syngenta-Foundation, 2011), Berhe, 1973; Ketema, 1991; Hundera *et al.*, 2000; Zhang *et al.*, 2001; Tefera *et al.*, 2003; Yu *et al.*, 2006).

A different cause for the currently low teff yields is bad seedling establishment. For homogenous seedling emergence and to better compete with weeds, teff requires a homogenous seedbed. Preparing a flat seedbed on Ethiopian vertisols using man power and draught animals (oxen) is tedious and laborious. Moreover, depending on water availability, teff requires temperatures higher than 10 °C in order to germinate. At lower temperatures growth is arrested and the plant is more susceptible to pests and diseases, e.g. damping off. In the Netherlands, water, nutrient and lack of proper seedbed preparations are unlikely to reduce yields. However, both poor seedling establishment at the start of the season resulting from low temperatures, and lodging can potentially restrict teff grain yield in the Netherlands.

In the period between 1995 and 2010 on average about 28% of the total Ethiopian acreage of the seven major cereals was occupied by teff, but teff only accounts for 20% of the overall grain production. These lower average teff yields compared to the other cereals can, to some degree, be explained by the crops ability to produce some yield where other crops fail (Ketema, 1997). Nevertheless, the yield of well-fertilized unsupported plants in 'on station' field experiments is 2.5 Mg·ha^{-1} on average (Tulema et al., 2005), which is half of the grain yields that can be obtained for wheat landraces (Erkossa et al., 2000).

If teff yields are indeed lower than other cereals in Ethiopia, then the question arises: 'For what reason(s) do the Ethiopians continue to grow teff?' Firstly, teff can be grown in areas prone to both droughts and excessive water. It withstands anaerobic conditions better than many other cereals, including wheat and sorghum (Ketema, S., 1991; Ketema, 1997). Secondly, teff has fewer disease or insect pest problems compared with other cereals growing in Ethiopia (Amogne *et al.*, 2000). Thirdly, nitrogen fertilizer requirements are low, i.e. no more than 60 kg·ha^{-1} N is recommended for obtaining yields in the order of 2.0 Mg·ha^{-1} (Kidanu *et al.*, 1999). Fourthly, and most importantly, teff receives relatively high

market prices for both its straw and its grain. This is because cattle prefers teff straw to straw of other cereals (Davy, 1913). And in the Ethiopian culture there is a strong preference for consuming teff instead of other cereals (Belay *et al.*, 2006). Ethiopians consume teff in the form of popular pancake-like bread, called enjera, and sometimes as a porridge or in the form of alcoholic drinks, called tela and katikala.

In summary the often sub-optimal growth conditions (e.g. moisture stress, lack of fertilizer or pesticides) and Ethiopian cultural preferences make teff a relatively safe and suitable crop for the average Ethiopian farmer.

Teff's growth environment

Dutch farmers possess more capabilities in terms of mechanised land preparation, drainage, nutrient management and pesticide management than Ethiopian farmers. It is therefore surprising that at the start of this project, teff grain yields in the Netherlands (1.0-1.5 $Mg{\cdot}ha^{-1}$) were comparable to the Ethiopian national average. The harvest index (which is the ratio of dry weight of grain, to dry weight of total above ground biomass) for teff in the Netherlands was also low (0.1 - 0.2) compared to that of wheat and barley (0.4-0.6). This low harvest index indicates that the crop produces sufficient biomass (5-15 $Mg{\cdot}ha^{-1}$), but a modest quantity of grains is harvested. Therefore it is unlikely that daily solar photosynthetically active radiation (PAR) and water stress are major yield constraints in the Netherlands. The more so because grain yields from well-irrigated Dutch experimental plots were comparably low (1.0-2.0 $Mg{\cdot}ha^{-1}$). However, several other reasons for the low Dutch grain yields can still be postulated, such as: slow progress through developmental phases resulting from sub-optimal temperature or day length, or severe lodging, or grain losses due to strong wind. Comparison of Dutch and Ethiopian temperatures, day lengths and wind forces during the growing seasons could help to identify the constraints on teff yield in the Netherlands.

The main production areas of teff in Ethiopia are in the central-west to the central-north, at altitudes between 1700 and 2400 m a.s.l., in particular the administrative regions of Shewa, Gojam, Gonder, Wello and Welega (Ketema, 1997)(CSA Ethiopia, survey 2005-2010). Temperature during the growing seasons in Ethiopia is, depending on altitude, mostly above 8 °C and below 27 °C with a daily average between 15 and 22 °C (NMA, 2011). Teff requires temperatures between 15 and 21 °C for optimal growth (Yizengaw & Verheye, 1994; Zerihun, 1996; Ketema, 1997). Our preliminary field trials showed that low temperatures (< 10 °C), especially at the beginning of the Dutch growing season, can cause bad seedling establishment or arrest crop development.

One of the major environmental differences between Ethiopia and the Netherlands is day length. In Ethiopia during the main growing season day length, here defined as civil twilight, is about 13 h at sowing and approximately 10 h at harvest. In the Netherlands the day length is approximately 17 h at sowing, peaks at 18.5 h on the 21^{st} of June and then decreases to approximately 14 h at harvest. As indicated by Gebreselassie (1985), we

expect that teff is a short-day plant. Consequently, the Dutch long days will prolong the time required to reach the heading stage. Late heading (flowering) will shift the harvest date to wetter and colder weather in September, which is undesirable, since cereals generally require a dry period to ripen properly.

The preliminary field trials in the Netherlands confirmed the Ethiopian observations (Ketema, 1983; Ketema, Seyfu, 1991) that the crop is susceptible to lodging. Average wind speeds during the growing season in the central highlands of Ethiopia (≈ 2 m·s^{-1}) are lower than in the Netherlands (≈ 3 m·s^{-1}) (NMA, 2011). However, maximum winds speeds during the growing season in the Ethiopia are equal, or even higher than in the Netherlands (MAQ, 2011; NMA, 2011).

Above, I have postulated several grain yield constraints for teff in the Netherlands. In relation to these constraints four research topics for this thesis have been chosen:

I: Viable teff seed can fail to germinate as a result of sub-optimal temperatures at the start of the season. This relates to the first research topic of this thesis: teff germination in response to temperature and water availability.

II: In both Ethiopia and the Netherlands teff is susceptible to lodging. This relates to the second research topic of this thesis: the analysis of lodging in teff.

III: The long day length in the Netherlands may increase the time to heading (flowering) in teff. This relates to the third research topic of this thesis on photoperiodism in teff.

IV: Relatively low average temperatures and long day lengths in the Netherlands could prolong the developmental stages of the crop, especially at the beginning of the growth season. A widely used indicator of the rate of development during the early stages is the time interval between the appearance of two successive leaves, the so-called phyllochron. This relates to the fourth research topic of this thesis: the variation in teff phyllochron.

General objectives and outline of this thesis

The general objective of the work described in this thesis was to examine some of the major yield constraints of teff, thus helping breeders to identify targets for breeding high yielding cultivars for cultivation in the Netherlands. The evaluation of preliminary Dutch field trials and comparison between the Dutch and the Ethiopian growth environment in the previous section, resulted in the identification of four research topics that are related to the most yield-restraining factors of teff. Ergo, the topics of this thesis are: seed germination, lodging, timing of leaf appearance (phyllochron) and time to heading. Apart from forming possible yield constraints and the common denominator "teff", the four chapters all share a strong quantitative component, assessing the environmental impact on seed or plant development. The chapters can, however, be read as independent units, but all references used are compiled at the end of this thesis.

In compliance with the regulations of our funders (STW (see section on funding), we choose the topics in consultation with breeders, farmers, food technologists, crop physiologists and agronomists. Aside from the practical objective of helping breeders to obtain a high-yielding cultivar, I also want to use this thesis to advance our current understanding of the seed physiology, phenology and morphology of teff in particular, and other cereals in general. Therefore, although the main model species in this thesis is teff, for several plant traits an explicit comparison will be made to wheat and rice, using published and newly gathered data.

Chapter 2: Germination of teff: Re-examining "hydro thermal time modelling"

For good seedling establishment in the field, teff germination has to take place close to the soil surface (van Delden *et al.*, 2010). Consequently, large variations in temperatures and water potentials commonly occur when growing this crop in the Netherlands. The Hydro thermal time model (HTT) of Alvarado & Bradford (2002) is the current standard to describe the interacting effects of temperature and water potential on the time required to germination of seeds in a population. We fitted this HTT model (Alvarado & Bradford, 2002) to an extensive germination data set of teff. This model did, however, not give a satisfactory fit to our data. In this chapter we present a modified modelling framework, that provides a better description of teff germination data than the model of Alvarado and Bradford (2002). This modified modelling framework introduces more flexibility in modelling of seed germination at all combinations of constant temperature and constant water potential.

Chapter 3: Analysing lodging of the panicle bearing cereal teff

Lodging, the permanent displacement of crop plants from their vertical due to root or shoot failure, is a major yield constraint of teff. The objective of this chapter is to analyse the causes of lodging of teff by using, modifying and validating conventional biomechanical models (for both root or shoot failure) and making comparisons to rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.).

Chapter 4: Variation in cereal phyllochron revisited

As a vital condition for making accurate predictions of crop development in a field, with continuously changing circumstances, the fundamental concepts on the timing of leaf appearance obtained under constant temperatures need to be correct. Yet, the theory on the phyllochron, i.e. time between the appearance of two successive leaves, is still controversial. Many studies have highlighted inaccuracies in predictions of the timing of successive leaves in the field. This chapter provides an accurate description of the fundamental concepts on the timing of leaf appearance in the cereals teff (*Eragrostis tef* (Zuccagni) Trotter), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.)

Chapter 5: Photoperiodism in teff: Analysis of ontogeny and morphology in response to photoperiod

This chapter analyses the degree to which teff's ontogeny and morphology is day-length sensitive and provides a detailed description and quantification of the response of teff to day length. Smooth logistic functions are presented that are generally applicable in short-day cereals. By using four distinct teff cultivars, we also studied the feasibility to breed for a teff genotype that is well adapted to northern latitudes.

Chapter 6: General Discussion

The study on this crop in the past several years created clear targets for breeding towards a high yielding cultivar in the Netherlands. This chapter summarizes the gained insights for both breeders and scientist. I start this chapter by discussing my view on modelling in plant biology.

Chapter

2

Seed germination of teff:

Re-examining 'hydro thermal time modelling'

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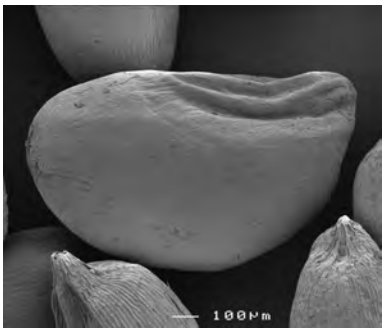

Abstract

• ***Background*:** Current hydrothermal time models describe seed germination curves well under conducive germination conditions, but they may fail at the extremes of the permissive range. In this Chapter we present a modified germination modelling framework, in which cardinal temperatures are a smooth function of water potential and, conversely, the cardinal water potential is a smooth function of temperature.

• ***Methods*:** Experimental data on seed germination of teff (*Eragrostis tef* (Zuccagni) Trotter) were obtained at 17 temperatures and 5 water potentials, using a complete factorial design with 3 replications, resulting in a highly discriminating data set.

• ***Key Results*:** We show that the postulate of a normal distribution of seed germination rate can explain the time course of seed germination at any combination of temperature and water potential. Final germination percentage and lag phase are emerging properties of the modelled mean germination rate and its variation among seeds in a population.

• ***Conclusions*:** The normal distribution provides an accurate and parsimonious summary of teff germination curves. The newly developed framework gives better predictions of seed germination than alternative models. The proposed modelling approach circumvents statistical problems of degree of freedom inflation and identifiability of spread in population parameters.

Key words:
Germination, teff (*Eragrostis tef* (Zuccagni) Trotter), water potential, temperature, model, hydrothermal time, normal distribution, stress.

Roman abbreviations used in this chapter

Roman symbols	Explanation	Units	Eqns.
a	Parameter characterising the slope of the relationship between ψ_b and T near T_b	$^oC^{-1}$	2.7
AIC	Akaike's information criterion, a measure of the goodness of fit		
b	Parameter characterising the slope of the relationship between ψ_b and T near T_c	$^oC^{-1}$	2.7
c	Shape parameter characterizing the rate of the change of θ_H with temperature at suboptimal temperatures		2.8
CV	Coefficient of variation		2.9
d	Shape parameter characterizing the rate of the change of θ_H with temperature at supra-optimal temperatures		2.8
g	Germination percentile	%	2.1, A2.2.13
$\widehat{g}(t)$	Predicted germination fraction as a function of time		2.2
ISTA	International Seed Testing Association		
K_T	The slope of increase of base water potential at $T > T_o$	°C·MPa^{-1}	2.1, 2.5
k	Observed number of germinated seeds of the replicate		2.3
n	Number of viable seeds per replicate		2.3
NTC	Negative temperature coefficient		
$r(g)$	Germination rate $r(g) = t(g)^{-1}$, $r(50)$ represents median germination rate	h^{-1}	2.2
T	Temperature	°C	2.1, 2.2, 2.5, 2.6, 2.7, 2.8, 2.9,2.10, 2.12
T_b	Base or minimum temperature for seed germination	°C	2.6, 2.7, A2.2.13
T_c	Ceiling or maximum temperature for seed germination	°C	2.7, A2.2.13
T_i	Temperature at the inflection point of the curves describing σ	°C	2.10
$T_o^{T_b(\psi)}$	the temperature where $T_b(\psi)$ does not further increase	°C	A2.2.13
$T_o^{T_c(\psi)}$	the temperature where $T_c(\psi)$ has its lowest value	°C	A2.2.13
T_o	Optimum temperature for seed germination	°C	2.1, 2.6
T_s	Temperature at which σ starts to increase with temperature	°C	2.10
$\overline{t}$	Mean time to germination of the seed population	h	2.2
$t(g)$	Time to germination of percentile g and $t(50)$ represents median time to germination	h	2.1, A2.2.13

Greek abbreviations used in this chapter

Greek symbols	Explanation	Units	Eqns.
α	Proportionality factor between σ_{min} and temperature	$^{\circ}C^{-1}{\cdot}h^{-1}$	2.9
μ	Mean germination rate, also applied as a function of both water potential and temperature, $\mu(\psi, T)$	h^{-1}	2.2, 2.4, 2.9
ψ_b	Median base water potential, i.e. minimal water potential required for 50% germination, also applied as a function of temperature, $\psi_b(T)$	MPa	2.1, 2.4, 2.5, 2.7, A2.2.13
ψ_b^{min}	Minimum value, over a range of temperatures, of the base water potential	MPa	2.7
σ	Standard deviation of germination rate, also applied as a function of both water potential and temperature $\sigma(\psi, T)$	h^{-1}	2.2, 2.9, 2.10
$\sigma_{min}(T)$	Minimal spread in seed germination rate, applied as a temperature dependent minimal spread	h^{-1}	2.10
$\sigma_{max}(\psi)$	Maximal spread in seed germination rate, applied as a water potential dependent maximum, thus $\sigma_{max}(0)$ represents σ_{max} when $\psi = 0$	h^{-1}	2.10
σ_{ψ_b}	Standard deviation of the base water potential	MPa	2.1, A2.2.13
θ_H	Hydro time, i.e. cumulative water potential, required for 50% germination	MPa·h	2.4, 2.8, 2.12
θ_{HT}	Hydrothermal time required for germination	MPa·°C·h	2.1, 2.6, A2.2.13
$\theta_H(T_o)$	The hydro time constant at the optimal temperature (T_o)	MPa·h	2.8
$T_o(\theta_H)$	The optimal temperature (C) of the hydro time constant (θ_H)	°C	2.8

Introduction

The time required by a seed to germinate depends on the interaction between environmental factors (including temperature, water and oxygen availability, nutrients, light, toxins and pathogens) and endogenous seed factors (including hormones, age, state of ripeness and dormancy). Seeds used as planting material in agriculture are preferably free from dormancy and uniform in size, age and history. In the absence of dormancy, oxygen shortage, toxins and pathogens, the main environmental factors that determine the time to germination are: temperature (T) (°C) and water availability (Hilhorst *et al.*, 1997). Water availability is best characterized by water potential (ψ) (MPa).

The relationship between temperature (T) and the reciprocal of time (t) to germination, i.e. germination rate (r), of a population percentile g is often taken to be linear. Therefore germination response to temperature can be described with the thermal time concept (Feddes, 1972; Bierhuizen & Wagenvoort, 1974). By combining the thermal time concept with probit analysis, Covell *et al.* (1986) were able to describe the time course of germination for a population of seeds at different temperatures.

Hegarty (1976) observed that germination rate (r) decreased linearly with the water potential (ψ) of the seed. Gummerson (1986), therefore, introduced the hydro time concept analogous to the thermal time concept. Gummerson (1986) combined the hydro time and thermal time concepts for the suboptimal range of temperature in one overarching model: the Hydrothermal time model (*HTT*). The "hydrothermal time constant" (MPa·°C·h) used in this model was considered to be a population constant and thus the same for all seeds. Essentially, the hydrothermal time approach assumes proportionality of the rate of seed germination with temperature and water potential, each as measured with respect to their cardinal values. This hydrothermal time approach was elaborated by several authors (Ni & Bradford, 1992; Dahal & Bradford, 1994; Bradford, 1995; Alvarado & Bradford, 2002; Rowse & Finch-Savage, 2003; Batlla & Benech-Arnold, 2010). Currently, *HTT* modelling for non-dormant seeds is based on the model of Alvarado and Bradford (2002) where the authors incorporated the effect of supra-optimal temperature into the *HTT* modelling approach.

We fitted this *HTT* model to an extensive germination data set of *Eragrostis tef* (Zuccagni) Trotter (common name teff), a small-seeded C_4 cereal species (dry seed size ≈0.59×1.0 mm) (Kebede *et al.*, 1989; Zewdu & Solomon, 2007) originating from Ethiopia (Vavilov, 1951). For good seedling establishment in the field, teff germination has to take place close to the soil surface (van Delden *et al.*, 2010). Consequently, extreme temperatures and water potentials commonly occur in its natural habitat.

The model of Alvarado and Bradford (2002) did not give a satisfactory fit to our data. As to be discussed in more detail in the materials and methods sections, many workers in the field encounter these problems. The overall objective of this chapter is to explain why the current models may not provide a satisfactory data fit for particular plant species. Here we propose a modified model framework that overcomes fitting problems of the hydrothermal time modelling approach for seed germination. The new model does not

contain discontinuous (i.e. broken) functions and accounts for both variation in cardinal temperatures and variation in the spread of germination within a population of seeds. The model is valid for the whole range of combinations of water potentials and temperatures at which seeds are able to germinate. Since our modified model framework focuses on modelling the rate of germination we propose the name "hydrothermal rate model" (*HTR*).

Materials and Methods

Background on the need for improving the *HTT* model

One of the most widely used models for hydrothermal time is that by Alvarado and Bradford (2002):

$$\text{Probit(g)} = \frac{\psi - \left(\frac{\theta_{\text{HT}}}{(T-T_{\text{b}})t(g)}\right) - \psi_{\text{b}}}{\sigma_{\psi_{\text{b}}}} \qquad \text{if} \quad T_{\text{b}} < T \leq T_{\text{o}} \qquad (2.1a)$$

$$\text{Probit(g)} = \frac{\psi - \left(\frac{\theta_{\text{HT}}}{(T_{\text{o}}-T_{\text{b}})t(g)}\right) - \psi_{\text{b}} - K_{\text{T}}(T-T_{\text{o}})}{\sigma_{\psi_{\text{b}}}} \qquad \text{if} \quad T > T_{\text{o}} \qquad (2.1b)$$

where Probit(g) is the germination percentage g, converted to probits, ψ (MPa) the water potential, T (°C) the temperature, θ_{HT} (MPa·°C·h) the hydrothermal time constant, T_{b} (°C) the base temperature, $t(g)$ (h) the time to germination of a percentile g, ψ_{b} (MPa), the median base water potential, and $\sigma_{\psi_{\text{b}}}$ (MPa) the standard deviation of base water potential. Application of the model is possible if base water potential is normally distributed. The fit is achieved in one big overarching probit regression. In order to obtain an accurate fit to our germination data on teff we will modify and extend this *HTT* model. These modifications were motivated by four difficulties with the current concept.

First, the *HTT* model assumes that the base water potential (ψ_{b}) is constant for the lower temperature range of germination (Fig. 2.1c). Accordingly, there is one common base temperature for germination (T_{b}) irrespective of water potential (Fig. 2.1a,c,e). There is increasing evidence, however, that cardinal temperatures and base water potential interact for several species, as summarized by Bradford (1995) and later confirmed in, e.g., *Orobanche aegyptiaca* (Kebreab & Murdoch, 1999), *Festuca rubra, Lolium perenne* (Larsen *et al.*, 2003), *Poa pratensis, Eurotia lanata* (Wang *et al.*, 2005) and *Arundo donax* (Graziani & Steinmaus, 2009). To the best of our knowledge this interaction at sub optimal temperatures has never been incorporated in the *HTT* model.

Second, the *HTT* model uses discontinuous linear relationships, to model the relationship between germination rate and temperature. Such discontinuous linear functions are subject to debate (Marshall & Squire, 1996; Hardegree, 2006), since the overall relation between temperature and development rate of a biological system is generally smooth and curvilinear (Schoolfield *et al.*, 1981; Labouriau & Osborn, 1984; Zwietering *et al.*, 1991; Yin *et al.*, 1995; Orozco-Segovia *et al.*, 1996; Yan & Hunt, 1999; Timmermans *et al.*, 2007).

Third, the *HTT* model attributes all variation within a seed population in time to germination to the differences among individual seeds in base water potential $\left(\sigma_{\psi_b}\right)$. This single variation parameter $\left(\sigma_{\psi_b}\right)$ is assumed to have a constant value regardless of water potential. Although this simplification of reality may be justified from a modelling perspective, from a biological perspective spread in base temperature (T_b) is not unlikely. The more so because, in experiments at constant water potential, cardinal temperatures have also been shown to vary; for example in seed populations of *Brassica napus* (Marshall & Squire, 1996), *Daucus carota* (Finch-Savage *et al.*, 1998), *Stellaria media* (Grundy *et al.*, 2000) and *Lithospermum arvense* (Chantre *et al.*, 2009). In other words it is hard to identify whether variation in time to germination is a result of the seed response to temperature or the response to water availability or both. Modelling the spread of the process rate itself as a function of both temperature and water potential will circumvent this problem.

The fourth problem of the current approach in *HTT* modelling is related to the statistical assumptions made. The *HTT* model is fitted using probit transformed data, whereby each observed germination percentile, g, is considered as an independent observation. However, subsequent observations in time of germination fraction within a single replicate constitute dependent data. Treating these as independent results in inflation of degrees of freedom, which can result in spurious significance claims. An appropriate data analysis should account for this statistical dependence by applying a data reduction step in which dependent data are summarized, by fitting growth curves, and subsequently analysing the parameters characterizing those curves (Keuls & Garretsen, 1982). Furthermore, germination data provide a classical example of heteroscedastic data for which a binomial error distribution is appropriate (Hilborn & Mangel, 1997). Moreover, transformation of germination percentages of 0 or 100% is not possible and observations below 5% and above 95% are recommended to be removed (Bradford, 1990) when fitting ordinary least squares. A modelling technique that allows inclusion of all available data, such as a maximum-likelihood weighted regression method as proposed by Bradford (1990) is desirable.

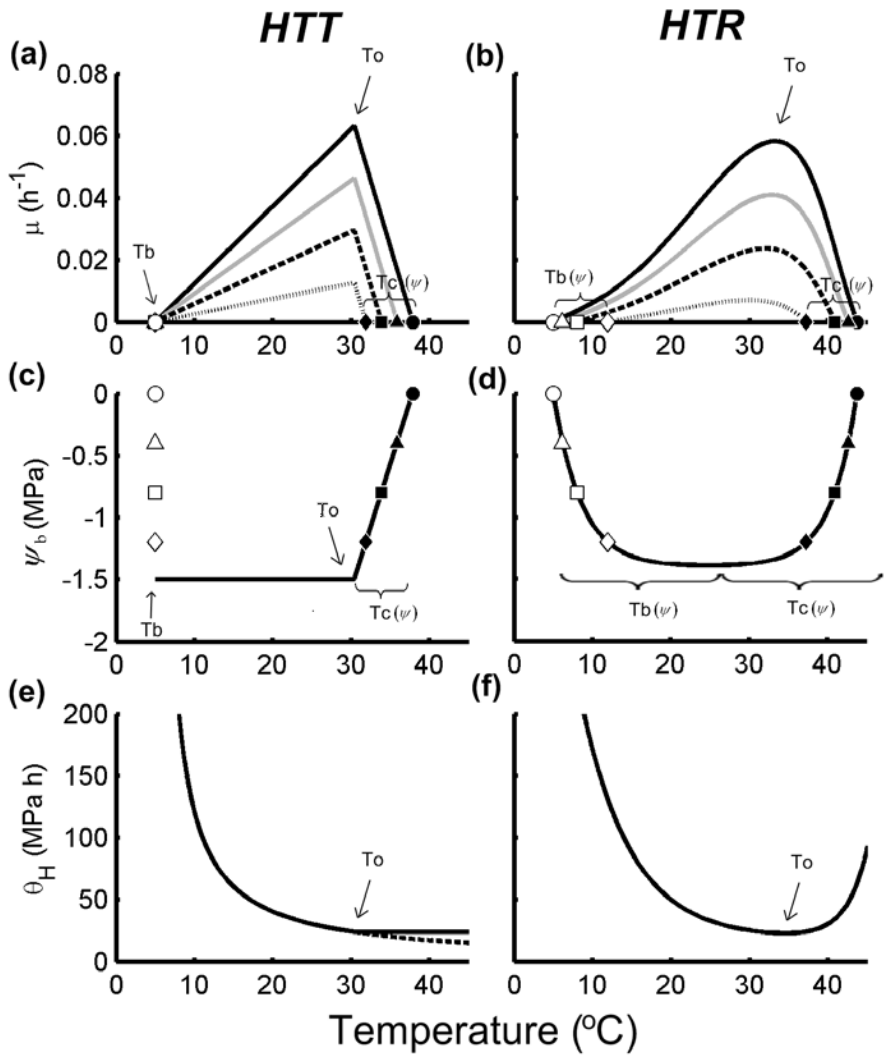

Fig. 2.1: Comparison of key relationships defining two models for seed germination: (1) the current hydrothermal time model (*HTT*) of Alvarado and Bradford (2002) (a, c, e) (eqn 2.1); (2) hydrothermal rate model (*HTR*) (b, d, f) (eqn 2.4, 2.7 and 2.8). The top two panels (a,b) describe for each of the two models the response of mean rate of seed germination to temperature at water potentials of 0 MPa (solid line), -0.4 MPa (grey line), -0.8 MPa (broken line) and -1.2 MPa (dotted line). The middle two panels (c,d) describe for each of the two models the response of base water potential to temperature and different markers represent cardinal temperatures T_b (open symbols) and T_c (closed symbols) at water potentials: 0 MPa (circles), -0.4 MPa (triangles), -0.8 MPa (squares) and -1.2 MPa (diamond). The two panels at the bottom (e,f) describe for each of the two models the response of hydrotime, θ_H, to temperature. The solid line in panel (e) represents the model of Alvarado and Bradford (2002) and the broken line the model of Rowse and Finch-Savage (2003).

Theory of the Hydro Thermal Rate model

Here, we present a modelling framework that overcomes the listed difficulties of the current *HTT* modelling approach for seed germination. The proposed data analysis is subdivided into three steps (Fig 2.2).

In step 1 the germination curves from individual replicates are summarized by fitting two parameters (i.e. mean germination rate and corresponding variation) for each replicate before further analysis. The error model used for fitting the germination data is binomial.

In step 2 the response of these summary measures to water potential and temperature is then further analysed using flexible smooth continuous functions that are fitted with non-linear regression, assuming a normal error model. These functions allow cardinal temperatures and base water potential to interact and vary over the whole temperature range of seed germination.

In step 3 two alternative models are proposed: one, using a coefficient of variation and two, using an empirical logistic function to model variation in germination rate within the seed population. In both models the spread in germination rate, is free to fluctuate as a function of water potential and temperature.

Step 1

The germination rate of a seed at given constant conditions is defined as the reciprocal of the time to germination, $\frac{1}{t} = r$. This rate (r) varies within the seed population. Some seeds germinate early and have a high rate, while others germinate late, having a low rate. When a cumulative percentile g has germinated at time t, we can equivalently state that a percentile g has a germination rate of at least $\frac{1}{t}$. For the distribution of the rate, several models might be used as a null model. Here, we choose the normal distribution to describe the variation in rate, because of the distributions' generality and strong theoretical foundation (Hilborn & Mangel, 1997). Moreover, this assumption is consistent with the assumption of a normal variability in the base water potential of the *HTT* model (Alvarado & Bradford, 2002). As a consequence, the cumulative normal distribution function $\left(\text{with population mean } (\mu) \text{ and spread } (\sigma)\right)$ can be used to describe the germination of

population quantile $\widehat{g}(t)$ as a function of time t:

$$\widehat{g}(t) = \Pr\left(\underline{t} \leq t\right) \tag{2.2a}$$

$$= \Pr\left(\frac{1}{\underline{t}} \geq \frac{1}{t}\right) \tag{2.2b}$$

$$= \Pr\left(-\frac{1}{\underline{t}} \leq -\frac{1}{t}\right) \tag{2.2c}$$

$$= \Phi\left(\frac{-\frac{1}{\underline{t}} - \left[-\mu\left(\frac{1}{\underline{t}}\right)\right]}{\sigma\left(\frac{1}{t}\right)}\right) \tag{2.2d}$$

$$= \Phi\left(\frac{-r + \mu(r)}{\sigma(r)}\right) \tag{2.2e}$$

where the $\underline{t}$ (h) is the stochastic variable time to germination, t (h) the actual time to germination of population quantile $\widehat{g}(t)$, r (h^{-1}) the germination rate, μ (h^{-1}) and σ (h^{-1}) the mean and standard deviation of the rate, and Φ the cumulative standard normal distribution function.

While the distribution of the rate $r(g)$ is assumed to be normal as a null model, the corresponding distribution of the time to germination will not be normal but skewed. The mean rate of germination (μ) is equal to the median rate of germination $(r(50))$ because these two parameters are equal in a normal distribution. Equality does not hold for the mean time $(\overline{t})$ and median time $(t(50))$ to germination. Due to the right-skewed distribution of time to germination, the mean $(\overline{t})$ is greater than the median $(t(50))$. It should be noted that the distribution of the rate allows negative values. Negative values in the left tail of the distribution of the rate represent seeds that do not germinate under the given conditions, i.e. the rate is effectively truncated at a value of 0. Equation 2.4 can be fitted to data by minimizing the negative log likelihood. Given the type of data, binomial likelihood is appropriate (Hilborn & Mangel, 1997):

$$LL = \sum\left(-k \cdot \log\left[\widehat{g}(t)\right] - (n-k) \cdot \log\left[1 - \widehat{g}(t)\right]\right) \tag{2.3}$$

where LL is the negative log likelihood, $\widehat{g}(t)$ the predicted germination quantile at time t, n the number of viable seeds of the replicate, and k the observed number of germinated seeds in the replicate. The dependent data of observation of germination fraction within a replicate sample are now summarized with two parameters, i.e. μ and σ, for each replicate.

Step 2

In the second step, the response of mean germination rate (μ) to water potential and temperature is modelled. In order to express the mean germination rate (μ) as a function of both water potential (ψ) and temperature (T), we rewrite the model of Alvarado and

Bradford (2002) (eqn 2.1) back to its basic form as used by Gummerson (1986):

$$\mu(\psi, T) = \frac{\psi - \psi_b(T)}{\theta_H(T)} \tag{2.4}$$

where $\mu(\psi, T)$ is the mean germination rate, ψ(MPa) is water potential, T(°C) is temperature, $\psi_b(T)$ (MPa) is the base water potential of the population median as a function of temperature, $\theta_H(T)$ (MPa·h) is the hydro time constant as a function of temperature. In the approach of Alvarado and Bradford (2002), $\psi_b(T)$ in eqn 2.4 (Fig. 2.1c), is represented by:

$$\psi_b(T) = \psi_b \qquad \text{if} \quad T_b < T \leq T_o \tag{2.5a}$$

$$\psi_b(T) = \psi_b + K_T(T - T_o) \qquad \text{if} \quad T_o < T \leq T_c \tag{2.5b}$$

where ψ_b (MPa) is the constant base water potential at suboptimal temperatures, T_b and T_c (°C) are, respectively, the base and ceiling temperature below and above which half of the population does not germinate, T_o (°C) the temperature at which germination rate is maximal, and K_T the slope of the relationship between temperature, T, and ψ_b. This relationship is only valid at temperatures above T_o. In the approach of Alvarado and Bradford (2002), $\theta_H(T)$ in eqn 2.4 (Fig. 2.1e), is represented by:

$$\theta_H(T) = \frac{\theta_{HT}}{(T - T_b)} \qquad \text{if} \quad T_b < T \leq T_o \tag{2.6a}$$

$$\theta_H(T) = \frac{\theta_{HT}}{(T_o - T_b)} \qquad \text{if} \quad T_o < T \leq T_c \tag{2.6b}$$

where θ_{HT} (MPa·°C·h) is the hydrothermal time constant, T_b the base temperature. Note that at supra optimal temperatures the value for hydrotime is fixed, i.e. $\theta_H(T) = \theta_H(T_o)$, (Fig. 2.1e).

Thus in the model of Alvarado and Bradford (2002), $\psi_b(T)$ and $\theta_H(T)$ are represented by eqn 2.5 and 2.8, respectively. To gain model flexibility we now propose two flexible smooth U-shaped functions, eqn 2.7 (Fig. 2.1d) and 2.8 (Fig. 2.1f), which replace eqn 2.5 (Fig. 2.1c) and 2.8 (Fig. 2.1e). To describe base water potential (ψ_b) as a function of temperature we propose:

$$\psi_b(T) = \psi_b^{min}\left(1 - e^{a(T_b - T)}\right)\left(1 - e^{b(T - T_c)}\right) \tag{2.7}$$

where ψ_b^{min} (MPa) is an asymptotic minimum base water potential (Fig. A1.2.12), T_b (°C) the base temperature at maximum water availability $(\psi = 0$ MPa$)$ and a (°C^{-1}) and b (°C^{-1}) characterize the slope of the relationship between base water potential and temperature in the sub-optimal and supra-optimal ranges, respectively.

To describe hydrotime (θ_H) as a function of temperature we propose:

$$\theta_H(T) = \theta_H(T_o) + \left(\frac{d}{c}\right)^{\frac{2c+d}{c+d}} \left(1 - e^{[c(T_o(\theta_H)-T)]}\right) + \left(\frac{d}{c}\right)^{\frac{c}{c+d}} \left(1 - e^{[d(T-T_o(\theta_H))]}\right) \quad (2.8)$$

where $\theta_H(T_o)$ (MPa h) is the hydro time constant at the optimal temperature T_o (°C), $T_o(\theta_H)$ is the temperature at which the hydro time required for germination is at its minimum, and shape parameters c and d determine the change in θ_H in the sub- and supra-optimal ranges of temperature, respectively.

In essence we have rewritten the hydro time equation of Gummerson (1986) as a function of temperature (eqn 2.4). We will refer to this model as the hydrothermal rate (*HTR*)-model.

Step 3

The third step involves modelling the spread in germination rate (σ) as a function of temperature (T) and water potential (ψ). We compared two approaches. The first approach assumes a constant coefficient of variation (CV) of seed germination rate when conditions are suitable for germination, and sets a temperature dependent minimum spread when the rate becomes low:

$$\sigma(\psi, T) = \max\left[\mathrm{CV} \cdot \mu(\psi, \mathrm{T}),\ \sigma_{\min}(\mathrm{T})\right] \quad (2.9a)$$

and

$$\sigma_{\min}(T) = \alpha \cdot T \quad (2.9b)$$

where $\sigma(\psi, T)$ and $\mu(\psi, T)$ (h^{-1}) are the standard deviation and mean of the germination rate at a certain combination of temperature (T) and water potential (ψ), CV is the coefficient of variation and, $\sigma_{\min}(T)$ (h^{-1}) is the minimum spread. The parameter α (°C·h^{-1}) characterizes the increase in minimal spread with temperature.

The second approach for modelling the spread (σ) uses a logistic relationship between standard deviation and temperature:

$$\sigma(\psi, T) = \frac{\sigma_{\max}(\psi) - \sigma_{\min}}{1 + e^{2\frac{(T_i - T)}{(T_i - T_s)}}} + \sigma_{\min} \quad (2.10a)$$

and

$$\sigma_{\max}(\psi) = \sigma_{\max}(0) \cdot e^{\psi} \quad (2.10b)$$

where σ (h^{-1}) is the population spread; $\sigma_{\min}$ (h^{-1}) the minimum of the observed spread; ψ (MPa) the water potential; T (°C) the temperature; $\sigma_{\max}(0)$ (h^{-1}) the asymptote to which maximum spread at $\psi = 0$ MPa approaches; $\sigma_{\max}(\psi)$ (h^{-1}) the asymptotic maximum spread as a function of water potential; T_s (°C) temperature at which σ (h^{-1}) starts to increase; and T_i (°C) the temperature at the inflection point of the curve.

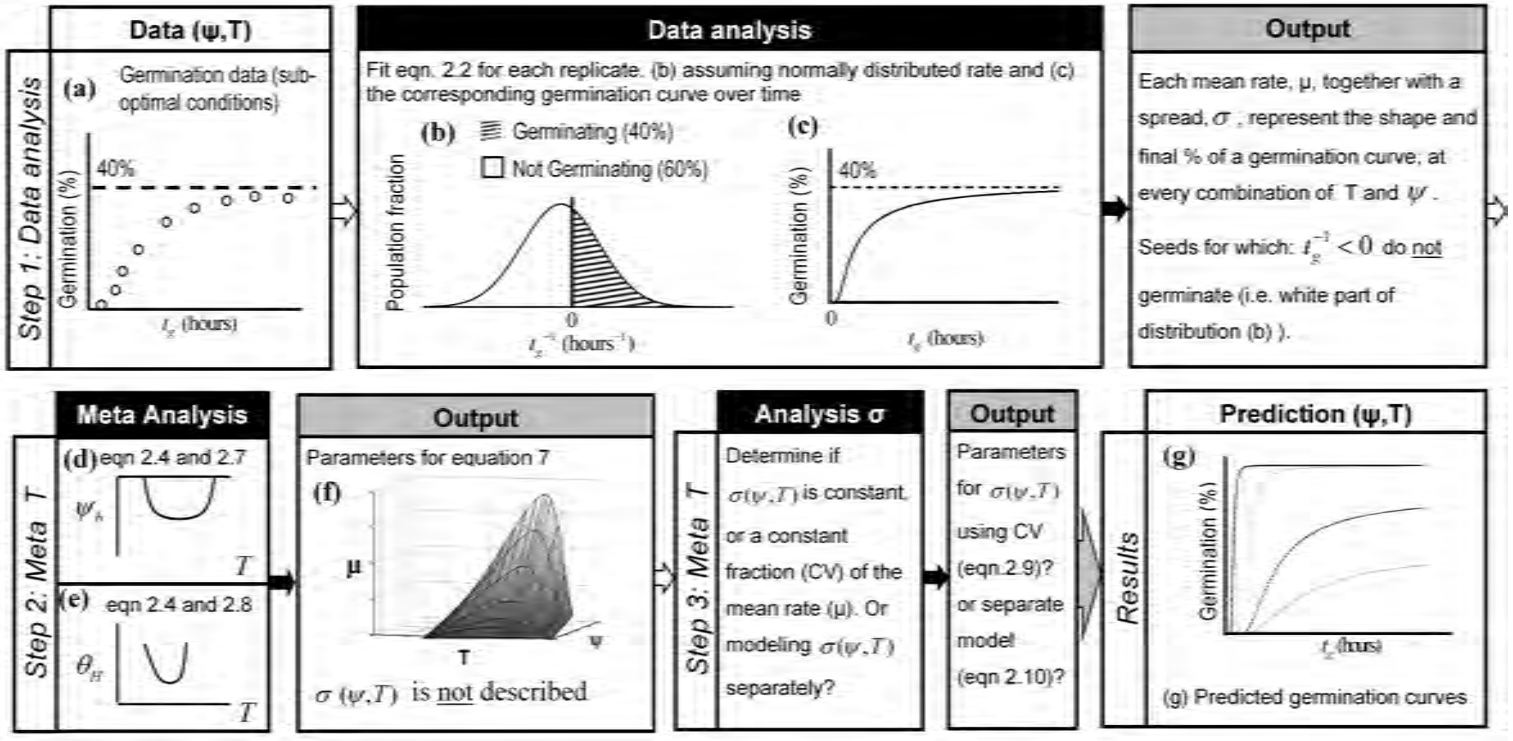

Fig. 2.2: Tableau illustrating the framework for model fitting. Step 1: Panel (a) shows a cumulative number of seeds that germinated over time. (b) In the model, it is a priori postulated that the germination rate is normally distributed within the seed population (eqn 2.2).(c) The corresponding cumulative distribution in time to germination is skewed. Step 2: In a single regression step mean rate (μ) is both linear related (eqn 2.4) to water potential (ψ) and curvilinear related (eqn 2.7 (d) and 2.8 (e)) to temperature (T). The result (f) of this fit (d-e) is a response surface of mean rate (μ) in water potential and temperature space $(\mu(\psi, T))$. Step 3: Now the spread (σ) obtained in step 2 remains to be modelled as a function of water potential (ψ) and temperature (T), either by assuming a temperature dependent minimum spread in combination with a coefficient of variation (CV) (eqn 2.9), or by modelling the spread separately (eqn 2.10). Together, the mean rate $(\mu(\psi, T))$ and the spread $(\sigma(\psi, T))$, can predict the germination curves (g) including final germination at any combination of water potential (ψ) and temperature (T).

Experimental setup

Home propagated seeds of teff (*Eragrostis tef* (Zuccagni) Trotter), cultivar DZ-Cr-255 (Gibe) of approximately 0.7-0.8 mm length were used. Teff seeds were germinated at 85 combinations of 17 temperatures (8, 10, 12, 14, 17, 21, 24, 27, 30, 33, 35, 36, 37, 39, 41, 43, 45 °C) and 5 water potentials (0, -0.4, -0.8, -1.2, -1.4 MPa). Every combination was conducted in triplicate, where every replicate consisted of a single Petri dish (diameter 85 mm, height 15 mm) with 100 seeds. Petri dishes were placed in an experimental facility with 100 separate, thermally isolated cells (diameter 102 mm, height 33 mm), each with a separately controlled temperature (accuracy ±0.2 °C, SMARTEC-sensors, Breda, The Netherlands) (Mclaughlin *et al.*, 1985; Timmermans *et al.*, 2007). This results in true replicates where each single Petri dish had an independent germination environment. Set temperatures were verified with NTC-thermistors in the germination medium. Water potentials (ψ) were obtained by combining poly ethylene glycol (PEG 8000) and pure milli-Q water ($\psi \approx 0$) (Sigma-Aldrich) according to the equation:

$$\psi = 0.13[\text{PEG}]^2 \cdot T - 13.7[\text{PEG}]^2 \text{ (Michel, 1983)} \tag{2.11}$$

as recommended by Hardegree and Emmerich (1990).

The Petri dishes were sealed with Para film to limit evaporation. To further prevent evaporation and also prevent dripping of condensate onto the seeds, a piece of water saturated filter paper (4.1 g each, diameter 85 mm product no. 3621, Schleicher & Schuell, Dassel, Germany) was put in the Petri dish lid at temperatures higher than 21 °C. At temperatures lower than 21 °C evaporation was very low and since PEG solutions are hygroscopic, a water saturated filter paper could increase the water potential of the solution. According to Hardegree and Emmerich (1990), filter paper contains a hydrophylic volume fraction that is inaccessible to high molecular weight polymers such as PEG 8000. Hardegree and Emmerich (1990) showed that water absorbed by filter paper fibres concentrates the polyethylene glycol solution and, as a consequence, lowered the intended water potential in solution-filter paper mixtures. Therefore seeds were not put on traditional filter paper but they made direct contact with the fluid lying on a slightly elevated sieve with a 5 mm plastic brim. To aerate the whole system and correct for evaporation, the sieve was lifted from the Petri dish at least two times per day and the evaporated water mass (determined by weighing) was added and thoroughly mixed with the fluid. Using sieves in this way avoids direct contact of seeds with added water or a poorly mixed PEG solution.

Seeds were scored as germinated when protrusion of the radicle exceeded 2 mm (ISTA); germinated seeds were removed from the system. Observation frequency depended on germination rate but was at least two times a day until maximal 1500 h after imbibition.

Statistical analysis

All calculations were performed in Matlab (MathWorks, Natick, Massachusetts, USA) version 7.8.0.347 (R2009a). Maximum likelihood estimation (eqn 2.3) and nonlinear least squares regression (all remaining fits) were performed with the simplex method, using the built-in optimisation function fminsearch. In order to prevent fminsearch to convergence to local minima the fit was repeated with 10 different combinations of randomly chosen initial parameter values within the biologically plausible range. Values for parameter θ_H and the right hand side of eqn 2.7 were log transformed for fitting the large range in parameter values. To compare model performance, Akaike's information criterion (AIC) and sums of squared standardized residuals were used. When residuals are standardized, the raw residuals (observation model prediction) are divided by their corresponding binomial standard errors $\sqrt{n \cdot p(1-p)}$ where, n is the total number of seeds and p the model prediction. These standardized residuals are more useful because binomial responses do not have constant variance.The AIC was calculated by taking twice the sum of the minimized negative log-likelihood (eqn 2.3) of the final model plus two times the number of parameters. A model with lower AIC is superior to a model with higher AIC (Hilborn & Mangel 1997). Generally, a difference in AIC of 10 or more between different models is taken as sufficient evidence that the model with the lower AIC provides a superior description of the data (Burnham & Anderson, 2002).

Results

The *HTT*-model (Alvarado & Bradford, 2002) (eqn 2.1), fitted in the usual way on the probit scale, gave a rather poor description of the data $r^2 = 0.645$. So did the less restricted model of Rowse and Finch-Savage (2003): $r^2 = 0.642$. To gain insight in the reason for insufficient fit, we here show the detailed results of the proposed three step modelling framework.

Step 1: Are germination curves the result of a normally distributed germination rate?

The postulate of a normally distributed germination rate was tested by fitting to germination data from each replicate, i.e. individual Petri dishes. Despite its simplicity, and having only two parameters (μ and σ) it described the entire range of germination curves (Fig. 2.3). It could also handle low final germination percentages of less than 10% (Fig. 2.3f). The overall fit was very good, the r^2 's for the 5^{th}, 25^{th}, 50^{th}, 75^{th} and 95^{th} percentile of all 198 individual fits were respectively 0.945, 0.971, 0.986, 0.994 and 0.999.

The mechanism by which the model accommodates for final germination percentages is by defining a mean germination rate below zero, such that only the right tail of the distribution has a positive germination rate. The area beneath this positive tail represents the final germination percentage.

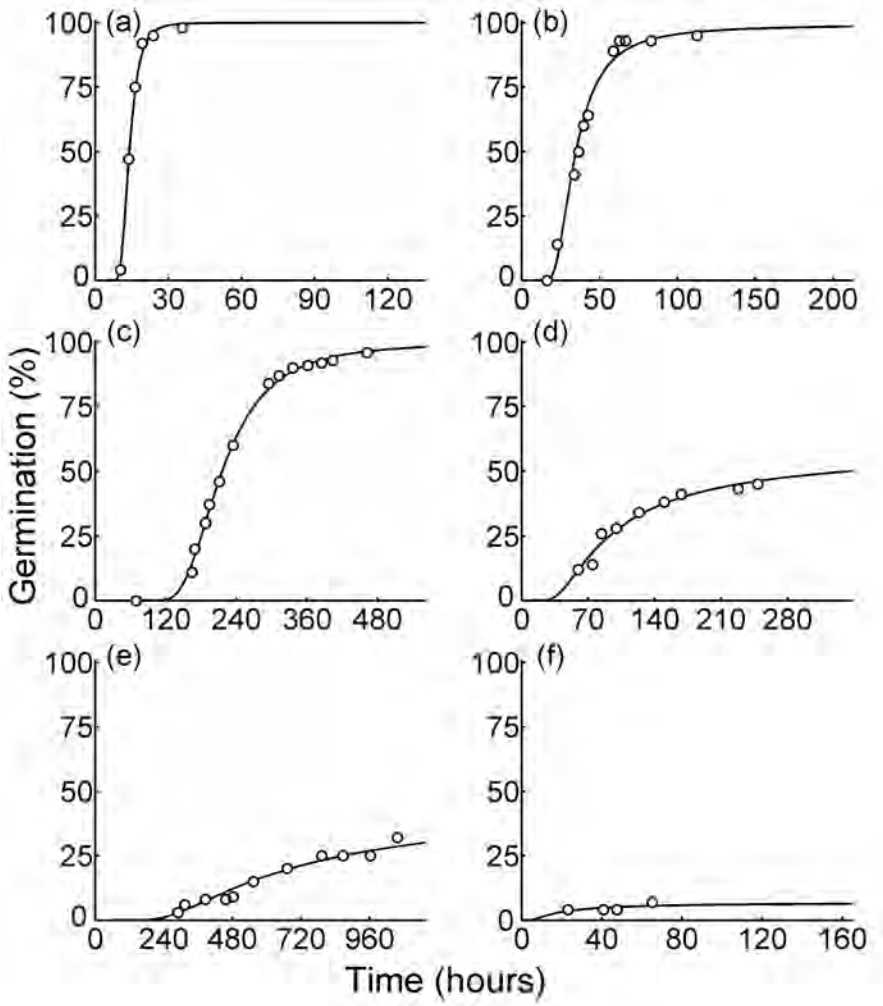

Fig. 2.3: Example fits of eqn 2.2 (solid lines) to data (circles) of six individual replicates selected from treatments with different combinations of temperature and water potential: (a) 33 °C and 0 MPa, (b) 35 °C and -0.8 MPa, (c) 17 °C and -1.4 MPa, (d) 35 °C and -1.4 MPa (e), 10 °C and -0.8 MPa, and (f) 43 °C and 0 MPa. Note the different time scales of the x-axes.

Step 2: Parameter μ as a function of water potential (ψ) and temperature (T)

In this second step we used the *HTR* model (eqn 2.4) to fit the response of mean germination rate to temperature (T) and water potential (ψ) (Fig. 2.4). The *HTR* model in combination with the new eqn 2.7 and 2.8 provided a better fit ($r^2 = 0.974$) for this response surface than the *HTT* model (eqn 2.1) ($r^2 = 0.934$) (Fig. 2.4). The intersection of the fitted response surface with the base plane (where the mean germination rate is 0) shows the relationship between the base water potential and temperature (Fig. 2.4). As water potential decreases, the ceiling temperature T_c decreases while the base temperature, T_b, increases, thus narrowing the range of temperatures at which germination takes place (Fig. 2.1, 2.4 and 2.5). Figure 2.4 and 2.5 make clear that the *HTT* model is a justified, but a less elegant way of describing the data. In the *HTT* concept the optimum temperature, T_o, is fixed whereas in the new *HTR* model, T_o increases subtly with water potential (Fig. 2.4). Cross sections through the response surface are given to accurately show the model fit of germination rate, μ, to temperature at different water potentials for the three models (Fig. 2.5).

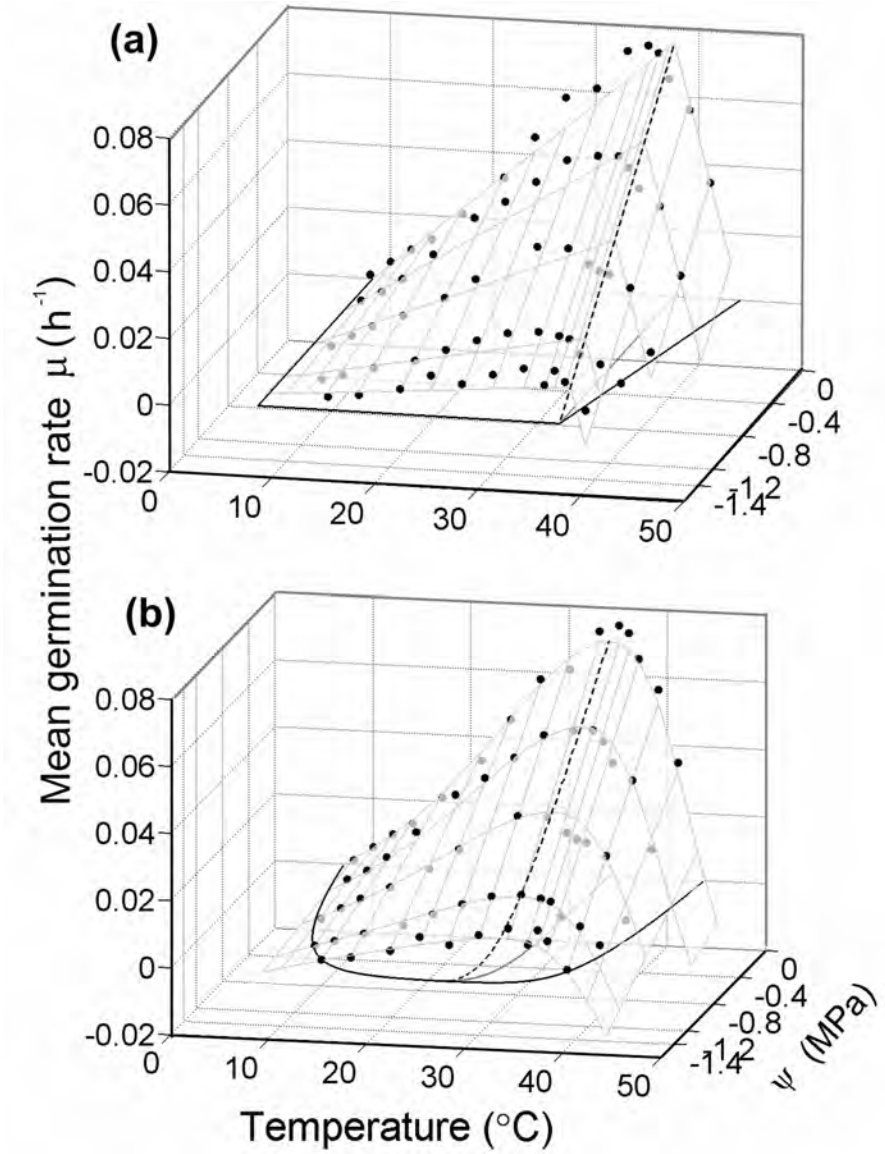

Fig. 2.4: Mean germination rate (μ) (dots) (z-axis; h^{-1}) as a function of water potential (ψ) (x-axis; MPa) and temperature (T) (y-axis; °C) for (a) the *HTT* model (eqn 2.1) and (b) the *HTR* model (eqn 2.4, 2.7 and 2.8). The gridded surface represents the modelled response surface. Black dots are above the surface whereas grey dots are below it. The U-shaped solid black line is the intersection between the response surface and the plane defined by $\mu = 0$. This U-shaped solid black line represents the relationship between base water potential and temperature $(\psi_b(T))$. The same U-shaped solid black line shows cardinal temperatures $T_b(\psi)$ and $T_c(\psi)$ as a function of water potential. The maximum germination rate at each water potential is represented by the broken black line. The corresponding optimum temperature T_o as a function of water potential (ψ) is represented by the solid grey line drawn at $\psi = 0$ in the (T, ψ) plane.

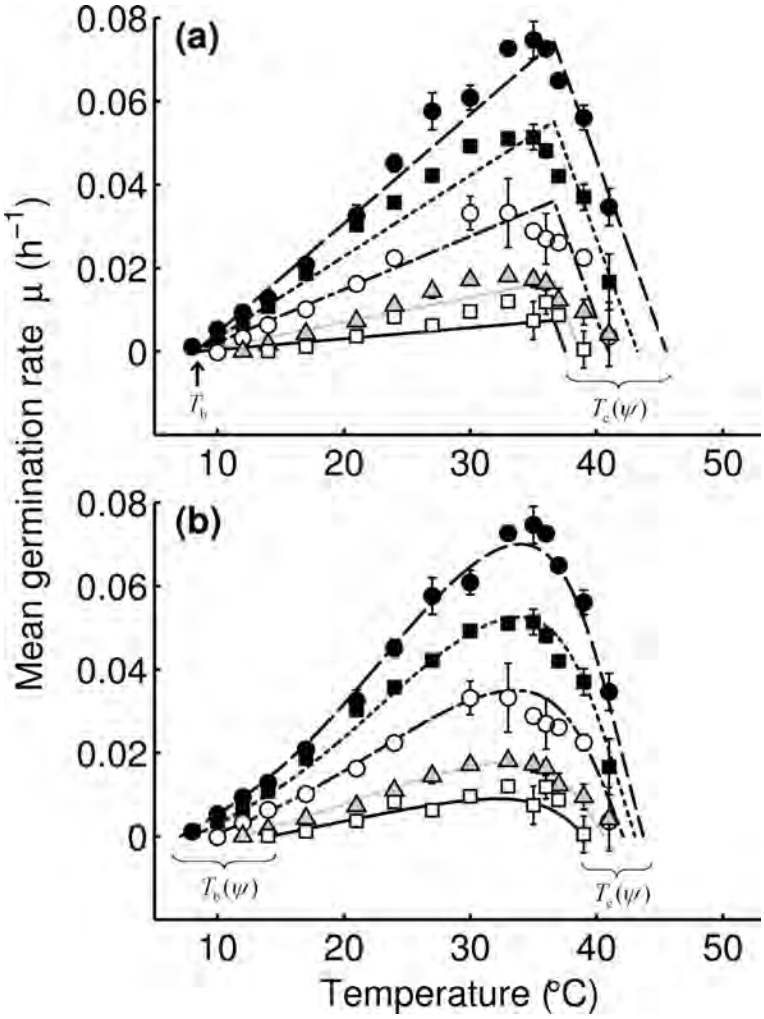

Fig. 2.5: Mean germination rate (μ) at five water potentials (n = 3, error bars represent sd; not visible when smaller than the marker) plotted against temperature for (a) the *HTT* model (eqn 2.1) $r^2 = 0.934$ (b) the *HTR* model (eqn 2.4, 2.7 and 2.8). $r^2 = 0.976$. Different markers represent water potentials: 0 MPa (closed circles), -0.4 MPa (closed squares), -0.8 MPa (open circles), -1.2 MPa (grey triangles) and -1.4 MPa (open squares). Lines represent the modelled mean rate $(\mu(T))$ at each water potential: 0 MPa (broken), -0.4 MPa (dotted), -0.8 MPa (broken dotted), -1.2 MPa (grey) and -1.4 MPa (solid).

We fitted the mean germination rate of all water potentials and replicates for each temperature using eqn 2.4, resulting in values for ψ_b and θ_H for each temperature. The *HTR* model provided a very good description of the two parameters, $\psi_b(T)$ ($r^2 = 0.973$; eqn 2.7) and $\theta_H(T)$ ($r^2 = 0.971$; eqn 2.8) (Fig. 2.6), that underpin the 3D response surface (eqn 2.4) (Fig. 2.4). In the *HTT* model the parameters $\psi_b(T)$ and $\theta_H(T)$ are not directly fitted. Instead the relationships shown in Fig. 2.6 for the *HTT* are derived from the overall *HTT* model, fitted on all the germination data. It appears that the *HTT* based relationships for ψ_b and θ_H as a function of temperature have leverage points at low (10-12 °C) and high (37-42 °C) temperatures resulting in a very low r^2's, in this case even below zero. The latter indicates that taking the average ψ_b and θ_H over all temperatures gave better predictions than the *HTT* model. We did obtain some improvement in goodness of fit for $\psi_b(T)$ and $\theta_H(T)$ when the *HTT* model was refitted on a restricted data set, i.e. when data of temperatures below 20 °C were removed, $\psi_b(T)$ $r^2 = 0.193$ and $\theta_H(T)$ $r^2 = 0.506$. By fitting on this restricted data set r^2 increased from 0.645 to 0.751, indicating that the *HTT* model is not well equipped to describe germination in the low suboptimal temperature range of teff.

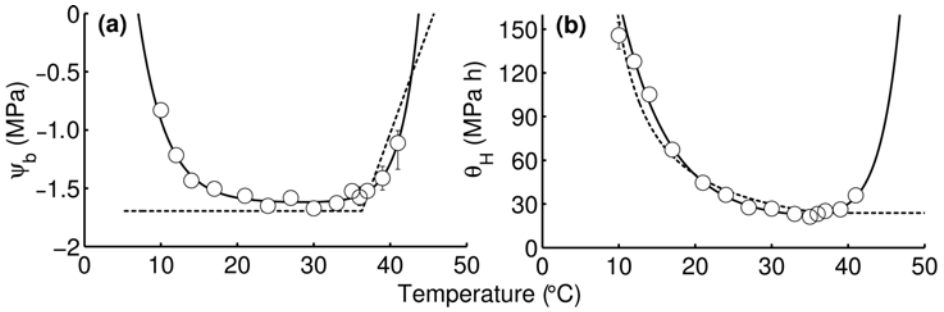

Fig. 2.6: (a) Base water potential (ψ_b) (circles) and (b) hydro time constant (θ_H) (circles) as a function of temperature. Error bars indicate data point extremes based on the highest and lowest values of mean germination rate (μ) fitted with eqn 2.4 for each single temperature. The solid line represents the fitted *HTR* model (a) eqn 2.7 $r^2 = 0.973$ and (b) eqn 2.8 $r^2 = 0.971$; the broken line represents the fitted *HTT* model (a) eqn 2.5 $r^2 < 0$ and (b) eqn 2.6 $r^2 < 0$. Parameters for eqn 2.5 and 2.6 are derived from the overarching probit regression model used for fitting eqn 2.1.

Step 3: Parameter σ as a function of water potential (ψ) and temperature (T)

In the third step we compare two alternative approaches to model the spread (σ) around the mean germination rate (μ). In the first approach we combine a coefficient of variation (CV) with a minimum value of the spread (σ_{min}) that is a function of temperature (eqn 2.9). Using a minimal value of spread is consistent with the data, as for each temperature the treatments of -1.2 and -1.4 MPa (Fig. 2.7) have approximately the same value for spread σ. In other words, the spread as a function of water potential $(\sigma(\psi))$ reaches a temperature dependent minimum value of σ at low water potentials. Equation 2.9 in combination with

the *HTR* model proved to be a good model ($r^2 = 0.793$) that only uses two parameters to describe the spread as a function of water potential $(\sigma(\psi))$ (Fig. 2.7). The bell shapes of the fitted function, however, did not entirely correspond with the pattern shown by the data; the fitted curves show an optimum, whereas data points indicate that σ approaches a constant value at high values of temperature (Fig. 2.7).

The second approach is based on an empirical logistic function (eqn 2.10), that approaches a constant value at high values of temperature (Fig. 2.7). The logistic eqn 2.10 yielded a better description of the relationship between the spread (σ) and predictor variables ψ and T in our data set ($r^2 = 0.900$), but at the cost of four (eqn 2.10) instead of two parameters (eqn 2.9) (Fig. 2.7).

The *HTT* model (eqn 2.1) uses only one parameter to describe the spread in time to germination, σ_{ψ_b}. On basis of eqn 2.1 the spread in base water potential, σ_{ψ_b}, can be expressed in the spread in germination rate, σ, by (Fig. 2.7):

$$\sigma = \frac{\sigma_{\psi_b}}{\theta_H(T)} \tag{2.12}$$

From eqn 2.12 and Fig. 2.7 we can see that the *HTT* model crudely assumes the same relation between σ and temperature as eqn 2.10. However, the current *HTT* model does not account for a spread that changes with water potential (Fig. 2.7).

Comparing model predictions of seed germination

Having obtained $\mu(\psi, T)$ (eqn 2.4, 2.7 and 2.8) and $\sigma(\psi, T)$ (eqn 2.9 or 2.10) the actual germination curves and the final germination percentages can be described at any combination of temperature (T) and water potential (ψ).

The total number of parameters of the new hydrothermal rate model (*HTR*) is 11 (when using eqn 2.9) or 13 (when using eqn 2.10) whereas the hydrothermal time (*HTT*) (eqn 2.1) of Alvarado and Bradford (2002) contains only 6 parameters. Therefore the question arises whether the model improvement is in statistical terms $(\overline{r^2} = 0.646$ for *HTT*, $\overline{r^2} = 0.842$ for *HTR* (eqn 2.9) and $\overline{r^2} = 0.874$ for *HTR* (eqn 2.10)$)$, worth the cost of the additional parameters. In order to answer this question the overall sum of squared residuals and Akaike's Information Criterion (AIC) were calculated (Table A3.2.2).

The plotted residuals for the *HTR* model were more homoscedastic than those of the *HTT* (Fig. 2.8). The residual histogram also shows that the *HTR* models had a narrower error range and smaller sum of squared standardized residuals (SSE) than the *HTT* model (Fig. 2.9).

The AIC was 1.686×10^5 for the traditional *HTT* model compared to 1.487×10^5 for the *HTR* model with 11 parameters, and 1.461×10^5 for the model with 13 parameters. These AIC values indicate a major improvement of the new over the old model. According to the AIC the *HTR* 13-parameter model (eqn 2.10) was a meaningful improvement over the *HTR* 11-parameter model (using eqn 2.9). Therefore on statistical grounds the 13-parameter

HTR was superior to the 6-parameter *HTT* and 11-parameter *HTR*, which is confirmed by comparing all fits (e.g. Fig. 2.10) to the data and inspection of residual plots (Fig. 2.8 and 2.9). The final parameter values for all parameters of these models are given in Table A4.2.3.

Final germination percentage at high or moderate water potentials (=-0.8 MPa) was well described by the reference *HTT* model as well as the *HTR* model (Fig. 2.11a-c). The main differences occurred at the high end of the temperature range. Both approaches showed minor lack of fit at supra-optimal temperatures. However, at a limiting water potential of -1.2 MPa, the *HTT* model substantially underestimated the final germination percentages in the intermediate temperature range whereas *HTR* model described the data very well (Fig. 2.11d). At the lowest water potential tested (-1.4 MPa), the *HTT* model showed major lack of fit at all temperatures whereas the *HTR* approach described the final germination data well (Fig. 2.11e).

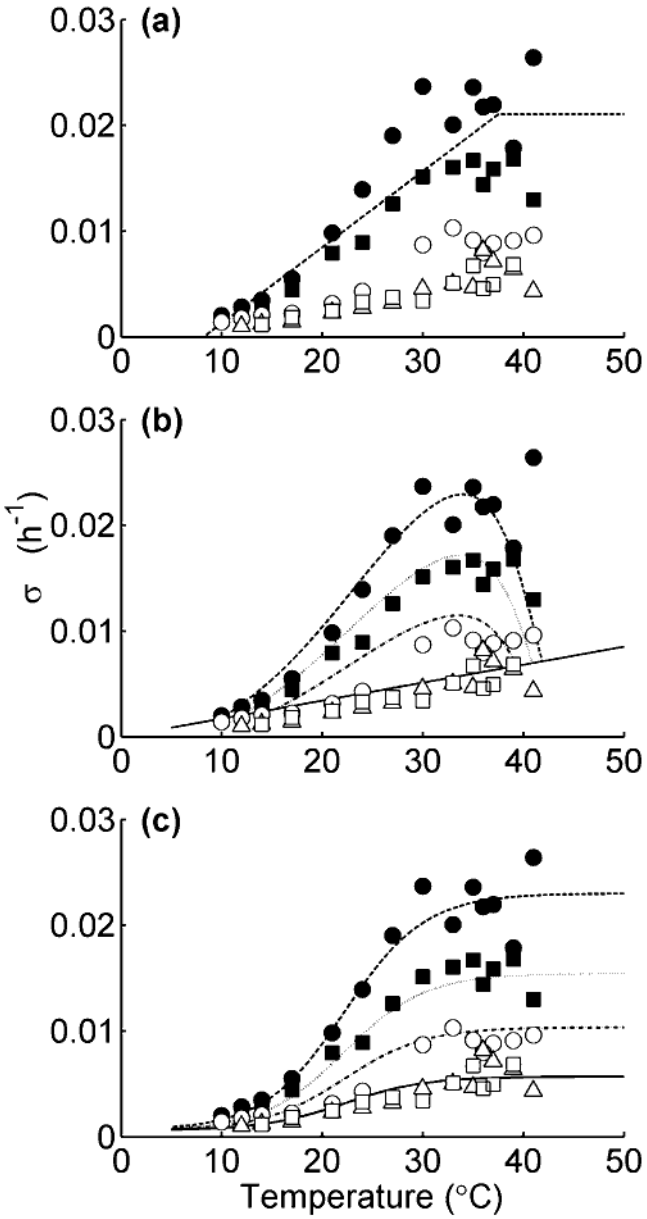

Fig. 2.7: Relationship of spread (σ) of germination rate (r) to temperature at five measured water potentials (n = 3, error bars are omitted for readability) for (a) *HTT* model where σ is calculated with eqn 2.12, $r^2 = 0.114$ (b) *HTR* model where σ is calculated with eqn 2.9 (CV), $r^2 = 0.793$ and (c) where σ is separately modelled with eqn 2.10, $r^2 = 0.900$. Different markers represent the water potentials: 0 MPa (closed circles), -0.4 MPa (closed squares), -0.8 MPa (open circles), -1.2 MPa (grey triangles) and -1.4 MPa (open squares). Lines represent the modelled spread (σ) for each water potential: 0 MPa (broken), -0.4 MPa (dotted), -0.8 MPa (broken dotted), -1.2 MPa (grey) and -1.4 MPa (solid).

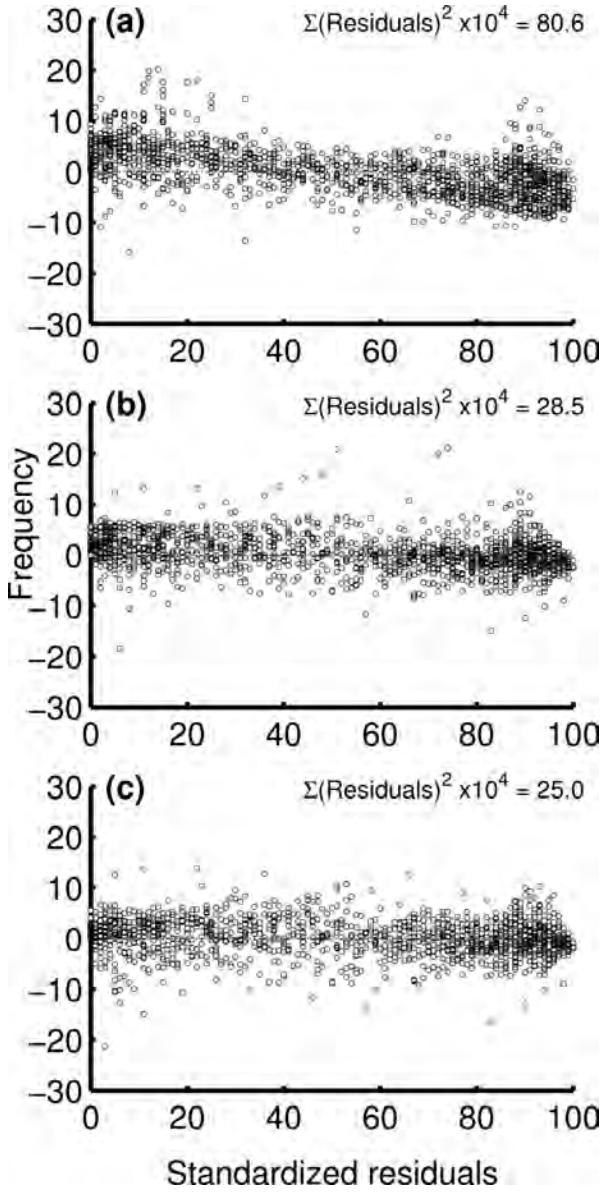

Fig. 2.8: Standardized residual germination for (a) the traditional *HTT* model (eqn 2.1), (b) *HTR* model fitted using eqn 2.4, 2.7, 2.8 and 2.9 (σCV) and (c) *HTR* model fitted using eqn 2.4, 2.7, 2.8 and 2.10 (σLogistic).

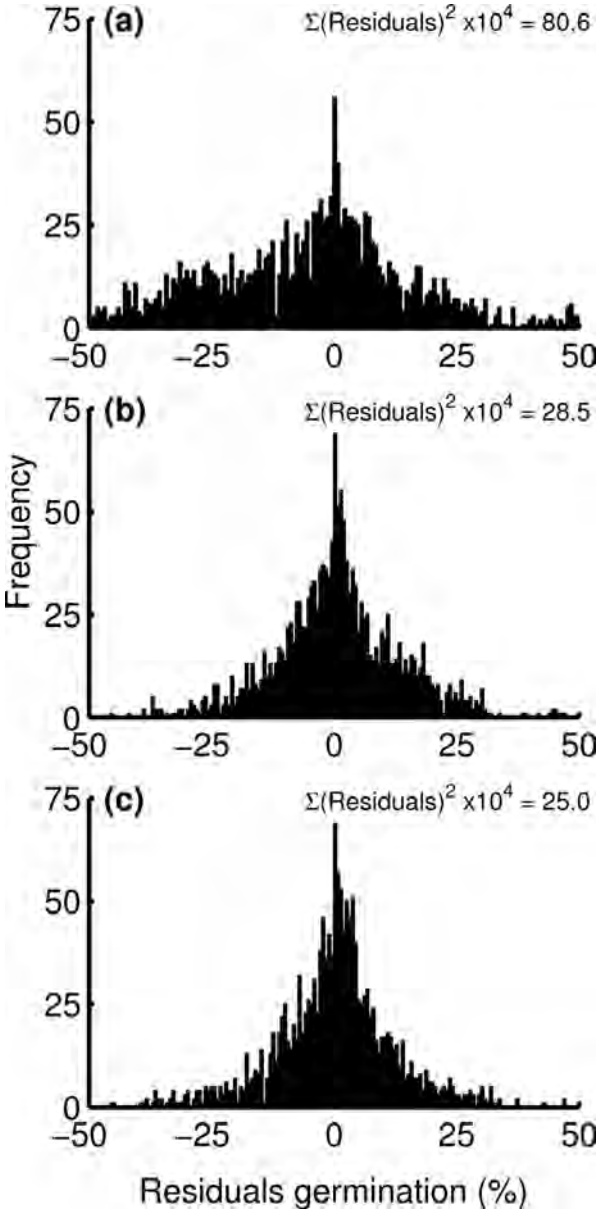

Fig. 2.9: Frequency distribution of residual germination for (a) the traditional *HTT* model (eqn 2.1), (b) *HTR* model fitted using eqn 2.4, 2.7, 2.8 and 2.9 (σCV) and (c) *HTR* model fitted using eqn 2.4, 2.7, 2.8 and 2.10 (σLogistic).

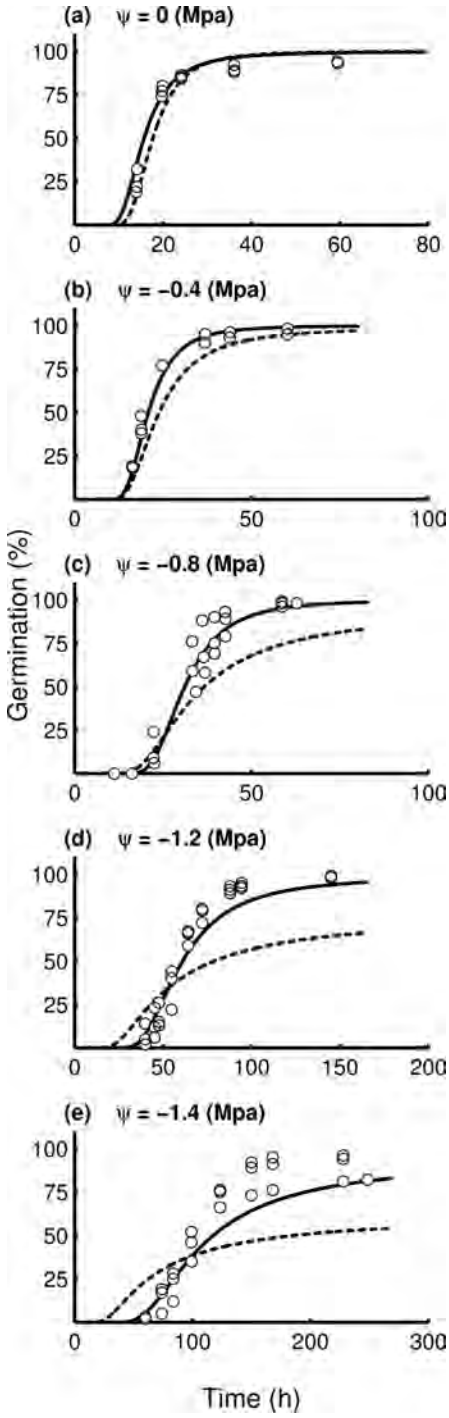

Fig. 2.10: Cumulative percentage of germinated seeds (circles) of 3 replicates plotted versus time. Lines represent germination curves as described by the overarching *HTR* (solid line) and *HTT* (broken line) models at 30 °C for 5 water potentials (a-e) of 0, −0.4, −0.8, −1.2 and −1.4 MPa.

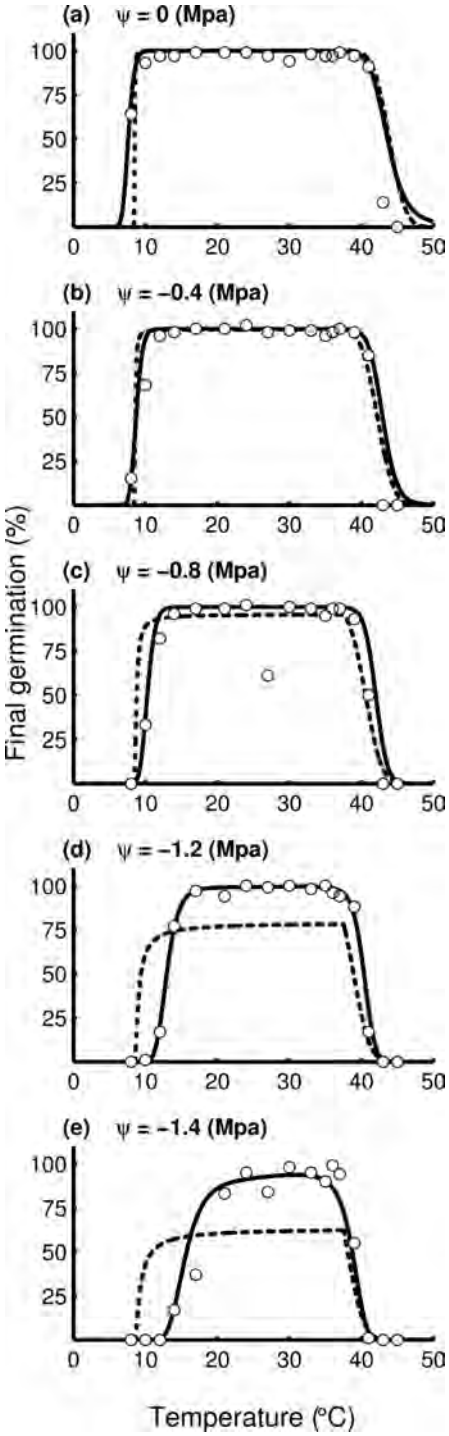

Fig. 2.11: Observed maximum final germination (circles) and two model predictions (lines) as a function of temperature (T) at five water potentials (a-e). The broken line represents the traditional *HTT* model (eqn 2.1). The solid black line represents the *HTR* model, using eqn 2.4, 2.7, 2.8 and 2.10 to model σ. The r^2 of each individual line per treatment is given in Table 2.1. At 27 °C and -0.8 MPa final germination suddenly dropped (c) although we did not find a clear cause, this outlier is probably due to an experimental error.

Table 2.1: The r^2's of two models for final germination shown in Fig. 2.11.

ψ	*HTT*[1]	*HTR*[2]
0	0.744	0.735
−0.4	0.623	0.785
−0.8	0.421	0.806
−1.2	0.247	0.934
−1.4	0.106	0.877

[1] 6 parameter *HTT* (eqn 2.1)
[2] 13 parameter *HTR* (eqn 2.4, 2.7, 2.8 and 2.10)

Discussion

This study provides a parsimonious germination curve (eqn 2.2) that is based on the premise of a normally distributed rate. This curve (eqn 2.2) is able to summarize germination data originating from a wide range of combinations of water potential and temperature, using only two parameters, i.e. the mean germination rate, μ, and spread, σ. Plotting these summary measures as function of water potential and temperature provided a clear insight in the lack of fit of the *HTT* model (eqn 2.1) for *Eragrostis tef* (Zuccagni) Trotter).

The transparency of the data analysis presented here enabled us to increase goodness of fit by, firstly, using a smooth curvilinear 3D response surface of mean germination rate to temperature and water potential (eqn 2.4, 2.7 and 2.8). And secondly, by making the spread a function of both temperature and water potential (eqn 2.9 and 2.10), $\sigma(\psi, T)$.

Temperature response

Plotting mean germination rate, μ, as a function of both water potential and temperature confirms the finding of Gummerson (1986): at a constant temperature mean germination rate decreases linearly with water potential. However, the response of mean germination rate to temperature shows that cardinal points (i.e. base (T_b), optimum (T_o), ceiling (T_c) temperature and base water potential (ψ_b)) are not constants but form a continuous, smoothly curving series of threshold points in the (T,ψ) space (Fig. 2.1, 2.4, 2.5 and 2.6). Therefore cardinal temperatures are a function of water potential: $T_b(\psi)$, $T_o(\psi)$ or $T_c(\psi)$ and conversely, the base water potential is a function of temperature $\psi_b(T)$. Hence, germination inhibition at a particular threshold combination of water potential and temperature should not be attributed exclusively to temperature falling below a minimum (T_b) or rising above a maximum (T_c), or water potential dropping below a minimum (ψ_b): it is the combination of temperature and water potential that matters. However, explicitly defining base water potential as a temperature dependent variable (eqn 2.7), $\psi_b(T)$, can provide a good description of the continuous, smoothly curving series of threshold points in the (T,ψ) space. In essence this is similar to what Alvarado and Bradford (2002) proposed for supra optimal temperatures; they expressed the ceiling temperature, T_c, by using eqn 2.5 to describe $\psi_b(T)$. However, by using eqn 2.7 instead of eqn 2.5 we include the sub optimal temperature range and make the model smooth and flexible.

Our results show that not only the threshold water potential, ψ_b, i.e. absolute amount of free available water required for germination, changes with temperature, but also the progress in germination per unit of free available water changes (i.e. hydrotime). An increasing hydrotime and thus decreasing effect per unit available water on germination rate, is commonly accepted (Alvarado & Bradford, 2002; Finch-Savage & Leubner-Metzger, 2006) for suboptimal temperatures (Fig. 2.1e). This study, however, shows that at supra optimal temperatures hydro time also tends to increase (Fig. 2.1f and 2.6b). In other words, towards temperature extremes the effect per unit of additional free available water on germination rate will decrease, which justifies the U shape used in the *HTR* model (eqn 2.8).

Spread as function of both water potential and temperature

Seed physiological parameters like T_b, T_c, ψ_b and θ_H are not constant and they are not a direct result of a single measurable molecular event. On the contrary, the values of these parameters are the cumulative result of the combination of seed physiological phenomena (e.g. gene transcripts, enzymes, hormones, solutes) and environmental factors (temperature, water potential, light, nitrate) (Finch-Savage & Leubner-Metzger, 2006). Each of these process driving factors most likely follows a distribution within the seed population. It is impossible, and in this study irrelevant, to disentangle these process driving distributions on basis of the process outcome alone, i.e. a distribution in time to germination. Therefore, we prefer using a distribution of the process rate itself, which can be directly derived from each individual germination curve, *viz.*: the distribution of the reciprocal of time to germination. By using this distribution, the impact of internal and external factors on germination rate can still be represented by physiological parameters. There is, however, no need to separately model variation in cardinal temperatures or base water potential when the variability in the process rate itself is being modelled.

The *HTT* model attributes all variation in time to germination within a population of seeds to one single physiological parameter, i.e. the spread in base water potential, σ_{ψ_b}. Although parameter, σ_{ψ_b}, used in eqn 2.1 has a constant single value, the model implicitly describes the actual spread in germination rate, σ, as a function of temperature only (eqn 2.12). Consequently, the spread in germination rate, σ, as described by eqn 2.1 with does not change under influence of water potential. However, according to our results on teff (Fig. 2.7), the spread in germination rate, σ, should be a function of water potential. But, by choosing σ_{ψ_b} to describe the spread in time to germination, one chooses a physiological parameter that changes with the same environmental factor that defines its existence, i.e. water potential. Modelling the process rate itself circumvents such an awkward relation.

Final germination

The *HTT* modelling approach intends to capture the intrinsic physiological linkage between the response of germination rate to temperature and water potential and the fraction of germinated seeds. However, confirming data from Grundy (2000), this study clearly illustrates that the *HTT* model as such did not accurately predict the final germination percentage (Fig. 2.11). The new *HTR* model predicted the final germination percentage substantially better, without including a dedicated parameter for final germination percentage. Thus, final germination is an emerging property of two parameters: mean germination rate, μ, and spread, σ.

An extended version of the *HTT* model

Brown and Mayer (1988) warned that models of increasing complexity (i.e. more parameters and flexibility) can provide a better fit, but at the risk of accommodating an increased proportion of random experimental error (i.e. overfitting). It is, therefore, appropriate to use AIC to assess whether improved model fit justifies the number of parameters (Hilborn & Mangel, 1997; Bolker, 2008). This risk of overfitting may be further reduced by analysing data from extensive and accurate laboratory experiments in which random error is minimal. Nevertheless, when the new model framework is used in further research with only a small number of data points and high experimental error, linear models can be a safer resort to avoid false claims of prediction accuracy. Therefore in appendix 1, we offer an extended *HTT* model that incorporates a spread and base temperature that changes with water potential. Note that this notation (eqn A2.2.13) accommodates for a spread in base temperature (T_b), because if $\psi = 0$, then $\frac{\sigma_{\psi_b}}{\theta_H(T_b)} = \sigma_{T_b}$ and similarly $\frac{\sigma_{\psi_b}}{\theta_H(T_c)} = \sigma_{T_c}$. Fitting this discontinuous model to all data in one single regression remains statistically incorrect. Nevertheless, this procedure has the advantage that the parameter combinations of the model can also hold information and therefore make the overall extended *HTT* model more parsimonious than the *HTR* model. However, according to the AIC, the goodness of fit of the *HTR* compared to the extended *HTT*, still justifies the use of 6 additional parameters in the *HTR* model compared to the extended *HTT* model.

Former and further research

For some species or seed batches the assumption of a normally distributed rate could be incorrect. In such a case eqn 2.2 will not provide a satisfactory fit to the data. When this occurs it would be necessary to implement other distributions like for example a Weibull distribution as proposed by Watt *et al.* (2010).

During the current study, data were gathered under well controlled laboratory conditions. Temperatures and water potentials were artificially kept constant. However, making agronomical field predictions requires a model in which both water potential (ψ) and temperature (T) can fluctuate over time. This will involve explicitly separating and modelling imbibition and priming at different temperatures and water potentials (Rowse *et al.*, 1999) and performing model validation in the field (Finch-Savage *et al.*, 2005).

Conclusion

The *HTT* model has widely recognized advantages, in particular: few parameters, physiological interpretation and a pre-packaged statistical framework that can readily be applied. Yet, this study showed significant drawback of that framework: the usage of broken functions which in this case is not biologically plausible, the violation of the requirements of the statistical model, and the regression model as such (eqn 2.1) is sort of a "black box" causing

loss of intuitively deriving reasons for lack of fit. Moreover, the *HTT* model is not suitable under extreme conditions.

The advantage of the stepwise *HTR* approach proposed here is transparency. The summary measures mean germination rate, μ, and spread, σ, provide clear insight in how the germination behaviour of a seed population reacts to water potential and temperature. By using variation in the process rate itself, variation in time to germination can directly be derived from the data. Although the number of parameters of the *HTR* model is significantly higher than the *HTT* model, the *HTR* model can fit the entire permissive range of temperature and water potential combinations, using smooth flexible continuous functions. It becomes, moreover, immediately clear if the model assumptions are violated when analysing a particular data set. Additionally, the equations presented here can simply be rewritten for a fit at sub- or supra-optimal temperatures only.

Appendix 1

The supplementary figure in this appendix provides an example shape of eqn 2.7. The purpose of this figure is to gain understanding in the logic of the function itself and the interpretation of its parameters.

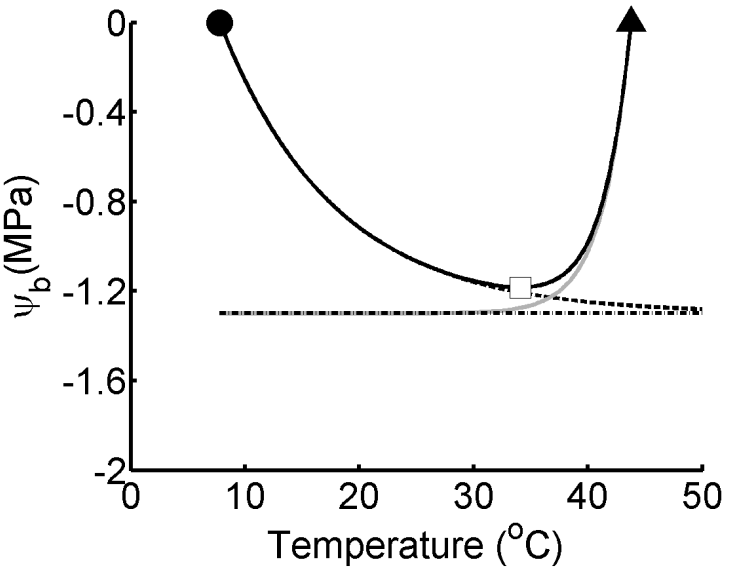

Fig. A1.2.12: Example shape of eqn 2.7: $\psi_b(T) = \psi_b{}^{min}\left(1 - e^{a\left(T_b - T\right)}\right)\left(1 - e^{b(T - T_c)}\right)$ (solid line). The black broken line describes the left part suboptimal temperatures $\psi_b^1(T) = \psi_b^{min}\left(1 - e^{a\left(T_b - T\right)}\right)$ and the grey solid line describes the right part (supra-optimal temperatures $\psi_b^2(T) = \psi_b^{min}\left(1 - e^{b(T - T_c)}\right)$). The dotted line at ψ_b^{min} represents the horizontal asymptote of the two part functions $\psi_b^1(T)$ and $\psi_b^2(T)$. The square represents the actual minimum, which is not necessarily the same as ψ_b^{min}. The circle represents T_b and the triangle T_c.

Appendix 2

This appendix provides a new modified version of the *HTT* model that can be fitted on all data in a single fit. To accommodate for changing T_b as function of water potential ($T_b(\psi)$) we introduce parameter $T_o^{T_b(\psi)}$, i.e. the temperature where T_b does not further increase with water potential. We than replace T_o with $T_o^{T_c(\psi)}$, i.e. the temperature where T_c as function of water potential has its lowest value (Fig. A2.2.13). Variation in germination speed is, furthermore, made a function of water potential by introducing an exponential scaling factor (e^{ψ}):

$$\text{Probit(g)} = \frac{\psi - \left(\frac{\theta_{HT}}{(T-T_b)t(g)}\right) + \psi_b \cdot \frac{(T-T_b)}{\left(T_b - T_o^{T_b(\psi)}\right)}}{\sigma_{\psi_b} \cdot e^{\psi}} \quad \text{if} \quad T_b < T \leq T_o^{T_b(\psi)} \tag{A2.2.13a}$$

$$\text{Probit(g)} = \frac{\psi - \left(\frac{\theta_{HT}}{(T-T_b)t(g)}\right) - \psi_b}{\sigma_{\psi_b} \cdot e^{\psi}} \quad \text{if} \quad T_o^{T_b(\psi)} < T \leq T_o^{T_c(\psi)} \tag{A2.2.13b}$$

$$\text{Probit(g)} = \frac{\psi - \left(\frac{\theta_{HT}}{(T-T_b)t(g)}\right) + \psi_b \cdot \frac{(T-T_c)}{\left(T_c - T_o^{T_c(\psi)}\right)}}{\sigma_{\psi_b} \cdot e^{\psi}} \quad \text{if} \quad T > T_o^{T_c(\psi)} \tag{A2.2.13c}$$

where Probit(g) is the germination percentage g, converted to probits, ψ (MPa) the water potential, T (°C) the temperature, the hydrothermal time constant, T_b (°C) the median base temperature, T_c (°C) the median ceiling temperature, $t(g)$ (h) the time to germination of a percentile g, ψ_b (MPa), the median base water potential, and σ_{ψ_b} (MPa) the standard deviation of base water potential. Application of the model is possible if base water potential is normally distributed. The fit is achieved in one big overarching probit regression.

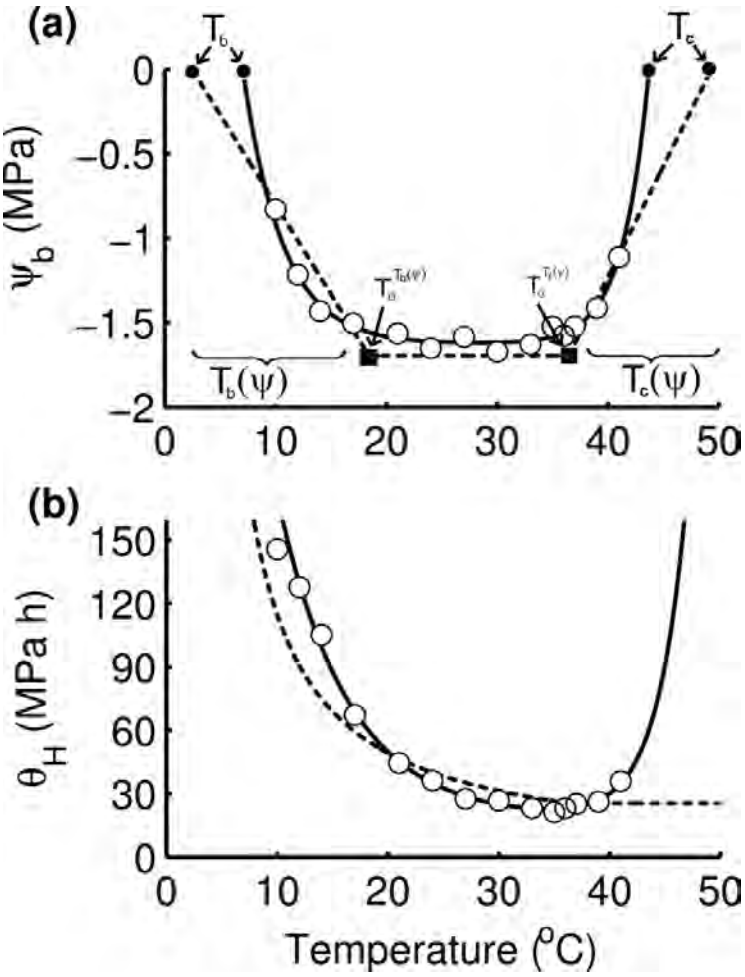

Fig. A2.2.13: (a) Base water potential (ψ_b) (circles) and (b) hydro time constant (θ_H) (circles) as a function of temperature. The solid line represents the fitted *HTR* model (a) Eqn 2.7 $r^2 = 0.973$ and (b) Eqn 2.8 $r^2 = 0.971$; the broken line represents the extended *HTT* model (a) Eqn A2.2.13 $r^2 < 0.754$ and (b) Eqn A2.2.13 $r^2 < 0.847$.

Appendix 3

Table A3.2.2: The goodness of fit for different model combinations

		Equations	*HTT* Alvarado and Bradford (2002)	*HTT* Rowse and Savage (2003)	*HTT* Extended (Appendix 1)	*HTT* Extended (Appendix 1)	*HTR*	*HTR*	*HTR*
Fit procedure			Single fit	Single fit	Single fit	Step wise	Step wise	Step wise	Step wise
						2.4	**2.4**	**2.4**	**2.4**
						2.5	**2.5**	**2.7**	**2.7**
Equations			**2.1**	**2.1**	**A2.2.13**	**2.6**	**2.8**	**2.6**	**2.8**
r^2	σ_{ψ_b}	**2.1**	0.645	0.642	0.801	-	-	-	-
	σCV	**2.9**	-	-		0.618	0.727	0.674	0.856
	$\sigma(\psi T)$	**2.10**	-	-		0.551	0.695	0.630	0.874
AIC $\times 10^5$	σ_{ψ_b}	**2.1**	1.686	1.687	1.533	-	-	-	-
	σCV	**2.9**	-	-		1.899	1.618	1.694	1.487
	$\sigma(\psi T)$	**2.10**	-	-		1.750	1.688	1.772	1.461
$\sum$(residual)^2 $\times 10^5$	σ_{ψ_b}	**2.1**	7.074	7.120	3.966	-	-	-	-
	σCV	**2.9**				7.880	5.493	6.571	2.845
	$\sigma(\psi T)$	**2.10**				9.402	6.155	7.484	2.505
Number of Parameters	σ_{ψ_b}	**2.1**	6	6	7	-	-	-	-
	σCV	**2.9**	-	-	-	7	9	8	11
	$\sigma(\psi T)$	**2.10**	-	-	-	9	11	10	13

Appendix 4

Table A4.2.3: Parameter estimates

Parameter	*HTT* Alvarado and Bradford (2002)	*HTT* Rowse and Savage (2003)	*HTT* Extended	*HTR*	*HTR*
Equations	**2.1**	**2.1**[*1]	**A2.2.13**	**2.4, 2.5, 2.6**	**2.4, 2.7, 2.8**
θ_{HT}	595.67	601.04	864.24	625.23	-
σ_{ψ_b}	0.43	0.43	0.62	-	-
K_T	0.17	0.19	-	0.11	-
$T_o, T_o(\theta_H)$ or $T_o^{T_c(\psi)}$	36.63	36.69	36.48	35.11	34.59
$\psi_b$or ψ_b^{min}	-1.55	-1.56	-1.68	-1.69	-1.63
a	-	-	-	-	0.28
b	-	-	-	-	0.42
T_b	8.27	8.26	2.47	8.54	7.04
T_c	-	-	49.36	-	43.72
$T_o^{T_c(\psi)}$	-	-	18.42	-	-
$\theta_H(T_o)$	-	-	-	-	22.77
c	-	-	-	-	0.16
d	-	-	-	-	0.39
CV	-	-	-	0.334	0.327
α x 10^{-4}	-	-	-	1.63	1.70
$\sigma_{max}(0)$	-	-	-	0.023	0.023
T_i	-	-	-	22.22	22.22
T_s	-	-	-	14.21	14.21
σ_{min}x 10^{-4}	-	-	-	6.01	6.01

[*1] Rowse and Savage (2003) use eqn 2.1 but model hydrotime at supra optimal temperatures with eqn 2.6a instead of eqn 2.6b as used by Alvarado and Bradford (2002).

Chapter

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3

Biomechanics of teff:

Analysing lodging of the panicle bearing cereal teff

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Abstract

• ***Background*:** Lodging is the permanent displacement of crop plants from their vertical due to root or shoot failure. Lodging is a major yield constraint in panicle bearing cereal teff (*Eragrostis tef* (Zuccagni) Trotter). The objective of this chapter is to analyse the causes of lodging of teff by using, modifying and validating conventional biomechanical models.

• ***Methods*:** The model parameters were obtained from a field trial with two contrasting teff cultivars, using novel *in situ* and laboratory measurements under wet and dry conditions. Cross species model validation was done with rice (*Oryza sativa* L.).

• ***Key Results*:** Teff is more susceptible to root lodging than to shoot lodging, although the data indicated that shoot strength is also insufficient. Hence, simultaneously breeding for both improved root anchorage and shoot strength is advocated.

• ***Conclusions*:** The study showed that the lodging model, derived for the spike-bearing cereals wheat, needed modifications in order to be able to deal with panicle-bearing plants like teff and rice. Water adhering to plants due to rain or dew increased lodging susceptibility. To prevent underestimation of lodging susceptibility, future lodging research should be done under completely wet conditions (water saturated soil and wetted shoots).

Key words:
teff (*Eragrostis tef* (Zuccagni) Trotter), lodging, water loading, rice, model, anchorage, biomechanics, safety factor.

Abbreviations used in this chapter

Roman Symbols	Explanation	Units	Eqns.
SF	safety factor		3.11
a_1 and a_2	major outer and inner diameter of the ellipse respectively	m	3.10
b	scaling parameter		3.11
c	shape parameter		3.11
b_1 and b_2	minor outer and inner diameter of the ellipse respectively	m	3.10
dF/dY	initial slope of the force/deflection curve	N/m	3.8
D	root cone diameter	m	3.6
E	Young's Modulus	Nm^{-2}	
EI	flexural rigidity	Nm^2	3.8 3.9
F_{max}	maximum force a stem can withstand before it fails	N	3.7
g	the acceleration due to gravity	$N{\cdot}kg^{-1}$	3.3 3.4 3.13 3.14
h_P	the height of the centre of gravity of the plant	m	3.4 3.11
h_S	the height of the centre of gravity of the shoot	m	3.3 3.13
I	second moment of area	m^4	3.9 3.10
k	dimensionless proportionality constant linking S_A to τ and D		3.6
L	distance between the supports of the three point bending test	m	3.7 3.8
M_P	self weight moment of the whole plant	Nm	3.1 3.4 3.5 3.14
m_P	fresh plant mass	kg	3.4 3.14
M_S	self weight moment of the shoot	Nm	3.2 3.3 3.5 3.13
m_S	fresh shoot mass	kg	3.3 3.13
R	the rate at which the function of h_P changes over time	$cm{\cdot}d^{-1}$	3.12
S_A	root anchorage strength	Nm	3.1 3.6
SF_A	safety factor against anchorage failure		3.1
SF_S	safety factor against stem failure		3.2
S_S	maximum self weight moment Nm before shoot failure		3.2 3.7
Greek Symbols	**Explanation**	**Units**	**Eqns.**
α	constant derived by regression		3.13
β	constant derived by regression		3.13
τ	soil shear strength	Nm^{-2}	3.6
θ	angle of the plant or shoot	°	3.3 3.4 3.13 3.14

Introduction

Teff (*Eragrostis tef* [Zuccagni] Trotter) is a panicle bearing C_4 cereal crop (Kebede *et al.*, 1989) originating from Ethiopia. Teff grains and flour do not contain gluten (Spaenij-Dekking *et al.*, 2005) and are rich in minerals, especially iron (Mengesha, 1966; Abebe *et al.*, 2007; Verdonschot *et al.*, 2008). These two characteristics make teff a desirable ingredient in health products, particularly for celiac disease patients. Teff can replace gluten containing cereals in products like pasta, bread, beer, cookies and pancakes. Ethiopia, where teff is the main cereal crop and food shortage a recurring phenomenon, exerts an export ban on teff. Therefore interest rose in growing teff outside Ethiopia. Teff was recently introduced in north western Europe (Hopman *et al.*, 2008). As in other regions where teff is cultivated, teff yields in the Netherlands are modest (1.0-1.5 Mg·ha^{-1}) and quality is often low. A major factor limiting yield and quality is lodging. Lodging can be defined as the permanent displacement of a plant from the vertical (Berry *et al.*, 2004). In teff, lodging frequently occurs before the grain filling period starts. Lodging prevents the crop from ripening properly and often results in mouldy panicles, inferior seed quality and sprouting seeds on the panicle.

In Ethiopia, lodging of teff is also a common phenomenon and one of the causes for the current low grain yields: the Ethiopian national average grain yield of teff is in the order of 0.8 Mg·ha^{-1} (Tulema *et al.*, 2005). This low national average is partly associated with constraints such as water logging, drought and nutrient limitation (Tulema *et al.*, 2005). The yield of well fertilized unsupported plants in 'on station' field experiments is on average in the order of 2.5 Mg·ha^{-1} (Tulema *et al.*, 2005). Teklu and Tefera (2005), however, reported yields up to 4.6 Mg·ha^{-1} for teff supported with nets to prevent lodging. Yizengaw and Verheye (1994) consider 4.6 Mg·ha^{-1} as a good approximation of the yield potential of teff under rainfed conditions in Ethiopia. The difference in yield between naturally growing teff and supported teff implies that solving teff's lodging problems would dramatically increase actual yield. Lodging resistance, therefore, is the main focus in several breeding programmes (Assefa *et al.*, 2011; Berhe, 1973; Ketema, 1991; Hundera *et al.*, 2000; Zhang *et al.*, 2001; Tefera *et al.*, 2003; Yu *et al.*, 2006).

Crook and Ennos (1994) developed simple equations to investigate lodging phenomena in cereals. These static equations predict a relative degree of susceptibility to anchorage failure and shoot failure, known as root and shoot lodging, respectively. According to Crook and Ennos (1994) lodging susceptibility in cereals depends on three factors: (i) the size and dynamics of the forces to which the plant is subjected (Pinthus and Brady, 1974); (ii) the bending strength of the shoot and its resistance to buckling; (iii) the anchorage strength of the root system. Although the static equations developed by Ennos and co-workers only take factors (ii) and (iii) into account, while neglecting influences by wind, the results corresponded well with field observations. Yet, these equations do not aim to predict the actual onset of lodging in the field as a result of weather conditions (Baker *et al.*, 1998).

In this study three main issues are addressed. The first and main objective of the study is to measure the biomechanical properties of teff in order to distinguish whether the plant is more susceptible to shoot or root lodging. To analyze the lodging susceptibility of teff during a Dutch growing season we designed an apparatus to assess *in situ* biomechanical properties of teff. Two morphologically contrasting cultivars were studied.

The second objective is to examine the applicability of the models of Crook and Ennos (1994) to the problem of assessing the effects of plant structure on lodging risk of teff and rice. These authors worked with wheat, i.e. shoots with erect spikes, whereas teff has a drooping panicle. Hence, for teff the self weight moment of an erect shoot is not zero as is assumed in these model equations. Moreover, a second model assumption is that the shoots as a whole behave as uniform rigid beams. This assumption is incorrect for most rice and teff cultivars, given that their tapering shoots are known to bend under their own weight.

The third aspect addressed is the modifying effects on lodging of water adhering to the shoots as a due to dew or rain. Lodging of cereals often occurs as a consequence of rainstorms (Berry *et al.*, 2004). The common perception is that the effect of rain water on lodging is primarily brought about via the lubricating effect on the soil, reducing root anchorage strength (Crook and Ennos, 1993; Baker *et al.*, 1998; Sposaro *et al.*, 2008). Wind gusts exercise forces on the shoot system which can lead to root lodging or shoot lodging in firmly anchored plants. We observed that root lodging of teff also occurred in the absence of wind when plants became wet due to dew or drizzle while the soil surface was practically dry (Video S1)[1]. This points at a direct physical effect of the weight of water adhering to the shoot on lodging. Though papers presenting more elaborate models like Baker *et al.* (1998), Berry *et al.* (2003) and Berry *et al.* (2007) mention a possible influence on lodging susceptibility of water adhering to stem, leaves and panicle, no data to verify this hypothesis have been published; neither is adhering plant surface water accounted for in any of the models for cereal lodging existing today.

This chapter elaborates first on the theoretical background of equations which were used and modified during the current work. Thereafter the experimental procedures and applied statistical methods are provided followed by the results of these experiments. The major findings of this work are summarised and the morphology and lodging characteristics of teff are compared with other cereals. The conclusions of this work are placed in context and recommendations are made for breeding and further research.

Materials and methods

Theory

Crook *et al.* (1994) were the first to apply the concept of 'factor of safety' to a phenomenon in plant science, namely lodging. The safety factor (SF) indicates the number of times a

[1]Video S1 [http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.2010.03224.x/suppinfo]

support organ can bear the self weight moment (M) of the organ it is supporting. Crook *et al.* (1994) defined a safety factor against anchorage failure (root lodging) and a safety factor against shoot failure (shoot lodging). The safety factor against anchorage failure (SF_A) is given by:

$$SF_A = \frac{S_A}{M_P} \tag{3.1}$$

where S_A (Nm) is the root anchorage strength, i.e. the maximum moment at $\theta°$ from the vertical that a root system can withstand before rotating further in the soil; M_P (Nm) is the self weight moment of the whole plant at $\theta°$ from the vertical (eqn 3.4).

Analogous to this, the safety factor against shoot failure (SF_S) is given by:

$$SF_S = \frac{S_S}{M_S} \tag{3.2}$$

where S_S (Nm) is the maximum self weight moment which the shoot can support before it fails and M_S (Nm) is the self weight moment at θ from the vertical (eqn 3.4).
Parameters M_S, M_P, S_A and S_S can be directly measured with a dedicated device ('lodging meter') or calculated with the following equations.

Under the assumption that the whole shoot behaves as a rigid beam, M_S (Nm) is given by:

$$M_S = \sin\theta \cdot h_S \cdot m_S \cdot g \tag{3.3}$$

where θ is the angle of inclination from the vertical, h_S (m) is the height of the centre of gravity of the shoot, m_S (kg) is the mass of the shoot and g (N·kg^{-1}) is the acceleration due to gravity. Similarly M_P (Nm) is given by:

$$M_P = \sin\theta \cdot h_P \cdot m_P \cdot g \tag{3.4}$$

where h_P (m) is the height of the centre of gravity of the plant and m_P (kg) is the mass of the plant. Since plants often have a whimsical, three dimensional structure, h_P can be hard to obtain; alternatively M_P is given by:

$$M_P = \sum_{i=1}^{n} M_{S_i} \tag{3.5}$$

where n is the number of tillers per plant and M_S (Nm) is the moment of an individual shoot at $\theta°$ from the plant's vertical.

Root system anchorage strength is influenced by four main factors: root strength and rigidity, the number of roots, the angle of inclination of roots and the soil shear strength

(Ennos, 1991). Crook and Ennos (1993) developed an equation which theoretically integrates these parameters. Baker (1998) replaced the theoretical proportionality constant k with an empirical constant derived by regression. Assuming the roots will not snap, S_A can now be estimated by:

$$S_A = \tau \cdot D^3 \cdot k \tag{3.6}$$

where τ (Nm^{-2}) is the soil shear strength, D (m) the root cone diameter and k is a dimensionless constant.

The bending strength of the base shoot section represents the maximum self weight moment (S_S) (Nm) which the stem base can support before it fails. S_S can be measured in a three point bending test (for measuring procedure see Materials and Methods section: "Bending tests and microscopic observations"). Considering the base stem section as a uniform beam, S_S is given by:

$$S_S = \frac{F_{max} L}{4} \tag{3.7}$$

where F_{max} (N) is the maximum force a stem will withstand before it fails and L (m) is the distance between the supports in the three point bending test.

According to Crook *et al.* (1994) the measure for stiffness of the stem section, i.e. flexural rigidity (EI) (Nm^2), is given by:

$$EI = \frac{L^3(dF/dY)}{48} \tag{3.8}$$

where dF/dY (N/m) is the initial slope of the force/deflection curve, obtained from the bending tests. Estimation of the slope (dF/dY) was restricted to the linear elastic part of the force/deflection curve. Young's Modulus (E) (Nm^{-2}), the measure for material elasticity for the stem as a composite (the higher value E, the stiffer the material), is given by:

$$E = \frac{EI}{I} \tag{3.9}$$

where I is the second moment of area (m^4) of a hollow, ellipse-shaped beam given by:

$$\mathrm{I} = \frac{\pi}{4(a_1^3 \cdot b_1 - a_2^3 \cdot b_2)} \tag{3.10}$$

where a_1 and a_2 are the large diameter of respectively the outer and inner ellipse (m), and b_1 and b_2 the short diameter of respectively the outer and inner ellipse (m).

Field conditions and plant material

Two contrasting cultivars, coded 04T19 and Ayana were obtained from Foundation Share (the Netherlands), cultivar 04T19 is thicker-stemmed, more robust, taller and later flowering cultivar than cultivar Ayana; at similar plant population density (200 m^{-2}) Ayana tillered more profusely than 04T19. Both genotypes have been developed by mass selection from landraces.

Seeds were hand sown in a 7 × 7 cm grid in approximately 8 mm deep holes, in a smooth sowing bed in a sandy soil near Wageningen (the Netherlands, lat 51°59'22"N, 5°39'38"E) on May 23 2008. Both cultivars were grown under two conditions: without and with support of plants through nets. Cultivars and netting treatments were set up in a completely randomized block design containing six blocks and four treatments (two cultivars times two levels of support).

At the two-leaf stage, plots were thinned and plants were occasionally transplanted to obtain one plant per grid hole. Transplanted plants were labelled and discarded from the actual observations. Plot dimensions were 0.98 m (15 plants) by 5.32 m (76 plants) resulting in a plant population density of approximately 219 plant m^{-2}.

Shortly after sowing a vertically moveable construction with two layers of 10 × 10 cm meshed gauze was installed in the netting treatments. Nets were raised according to the crop height, to keep the plants as stable as possible. The outer four rows of the plots were not sampled in consideration of atypical border effects (Scott *et al.*, 2005). With these restrictions, each week, plants were randomly selected from a randomly selected spot at the top or bottom end of the plot (in order to keep the crop structure in the middle of the plot intact during the whole experiment). Plots were bordered with at least 2 m of land planted to maize, starting at 0.5 m from the edge of the plot. These maize plants were trimmed to the crop height of teff.

Soil tests indicated more than sufficient availability of Potassium (K) and Phosphorus (P). Nitrogen (N) fertilizer was applied at a rate of 35 kg·ha^{-1} resulting in free available mineral N of approximately 60 kg·ha^{-1} including dry and wet nitrogen deposition. As a precaution to prevent manganese deficiency, sulphur-manganese (0.35 kg·ha^{-1} $MnSO_4$) was sprayed on the foliage on June 18 and July 2 2008.

Rice (*Oryza sativa* L.) cultivar Quinai was used for a cross-species equation validation (eqn 3.13). Seeds were obtained from Qingdao Agricultural University in China. Plants were cultivated in containers with approximately 220 plants per m^2 on a half strength Hoagland nutrient solution in a climate chamber at 26/23°C night/day temperature and 12 hours day length under a mixture of SON-T and HPI (500 μmol·m^{-2}·s^{-1}).

Measurements on whole plants, shoots and panicles

The percentage of plants lodged by more than 45° was estimated twice per week. Crop height, defined as the distance between the soil and average plant height on a plot, was measured in the field before each sampling. Directly after each sampling the plants were

placed in a cooled container, individually placed in moist plastic bags that were loosely wrapped around the plants. Within 20 minutes after harvest the plants were ready for examination in the lab. When measurements required plants to be dry, plants were dried with strongly absorbing soft paper towels.

Following the measurements on the whole intact plants, the three biggest individual shoots were cautiously removed from the plant. Centre of gravity of the whole plant and of separate shoots were determined by balancing them on a thin smooth metal tube and measuring the distance between the balance point and their base end. The gravitational moment of plants, shoots and panicles under 0°, 30°, 45° and 60° was established with a custom constructed lodging meter (Fig. 3.1), built from a sensitive digital "torque screwdriver" (reading up to 1.5 Nm in 0.001 Nm intervals; Mecmesin Ltd, Broadbidge Heath, UK). Plant or separate shoot fresh weight and the lengths of stem, peduncle and panicle were recorded.

To study the impact of adhering water, plants and separated shoots were sprayed with a plant spray until they showed water runoff. Under these standardized wet conditions, fresh weight, the centre of gravity and gravitational moment were measured again for intact plants and individual shoots. Wetting and drying the same stem repeatably demonstrated that the weight increase by wetting had a maximum deviation of ±7%. All measurements made on both teff cultivars were also made on rice stems.

The number of heading plants (i.e. cereal plants that show the tip of their inflorescence) was scored each subsequent day when heading was expected. Teff plants are mainly self-pollinating and heading and flowering almost coincide in teff (Mengesha and Guard, 1966). We will use the more general term flowering instead of heading; and defined the moment of flowering for a cultivar when 50% of all the plants showed the tip of their panicle.

Bending tests and microscopic observations

After the measurements on the whole shoot the plants were kept overnight in a plastic bag with about 10 cm water at 4 °C to bring the plants to a standardized turgor level. For shoots larger than 10 cm, sections of 10 cm were taken at the basis, the geometrical midst, 80% of the stem length and of the peduncle just above the leaf sheath collar. These four sections were subjected to a standard three-point bending test, analogous to (Oladokun and Ennos, 2006), using a Zwick Universal Testing Machine (model BZ2.5/TS1S with positioning, repetition accuracy ± 2 μm and accuracy of the set speed 0.02 % of V_{nom}). Stem sections were placed on two supports set 60 mm apart, while a blunt rubber probe with a diameter of 20 mm, attached to the crosshead of the Zwick, was moved down at a speed of 50 mm·min^{-1}, touching the stem midway between the supports and bending it. A force/displacement graph was simultaneously recorded by a connected computer and was used to calculate the mechanical properties of the stem section.

After the bending test, cross sections were made from all stem parts near the location of impact by the probe. Stem cross sections were coloured with phloroglucinol (Jensen,

1962); high resolution digital images were made under a stereo microscope. The stem tissue layers were distinguishable and their dimensions were measured using ImageJ, version 1.42 (Rasband, 2009).

Measurements on the root system

The evening before measuring the root system, only the relevant plot locations were watered to soil saturation. Next morning, before the actual measurements began, these plot locations were watered again and allowed to drain to field capacity under gravity for at least one hour. This gave simulated soil conditions typical of conditions after rain and comparable to other studies (Crook *et al.*, 1994; Baker *et al.*, 1998; Oladokun and Ennos, 2006; Sposaro *et al.*, 2008). Soil shear strengths (i.e. the maximum resistance of a soil to shearing stresses) were measured with a shear-vane; the average was 13.1(standard error 1.7) kPa. Plants were cut at 11 cm height. To make the remaining shoot parts (stubble) behave like a rigid beam, a pointed light-weight hollow metal pin was placed in the middle of each stem base joining at the bottom of the plant. Next, all stem bases and the metal pin were bundled together with a fastener. Lastly, the stubble-pin combination was slowly pushed to an angle of respectively 30°, 45° and 60° from the vertical while the lodging meter (Fig. 3.1) recorded the maximum resistance of the rooting system. Measurements were corrected for the self weight moment of the pushing device and stubble-pin combination.

The soil core containing the bristles and rooting system was dug up and preserved in a cooling box. In the lab, cores were pushed on a 2 cm grid pinboard and soil was gently washed away exposing the rooting structure. Photographs were made from the root system and root dimensions were measured with ImageJ version 1.42 (Rasband, 2009). Root plate diameter was measured at the theoretical rotation point of the root-soil cone. Like Crook and Ennos (1993) we estimated this rotation point to be half the plant base diameter, the diameter of merging tillers at the soil surface, below the shoot to root transition.

Statistical analysis

SAS version 9.1.3 SP4 was used when data were statistically analysed. To test whether the safety factor (SF) of wetted plants deviated from that of the dry plants, the data were modelled with the PROC MIXED procedure of SAS taking wetting and time as main effects and regarding blocks and differences between shoots from the same plant as random effects:

$$SF = \text{time} + \text{wet} + \text{time} \cdot \text{wet} \tag{3.11}$$

Assessment for significant differences was done with the least square means (LSMEANS) statement (p=0.001).

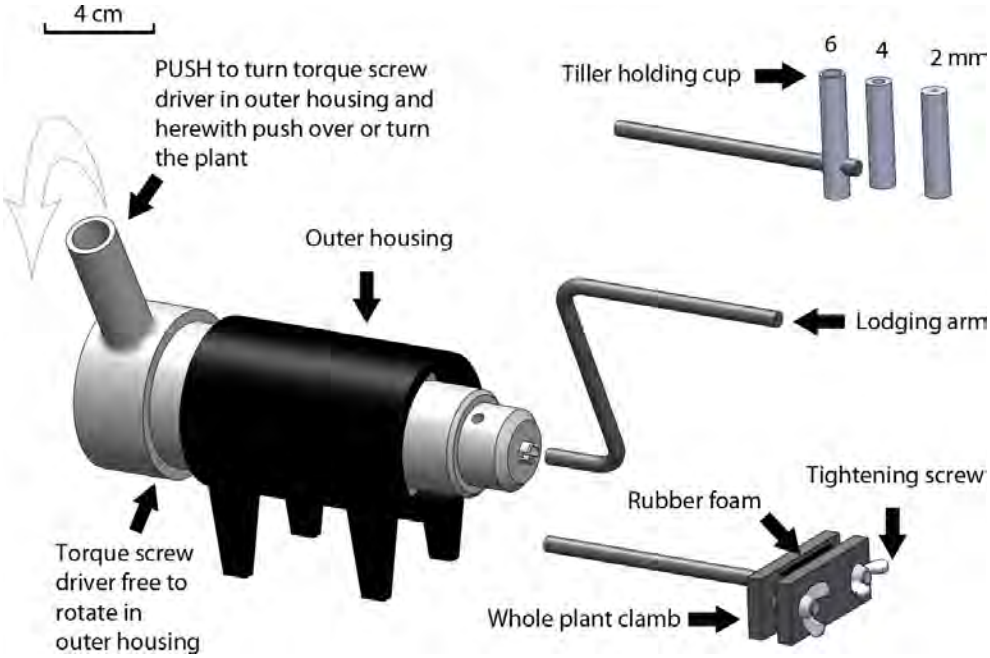

Fig. 3.1: Plan of the lodging meter parts are exchangeable. By pushing the turning arm the torque screwdriver rotates in its outer housing. Rotating the screwdriver also rotates attached lodging arm or tiller holding cup or whole plant clamp. Security spikes fix the meter in the soil. Concept based on (Crook and Ennos, 2000).

The increase in height of the centre of gravity (h_P) (cm) of wet and dry plants with time (t) (d) was described by Richards sigmoids (Berry *et al.*, 1988):

$$h_p(t) = h_{pMAX}\left(1 - b \cdot \exp\{R \cdot t\}\right)^{\left(\frac{1}{1-c}\right)} \tag{3.12}$$

where h_{pMAX} (cm) is the asymptotic maximum of h_P, b is a scale-dependent parameter, R is the rate at which the function of h_P changes (cm·d^{-1}) and c is the shape parameter. Richards sigmoids and power regression lines (eqn 3.13 and 3.14) were estimated using PROC NLIN procedure. Whether the data of wet plants could be described by a Richards sigmoid fitted for dry plants or whether other parameter values were necessary for wet plants was tested with an F-test on the residual sum of squares of both models. Means in the text are followed by their standard error in parenthesis.

Results

Crop and plant morphology

After a precipitation event on 17 July 2008, the plants of cultivar Ayana started to deviate from the vertical but did not exceed a displacement of 30°. On the 30th of July 2008 we observed severe lodging in the unsupported plots of Ayana (Fig. 3.2). Then, also some of the unsupported plots of 04T19 started to deviate from the vertical. Within two weeks after the start of lodging the plants in the unsupported plots of both cultivars Ayana and 04T19 were totally root lodged, whereas plants in the supported stands were undamaged and upright.

Crop height (Fig. 3.2) and the plant's centre of gravity (Fig. 3.3) displayed a similar pattern of change over the season. After both crop height (Fig. 3.2) and plant fresh weight (Fig. 3.4a) had reached their plateau level, the height of the plant's centre of gravity still kept increasing (Fig. 3.3) because of the continued accumulation of dry matter in the top of the plant.

The relative weight increase over time, due to wetting was not constant but followed a quadratic polynomial. The relative weight increase due to wetting was 25 % at the beginning of the season,15 % in the middle and 25 % at the end of the season. The seasonal average of the relative weight increase by wetting of cultivars Ayana and 04T19 was 18% and 15%, respectively. Wetting also significantly increased the plant's centre of gravity ($p>0.001$) (Fig. 3.3), although the magnitude of the effect was on average 5%.

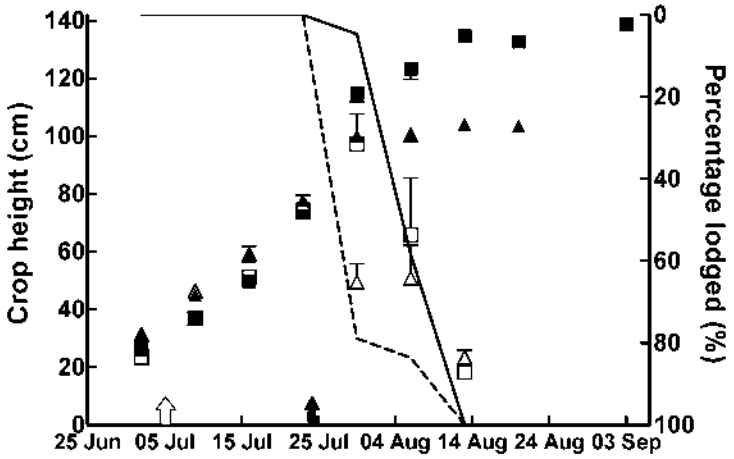

Fig. 3.2: Development of crop height (cm) over time of cultivars Ayana (triangles) and 04T19 (squares), either supported (closed symbols) or unsupported (open symbols). Flowering time defined as 50% flowering plants is shown for cultivar Ayana (open arrow) and cultivar 04T19 (closed arrow). Percentage of lodged plants is indicated with the broken line for Ayana and the solid line for 04T19. Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

Shortly after flowering tillering ceased, tiller number reaching values of 9-10 tillers per plant for Ayana and 5 to 6 tillers per plant for 04T19. From this moment onwards the number of viable tillers started to decrease resulting in about 7 mature basal shoots per plant for Ayana and 3 to 4 for 04T19.

Until the lodging started in unsupported plants there were no significant differences in the trends between the supported and unsupported plant stands for any of the characteristics measured.

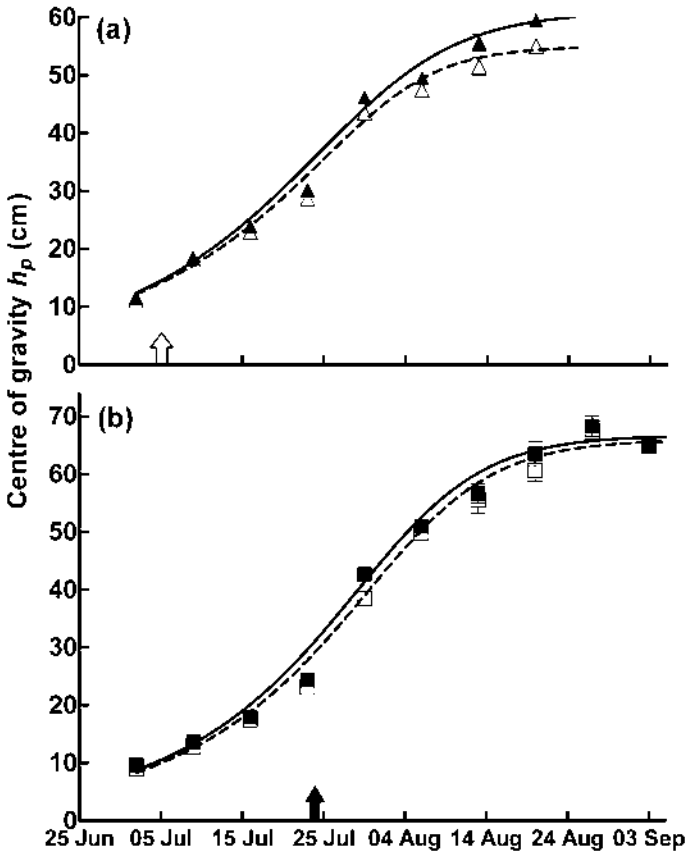

Fig. 3.3: Height of the centre of gravity (cm) and fitted Richards sigmoids of wet (closed symbols, drawn line) and dry (open symbols, broken line) plants; for (a) cultivar Ayana and (b) cultivar 04T19. Flowering time defined as 50% flowering plants is shown for Ayana (open arrow) and 04T19 (closed arrow). Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

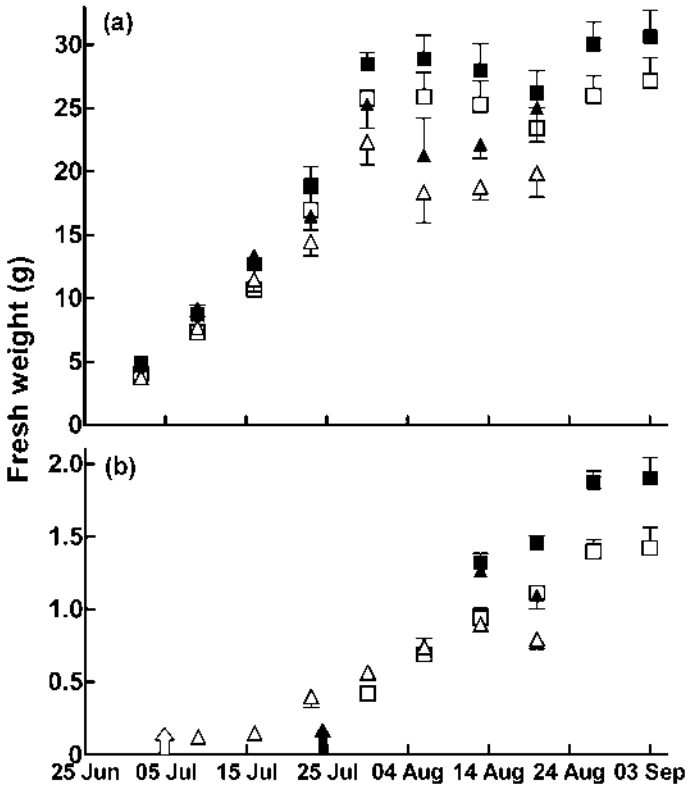

Fig. 3.4: (a) Average total shoot fresh weight for cultivar Ayana wet (closed triangle) and dry (open triangle) and cultivar 04T19 wet (closed square) and dry (open square) and; (b) fresh weight of the wet and dry panicles separately; same symbols as in (a). Flowering defined as 50% flowering plants is shown for Ayana (open arrow) and 04T19 (closed arrow). Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

Morphological and mechanical properties of the shoot

Teff shoots are known to bend easily under their own weight; and in combination with the drooping panicles this will generate a gravitational moment even if the shoot base is standing up straight. This complicates the usage of eqn 3.3 and 3.4, because these equations assume M_S and M_P to be zero in a perfectly upright position. Nevertheless, measuring the actual moment of plants or separated shoots and plotting them against the calculated moment (eqn 3.3 and 3.4) at a particular $\theta°$ did reveal a systematic deviation (Fig. 3.5a). Plotting the deviation from the equation as a function of $\theta°$ provided a correction for the estimated M_S and M_P given by:

$$M_S = \alpha \cdot (\sin\theta \cdot h_S \cdot m_S \cdot g)^{\beta} \tag{3.13}$$

and M_P is given by:

$$M_P = \alpha \cdot (\sin\theta \cdot h_P \cdot m_P \cdot g)^{\beta} \tag{3.14}$$

where α and β are constants derived by regression analysis. Rice and teff showed strikingly similar values for α and β (Fig. 3.5b).

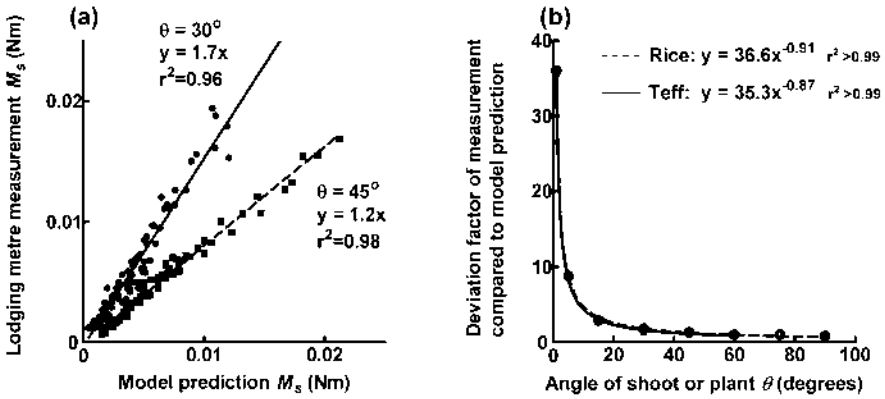

Fig. 3.5: (a) Scatter plots and regression lines of measured shoot selfweight moment (M_S) versus predicted moment of M_S (eqn 3.3) at angles (θ) of 30° (closed circle; solid line) and 45° (closed square; broken line). (b) Deviation factor of measured M_S compared to equation prediction of M_S over a range of angles (i.e. data points are slopes like presented in Fig. 3.2 a for teff plants (closed triangle), teff shoots (closed circle) or rice shoots (open circle). Where x is represented by eqn 3.3 (shoot) and 3.4 (plant) and y= $\alpha \cdot x^{\beta}$ is represented by eqn 3.13 (shoot) and 3.14 (plant).

Between the two cultivars, there were marked differences in measured parameter values for: bending strength (S_S) (eqn 3.2 and 3.7), flexural rigidity (EI) (eqn 3.8 and 3.9), Young's Modulus (E) (eqn 3.8) and stem dimensions (eqn 3.10) (Fig. 3.6). Bending strength (S_S) and flexural rigidity (EI) increased rapidly with time but levelled off approximately 8 days after flowering in both cultivars (Fig. 3.6a,b). Shoots often failed instead of breaking and therefore bending strength and flexural rigidity were strongly correlated (r^2=0.92).

Cultivar 04T19 showed values for both bending parameters S_S and EI that were four times higher than values measured in Ayana. In contrast, Ayana showed higher tissue stiffness (Young's Modulus, (Fig. 3.6c) than 04T19. Therefore predictably (eqn 3.9 and 3.10), the second moment of area was higher for 04T19 than for Ayana as was the independently measured stem diameter (Fig. 3.6d). Dry weight per volume of tissue (i.e. "tissue density") was on average higher for Ayana 0.060 (0.017) g·cm^{-3} than for 04T19 0.046 (0.020) g·cm^{-3}. In both cultivars the increase in Young's Modulus over time coincided with an increase in "tissue density" (r^2=0.76 for Ayana and r^2=0.83 for 04T19) (data not shown).

Stem cross sections showed an elliptic shape. The smallest width of the ellipse (Fig. 3.6d) showed a seasonal pattern of increase comparable to the largest stem width (not shown); the largest width of the ellipse was on average for Ayana 1.071 (0.08) times larger than the smallest width and for 04T19 1.22 (0.14) times so.

Microscopic analysis of stem cross sections stained with phloroglucinol, showed the lignified surface of epidermal, sclerenchymatic and parenchymatic tissue. The total lignified tissue surface was not correlated to the Young's Modulus (r^2 <0.05 data not shown) for both cultivars. Neither was the Young's Modulus correlated with the cross-sectional surface area of any of the three individual tissues. Results did not improve if cross-sectional areas were expressed as fractions of the total stem tissue surface area (r^2 <0.05 data not shown). There was also no significant correlation between the number of vascular bundles per unit surface area and the Young's Modulus (r^2 <0.05 data not shown).

The absolute weight increase by wetting individual shoots showed a linear increase with time (r^2 >0.96 for both cultivars; data not shown). The panicle of 04T19 contained about 56% of the plant's total adhering water and for Ayana this was 64%. The fraction of adhering water present in the panicle was larger than would be expected on basis of its length or fresh weight. Panicle length was about 30% of the total stem length in both cultivars, the fraction of shoot fresh weight present in panicles was 0.3 for Ayana and 0.2 for 04T19.

For both cultivars the safety factor against shoot lodging (SF_S) (eqn 3.2) dropped to values close to one halfway the growth cycle (Fig. 3.7a). The shoot self weight moment (M_S) of cultivar 04T19 kept increasing whereas plant bending strength (S_S) did not increase. Therefore SF_S declined to low values during the season; moreover SF_S was systematically reduced by wetting the plants. However, the absolute critical SF_S value of one was not reached (Fig. 3.7a). This implies it is unlikely that gravitational forces alone could break the basal region of the shoots.

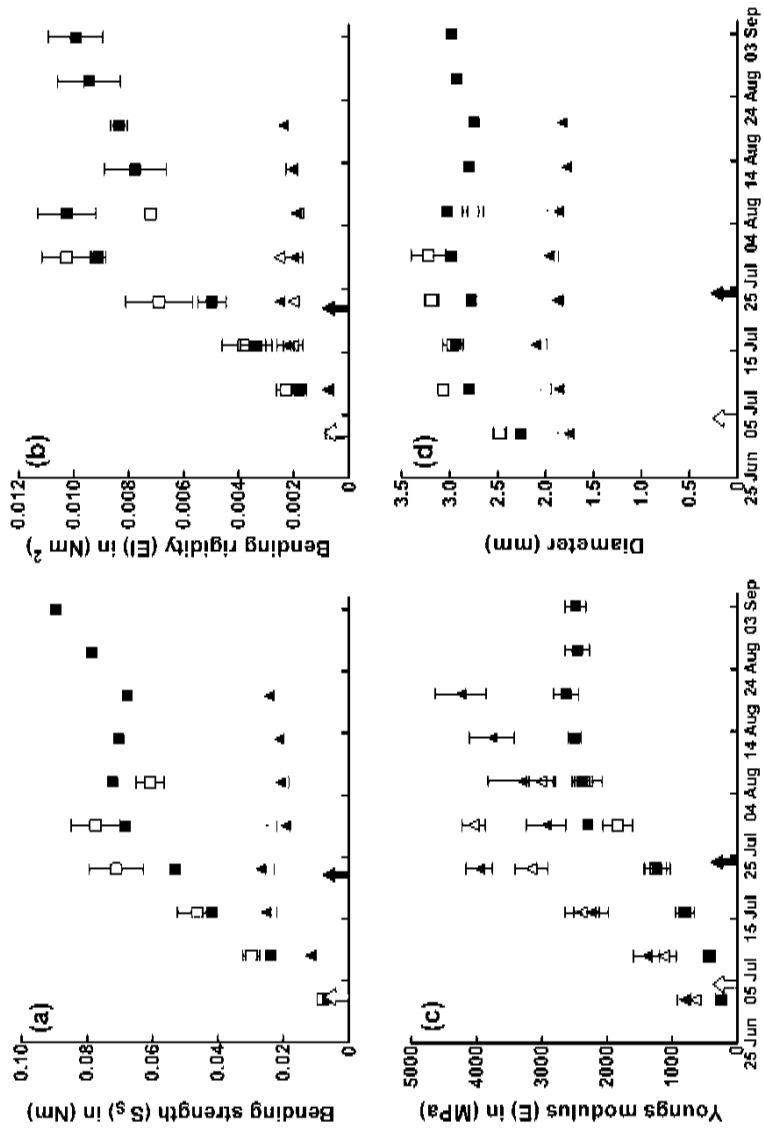

Fig. 3.6: Mechanical properties of the basal region of the shoots during the growing season. (a) Bending strength (S_S) (eqn 3.7), (b) Flexural rigidity (EI) (eqn 3.8), (c) Young's Modulus (E) (eqn 3.9) and (d) Smallest shoot diameter at 5 cm height. For cultivar Ayana supported (closed triangle) and unsupported (open triangle) and cultivar 04T19 supported (closed square) and unsupported (open square). Flowering time defined as 50% flowering plants is shown for cultivar Ayana (closed arrow) and cultivar 04T19 (open arrow). Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

The safety factor against breaking of the peduncle by its own weight was 2-3 times higher than the SF_S (data not shown).

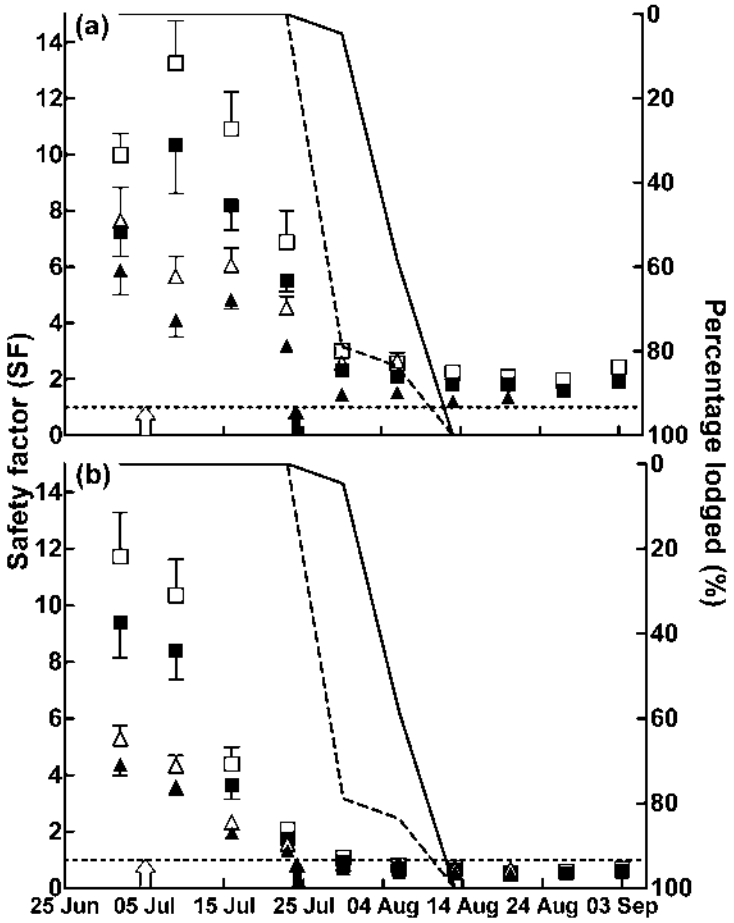

Fig. 3.7: Factor of safety (symbols) for (a) shoot (SF_S) and (b) root (SF_A), during the season; for cultivar Ayana wet (closed triangle) and dry (open triangle) and cultivar 04T19 wet (closed square) and dry (open square). Observed lodging percentage in unsupported fields for Ayana (broken line) and 04T19 (solid line). Horizontal dotted line is the critical safety factor (SF=1). Flowering time defined as 50% flowering plants is shown for Ayana (open arrow) and 04T19 (closed arrow). Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

Morphology and mechanical properties of the roots

Because of its small seeds teff has to be sown at a maximum depth of 10 mm, resulting in crown roots that emerge at or above the soil surface. These crown roots form a bundle of around 23 initially vertically growing lignified roots (Table 3.1). Approximately 1 cm below the soil surface these root bundles started to spread out at an angle of 35° (Ayana) and 45° (04T19) from the vertical. During a short period early in the season the crown roots became thicker (Fig. 3.8); these changes coincided with a measured increase in anchorage strength (Fig. 3.8). In 04T19 we observed thick, rigid roots compared with the thinner, flexible roots of Ayana. The anchorage strength of Ayana showed a low correlation ($r^2 = 0.14$) with the product of the cube of root plate diameter and shear strength (eqn 3.6), whereas in 04T19 the correlation was higher ($r^2 = 0.55$) (Fig. 3.9). This suggests that unlike Ayana (thin roots), 04T19 (thick roots) was able to form a root-soil cone to some extent.

Until flowering, the safety factor against root lodging (SF_A) was sufficiently high for both cultivars to support the plants. Shortly after flowering, however, the safety factor dropped below the threshold value of one (Fig. 3.7b), implying root anchorage was too weak to prevent lodging even while only gravitational forces are taken into account, so disregarding events of wind for instance. This calculated point in time when the average SF_A dropped below one coincided with the independently observed onset of lodging in the field (Fig. 3.7b).

Table 3.1: Overview of estimated plant characteristics during late development of two genotypes of teff (this study), winter wheat (Crook *et al.*, 1994) and (Crook and Ennos, 1993)[*1], and rice (Oladokun and Ennos, 2006) and (Chuanren *et al.*, 2004)[*2].

	Teff		Winter wheat	Rice
	Ayana	**04T19**		
Flexural rigidity (EI) in (Nm^2)	0.002	0.01	0.04	1.8
Young's Modulus (E) in ($\times 10^{-3} Nm^{-2}$)	3.8-4.2	2.4-2.7	1.8-2.6	1.2-3.0[*2]
Shoot diameter (mm)	1.8	3.2	4.5	6.6
Bending strength (S_S) in (Nm)	0.02	0.07	0.16	2.5
Shoot length (m)	1.3	1.7	0.8	1.0
Factor of safety shoot (SF_S)	1.3	2.2	6	-
Plant base diameter	10.7	13.9	-	-
Angle of root inclination ($\theta°$)	37	45	93-103[*1]	-
Lignified crown roots	23	23	6-9	100
Root plate diameter (mm)	11.3	14.9	26.3-29.5[*1]	-
Factor of safety root (SF_A)	>1	>1	3	5

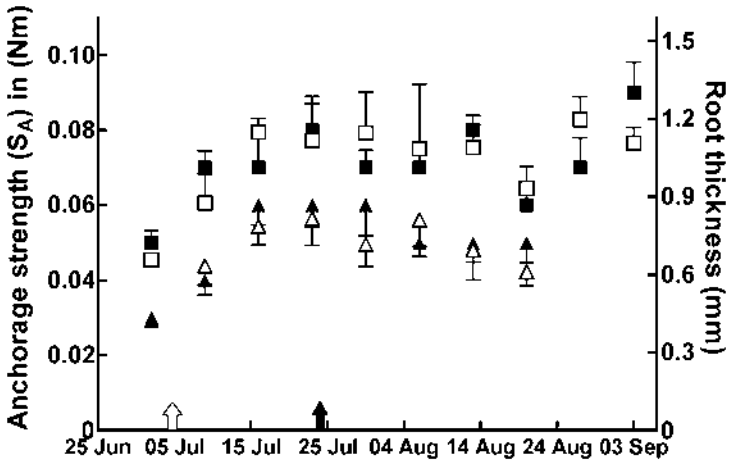

Fig. 3.8: Anchorage strength (S_A) (closed symbols, left vertical axis) and root thickness of six thickest roots (open symbols, right vertical axis), during the growing season. Triangles represent cultivar Ayana and squares cultivar 04T19. Flowering time defined as 50% flowering plants is shown for Ayana (open arrow) and 04T19 (closed arrow). Error bars indicate standard error (n = 6) and when not visible fall within the symbol.

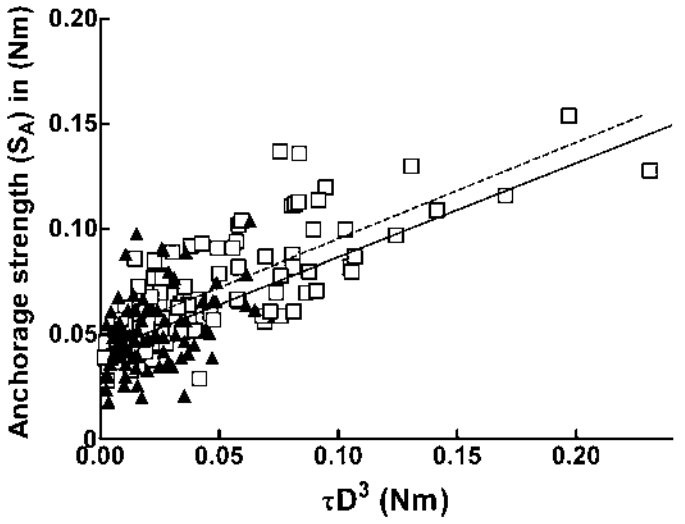

Fig. 3.9: Measured anchorage strength at 45° versus calculated anchorage strength [shear strength (τ) · root plate diameter 3 (eqn 3.6)]; for cultivar 04T19 (open square, broken line: $y = 0.46 + 0.049r^2 = 0.55$, n=89) and cultivar Ayana (closed triangle, solid line: $y = 0.45 + 0.042$ $r^2 = 0.14$, n=70).

Discussion

The results of this study show that like wheat (Crook and Ennos, 1994) and rice (Oladokun and Ennos, 2006), teff is also most susceptible to root lodging. This study explains why lodging was observed to occur during all years of practice since the introduction of teff in the Netherlands (2002). Already early during development teff root anchorage strength (S_A) reached its maximum (Fig. 3.8). About two weeks later plant fresh weight reached its plateau level (Fig. 3.4). The plant's centre of gravity, however, kept increasing in height due to panicle emergence, and later on grain filling (Fig. 3.3). This caused an increase in whole plant moment (M_P) until two weeks before harvest. The absolute critical safety factor against anchorage failure of one (i.e. the moment when lodging of unsupported plants is inevitable), was reached shortly after root anchorage strength, S_A reached its maximum but long before the maximum value of the whole plant moment, M_P was reached Therefore, the current *in situ* measurements of the biomechanical properties of the teff cultivars Ayana and 04T19 indicate that, regardless of wind force, lodging of unsupported teff plants is inevitable on sandy soils.

Plant base diameter, the diameter of joining tillers at the soil surface, and the average root plate diameter were small compared with other cereals (Table 3.1). The plant base diameter was, furthermore, frequently larger than the root plate diameter in both cultivars. The root bundles did not spread out from their onset but did so after approximately 1 cm below the soil. Since the plant base was in general larger than the root onset there was a narrowing in plant shape at the shoot-root transition. Therefore we hypothesise that plants were not well anchored: firstly because they were standing on bundles of flexible vertical roots; secondly the stem did not penetrate the soil and therefore crown depth was shallow which most likely reduces anchorage strength as Berry *et al.* (2000) have argued similarly for wheat.

The value of 0.46 in teff for the dimensionless constant k (eqn 3.6) (Fig. 3.9), a scalar serving to link shear strength and root plate diameter with anchorage strength, is similar to values found in other cereal species: 0.43 (Baker *et al.*, 1998) and 0.39 (Berry *et al.*, 2006) for wheat and 0.58 for barley (Berry *et al.*, 2006). Each of these authors forces the line relating anchorage strength to τD^3 through the origin (Fig. 3.9). However, an intercept of zero would theoretically imply no anchorage strength without a root plate. This is inconsistent with reality, since the joined shoots of cereals at the bottom of the plant can form a small plateau (plant base) in or on the soil which provides a certain degree of anchorage strength. Reanalyzing the published data of Baker (1998) and Berry (2006) revealed intercepts of 0.058 Nm (with $k_{new} = 0.36$) and 0.039 Nm (with $k_{new} = 0.36$) for wheat and 0.045 Nm (with $k_{new} = 0.54$) for barley. These values are comparable to the intercepts of 0.042 Nm (Ayana) and 0.049 Nm (04T19) which we found for the teff cultivars. In this line of thinking a higher intercept would need to be found for the thicker stems of sunflower compared to the smaller cereal stems; reanalysis of data of Sposaro (2008) confirmed this: an intercept value of ca. 3.9 (Nm) was found. Therefore we argue that not only the root plate but also the

plant base diameter can have a significant contribution to the plant's anchorage strength. This suggests that an additional component must be added to the equation as well as the resistance of the root soil cone: the resistance of the plant's stem base to being pushed through the soil and possibly help shaping the cone. This component seems to be similar in all the cereal species tested: around 0.05 Nm. Consequently an intercept should be included in eqn 3.6. As a practical consequence breeding for thicker stems will most likely also contribute to an increase of root anchorage strength.

Although teff is clearly susceptible to root lodging, the safety factor against shoot failure for both cultivars is also low. When during a previously conducted pilot field experiment the shoot bases were supported, the moment of lodging was postponed (unpublished results). Such supported plants did not break but were severely bent before harvest. This observation is in line with the current results and provides a further indication that both the root system and the shoots should be improved in order to enhance lodging resistance.

As in wheat, teff's bending strength and flexural rigidity rapidly increased during initial crop development. The values of bending strength and flexural rigidity, though, are very low compared with wheat and rice (Table 3.1). However, the Young's Modulus is higher in teff than in wheat, while the stem diameter is smaller in teff than in wheat. Therefore stem rigidity (EI) could be enhanced by increasing the stem diameter and thus the second moment of area (I) of teff, while preserving the current tissue density (i.e. Youngs Modulus (E)). Furthermore teff flexural rigidity and bending strength were strongly correlated; teff shoots are failing instead of breaking, suggesting that an increase in stem rigidity will also increase stem bending strength.

Adhering water on stem, leaves and especially the panicle significantly reduced both the shoot safety factor (on average Ayana 31% and 04T19 23%) and the anchorage safety factor (on average Ayana 16% and 04T19 18%). The calculated self weight moment (eqn 3.3 and 3.4) for plant angles (θ) smaller than 50°underestimated the actually measured self weight moment of both the whole plant and separated shoots (Fig. 3.5b). Since teff does not grow a straight spike but has a panicle type of inflorescence the simple classical lodging equations developed for wheat (Crook and Ennos, 1994) and rice (Oladokun and Ennos, 2006) are not entirely valid for the panicle bearing crops and need adjustment (eqn 3.13) or need to be replaced by more sophisticated models such as a modified version of Berry *et al.* (2006). In our opinion a future model should be able to calculate the safety factor for wet plants at any given point along the shoot. Considering the safety factors of different sections of the whole shoot is important as the plant's base is not necessarily the weakest point of the stem. According to Lebrowski (1999) the mechanical behaviour of the stems is a result of rather complex shoot-wind interactions, where dynamic loads and thus oscillations are involved. Therefore to be able to accurately predict the lodging moment in the field a future model should include the effect of the forces encountered during strong winds, so integrating the effects of wind drag that cause large deflections in any cereal. This approach can be used to calculate the appropriate shoot dimensions for panicle bearing cereals in

general. The model calculations of self weight moment at any point along the stem can be verified with measurements of our lodging metre.

Based on field observations in teff we presupposed that the shoot base is the most vulnerable shoot part in terms of lodging susceptibility. Our measurements confirmed that the safety factor against peduncle failure was significantly higher than the safety factor for the shoot base in both cultivars. In general the strength of the plant's base, however, is not necessarily the critical point for lodging. In barley (Berry *et al.*, 2006) the peduncle is viewed as being the weakest point along the shoot. Breaking peduncles has also been reported in a few teff cultivars that are commonly not cultivated (Ketema, 1991).

In conclusion the *in situ* field measurements were able to point out the main lodging causes of teff. To the best of our knowledge all published work on lodging in teff was focused on the shoots; here we show that enhancing the anchorage strength of the roots has priority over stem enhancement. Nevertheless breeding efforts should not only focus on a wider root plate diameter and more rigid horizontally growing roots but also on shorter and thicker stems. This study also infers that the high safety factor against shoot lodging reported for rice (Oladokun and Ennos, 2006) is probably too optimistic. This is concluded from both the data on rice reported in this study and from a study by Ishimaru (2008) showing shoots rather than the roots failed during severe rain and winds.

We advocate that further biomechanical lodging research should be done under totally wet conditions a wetted root-system as well as wetted stems, leaves and inflorescences. We surmise that the lack of incorporation of plant surface water partially explains the mismatch between lodging model predictions and reality (Berry *et al.* 2003).

Chapter

4

Photoperiodism in teff:

Analysis of ontogeny and morphology in response to photoperiod

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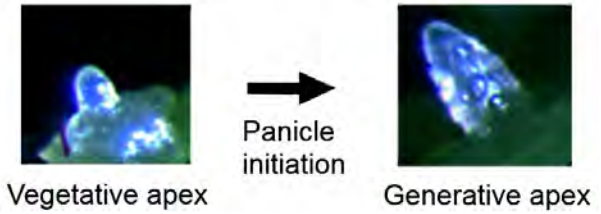

Abstract

• ***Background*:** The Ethiopian cereal teff (*Eragrostis tef* (Zuccagni) Trotter), traditionally grown under short days (11-13 h) (SD), is currently introduced into NW Europe where the early phases of the growing season are characterized by long days (LD) (15-17 h). This chapter analyzes to what degree teff's ontogeny and morphology is day length sensitive.

• ***Methods*:**To describe the day length response of teff regarding panicle initiation, time to heading, number of phytomers, plant height and biomass, phytotron and greenhouse experiments were conducted. In these experiments two Ethiopian cultivars, Gibe and Ziquala, and two cultivars from a Dutch breeding programme, Ayana and 04T19, were exposed to day lengths of 9, 10.5, 12, 13.5, 15, 16.5 and 18 h.

• ***Key Results*:** The Ethiopian cultivars of teff showed a stronger photoperiod response than the Dutch cultivars. For example, time to heading for Ziquala was 88 d under LD but 37 d under SD whereas it was for Ayana 45 d under LD and 29 d under SD. Time to heading could be described using a smooth logistic function consisting of four biologically interpretable parameters. Application of the same function to literature data of rice showed the broader applicability of the approach for cereals. Not only panicle initiation, but also development and outgrowth of the panicle were influenced by photoperiod. Plant-to-plant variation in time to heading, the total number of phytomers per shoot, the number of elongated internodes and biomass were higher in LD than in SD treatments for all cultivars.

• ***Conclusions*:** This chapter provides a detailed description and quantification of the response of teff to day length. The smooth logistic function presented is generally applicable in short day cereals. Our findings suggest that it is feasible to breed for a teff genotype which is well adapted to northern latitudes.

Key words:
teff (*Eragrostis tef* (Zuccagni) Trotter), flowering, heading, photoperiod, day length, phenology, morphology, genetic variation, adaptation

Abbreviations used in this chapter

Symbols	Explanation	Units	Eqns.
D	day length	h	4.1 4.1
D_c	critical day length, after which day length starts to have an impact on time to heading	h	4.1 4.2
D_Δ	day length at which alteration of day length has the strongest impact on change in progress to heading	h	4.1
D_m	maximum day length at which an increase in day length increases time to heading in SDPs	h	4.2
$t(D)$	time to heading	d	4.1 4.2
t_{max}	maximal time to heading	d	4.1
t_{min}	minimal time to heading	d	4.1
${t_{max}}^{-1}$	the inverse of the maximal time to heading	d^{-1}	4.2
${t_{min}}^{-1}$	the inverse of the minimal time to heading	d^{-1}	4.2
LD	long day	h	
LDP	long day plant		
PI	panicle initation, occurrence of a generative apical meristem in 50% of the cases in a sample		
SD	short day	h	
SDP	short day plant		
SSE	sum of squared error (i.e. residual) Σ(data − model)^2		

Introduction

Teff (*Eragrostis tef* (Zuccagni) Trotter) is a C_4 cereal crop species, originating from Ethiopia. Teff grains are rich in minerals, especially iron (Mengesha, 1966; Abebe *et al.*, 2007; Verdonschot *et al.*, 2008) and teff flour does not contain gluten (Spaenij-Dekking *et al.*, 2005). Gluten is a multi-protein complex in grains that can cause celiac disease in genetically predisposed humans (Di Sabatino & Corazza, 2009). For this group and other consumers the multipurpose grain teff is a valuable ingredient in health products. For this reason there is an interest in growing the crop outside Ethiopia for specialty food markets. Teff can be grown in the temperate climates of NW Europe, such as in The Netherlands. Grain yields, however, are modest (1.0-1.5 Mg·ha^{-1}), the harvest is (too) late in the season (approximately end of September) and the crop is sensitive to lodging (van Delden *et al.*, 2010). In Dutch field conditions the harvest index rarely exceeds 0.25 (personal communication from breeders and growers). Growing teff in NW Europe would be economically more attractive if grain yield could become at least 2.5 Mg·ha^{-1}.

Summerfield *et al.* (1997) postulated that most plants originating from tropical latitudes are short-day plants (SDPs). Since teff originates from Ethiopia, an African country near the equator (4-14 °N, 33-47 °E), teff is most likely a SDP. When SDPs are grown under the long day (LD) conditions of the temperate climates of NW Europe, flowering will be postponed (facultative SDP) or impeded (obligate SDP). Later flowering in cereals coincides with a larger final number of leaves, i.e. more vegetative phytomers per shoot (Hay & Kirby, 1991; Yin & Kropff, 1996). This could result in a higher number of elongated internodes, creating longer plants that are more susceptible to lodging (van Delden *et al.*, 2010). Early flowering could advance the grain filling period and harvest time to *ca* mid-August, thus avoiding exposure to the more wet and humid conditions later in the season, associated with increased chances of grain losses caused by grain shedding, diseases and lodging. We postulate that day length sensitivity is the main cause of the late harvest and greatly contributes to low grain quality and yield. Therefore the first objective of this chapter is to analyze to what degree teff's ontogeny and morphology are photoperiod sensitive and the second objective is to assess whether there is sufficient genetic variation in response to photoperiod within four selected, contrasting teff cultivars to encourage breeding for an early flowering, day length neutral, cultivar.

If data on time to flowering is transformed to rate to flowering (i.e. time^{-1}) before data analysis, then discontinuous linear photoperiod response models provide a good representation of the data (Roberts & Summerfield, 1987; Summerfield *et al.*, 1997). However, discontinuous linear (i.e. broken stick models) are in general a crude way of describing the response of a biological process to its environment, smooth curvilinear lines often provide a better description of the data (Schoolfield *et al.*, 1981; Labouriau & Osborn, 1984; Zwietering *et al.*, 1991; Yin *et al.*, 1995; Orozco-Segovia *et al.*, 1996; Yan & Hunt, 1999; Timmermans *et al.*, 2007). It would furthermore be convenient if the model could be fitted directly to the data, instead of first transforming time to heading to rates of progress toward

heading. For these reasons our third objective is to develop a smooth curvilinear function with biologically interpretable parameters that fits the data without the need of data transformation. This curvilinear model will be compared to the conventional linear model (Roberts & Summerfield, 1987). For this comparison not only newly gathered teff data is used but, also literature data on rice (Best, 1961) is used as cross-species model validation.

In theory grain yields of early flowering teff cultivars do not necessarily equal grain yields of late flowering plants, because the larger vegetative biomass produced under long day (LD) conditions may promote the potential yield (Adams & Langton, 2005). Adams and Langton (2005) argued that a potential yield increase, by intentionally mismatching crop photoperiodism and growth environment, is hardly explored by breeders and farmers. This may be for good reasons, because higher shoot biomass accumulation does not necessarily translate into higher grain yields. Our fourth objective, therefore, is to assess whether grain biomass and the ratio between grain and vegetative biomass is influenced by day length under non-stress controlled conditions.

Choosing the optimal harvest time for teff is hard, because there is extensive temporal variation in ripening within the crop. This variation is possibly related to spread in flowering and ripening between tillers on one plant (personal communication from teff growers). Our fifth objective, therefore, is to pinpoint the probable causes of variability in flowering and ripening within the teff crop.

In summary this chapter provides a detailed description of the effect of day length on ontogeny (time to panicle initiation, heading), morphology (final number of leaves, number of internodes, tillers) and growth (shoot biomass, grain biomass, length) of four contrasting cultivars of *Eragrostis tef* (Zuccagni) Trotter). The chapter, furthermore, compares a traditional linear model (Roberts & Summerfield, 1987) and a novel smooth curvilinear model to describe time to flowering in response to photoperiod in cereals.

Materials and Methods

Plant material and growth environment

Four contrasting cultivars coded: Gibe (cultivar DZ-Cr-255), Ziquala (cultivar DZ-Cr-358), Ayana and cultivar 04T19 were obtained from Millets Place (the Netherlands). Cultivars Ayana and 04T19 resulted from a Dutch breeding programme by mass selection from landraces. Cultivars Gibe and Ziquala originate from Ethiopian breeding programmes. Cultivars Gibe and Ayana have a similar appearance, cultivar 04T19 is a thicker-stemmed, more robust, taller cultivar and the appearance of Ziquala is intermediate between Ayana and 04T19.

Two experiments were conducted: a growth chamber experiment with six day lengths (of 9, 10.5, 12, 13.5, 15 and 16.5 h) and a greenhouse experiment with four day lengths (9, 12, 15 and 18 h). This chapter is primarily based on the growth chamber experiment; data from the greenhouse experiment were used to verify the repeatability of the results.

Plants in the six growth chambers received 8 h and 45 minutes of artificial assimilation light provided by 400 Watt SON-T Agro Philips lamps and 400 Watt HPI-T Plus Philips lamps (3.5 lamps·m^{-2}). The photosynthetically active radiation (PAR) provided by the assimilation lights was 475 ±15 μmol·m^{-2}·s^{-1} at canopy level. To maintain constant radiation at the canopy level pots were placed on a plateau that was adjustable in height. Plants in the four greenhouses received 8 hours and 45 minutes of natural day light, after which the greenhouses were covered with a metal hood. In both the climate chambers and the greenhouses day length extending light was from incandescent (Philips Classictone 75 W No. 011503) and fluorescent (Philips Master TLD 58/840 reflex new generation) lamps, positioned in such a way that all plants received at least 14 ±15 μmol·m^{-2}·s^{-1}, well above the threshold of 10±15 μmol·m^{-2}·s^{-1} for full photoperiod response (Foggo & Warrington, 1989). The ratio of red : far-red (660 nm : 730 nm) light established by these day length extending lights was 1.45. In all facilities the day length extending lights were turned on 5 min before the assimilation lamps. For the shortest day length (9 h) the day length extending lights were turned off 10 min after the growth lights. The period of assimilation light was prolonged with day length extending light to establish day length treatments of 10.5, 12, 13.5, 15, 16.5 and 18 h.

All facilities were temperature controlled by either cooling or heating, temperature was set to 16 °C during the 11-h night period and to 23 °C for the 9-h day period that coincided with the assimilation light period. Switching from night to day temperature and vice versa was gradual and took 2 h, resulting in an average daily air temperature of 19.8 °C.

Plants were grown in round plastic pots (diameter 212 mm; height 144 mm) filled with potting soil. Osmocote® controlled release fertilizer was mixed with the potting soil at a rate of 7.7 g·pot^{-1}. Approximately five teff grains were sown in each of ten holes, evenly distributed in a grid over the pot surface. Two days after emergence the plants were thinned to one plant per hole, i.e. ten plants per pot. Each cultivar had six replicates within a day length treatment. This resulted in approximately 200 plants per m^2. To prevent lodging each pot was equipped with a plastic ring, fixed above the pot with a thin wooden stick that was planted in the middle of a pot; its height was adjusted during plant growth.

Observations

For practical reasons most plant characteristics (i.e. time to heading, panicle initiation, leaf number, internode number) regarded the plant's main stem. Under short days the main stem was without exception the first heading shoot, under long days (i.e. 15 h and beyond) the two largest tillers might be heading one or two days earlier than the main stem (Ketema, 1983). For plants in the growth chambers all characteristics that were recorded for the main stem were also recorded for the third tiller. Depending on plant density and cultivar, the third tiller is the tiller at the highest ranked phytomer that is still of agronomic importance.

Every second day, six plants at each of the day lengths of 9, 13.5, 15 and 16.5 h were harvested from the growth chambers. Samples were frozen. Later microscopic observations

were done on thawed samples, determining the physiological status of the growing tip of main stem and third tiller. The definition adopted for panicle initiation (PI) was the occurrence of a generative apical meristem in 50% of the cases in a sample.

Teff plants are mainly self-pollinating and heading and pollination almost coincide in teff (Mengesha & Guard, 1966). We will use the term heading instead of flowering. Heading was defined as emergence of the tip of the inflorescence from the sheath of the flag leaf. When three or more main stems within a single pot were heading the pot was noted to be heading. To determine time to heading six of the ten plants per pot were labelled and monitored daily, however, after 120 days observations became weekly and were discontinued when all main stems and third tillers had senesced.

To facilitate leaf counting, every fourth leaf was delicately marked with a water resistant inkmarker. A pilot study showed that there was discernable effect of plant markings on plant development or growth.

Final main stem length was measured from the root-shoot transition to the pedicel of the first spikelets on the peduncle. The distance between the first pedicel and the tip of the panicle was defined as panicle length. The crop height was defined as the estimated distance between the soil and the average top of the crop canopy (leaves or bent panicle).

To obtain plant dry weight and grain yield plants were dried at 70 °C until constant weight. Grain and shoot biomass were separated using a laboratory threshing machine with 3×9 mm sieve.

Data analysis

Two models to describe time to heading in response to day length were compared in this study: a Gompertz curve (eqn 4.1) and a broken linear function (eqn 4.2). The logistic Gompertz curve was derived from Zwietering *et al.* (1990):

$$t(D) = (t_{\max} - t_{\min})\, e^{-e^{-\frac{D_c - D}{D_c - D_\Delta} + 1}} + t_{\min} \tag{4.1}$$

where variable $t(D)$ (d) is the time to heading, variable D (h) the day length, D_Δ (h) the day length at which alteration of day length has the strongest impact on change in progress to heading, $t_{\min}$ (d) the minimal time to heading, $t_{\max}$ (d) the maximal time to heading, parameter D_c (h) the critical day length, i.e. the minimum day length at which day length starts to have an impact on time to heading. Note that $t_{\min}$ and $t_{\max}$ are asymptotes and thus parameter values are theoretical approximates of observed data.

Previously, discontinuous linear models (e.g. (Summerfield *et al.*, 1997)) were used to describe time to flowering. In order to fit such a linear model, data on time to flowering have to be transformed to rate of progress toward flowering (d^{-1}). Equation 4.2 is essentially the same linear model as used by Summerfield *et al.* (1997) but, is rewritten to describe rate of progress toward flowering at a single temperature with biologically interpretable

parameters:

$$t(D)^{-1} = {t_{\min}}^{-1} \quad \text{if} \quad D < D_c \tag{4.2a}$$

$$t(D)^{-1} = \frac{D\left({t_{\max}}^{-1} - {t_{\min}}^{-1}\right)}{(D_m - D_c)} + \frac{D_c\left({t_{\max}}^{-1} - {t_{\min}}^{-1}\right)}{(D_c - D_m)} + {t_{\min}}^{-1} \quad \text{if} \quad D_m \leq D \leq D_c \tag{4.2b}$$

$$t(D)^{-1} = {t_{\max}}^{-1} \quad \text{if} \quad D > D_m \tag{4.2c}$$

$t\,(\mathrm{D})^{-1}$ (d^{-1}) is the inverse of time to heading, variable D (h) the day length, D_c (h) the critical day length at which the variable day length (D) starts to influence time to heading, D_m (h) maximum day length at which an increase in day length increases time to heading in SDPs, ${t_{\min}}^{-1}$ (d^{-1}) the inverse of the minimal time to heading, ${t_{\max}}^{-1}$ (d^{-1}) the inverse of the maximal time to heading.

Equation 4.1 was directly fitted on data of days until heading. Before fitting eqn 4.2, however, data were transformed to rates of progress (d^{-1}) toward heading (Summerfield *et al.*, 1997). The purpose of these models is to describe the time to heading. Thus for statistical and graphical comparison, eqn 4.2 fitted on transformed data, *viz* rates of progress (d^{-1}), were converted back to time (d) to heading.

Nonlinear least squares regression was used to estimate the parameters of eqn 4.1 or 4.2 in one single fitting routine. Nonlinear regression was performed with the simplex method, using the built-in optimisation function 'fminsearch' of Matlab (MathWorks, Natick, Massachusetts, USA) version 7.8.0.347 (R2009a). SAS version 9.1.3 SP4 (SAS Institute Inc., Cary, NC, USA) was used for all comparisons between means. Using the PROC MIXED procedure of SAS, day length effects on time to heading and all plant morphologic characteristics were analysed. Assessment for significant differences was done with the least square means (LSMEANS) using Tukey's honest significance test ($p \leq 0.05$). Means in the text are followed by their standard error in parenthesis. If the standard deviation instead of the standard error is given, then the number in parenthesis is preceded by 'sd'.

Results

Effects of day length on time to heading

In both the growth chamber and the greenhouse experiment all four cultivars showed a delay in time to heading with increasing day length (Table A1.4.5), day lengths between 12 and 13.5 h caused the strongest change in delay (Figs. 4.1 and 4.2). The Ethiopian cultivars Gibe and Ziquala showed a more pronounced response to day length than the new Dutch cultivars Ayana and 04T19 (Figs. 4.1 and 4.2; Table A1.4.5 and A2.4.6). Cultivar Ayana demonstrated the shortest time to heading of all tested cultivars at day length longer than 12 h (Fig. 4.1; Table A1.4.5 and A2.4.6).

Heading was notably postponed by day length treatments longer than 10.5 h, thus teff behaved as a facultative (quantitative) short day plant. However, at day lengths of 15 and

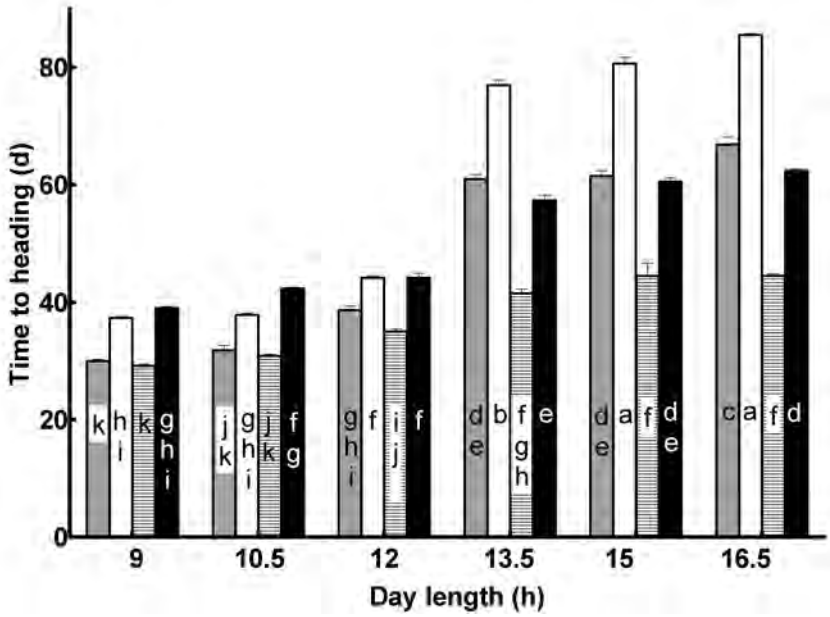

Fig. 4.1: Time to heading of the main stem of four cultivars: Gibe (grey bar), Ziquala (white bar), Ayana (hatched bar) and 04T19 (black bar), for six different day lengths. Treatments with the same letter are not significantly different. Error bar represents the standard error (n = 6).

16.5 h the Ethiopian cultivar Ziquala did show obligate (qualitative) SDP characteristics, like nubbin or barren panicles. At these LD treatments, moreover, a sizable fraction of the main stems of the Ethiopian cultivars did not produce a panicle at all: for Ziquala this amounted to 44% at 15 h and 28% at 16.5 h while for Gibe 6% of the main stem were without panicle both at 15 h and 16.5 h day length. All main stems of the Dutch cultivars had a panicle (Table 4.1). Teff has apparently no obligate vernalization requirement since all plants became generative while temperatures were always above 15 °C from sowing to heading.

Within day length treatments, time to heading was not significantly different between the growth chamber and greenhouse experiments, except for the 15 h day length treatment (Table A2.4.6). Post experiment measurements identified light leakage and a slightly lower temperature in the greenhouse with 15 h day length, which is probably the cause for the delayed flowering compared to the 15 h day length in the growth chambers. Comparing data from the greenhouse and the growth chamber experiments revealed that times to heading were not significantly different between the treatments with the longest day length, i.e. 16.5 h in the growth chamber equalled 18 h in the greenhouse. An exception to this rule was found for cv. Ziquala, which flowered on average 5 days later in the greenhouse (18 h) than in the growth chambers (16.5h).

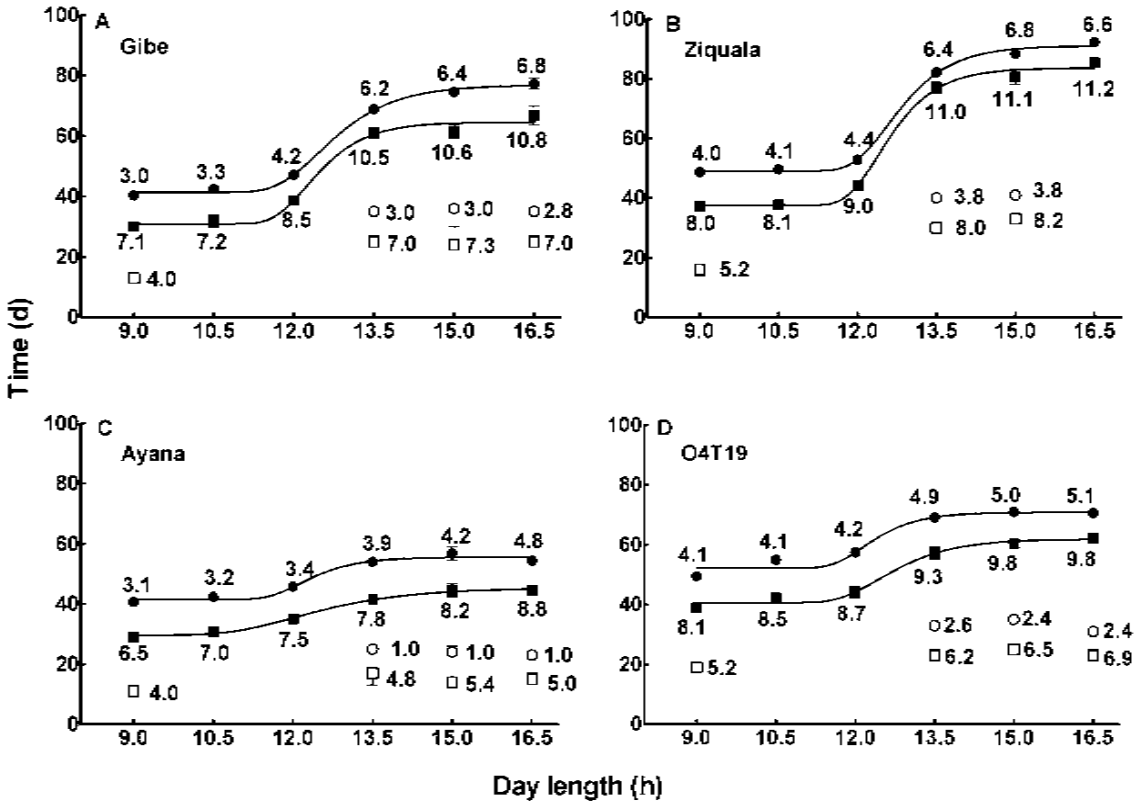

Fig. 4.2: Day length effects on time to heading (closed symbols), panicle initiation, PI (open symbols) of main stem (squares) and third tiller (circles) and the associated numbers of appeared leaves at time of heading or PI for cultivars (a) Gibe, (b) Ziquala, (c) Ayana and (d) O4T19. Error bar of closed symbols represents the standard error (n = 6) and when not visible fall within the symbol. Error bars of open symbols represent ranges between the first and last observed generative meristem and when not visible fall within the symbol.

Table 4.1: Spread in time to heading for the main stem or the third tiller; spread in time to heading between the main stem and the third tiller; and the percentage of final heading main stems and third tillers per pot (growth chamber). Means are followed by their standard error between brackets.

Cultivar	Day length (h)	Time between first and last heading main stem within one pot (d)	Time between heading of the main stem and third tiller (d)	Percentage of heading main stems	Time between first and last heading third tiller in a pot (d)	Percentage of heading third tillers
Gibe	9	5.8(0.3)	100	10.2(0.3)	5.7(0.5)	97
	10.5	3.8(0.5)	100	10.7(0.3)	7.8(0.9)	100
	12	4.5(0.5)	100	8.5(0.3)	5.5(0.6)	97
	13.5	8.2(0.6)	97	7.8(0.5)	7.8(0.6)	92
	15	11.4(1.3)	94	12.5(0.4)	7.8(0.9)	89
	16.5	15.2(1.1)	94	9.7(0.6)	13.8(1.1)	100
Ziquala	9	1.5(0.3)	100	11.3(0.3)	6.7(0.7)	97
	10.5	2.2(0.3)	100	11.8(0.5)	7.0(1.2)	94
	12	3.5(0.3)	100	9.2(0.5)	5.7(0.6)	94
	13.5	6.0(0.2)	89	5.0(0.3)	22.5(0.7)	92
	15	∞	56	∞	∞	83
	16.5	∞	72	∞	∞	72
Ayana	9	4.5(0.6)	100	11.5(0.2)	6.7(0.4)	100
	10.5	5.8(0.6)	100	12.0(0.5)	7.5(0.9)	97
	12	5.3(0.4)	100	11.0(0.3)	4.5(0.8)	97
	13.5	21.0(2.2)	100	12.0(0.6)	16.3(2.7)	94
	15	17.3(2.2)	100	12.3(1.2)	19.3(1.8)	100
	16.5	26.7(1.6)	100	9.7(0.6)	23.0(1.5)	100
04T19	9	1.3(0.2)	100	10.5(0.2)	3.5(0.5)	100
	10.5	1.5(0.2)	100	12.7(0.7)	6.0(0.9)	86
	12	2.5(0.4)	100	13.3(0.3)	8.5(1.3)	94
	13.5	6.7(0.3)	100	11.8(0.5)	8.2(0.6)	89
	15	4.5(0.8)	100	10.8(0.5)	8.3(0.2)	94
	16.5	4.7(0.5)	100	8.3(0.4)	11.3(1.9)	86

Table 4.2: Parameter values of eqn 4.1, eqn A3.4.3, eqn 4.2 and goodness of fit*1 for describing time to heading per cultivar; for the main stem and the third tiller of teff.

Cultivar	Equation	t_{min}	t_{max}	D_c	D_m	D_Δ	Sum of squared residual*2	r^2
Main stem								
Gibe	4.2	30.8	64.0	11.0	13.6		220.5	0.974
	4.1	30.9	64.6	11.6		12.2	211.0	0.975
	A3.4.3	30.4	64.8	11.1		12.2	212.0	0.975
Ziquala	4.2	37.6	83.0	11.4	13.7		128.8	0.992
	4.1	37.6	83.6	11.8		12.4	114	0.993
	A3.4.3	37.5	83.2	11.6		12.4	122.6	0.992
Ayana	4.2	29.2	44.2	9.9	14.1		169.6	0.892
	4.1	28.7	45.3	10.8		12.0	175.6	0.888
	A3.4.3	28.9	44.8	10.2		12.0	169.6	0.892
04T19	4.2	40.6	61.4	11.4	13.8		103.9	0.968
	4.1	40.7	62.0	11.7		12.5	98.2	0.969
	A3.4.3	40.1	62.0	11.3		12.6	95.5	0.970
Third tiller								
Gibe	4.2	41.3	76.8	11.3	13.8		205.5	0.976
	4.1	41.4	76.4	11.7		12.5	216.5	0.975
	A3.4.3	41.0	76.4	11.4		12.5	194.5	0.977
Ziquala	4.2	49.1	91.1	11.7	13.7		-	-
	4.1	49.1	90.4	11.9		12.6	174.8	0.986
	A3.4.3	49.0	90.4	13.5		12.7	187.2	0.986
Ayana	4.2	41.5	55.5	11.0	13.7		293.9	0.827
	4.1	41.1	55.4	11.5		12.1	289.2	0.829
	A3.4.3	41.1	55.4	11.1		12.2	285.7	0.832
04T19	4.2	52.0	70.8	11.1	13.7		200.9	0.926
	4.1	52.3	70.9	11.6		12.1	200.5	0.926
	A3.4.3	48.6	72.5	9.4		11.7	185.6	0.931

*1 Goodness of fit was calculated for the actual number of days to heading, thus after back transformation of the linear model.
*2 Sum of squared residual = $\Sigma(\text{data} - \text{model})^2$

Modelling the effects of day length on time to heading

For teff the Gompertz curve (eqn 4.1) or the logistic curve (eqn A3.4.3 in Appendix 3) described the time to heading in response to day length better than the linear model (eqn 4.2). Based on total sum of squares the logistic model was slightly better: overall logistic $\Sigma(\text{data}-\text{model})^2 = 1453$ (eqn A3.4.3), Gompertz $\Sigma(\text{data}-\text{model})^2 = 1480$ (eqn 4.1) versus linear model $\Sigma(\text{data}-\text{model})^2 = 1839$ (eqn 4.2) (Table 4.2). Cross-species model validation on literature data of the SDP rice cultivar Tjiana from Best in the same day length range as teff (i.e. 8-24 h), again showed that the smooth curvilinear model (eqn 4.1) was superior to the linear model on rates (Fig. 4.3). However, for this rice cultivar the Gompertz curve (eqn 4.1) was superior to the logistic function (eqn A3.4.3) and improved the fit statistics from $r^2 = 0.993$ to r^2 0.996. Comparing the actual fits of eqn 4.1 and eqn A3.4.3 to the data shows that the improved fit is due to the more appropriate shape assumptions of the Gompertz model than the logistic model (Fig. 4.3; eqn A3.4.3). Because in teff eqn 4.1 and eqn A3.4.3 fitted almost equally well, for the sake of uniformity the Gompertz model was plotted for both species (Figs. 4.2 and 4.3).

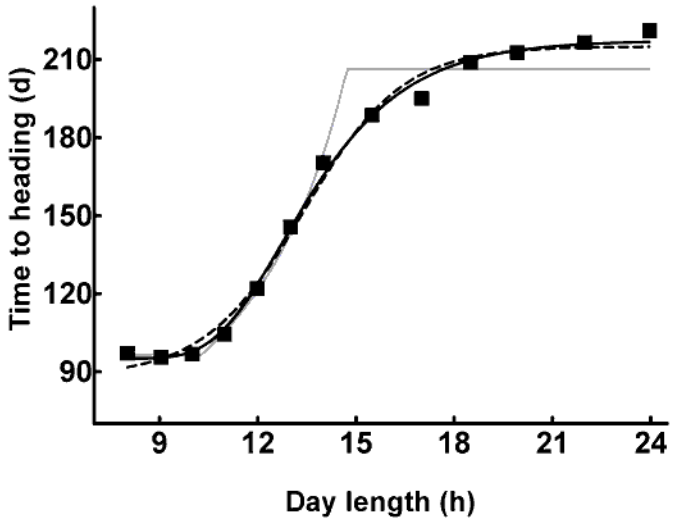

Fig. 4.3: Day length effects on time to heading of rice cultivar Tjiana (symbols), data of Best (1961). Lines represent three regression models: Gompertz curve eqn 4.1 (black solid line; r^2 0.996, SSE 102), logistic curve eqn A3.4.3 (black broken line; r^2 0.993, SSE 191), back transformed linear model eqn 4.2 (grey solid line; r^2 0.969, SSE 825).

Effects of day length on time to panicle initiation (PI) and final leaf number

Compared to the obvious day length response of time to heading (Fig. 4.2 closed symbols), the day length response of time to panicle initiation (PI) was less obvious (Fig. 4.2 open symbols). The differences in time to PI between SD (9 h) and LD (16.5 h) were more pronounced for the Ethiopian than for the Dutch cultivars: for Gibe the difference was 15.5 d and for Ziquala it was 11.7 d versus 4.3 d and 4.7 d for the Dutch cultivars Ayana and 04T19, respectively. The time from PI to heading was longer under LD conditions than under SD conditions. Consequently, day length influenced both the transition to a generative phase and the further development and elongation of the panicle. A reason for this could be that the flag leaf sheaths are longer at longer day length. Cultivar Ziquala had on average the biggest difference in sheaths length between SD 158(0.8) mm and LD 177(3.8) mm. Yet, a difference of 19 mm in sheaths length is unlikely to be the main explanation for the time difference in panicle outgrowth between LD and SD. Because, looking at the tremendous speed of panicle outgrowth (Fig. 4.4) during the days just before heading, a distance of 19 mm will delay heading a day or two at most but not 11.7 d. To scrutinise the phenomena of slow panicle outgrowth at LD in more detail we counted the number of spikelets for 04T19 under SD (9h) and LD (18h) conditions. The number of spikelets produced under LD was 961(21) (n=36) which was substantially more than 659(12) spikelets produced under SD (n=35). The number of spikelets produced per day, averaging over time from PI to heading was almost equal, i.e. 28 per day for LD treatments and 30 spikelets per day for SD treatments. Even small reductions in time to heading coincided with an on average lower number of final leaves, indicating that plant development was affected by photoperiod (Fig. 4.2). At the moment that the apical meristem switches from a vegetative, leaf constructing, meristem to a generative, inflorescence constructing meristem, the final number of leaves (phytomers) is determined. Postponed PI was indeed associated with an increase in final leaf number. Namely, under LD (16.5 h) compared to SD (9 h) extra leaves amounted to 3.7 in Gibe and 3.2 in Ziquala versus 2.3 in Ayana and 1.7 in 04T19 leaves.

Variation in time to harvest

Here we list three sources of variation that were observed regarding time to harvest under different day lengths.

First, teff plants within a pot were not heading at the same time (Table 4.1). The time between the first and last heading plant within a pot was higher at long days than at short days. There was, however, no significant correlation between the mean time to heading and the spread within a pot.

Second, teff shoots of the same plant were not heading synchronously (Fig. 4.2, Table 4.1). The third tiller was on average heading 10.7(0.54) days later than the main stem under almost all day lengths (Fig. 4.2, Table 4.1). In the greenhouse the numbers of tillers were counted both at flowering and at harvest (Table A2.4.6). We, furthermore,

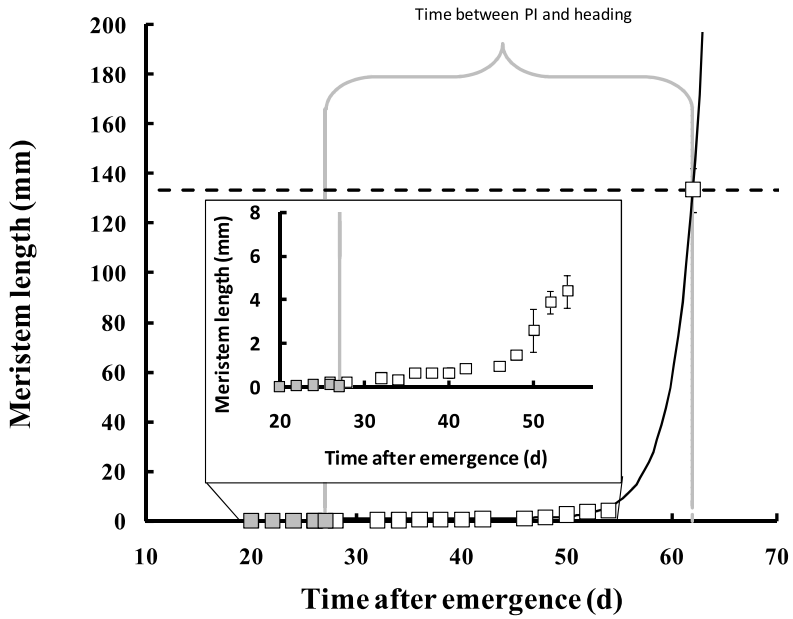

Fig. 4.4: Time after seedling emergence versus meristem length of cultivar 04T19; vegetative meristem is represented by grey squares; generative meristem by open squares and the solid line represents the exponential trend. The size the generative meristem (i.e. panicle) has to possess in order to appear from the final leaf sheath is represented by the broken line. The inset is a magnification of the data of the early growth stages.

distinguished between big agronomically important tillers and the remaining smaller tillers. The plants kept on producing tillers and panicles until the harvest date, also when the main stem had already senesced. These were mostly small auxiliary tillers that were formed after flowering. Cultivars Gibe with 23 and Ayana with 22 tillers were the most extensively tillering cultivars, whereas 04T19 with 8 tillers had overall the lowest number of tillers. This cultivar, 04T19, was also more uniform in ripening of the panicles. There was, furthermore, little effect of day length on tillering in any of the cultivars (Table A2.4.6).

Third, the developing panicle as such possessed a gradient in ripeness. Grains at the panicle tip were shedding while grains close to the peduncle were still in their grain filling stage (Photo P1.4.6 and P1.4.7).

Growth: biomass and lengths

Compared to the shortest day length treatments, all cultivars showed larger main stem shoot biomass at day lengths between 12 and 15 h (Table 4.3). In contrast to the shoot biomass, the biomass of the panicle (Table 4.3) and the actual grain yield in the greenhouse (Table 4.3) were not significantly different between short and long days.

The third tiller, which we defined as the highest ranked tiller on the main stem that was still of agronomic importance, had an average relative contribution to grain yield of only 15% (1%).

Gibe showed a significantly shorter main stem shoot length at short days (i.e. 9 and 10.5 h) compared to long days (i.e. 15 and 16.5 h) (Table 4.4). Longer main stem shoot length at long days than at short days was not observed for the other cultivars. However, the maximal crop height, which is an average over all tillers and plants per pot, did show a systematic increase with day length for all cultivars (Table 4.4).

Table 4.3: Day length effects on plant and main stem organ weight per cultivar. Means per cultivar with the same group letter were not significantly different ($p \leq 0.05$) (growth chamber). Means are followed by their standard error between brackets.

Cultivar	Day length (h)	Main stem DW (g)	Group letters	Main stem panicle DW (g)	Group letters	DW all stems + leaves per plant (g)	Group letters	DW of all panicles per plant (g)	Group letters	Total plant above ground DW (g)	Group letters
Gibe	9	0.6(0.1)	C	1.3(0.1)	AB	4.8(0.3)	B	7.4(0.4)	BC	12(0.7)	BC
	10.5	0.5(0.0)	C	1.0(0.1)	B	4.6(0.6)	B	5.7(1.1)	C	10(1.7)	C
	12	1.0(0.0)	B	1.8(0.1)	A	6.8(0.3)	B	9.0(0.8)	AB	16(1.0)	B
	13.5	2.0(0.1)	A	1.7(0.4)	AB	13(0.6)	A	9.9(1.0)	AB	23(1.6)	A
	15	2.0(0.1)	A	1.9(0.2)	A	14(0.8)	A	11(0.9)	A	24(1.7)	A
	16.5	2.0(0.1)	A	1.4(0.3)	AB	12(0.5)	A	9.2(0.5)	AB	22(0.8)	A
Ziquala	9	1.1(0.0)	D	2.2(0.1)	A	6.3(0.3)	C	8.6(0.6)	A	15(0.8)	BC
	10.5	1.1(0.1)	D	1.7(0.1)	B	6.4(0.6)	C	6.8(0.6)	B	13(1.2)	C
	12	1.6(0.1)	C	2.2(0.2)	A	8.2(0.6)	C	8.0(0.5)	AB	16(1.0)	ABC
	13.5	2.9(0.1)	A	0.4(0.1)	C	15(0.4)	B	4.0(0.4)	C	19(0.5)	AB
	15	2.5(0.2)	AB	0.0(0.0)	D	18(1.9)	AB	2.4(0.3)	CD	20(2.0)	A
	16.5	2.4(0.1)	B	0.0(0.0)	D	19(1.8)	A	1.0(0.2)	D	20(1.8)	A
Ayana	9	0.4(0.0)	C	1.1(0.1)	A	4.3(0.3)	C	6.9(0.7)	AB	11(0.9)	BC
	10.5	0.5(0.1)	C	1.0(0.1)	A	4.2(0.4)	C	4.4(0.6)	B	8.6(1.0)	C
	12	0.8(0.1)	BC	1.7(0.1)	A	6.1(0.5)	BC	7.6(0.7)	A	14(1.2)	AB
	13.5	1.1(0.1)	AB	1.0(0.3)	A	8.8(0.8)	AB	8.4(0.7)	A	17(1.4)	A
	15	1.3(0.2)	AB	1.3(0.4)	A	9.2(1.0)	A	8.4(1.1)	A	18(2.0)	A
	16.5	1.5(0.2)	A	1.5(0.2)	A	8.4(1.0)	AB	8.3(0.5)	A	17(1.3)	A
04T19	9	1.8(0.0)	C	2.3(0.1)	A	7.3(0.3)	C	6.9(0.4)	A	14(0.7)	C
	10.5	2.1(0.1)	BC	2.4(0.1)	A	7.8(0.5)	C	6.3(0.5)	A	14(1.0)	C
	12	2.6(0.1)	B	2.6(0.2)	A	9.6(0.6)	BC	7.5(0.4)	A	17(1.0)	BC
	13.5	3.1(0.2)	A	2.0(0.5)	A	13(0.9)	A	8.2(0.4)	A	22(1.1)	A
	15	3.4(0.2)	A	2.6(0.2)	A	13(0.4)	A	8.1(0.4)	A	21(0.6)	AB
	16.5	3.1(0.1)	A	2.3(0.2)	A	11(0.7)	AB	7.4(0.7)	A	18(1.4)	AB

Table 4.4: Day length effects on plant and main stem organ length per cultivar. Means per cultivar per temperature with the same letter were not significantly different ($p \leq 0.05$) (growth chamber). Means are followed by their standard error between brackets.

Cultivar	Day length (h)	Number of elongated internodes		Stem length till panicle (cm)		Panicle length (cm)		Stem length + panicle (cm)		Maximum crop height (cm)	
Gibe	9	4.0(0.0)	C	77(1.3)	C	39(1.8)	A	116(2.6)	C	124(1.5)	C
	10.5	4.3(0.1)	C	83(2.3)	C	41(1.4)	A	124(3.4)	BC	152(1.1)	C
	12	5.2(0.1)	B	98(3.5)	B	48(2.0)	A	146(5.4)	AB	150(1.3)	B
	13.5	6.7(0.2)	A	117(3.4)	A	36(8.0)	A	153(11)	A	151(3.7)	A
	15	6.8(0.2)	A	109(3.1)	AB	40(5.6)	A	149(6.2)	A	92(1.1)	A
	16.5	6.7(0.2)	A	107(2.7)	AB	34(7.3)	A	141(8.6)	AB	118(2.1)	A
Ziquala	9	3.9(0.1)	C	99(4.0)	C	54(0.9)	A	153(4.5)	A	134(1.5)	C
	10.5	3.9(0.1)	C	101(2.1)	C	53(0.9)	A	154(2.3)	A	140(4.1)	B
	12	4.1(0.1)	C	112(1.6)	B	59(0.7)	A	171(2.1)	A	92(1.1)	B
	13.5	5.8(0.1)	A	136(3.9)	A	26(6.3)	B	162(9.8)	A	114(1.5)	A
	15	5.8(0.2)	AB	107(1.6)	BC	2(1.3)	C	109(1.1)	B	115(2.6)	A
	16.5	5.3(0.2)	B	103(4.0)	BC	2(1.7)	C	105(5.2)	B	123(2.1)	A
Ayana	9	3.4(0.2)	B	71(3.4)	A	40(3.3)	A	111(3.4)	A	93(1.1)	C
	10.5	3.9(0.2)	B	78(4.6)	A	43(1.0)	A	121(4.5)	A	109(2.0)	C
	12	4.5(0.1)	AB	84(1.9)	A	47(4.3)	A	132(4.0)	A	102(2.1)	B
	13.5	4.7(0.2)	AB	79(4.6)	A	32(8)	A	109(11)	A	104(4.4)	A
	15	5.3(0.4)	A	83(5.7)	A	45(6.2)	A	128(7.5)	A	117(2.1)	A
	16.5	5.7(0.5)	A	91(7.5)	A	49(2.2)	A	136(9.6)	A	134(3.5)	A
04T19	9	3.8(0.2)	C	119(2.3)	AB	52(4.7)	AB	170(3.4)	B	107(1.1)	D
	10.5	3.9(0.1)	C	127(2.1)	AB	59(2.0)	AB	186(3.7)	AB	103(1.1)	C
	12	4.2(0.2)	BC	130(2.4)	A	63(2.1)	A	194(4.3)	A	121(0.8)	C
	13.5	4.3(0.1)	BC	127(4.7)	AB	48(4.2)	B	175(8.3)	AB	145(2.2)	B
	15	4.9(0.1)	A	126(4.2)	AB	56(4.8)	AB	181(7.4)	AB	143(1.1)	B
	16.5	4.6(0.2)	AB	116(2.7)	B	63(1.6)	A	179(3.7)	AB	144(2.6)	A

Discussion

Teff is a facultative (quantitative) short day plant, as shown in two independent experiments in two different environments (i.e. greenhouses versus growth chambers). Time to panicle initiation (PI) and flowering (heading) were notably postponed by day length treatments longer than 10.5 h. The strongest day length response in terms of time to heading was between 12 and 15 h for all cultivars. The Ethiopian cultivar Ziquala did show some characteristics of an obligate (qualitative) short day response exemplified by absent or sterile panicles and lower grain yields under long day length regimes of 15 and 16.5 h. This cultivar, Ziquala, also showed the most pronounced day length response, heading occurred at SD (9 h) as early as 37.3(0.2) days, whereas at LD (16.5 h) heading occurred first after 88.3(1.7) days. Among the cultivars there was significant variation in phenology, especially at LD (16.5 h) time to heading varied considerably between Dutch and Ethiopian cultivars, *viz.* from as early as 44.7(0.4) d (Ayana) to not until 88.3(1.7) d (Ziquala).

Compared to the traditional linear models on rate of progress towards flowering, the novel curvilinear model (eqn 4.1) presented here provides a description with higher accuracy of time to flowering in response to photoperiod. Using the same number of parameters the Gompertz curve (eqn 4.1) explains a higher portion of variance than the traditional linear model (eqn 4.2). The novel curvilinear model (eqn 4.1) can be directly fitted to the data without transformation to rates. This is in contrast to the linear model (eqn 4.2), where data transformation is required to obtain an accurate fit. The superiority of the curvilinear model over the linear model was confirmed when these models were fitted to the data of the SDP rice (Best, 1961). Note that we only use day lengths longer than 8 h because shorter day lengths are irrelevant to our research question and most likely cause stress due to the short diurnal period of photosynthesis. Fitting both models to rice data transformed to rates shows that curvilinear model (eqn 4.1) still out performs the linear model (eqn 4.2 and Fig. 4.5). As shown in Appendix 3 (eqn A3.4.4) the Gompertz curve and the logistic curve have some shape assumptions, for teff these models fit equally well, but for rice the Gompertz model was superior. Regardless of the shape assumptions our novel model requires sufficient number of data points over the whole range of day lengths. When data are only available over a limited photoperiod range, a linear model (eqn 4.2) would be safer resort. Long days not only increased time to heading, but also number of main stem leaves (i.e. vegetative phytomers), plant biomass, inter-plant variation, number of elongated internodes and crop height increased under long days. In contrast to the shoot biomass the grain biomass did not increase with day length.

At the moment that the apical meristem switches from a vegetative, leaf constructing meristem, to a generative, inflorescence constructing meristem, the final leaf number is determined. Assuming a constant plastochron, i.e. time between initiated leaves, a delay in switch would result in a higher final leaf number. Hence, when the LDP wheat is grown under SD conditions, then the final main stem leaf number will increase (Hay & Kirby, 1991). Similarly, when the SDP rice is grown under LD conditions, then the final

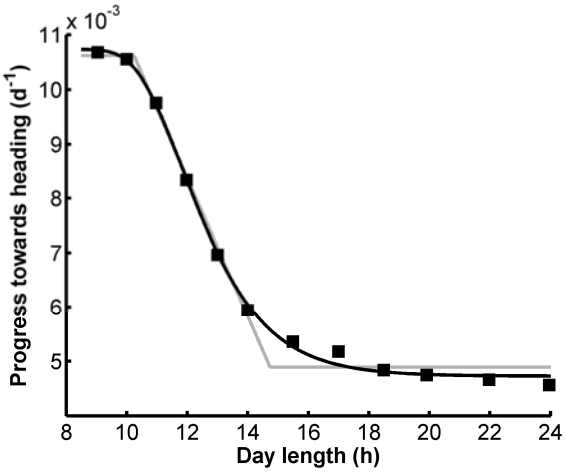

Fig. 4.5: Day length effects on progress towards heading of rice cultivar Tjiana (symbols), data of Best (1961). Lines represent two regression models: Gompertz curve eqn 4.1 (black solid line; r^2 0.998, SSE 1.19 x 10^{-7}, linear model eqn 4.2 (grey solid line; r^2 0.992, SSE 5.34 x 10^{-7}).

leaf number will also increase (Yin & Kropff, 1996). We found corresponding results for the SDP teff, with increasing day length the number of leaves (i.e. phytomers) increases Fig. 4.2). Correspondingly, the early flowering teff cultivars created fewer leaves than the late flowering cultivars. The number of tillers, however, did not increase with day length.

To harvest cereal grains in one single operation, to prevent the need for post harvest drying and to receive high market prices, all grains within the crop need to ripen simultaneously. Wheat is a very uniform crop; environment, including day length, has little or no influence on uniformity of ripening (Hay & Kirby, 1991). This is in contrast to teff grown under LD conditions, which is not a uniform crop. There was a gradient in ripeness within a panicle, large variation between panicles and the plant keeps on producing small tillers even when the main stem has already senesced. Long day conditions increased plant-to-plant variation in time to heading in Gibe, Ziquala and Ayana, but LD effects on variation in time to heading for 04T19 were small. Synchrony of agronomically important tillers within the plant did, however, not change much with day length for all tested cultivars.

Biomass losses by seed shedding was very high, grains were easily removed from the panicle. Therefore, when plants were harvested they were carefully enclosed in a bag in order to prevent grain losses and acquire accurate estimations of total grain biomass. In a field situation seed shedding will, without a doubt, result in major yield reduction. Moreover, also here the asynchrony in teff would increase the losses in a field situation, as the first grains start to shed when a significant number of grains within the crop still needs to be filled.This is not only due to plant-to-plant or shoot-to-shoot (Photo P1.4.6) variation but also to variation within the panicle as such (Photo P1.4.7).

On the basis of this study several teff breeding recommendations can be made. Early flowering will reduce crop height and main stem final leaf number, thus reducing lodging susceptibility (van Delden *et al.*, 2010), especially under long day conditions. In NW Europe early flowering will, moreover, circumvent the adverse weather conditions at the end of the growing season. This study shows that breeding for early flowering under long days does not necessarily coincide with lower grain biomass. Grain shedding should be reduced, as was already achieved via breeding for current wheat and rice cultivars (Doust, 2007). Teff plants make, furthermore, numerous auxiliary tillers that contributed < 4% (Table A2.4.6) to the final grain yield. These auxiliary tillers are attached to higher node ranks of the main stem and the most productive tillers. Their centre of mass deviates from the vertical, hence their presence makes the plant much more susceptible to lodging (van Delden *et al.*, 2010). Therefore breeding should aim at reducing the number of emerging auxiliary tillers. The modestly tillering cultivar 04T19 was also the cultivar which exhibited most uniform grain ripening, within the panicle and between shoots and plants. Ayana with a similar day length response, in contrast, showed both abundant tillering and non-uniform ripening (Photo P1.4.7). These large genetic differences between cultivars suggest there is ample genetic variation in all these traits; therefore the basis for breeding for high yielding cultivars seems to be present.

Appendix 1

Table A1.4.5: Day length effects on time to heading and final leaf number of the main stem and the third tiller per cultivar. Means per cultivar with the same group letter were not significantly different ($p \leq 0.05$) (growth chamber). Means are followed by their standard error between brackets.

Cultivar	Day length (h)	Main stem time to heading (d)		Main stem final leaf number		Third tiller time to heading (d)		Third tiller final leaf number	
Gibe	9	30.0(0.3)	D	7.1(0.1)	C	40.7(0.8)	D	3.0(0.0)	D
	10.5	32.2(0.9)	D	7.2(0.1)	C	42.8(0.9)	CD	3.3(0.1)	D
	12	38.7(0.7)	C	8.5(0.1)	B	47.2(0.8)	C	4.2(0.1)	C
	13.5	61.5(0.9)	B	10.5(0.1)	A	69.3(0.9)	B	6.2(0.1)	B
	15	62.0(1.0)	B	10.6(0.1)	A	74.5(1.0)	A	6.4(0.2)	AB
	16.5	68.3(2.1)	A	10.8(0.1)	A	78.0(2.0)	A	6.8(0.1)	A
Ziquala	9	37.3(0.2)	E	8.0(0.0)	C	48.7(0.6)	E	4.1(0.1)	CD
	10.5	37.8(0.3)	E	8.1(0.1)	C	49.7(1.1)	DE	4.0(0.0)	D
	12	44.2(0.3)	D	9.0(0.0)	B	53.3(1.0)	D	4.4(0.1)	C
	13.5	77.2(0.9)	C	11.0(0.0)	A	82.2(0.7)	C	6.4(0.1)	B
	15	83.8(1.4)	B	11.2(0.1)	A	88.3(1.1)	B	6.8(0.1)	A
	16.5	88.3(1.7)	A	11.1(0.1)	A	94.2(1.1)	A	6.6(0.1)	AB
Ayana	9	29.2(0.3)	C	6.5(0.1)	D	40.7(0.4)	C	3.1(0.1)	C
	10.5	30.8(0.3)	C	7.0(0.2)	CD	42.8(0.9)	BC	3.2(0.1)	C
	12	35.0(0.4)	B	7.5(0.1)	BCD	46.0(0.4)	B	3.4(0.1)	BC
	13.5	42.0(0.7)	A	7.8(0.3)	ABC	54.0(0.9)	A	3.9(0.2)	BC
	15	44.5(2.1)	A	8.2(0.3)	AB	56.8(2.3)	A	4.2(0.3)	AB
	16.5	44.7(0.4)	A	8.8(0.4)	A	54.3(1.3)	A	4.9(0.4)	A
04T19	9	39.0(0.3)	D	8.1(0.1)	D	49.5(0.6)	C	4.1(0.1)	B
	10.5	42.3(0.2)	C	8.5(0.1)	C	55.0(1.3)	B	4.0(0.1)	B
	12	44.2(0.8)	C	8.7(0.1)	C	57.5(1.0)	B	4.1(0.1)	B
	13.5	57.3(0.8)	B	9.3(0.1)	B	69.2(0.4)	A	4.8(0.1)	A
	15	60.5(0.7)	A	9.8(0.1)	A	71.3(0.6)	A	5.1(0.2)	A
	16.5	62.3(0.3)	A	9.8(0.1)	A	70.7(0.6)	A	5.1(0.1)	A

Appendix 2

Table A2.4.6: Time to heading, final leaf number and number of tillers. Means per cultivar with the same group letter were not significantly different ($p \leq 0.05$) (greenhouse). Means are followed by their standard error between brackets.

Cultivar	Day length (h)	Time to heading (d)		No. of tillers per plant at pot flowering		Final no. of leaves		Final no. of big tillers per plant		Final no. of all tillers per plant		Relative grain biomass contribution big tillers (%)	Relative grain biomass contribution small tillers (%)
Gibe	9	31.3(0.7)	B	6.0(0.2)	C	7.0(0.0)	C	5.8(0.4)	A	36.9(5.6)	A	11.7	1
	12	40.8(0.8)	B	7.5(0.8)	BC	8.2(0.2)	B	4.7(0.2)	A	22.9(2.9)	B	14.5	1.7
	15	71.7(1.3)	A	10.7(0.5)	A	11.0(0.2)	A	4.5(0.5)	A	17.2(2.9)	B	19.5	1
	18	71.7(1.2)	A	8.5(0.7)	AB	11.0(0.0)	A	5.0(0.3)	A	15.4(1.6)	B	17.9	1
Ziquala	9	38.8(0.4)	C	6.6(0.4)	B	8.0(0.0)	B	4.4(0.4)	A	20.1(3.1)	A	14.9	2.2
	12	45.3(0.7)	B	7.3(0.3)	AB	8.7(0.2)	B	3.6(0.3)	A	12.2(2.3)	AB	25.5	1
	15	95.8(0.7)	A	9.9(1.3)	A	11.0(0.2)	A	4.6(0.5)	A	14.1(0.7)	AB	15.7	2.9
	18	93.3(0.9)	A	8.0(0.6)	AB	11.0(0.2)	A	4.6(0.4)	A	11.2(1.2)	B	16.2	3.9
Ayana	9	30.5(0.5)	C	5.9(0.1)	B	6.5(0.2)	B	4.6(0.4)	A	24.2(3.2)	A	14.2	1.8
	12	35.5(1.0)	B	6.2(0.5)	B	7.2(0.3)	AB	5.3(0.3)	A	23.8(2.4)	A	17.3	0.5
	15	40.2(1.4)	A	7.6(0.4)	AB	7.7(0.6)	AB	3.4(0.9)	B	18.5(2.3)	A	21.3	1.8
	18	39.3(1.4)	AB	8.2(0.5)	A	9.2(0.7)	A	5.4(1.9)	A	20.5(2.3)	A	13.8	1.7
04T19	9	39.3(0.3)	C	4.5(0.3)	AB	8.2(0.2)	B	*-		*-		*-	*-
	12	42.3(0.6)	B	4.1(0.3)	B	8.3(0.2)	B	3.0(0.2)	A	9.1(0.5)	A	30.1	1.6
	15	63.3(1.5)	A	5.0(0.3)	AB	9.5(0.2)	A	3.3(0.3)	A	7.2(0.7)	A	30.1	0.2
	18	62.9(1.0)	A	5.7(0.6)	A	10.0(0.0)	A	3.6(0.2)	A	7.3(1.0)	A	27.5	0.2

Appendix 3

Instead of using the implicit non-symmetrical shape assumption of the Gompertz curve, we can also assume that the day length response is symmetrical around parameter D_Δ, i.e. the inflection point of eqn 4.1. If the day length response is symmetrical around parameter D_Δ, then a logistic curve derived from Zwietering *et al.* can be used:

$$t(D) = \frac{t_{max} - t_{min}}{1 + e^{-2 \cdot \frac{(D - D_\Delta)}{(D_\Delta - D_c)}}} + t_{min} \tag{A3.4.3}$$

where variable t(D) is the time to heading (d), variable D the day length (h), D_Δ the day length at which alteration of day length has the strongest impact on change in progress to heading (h), t_{min} the minimal time to heading (d) , t_{max} the maximal time to heading (d), parameter D_c the critical day length, i.e. the minimum day length at which day length starts to have an impact on time to heading (h). Note that t_{min} and t_{max} are asymptotes and thus parameter values are theoretical approximates of observed data.

The difference between the logistic curve (eqn A3.4.3) and Gompertz curve (eqn 4.1) is that the inflection point, D_Δ, of eqn A3.4.3 is at the symmetric midst of the curve:

$$t(D_\Delta) = \frac{(t_{max} - t_{min})}{2} + t_{min} \tag{A3.4.4a}$$

and of eqn 4.1 D_Δ is below the curves midst, resulting in an asymmetric logistic curve:

$$t(D_\Delta) = \frac{(t_{max} - t_{min})}{e} + t_{min} \tag{A3.4.4b}$$

Appendix 4

Table A4.4.7: Shoot and grain biomass per plant. Means per cultivar with the same group letter were not significantly different ($p \leq 0.05$) (greenhouse). Means are followed by their standard error between brackets.

Cultivar	Temperature (°C)	Day length (h)	Shoot DW (g)		Grain DW (g)		Total DW (g)		Harvest index (g per g)	
Gibe	18	9	3.6(0.12)	C	1.6(1.9)	AB	5.2(2.8)	B	0.31(0.020)	A
		12	4.3(0.11)	C	2.6(2.5)	A	6.9(3.5)	AB	0.37(0.019)	A
		15	6.8(0.35)	A	1.8(2.8)	AB	8.6(5.5)	A	0.21(0.021)	B
		18	5.5(0.39)	B	1.5(2.6)	B	7.0(5.9)	A	0.21(0.021)	B
Ziquala	18	9	4.1(0.11)	B	2.1(1.5)	A	6.2(1.5)	B	0.34(0.018)	A
		12	4.1(0.44)	B	1.8(4.3)	A	5.9(8.0)	B	0.28(0.046)	AB
		15	9.0(0.69)	A	2.3(5.4)	A	11.3(12)	A	0.19(0.029)	B
		18	8.6(0.87)	A	2.0(2.9)	A	10.6(9.1)	A	0.19(0.025)	B
Ayana	18	9	2.8(0.22)	B	1.4(2.2)	A	4.2(4.3)	A	0.31(0.026)	A
		12	3.7(0.18)	AB	1.9(3.2)	A	5.6(4.6)	A	0.32(0.032)	A
		15	5.2(0.58)	A	1.5(2.2)	A	6.7(7.2)	A	0.22(0.022)	A
		18	4.5(0.67)	AB	1.4(3.5)	A	5.9(10)	A	0.21(0.030)	A
04T19	18	9	5.9(0.50)	B	3.1(1.7)	A	9.0(6.2)	A	0.35(0.016)	A
		12	6.0(0.19)	B	3.0(2.3)	A	9.0(3.7)	A	0.33(0.015)	A
		15	7.7(0.46)	A	2.8(2.0)	A	10.5(5.8)	A	0.26(0.014)	B
		18	7.5(0.49)	AB	2.7(2.1)	A	10.2(6.3)	A	0.26(0.013)	B

Photos P1

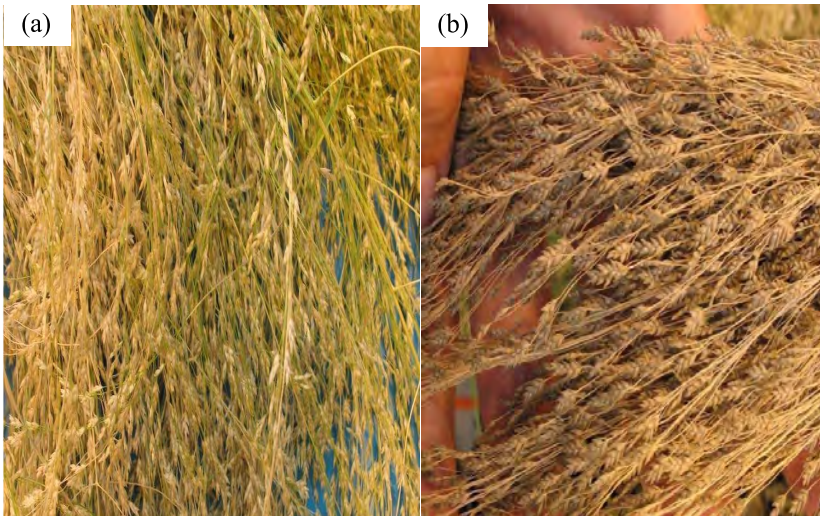

Fig. P1.4.6: (a) Panicles of Ayana and (b) Panicles of cultivar 04T19. Cultivar 04T19 headed later but reached grain maturity earlier and therefore was more uniform than cultivar Ayana.

Fig. P1.4.7: The gradient of ripeness of an average teff panicle (cultivar Ziquala) inset shows the difference in grain filling stage and maturity from seeds at the top of the panicle and at the bottom.

Chapter

5

Variation in cereal phyllochron revisited

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Abstract

• ***Background*:** The theory on the phyllochron under constant diurnal temperature and day length conditions is still controversial. Many studies have highlighted inaccuracies in predictions of the timing of appearance between two successive leaves (i.e. phyllochron) in the field. This chapter provides an accurate description of the fundamental concepts on the timing of leaf appearance in the cereals teff (*Eragrostis tef* (Zuccagni) Trotter), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.).

• ***Methods*:** We grew four teff cultivars under constant temperature conditions at six different day lengths. To assess the effect of temperature on rice phyllochron, we re-analysed literature data on four rice cultivars grown at five temperatures. Furthermore newly gathered data on timing of leaf appearance of outdoor-grown wheat was analysed.

• ***Key Results*:** There are two consecutive phases differing in phyllochron, phyllochron 1 (p_1) and phyllochron 2 (p_2), with $p_1 < p_2$. The effect of temperature on p_1 and p_2 can be normalised with the linear thermal time concept.

• ***Conclusions*:** Day length has no significant effect on the values of p_1 and p_2. The switch from p_1 and p_2 is abrupt and the difference between soil and air temperature can not account entirely for difference between p_1 and p_2.

Key words:
teff (*Eragrostis tef* (Zuccagni) Trotter), rice, wheat, leaf appearance, phyllochron, development rate, morphology.

Abbreviations used in this chapter

Roman Symbols	Explanation	Units	Eqns.
L	number of appeared leaves		5.1
L_c	number of leaves at the time of the phyllochron switch		5.1
L_s	initial (start) leaf number		5.1
p	phyllochron, i.e. duration between the appearance of two successive leaves	d	5.3
P	phyllotherm, i.e. duration between the appearance of two successive leaves	°Cd	
p_1	phyllochron 1	°Cd	5.1
P_1	phyllotherm 1	d	5.1
p_2	phyllochron 2	°Cd	5.1
P_2	phyllotherm 2	d	5.1
PI	panicle initation, occurrence of a generative apical meristem in 50% of the cases in a sample		
p_o	time to leaf appearance at the optimum temperature	d	5.4
T_b	base temperature for leaf appearance	°C	5.3 5.4
T_c	ceiling temperature for leaf appearance	°C	5.4
T_Δ	the theoretical difference between air and soil temperature	°C	5.5
T_D	day temperature	°C	
$t(L)$	time to appearance of leaf L	d	5.1
T_o	optimum temperature for leaf appearance	°C	5.4
T_N	night temperature	°C	
Z	dummy variable		5.2
Greek Symbols	**Explanation**	**Units**	**Eqns.**
β_0	intercept in a linear regression equation relating day length and phyllotherm	°Cd	5.6
β_1	slope of change in a linear regression equation relating day length and phyllotherm	°Cd·h^{-1}	5.6
β_{P_2}	exact prediction for the time to final leaf rank (regression variable)	d	5.2
θ_T	thermal time constant	°Cd	5.3

Introduction

In cereals, duration in days (d) between the appearance of two successive leaves is commonly termed phyllochron (symbolised with small letter '*p*'); phyllotherm (symbolised with capital letter '*P*') is the corresponding time in degree days (°Cd) above a threshold minimum or base temperature, T_b, and below an optimum temperature, T_o (Cao & Moss, 1989a; Bonhomme, 2000). Above the optimum temperature, T_o, phyllotherm can be calculated using a threshold maximum or ceiling temperature, T_c (Porter & Gawith, 1999). In the temperature range between base and optimum temperature it is commonly assumed that the phyllochron for all successive leaves is constant under constant temperature conditions. It is furthermore assumed that the phyllotherm is constant under variable temperature conditions (White *et al.*, 1990; Ellis *et al.*, 1993; Jamieson *et al.*, 2008). On the basis of this assumption one linear function should be able to describe the increase in number of appeared leaves over time expressed in degree days (°Cd). However, Cao and Moss (1991) concluded that: 'the use of a constant value for phyllochron in dynamic crop models of wheat leaf development is not appropriate for all circumstances.' Earlier, the suggestion was made that phyllochron is related to the rate of photoperiod change at seedling emergence (Baker et al., 1980) or that the thermal/photo ratio (the ratio of daily degree-days to day length) for the week following seedling emergence (Cao & Moss, 1989b) affected the phyllochron. Jame et al. (1998) concluded for wheat and barley that the mismatch between field observations and simulated leaf appearance can be solved by incorporating a curvilinear relation between effects of temperature and photoperiod on leaf appearance rate. However, several studies found a sudden, instead of a gradual, change in cereal phyllochron. This sudden change was observed around the time the apical meristem switches from vegetative to generative development, e.g. for rice (*Oryza sativa* L.) (Nagai, 1963; Baker et al., 1990) and for wheat (*Triticum aestivum* L.) (Baker et al., 1986; Boone et al., 1990).

The observed change in phyllochron could be a direct result from a change in temperature experienced by an apex. Because, an apex below or close to the soil surface can experience a temperature that significantly differs from the air temperature (Jamieson *et al.*, 2008). Hence, soil temperature may be the better predictor of phyllochron until internode extension lifts the apex above ground. Consequently wrong assumptions on meristem temperature lead to inaccurate predictions of development rate.

Although Baker *et al.* (1980), Jame *et al.* (1998) and Jamieson *et al.* (2008) gave different reasons for variation in phyllochron, all these studies assume that phyllochron is constant under conditions of constant diurnal day length and temperature. This contradicts the findings that Yin and Kropff (1996) made in a pot experiment with submerged rice (*Oryza sativa* L.). They showed that under conditions of constant day length and temperature, i.e. pot water temperature equalled air temperature, phyllochron was not constant during plant development. Streck *et al.* (2008) acknowledged this observation and correspondingly modelled retardation of leaf appearance rate as a smooth function of plant age. However,

considering their residual plots, the model adjustment of Streck *et al.* (2008) only accounted for part of the systematic gap between data and model predictions.

In this chapter, we postulate that the switch from vegetative to generative development relates to a slower outgrowth of leaves. The biological background for this hypothesis is as follows. Florigen, is a putative mobile signal that moves from an induced leaf to the apical meristem and causes flowering (Corbesier & Coupland, 2006). A candidate for encoding florigen is the FLOWERING LOCUS T (FT) gene found in the LDP Arabidopsis (Kobayashi *et al.*, 1999). An ortholog of the FT gene, termed Heading date 3a (Hda3), is the florigen candidate in the SDP rice (Tamaki *et al.*, 2007). Not only does FT expression result in early flowering, it also reduces leaf growth in both tomato (Lifschitz *et al.*, 2006) and Arabidopsis (Teper-Bamnolker & Samach, 2005). Lifschitz *et al.* (2006) suggested that 'floral transition and growth attenuation, instead of being the consequence of one another, are two facets of the same cellular responses'. If this postulate is applicable to cereals, i.e. leaf growth is attenuated when the plant reaches a generative stage, then the phyllochron would increase at the moment of panicle initiation. This hypothesis contradicts the current concept of a constant phyllochron.

Therefore in this chapter we will first test the hypothesise that there are two consecutive phases differing in phyllochron, called phyllochron 1 (p_1) and phyllochron 2 (p_2), with (p_1) < (p_2). In order to test this hypothesis we develop a model framework that can identify putative changes in phyllochron under constant environmental conditions.

If indeed under constant conditions the developmental response changes, then the base temperature (T_b) and thermal time constant (θ_T) of the two phyllochrons would also differ. Therefore our second hypothesis is that the impact of temperature on p_1 and p_2 can be normalised with the linear thermal time concept. However, if indeed there are two phyllochrons, then the thermal time parameters T_b and θ_T will differ between p_1 and p_2. To test this hypothesis literature data of four rice cultivars grown at five temperatures (Yin & Kropff, 1996) were re-examined.

Third, we hypothesise that even if the correct apex meristem temperature is used, cereal phyllotherm is not constant under constant conditions. The difference between soil and air temperature could nullify a putative change in phyllotherm. Therefore we will calculate the temperature difference required to equalize phyllotherm 1 (P_2) and phyllotherm 2 (P_2). This temperature difference will then be compared to the difference between soil and air temperature measured under controlled conditions in teff.

Fourth, we hypothesise that day length has no significant effect on the value of the phyllochron or phyllotherm itself. The time of panicle initiation is often under the regime of day length. Therefore day length may have an effect on the timing of the switch between P_1 and P_2. To this hypothesis leaf appearance data and data on timing of panicle initiation from four teff cultivars grown under six constant day length regimes were used.

In summary, over the past decades many studies have highlighted inaccuracies in predictions of the timing of appearance of successive leaves (Cao & Moss, 1991; Kirby, 1995) (McMaster & Wilhelm, 1995; Jame *et al.*, 1998; Jamieson *et al.*, 2008). As a vital condition for

making accurate predictions in the field, the fundamental concepts on the timing of leaf appearance obtained under constant temperatures need to be correct. Yet, as explained, divergent views exist on this matter. Towards finding a biological mechanism; the main objective of this chapter is to provide an accurate description of the fundamental concepts on the timing of leaf appearance under conditions of constant diurnal temperature and day length. Note that the approach used in this study will not identify a biological mechanism on a molecular level. For molecular biologist this study will merely point in a direction to look. For plant crop physiologist and crop modelers the study outcome can contribute to a better conceptualization of crop behaviour. We use new data on teff (*Eragrostis tef* (Zuccagni) Trotter) and previously published data of Yin and Kropff (1996) on rice, both crops are grown under constant diurnal temperature and day length conditions. Additionally, we examine spring wheat grown outdoors (thus under varying temperature and photoperiod conditions) during the summer in the Netherlands.

Materials and Methods

Plant material and growth environment

Teff

Four teff cultivars (i.e. Gibe (cv. DZ-Cr-255), Ziquala (cv. DZ-Cr-358), Ayana and cv. 04T19 obtained from Millets Place (the Netherlands)) were grown in pots in growth chambers with day lengths of 9, 10.5, 12, 13.5, 15 and 16.5 $h \cdot d^{-1}$. All facilities could be cooled and heated, day temperature (T_D) of 23 °C was set to coincide with the assimilation light period of 9 h hours and night temperature (T_N) was set to 16 °C for 11 $h \cdot d^{-1}$. Transitions from night to day temperature and vice versa were gradual and took 2 $h \cdot d^{-1}$ per transition, resulting in an average daily air temperature of 19.4 °C. The soil temperature was measured but not controlled, irrigation water was regulated to be at the same temperature as the air in the growth chamber.

Rice

Published data of Yin and Kropf (1996) on four rice cultivars were used. Main treatment characteristics are summarized here; for more details we refer to (Yin, Kropff & Goudriaan, 1996) and (Yin & Kropff, 1996). Four rice cultivars (IR36, IR72, IR64616H and Nipponbare) were grown at five constant temperature regimes (22, 24, 26, 28 and 32 °C) and four diurnally fluctuating T_D/T_N temperature regimes (26/22, 30/22, 22/26 and 22/30 °C). In each diurnally fluctuating regime, T_D and T_N were imposed for 12 h d^{-1}. The photoperiod was maintained throughout the experiment at 12 h d^{-1}, coinciding with the period of T_D. Rice plants were grown in pots in each environment from seedling emergence to flowering. The pot water temperature was controlled to be the same as the air temperature.

Wheat

In an outdoor experiment conducted under natural weather conditions in Wageningen, the Netherlands (51°58' N) from April to August 2006, spring wheat (*Triticum aestivum* L., cv. Minaret) plants were grown in containers of 70 × 90 cm, holding a soil layer approximately 35 cm deep. The containers were arranged closely together to ensure canopy homogeneity, and surrounded by guard containers to prevent border effects. Both soil and air temperature were measured.

Observations

For teff, each regime contained per cultivar six pots with ten plants each. Three plants per pot were used for observations. For rice each regime × cultivar combination contained two pots in each of which one plant was measured. For wheat, 10 plants per treatment were used for leaf appearance observations. In all species leaf appearance rate was based on the plant's main stem. A leaf was recorded as appeared when the tip of the leaf had emerged from the sheath of the preceding leaf. Leaf appearance was recorded daily during initial stages of development and the frequency was reduced to at least twice a week in later stages of plant development. Final leaf number is defined as the number of leaves that have appeared on a particular shoot until the shoot was heading (i.e. final leaf number is the number of vegetative phytomers on a shoot).

Microscopic observations in teff on the transition of the apical meristem from vegetative to generative, i.e. panicle initiation (PI) were done every second day on 6 plants per regime × cultivar combination. For these destructive observations additional pots were randomized within the experimental setup of teff. For more detail on observations of PI we refer to the materials and methods section in Chapter 4.

Modelling framework and statistical analysis

In this chapter we will abbreviate the units of phyllochron from d·leaf^{-1} to d, and for phyllotherm from °Cd·leaf^{-1} to °Cd.

In order to calculate the phyllotherm in cereals, a base temperature (T_b) is needed. For rice the base temperature was set to 10 °C (Yin & Kropff, 1996). For wheat the base temperature was set to 0 °C (Amir & Sinclair, 1991). The base temperature for teff was estimated to be 7.8 °C. This estimation was done by plotting the development rate of the first 5 leaves of cv. Ayana against temperatures of 16.3, 18.3, 18.8, 19.3, 19.8 and 24.3 °C. Linear extrapolation to a development rate of 0 identified the base temperature (Appendix 1 Fig. A1.5.9).

To test our first hypothesis, time required for each individual leaf to appear was described using:

$$t(L) = P_1 \cdot L - P_1 \cdot L_s \qquad \text{if } L_c < L \tag{5.1a}$$

$$t(L) = t(L_c) + P_2 \cdot L - P_2 \cdot L_c \qquad \text{if } L_c \geq L \tag{5.1b}$$

if P_2 is detected and thus both eqns 5.1a and 5.1b apply, then it can further be derived that:

$$P_1 = \frac{t(L_c)}{L_c - L_s} \tag{5.1c}$$

where variable $t(L)$ is the time in °Cd to appearance of leaf L; P_1 the first phyllotherm (°Cd); L the number of appeared leaves; P_2 the second phyllotherm (°Cd); L_c the number of leaves at the time of the phyllochron switch $t(L_c)$, L_c may be beyond the final leaf number in which case the regression is restricted to eqn 5.1a. Appearance of the first leaf was not recorded for each individual plant and since not all plants showed their first leaf exactly at $t = 0$, estimation of the parameter for the initial (start) leaf number, L_s, was allowed to vary between values of 0.5 and 1.5. Note that eqn 5.1 can be expressed both in degree days and in chronological time. In the first case phyllotherm (P in °Cd) is calculated and in the second case phyllochron (p in d). Note, furthermore, that we describe time to leaf appearance for individual plants rather than averages or lumped data of a number of plants.

An F-test (Appendix 2; A2.5.7) was used to determine whether the data could best be described using one phyllotherm (eqn 5.1a), or two phyllotherms (both eqns 5.1a and 5.1b). In order to obtain a reliable parameter value for P_2, appearance data from at least two successive leaves needed to be available. When the parameter value for P_2 was based on one leaf rank only, no value was assigned to P_2, but still the presence of a switch in phyllotherm was recorded. If the data were best described with one phyllotherm (eqn 5.1a) then a test was done to investigate whether the time of appearance of the last leaf deviated significantly from the regression line describing leaf appearance versus time. This was done by including an exact prediction (β_{P_2}) for the time to final leaf rank in eqn 5.1a. Using a dummy variable (Z) equal to one for the final leaf rank and equal to zero for all preceding leaves, the value of parameter β_{P_2} was estimated using regression analysis. If a student t-test indicated (Appendix 2; A2.5.8) that parameter β_{P_2} was significantly higher than zero then, the time of appearance of the last leaf deviated significantly from the average phyllotherm.

$$t(L) = P_1 \cdot L - P_1 \cdot L_s + Z \cdot \beta_{P_2} \tag{5.2}$$

Additionally we recorded whether the residual of the predicted time to appearance of the final leaf was smaller (negative) or bigger (positive) than predicted by eqn 5.1a.

To test our second hypothesis, the relation between temperature and the parameters of eqn 5.1 was described by rewriting the linear thermal time model as:

$$p = \frac{\theta_T}{(T - T_b)} \tag{5.3}$$

where p represents variable p_1 or p_2 (d) of eqn 5.1, T_b is the base temperature (°C) where time to leaf appearance is infinite, θ_T is the thermal time constant (°Cd), i.e. phyllotherm 1 or 2.

If permitted on basis of an F-test the 3 parameter beta function instead of the linear thermal time model (eqn 5.3) was used:

$$p(T) = p_o \left[\left(\frac{T - T_b}{T_o - T_b} \right)^{\frac{T_o - T_b}{T_c - T_b}} \left(\frac{T_c - T}{T_c - T_o} \right) \right]^{\alpha} \tag{5.4}$$

where p represents variable p_1 or p_2 (d) of eqn 5.1; T the temperature (°C); T_b the base temperature (°C) set to 10 °C for rice (Yin & Kropff, 1996); T_c the ceiling temperature (°C) set to 42 °C for rice (Yin & Kropff, 1996); T_o the optimal temperature (°C) at which the largest value i.e. p_o of variable p is reached; α is a shape parameter. Note that both paramters T_b and T_c are fixed, consequently this eqn 5.4 has only three variables, i.e. T_o, p_o and α.

To test our third hypothesis, the time in 'normalised days' phyllotherm was calculated and divided by the average temperature. This was appropriate because, T_b of wheat is generally assumed to be zero (Amir & Sinclair, 1991) and during the experiment temperatures did not drop below 7 °C. For both teff and rice it was possible to express the time to leaf appearance (phyllochron) in days (d) instead of degree days (°Cd) for the reason that the experimental facilities maintained the same average day temperature throughout the entire experiment. The temperature increase (T_Δ) needed to equalize the slope of phyllotherms, P_1 and P_2, was estimated by:

$$T_\Delta = \frac{p_2 - P_1}{p_1} \tag{5.5}$$

where T_Δ is the theoretical difference between air and soil temperature ($T_\Delta = T_{soil} - T_{air}$) (°C), p_2 is the second phyllotherm (°Cd), P_1 the first phyllotherm (°Cd) and p_1 phyllochron (d).

To test our fourth hypothesis the relation between day length and the parameters of eqn 5.1, were described using a standard linear model:

$$\theta_T = D \cdot \beta_1 + \beta_0 \tag{5.6}$$

where the thermal time constant, θ_T, represents the value of parameter P_1 or P_2 (°Cd) of eqn 5.1 and D the day length in hours, β_1 the slope of change (°Cd·h^{-1}) and β_0 the intercept

(°Cd). Note that when parameter β_1 is not significantly different from zero there is no day length effect and thus β_0 equals θ_T.

Nonlinear least squares regression was performed with the simplex method, using the built-in optimisation function 'fminsearch' of Matlab (MathWorks, Natick, Massachusetts, USA) version 7.8.0.347 (R2009a). In order to prevent fminsearch to convergence to a local minimum the fit was repeated with 10 different combinations of initial parameter values. These 10 combinations were based on a selection of the lowest sum of residuals in a population of 10^4 randomly chosen parameter combinations (all within logical biological boundaries). SAS version 9.1.3 SP4 (SAS Institute Inc., Cary, NC, USA) was used for all comparisons between means. Assessment for significant differences was done with the least square means (LSMEANS) using Tukey's honest significance test ($p \leq 0.05$). Means in the text are followed by their standard error in parenthesis.

Results

Phyllochron is not constant during plant development

Plotting leaf rank of teff, rice and wheat against time required for an individual leaf to appear, revealed that duration between the appearances of two successive leaves (phyllochron (d) or phyllotherm (°Cd)) is not constant for all leaf ranks (Fig. 5.1). For lower ranked leaves, however, duration between the appearance of two successive leaves was constant (phyllochron 1 (p_1) or phyllotherm 1 (P_1)). And similarly there was a constant duration between the appearance of two successive higher ranked leaves (phyllochron 2 (p_2) or phyllotherm 2 (P_2)). Equations 5.1a and 5.1b gave a good description of the duration (°Cd) between the appearance of two successive leaves, *viz.* for teff on average $\overline{r^2} = 0.995$, rice on average $\overline{r^2} = 0.998$ and for wheat on average $\overline{r^2} = 0.995$ (e.g. Fig. 1). The good fit on this leaf appearance data of individual plants, confirmed our first hypothesis *viz*: the existence of two phyllotherms. Whether or not an actual value was attributed to phyllotherm 2 (P_2) or phyllochron 2 (p_2) depended on the leaf rank at which the switch occurred (L_c) and the final main stem leaf number. For teff the final leaf number depended, on day length and cultivar (Table 5.1 and Fig. 5.2).

Here we explain the testing of first hypothesis in more detail. Our identification method for a putative second phyllotherm requires a sufficient number of degrees of freedom (Fig. 5.3). The number of degrees of freedom is directly related to the final leaf number. Therefore at lower final leaf numbers the second phyllochron might remain undetected. In fact, in the majority of the cases where only one phyllochron was detected, the residual of the last leaf was positive. On basis of a normally distributed error this was not to be expected. In other words, depending on temperature and day length our measuring and modelling approach did not always allow us to observe or quantify phyllotherm 2 (Fig. 2). However, the existence of parameter β_{P_2} and the systematically higher and positive residuals of the last leaf are a strong indication of an increase in time to leaf appearance

for the higher ranked leaves (Fig. 5.4). The switch between phyllotherm 1 and 2 was for all cultivars and species around leaf rank 6 to 8 with exception of rice cultivar Nipponbare where the phyllochron switch occurred at leaf rank 4 to 5 (Table 5.2). For all individual wheat and rice plants a value for phyllotherm 2 (P_2) could be assigned (Table 5.3 and A3.5.6), except for the rice cultivar Nipponbare. For this rice cultivar attribution of a value to parameter was not possible to one plant at 24 °C and one at 26 °C. For this rice cultivar attribution of a value to parameter p_2 was not possible to one plant at 24 °C and one at 26 °C. For teff the number of times a value for parameter P_2 could be obtained increased with increasing leaf number and increasing leaf number was itself a function of day length (Table 5.1 and Fig. 5.2).

The temperature response of parameters p_1 and p_2

Our second hypothesis was that the impact of temperature on parameters p_1, and p_2 can be normalised with the linear thermal time concept. But that the thermal time parameters T_b and θ_T will differ between p_1 and p_2. Equation 5.3 provided a good description of the relation between the parameters p_1 and p_2, and temperature (Fig. 5.5 and Table 5.4). The good fit of eqn 5.3 indicates that phyllochron at different temperatures can be normalised using the thermal time concept. However, since there were two phyllochrons we obtained two values for θ_T. A value resulting from fitting parameter p_1, in which case $\theta_T = P_1$, and a value resulting from fitting parameter p_2, in which case $\theta_T = P_2$ (Table 5.4). The values of the thermal time parameters T_b and θ_T, moreover, not only differed between the first and second phyllochron but also differed between cultivars (Fig. 5.5 and Table 5.4).

A change in apex temperature can not explain the phyllochron switch

Our third hypothesis was that the difference between soil and air temperature ($T_\Delta = T_{soil} - T_{air}$) could not nullify a putative change in phyllotherm. Therefore the temperature difference (T_Δ) required to equalize phyllotherm 1 (P_1) and phyllotherm 2 (P_2) was calculated for each species with eqn 5.3. For teff we used a base temperature, T_b, of 7.8 °C to calculate phyllotherm. The resulting average value of T_Δ was 10.0(0.39) °C, calculated using all day lengths and teff cultivars (Table 5.1). In other words the air temperature should have differed by 10.0 (0.39) °C from the soil temperature to provide a single phyllotherm. If we used a rather high T_b e.g. 10 °C, then we still obtained a value for T_Δ of 9.2(0.31) °C. The measured average difference between soil and air temperature was 0.39 °C. For rice, water temperature ($T_{water} = T_{soil}$ in eqn 5.3) equalled the air temperature, therefore the actual value of T_Δ should be close to zero. In rice, however, the calculated value of T_Δ ($T_b = 10$°C) to explain phyllotherm differences was 18.2(1.6) °C, averaged over all temperature regimes and cultivars (Table 5.2). In wheat the value of T_Δ ($T_b = 0$°C) was estimated to be 11.8(1.7) °C on average.

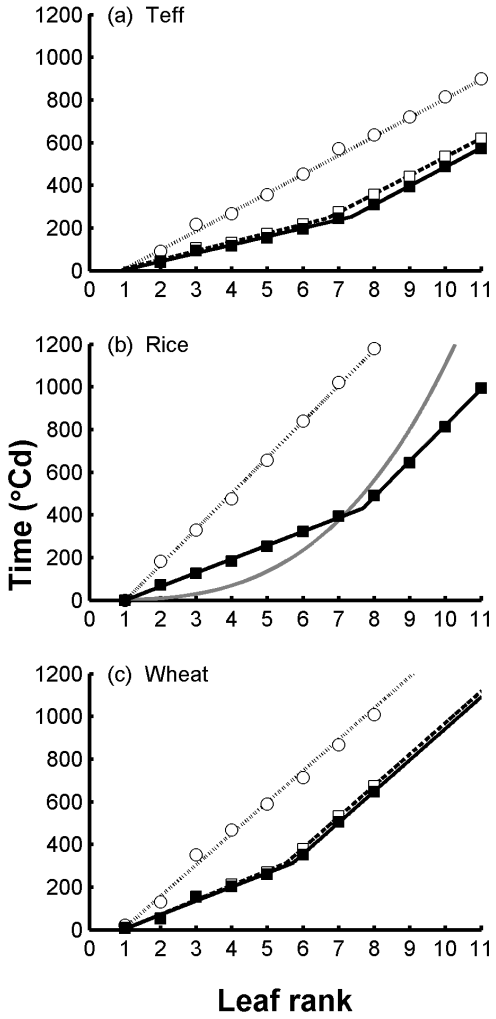

Fig. 5.1: Time required for a leaf to appear, in °Cd calculated with $(T - T_b) \cdot p$ ((a) for teff $T_b = 7.8$ °C,(b) for rice $T_b = 10$ °C and (c) for wheat $T_b = 0$ °C), plotted against leaf rank counted acropetally. Data points of each species represent leaf appearance data from a single representative main stem. Data points represented by closed squares are based on air temperature and are described with eqn 5.1a and 5.1b (solid line); open squares represent time required on basis of both soil and later air temperature (broken line eqn 5.1a and 5.1b); open circles are equal to the closed squares under the assumption that the second phyllochron, i.e. experiencing air temperature, is the representative pace of leaf appearance (dotted line calculated using eqn 5.5). The temperature required to lift the data points of the first phyllochron to the dotted line is the theoretical difference between air and soil temperature, T_Δ. The grey line in (b) is the model $y = x^b$ from Yin and Kropf (1996), expressing time in days in contrast to degree days as in the original publication.

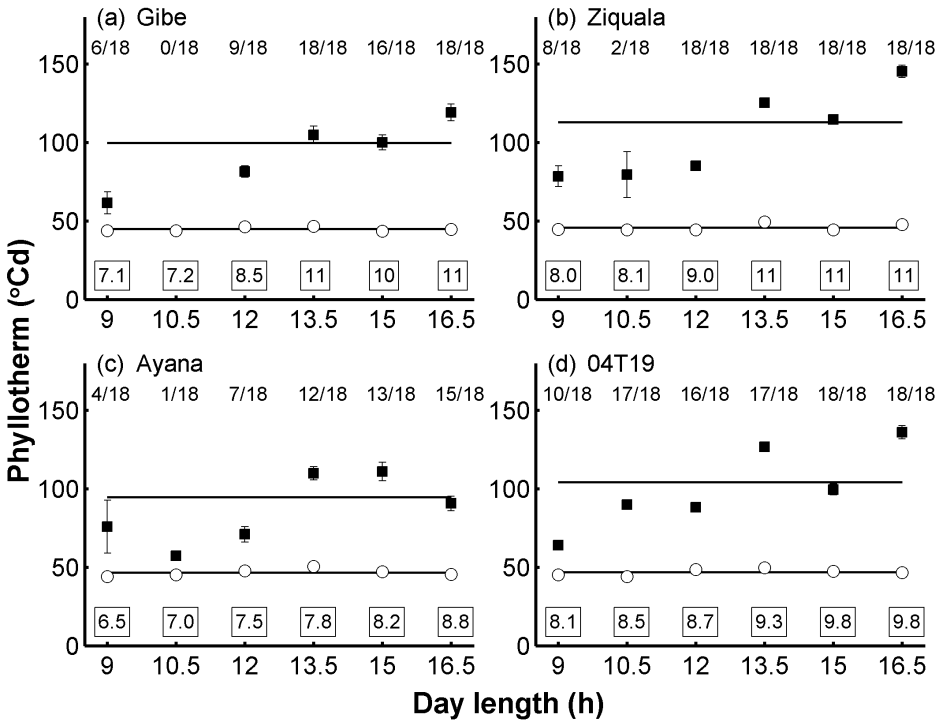

Fig. 5.2: Phyllotherm values in °Cd for phyllotherm 1 (P_1) (open circles) and for phyllotherm 2 (P_2) (closed squares) plotted against day length for four teff cultivars (a-d). Average final number of leaves is shown in boxes. For parameter P_1 n = 18, for parameter P_2, n is given at the top of each panel as a fraction of the total population. Parameter values and goodness of fit are given in Table 5.5.

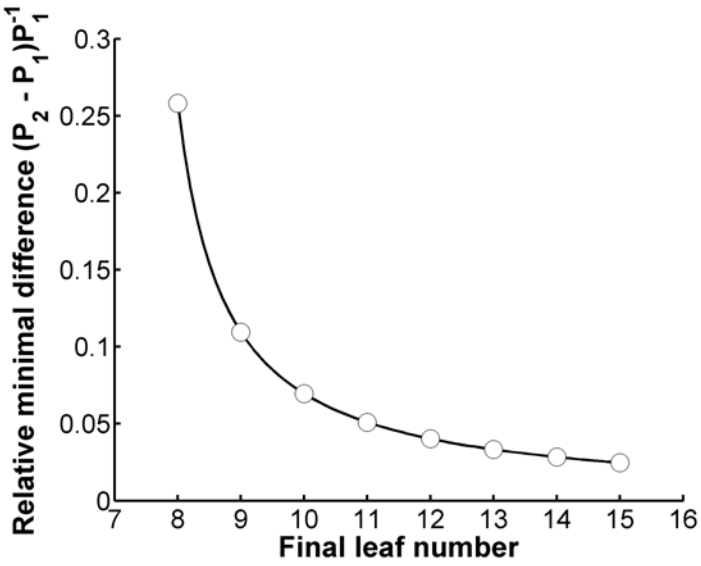

Fig. 5.3: Example of the relative minimal difference between phyllotherm 1, P_1, and phyllotherm 2, P_2, required within our model framework to assign a value to the second phyllotherm, P_2, as a function of final leaf number for teff cultivar cv. 04T19.

The effect of day length on phyllotherm

Our statistical analysis indicated that day length in teff did not affect the duration of either phyllotherm 1 (P_1) or phyllotherm 2 (P_2) (Fig. 5.2). As the temperatures in all greenhouses were the same, the data expressed in phyllochron (Table 5.1) are comparable to the data expressed in phyllotherm (Fig. 5.2). There was furthermore no significant systematic relation between day length and parameters L_c and $t(L_c)$. However, if the weight of the individual data points (i.e. number of observed switches per day length) was neglected, then phyllotherm seemed to increase with day length (Fig. 5.2). Moreover based on such an analysis of trend, the number of leaves at the switch, L_c, seemed to increase as did the time needed for the switch, $t(L_c)$, to appear. Phyllochron 1, in contrast, is even in such an analysis of trend unaffected by day length.

Clustering parameter values per cultivar showed that there was no significant difference between cultivars in parameter P_1 or P_2 in teff. However, the Dutch cultivars, Ayana (265(7.7) °Cd) and cv. 04T19 (265(5.6) °Cd), needed significantly less time to switch phyllochrons ($t(L_c)$) compared to the Ethiopian cultivars, Ziquala (293(6.2) °Cd) and Gibe (289(7.0) °Cd). The average number of leaves (L_c) at the moment of the switch was also significantly smaller for Ayana (6.9(0.14)) and 04T19 (6.9(0.10)) compared to Ziquala (7.5(0.11)) and Gibe (7.6(0.12)).

Since the phyllotherm switch could also be a consequence of the meristem switching from vegetative to generative development, i.e. panicle initiation (PI), the time to PI at each

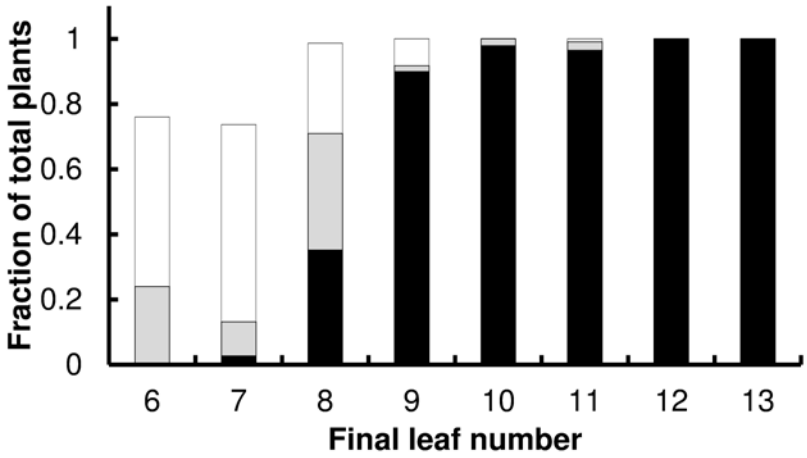

Fig. 5.4: Fraction of teff plants, of all cultivars, with a putative phyllotherm 2 (P_2). The fractions are grouped per final leaf number and consist of three categories: (i) plants for which a second phyllotherm was identified (black part of the bar), (ii) plant for which an additional parameter, β_{P_2} (eqn 5.2), was justified to describe the time to appearance of the last leaf (grey part of the bar) and (iii) plants for which parameters P_2 and β_{P_2} were not justified but for which eqn 5.1a, P_1, underestimated the last leaf (positive residuals) (white part of the bar).

day length was plotted against time to phyllotherm switch ($t(L_c)$). However, no significant one to one correlation between $t(L_c)$ and time to panicle initiation (PI) could be found. The moment the plant started elongating its internodes was also not correlated with time to phyllochron switch ($t(L_c)$). We did find, however, a systematic one to one relation between parameter L_c (eqn 5.1) and the number of appeared leaves observed at PI plus one leaf rank, $r^2 = 0.991$ (Fig. 5.6). This indicates that after the first leaf following PI, leaves appeared slower than the leaves before PI.

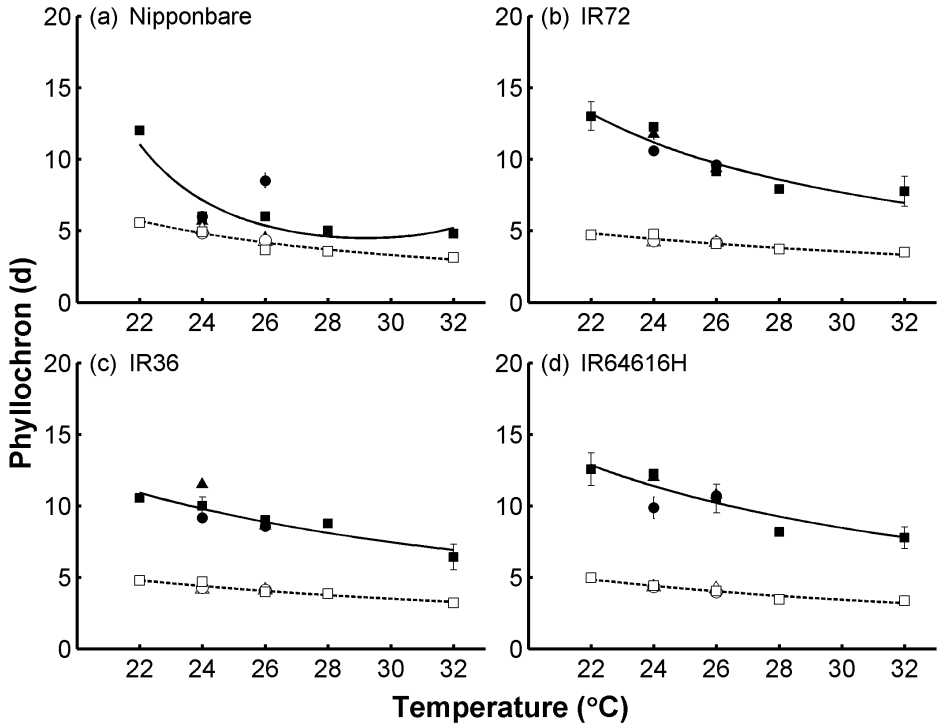

Fig. 5.5: Phyllochron values in days for phyllochron 1 (p_1) (open symbols) and for phyllochron 2 (p_2) (closed symbols) plotted against temperature for four rice cultivars (a-d). Constant temperatures are represented by squares, both averages of 24°C and 26°C represent weighted means of diurnally fluctuating temperatures (T_D/T_N) with low day temperature 22/26 or 22/30 °C (triangles) and high day temperature 26/22 or 30/22 °C (circles). All groups of data were best described using a linear thermal time model (eqn 5.3), except p_2 of Nipponbare (panel (a) closed symbols), where the additional parameter of the beta model (eqn 5.4) was justified. For all individual rice plants a value for phyllotherm 2 could be assigned, except for one Nipponbare plant at 24 °C and one at 26 °C. Thus n =2 for all data points except for p_2 of Nipponbare at 24 °C and 26 °C, where n = 1. Final leaf numbers are given in Table 5.2, parameter values and goodness of fit are given in Table 5.4.

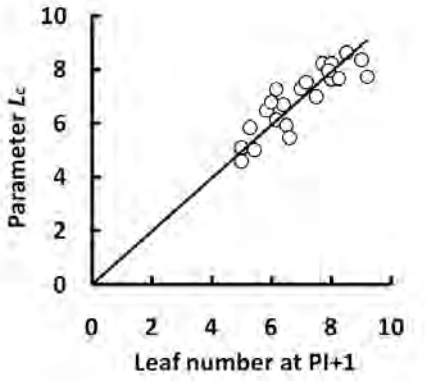

Fig. 5.6: Leaf rank after panicle initiation (PI) plus one versus calculated leaf rank at phyllochron switch (parameter L_c) estimated by eqn 5.1 (circles) for four teff cultivars at four day length regimes (9, 13.5 15 and 16.5 h) and 2 temperature regimes (16.3 and 19.4 °C) $r^2 = 0.991$.

Table 5.1: Final number of leaves and parameter values of eqn 5.1 and 5.5 for four teff cultivars at six day length regimes. Means per cultivar with the same letter are not significantly different ($p \leq 0.05$). For symbol explanation see footnote Table 5.2

Cultivar	Day length (h)	p_1 (d)		p_2 (d)[*1]		L_c (d)		$t(L_c)$ (d)		T_Δ (°C) at $T_b = 7.8$°C		Final leaf no.		Switch count[*1]
Gibe	9	3.8 (0.15)	A	5.4 (0.61)	B	5.0 (0.48)	B	13.6 (2.03)	B	4.1 (2.15)	B	7.1 (0.07)	C	6
	10.5	3.8 (0.05)	A	–		–		–		–		7.2 (0.07)	C	0
	12	3.8 (0.08)	A	6.7 (0.36)	B	7.1 (0.33)	A	20.4 (2.56)	AB	7.2 (1.96)	AB	8.5 (0.14)	B	9
	13.5	4.1 (0.11)	A	6.4 (0.53)	B	7.6 (0.23)	A	19.6 (3.37)	AB	10.2 (1.96)	AB	10.5 (0.07)	A	18
	15	4.0 (0.11)	A	7.7 (0.64)	AB	7.8 (0.34)	A	21.3 (3.44)	AB	10.1 (1.96)	AB	10.5 (0.20)	A	16
	16.5	4.0 (0.12)	A	9.8 (1.14)	A	8.2 (0.19)	A	27.3 (0.74)	A	14.1 (1.96)	A	10.8 (0.11)	A	18
Ziquala	9	3.9 (0.05)	BC	6.8 (0.66)	AB	6.0 (0.35)	C	12.7 (2.39)	A	7.7 (1.79)	BC	8.0 (0.00)	C	8
	10.5	3.8 (0.05)	C	6.8 (0.76)	AB	7.1 (0.25)	ABC	23.9 (1.00)	A	2.5 (2.83)	C	8.1 (0.06)	C	2
	12	3.7 (0.09)	C	7.1 (0.23)	AB	7.1 (0.16)	B	18.9 (1.15)	A	7.9 (1.63)	BC	9.0 (0.00)	B	18
	13.5	4.3 (0.13)	A	8.7 (1.12)	AB	8.4 (0.18)	A	19.1 (4.60)	A	14.2 (1.63)	AB	11.0 (0.00)	A	18
	15	4.0 (0.13)	ABC	6.4 (0.78)	B	7.7 (0.19)	AB	20.3 (2.71)	A	12.0 (1.63)	BC	11.2 (0.11)	A	18
	16.5	4.3 (0.11)	AB	10.5 (1.14)	A	8.3 (0.19)	A	21.7 (4.14)	A	19.6 (1.63)	A	11.1 (0.06)	A	18
Ayana	9	3.9 (0.04)	B	6.7 (1.16)	A	5.3 (0.17)	AB	15.5 (0.68)	AB	7.6 (2.58)	AB	6.5 (0.11)	C	4
	10.5	3.9 (0.09)	B	5.5 (.)	A	3.8 (0.84)	B	11.2 (2.46)	AB	2.7 (4.47)	AB	7.0 (0.15)	BC	1
	12	3.9 (0.10)	B	5.9 (0.43)	A	5.8 (0.26)	AB	11.1 (2.78)	B	3.7 (1.83)	B	7.5 (0.11)	BC	7
	13.5	4.4 (0.10)	A	7.8 (1.02)	A	6.6 (0.29)	A	18.3 (1.08)	AB	12.8 (2.00)	A	7.8 (0.27)	ABC	12
	15	4.2 (0.16)	AB	7.7 (1.43)	A	6.8 (0.25)	A	22.1 (1.21)	A	13.5 (1.83)	A	8.2 (0.37)	AB	13
	16.5	3.9 (0.11)	B	7.8 (0.64)	A	6.8 (0.45)	A	17.6 (3.40)	AB	8.9 (1.83)	AB	8.8 (0.53)	A	15
04T19	9	4.0 (0.12)	B	5.6 (0.18)	B	5.7 (0.28)	C	16.3 (1.19)	A	3.2 (1.73)	C	8.1 (0.06)	C	10
	10.5	4.2 (0.07)	AB	7.5 (0.35)	AB	6.9 (0.19)	B	20.8 (1.55)	A	7.5 (1.58)	BC	8.5 (0.11)	BC	17
	12	4.1 (0.04)	AB	7.3 (0.18)	AB	6.8 (0.20)	B	19.8 (1.43)	A	5.7 (1.58)	C	8.7 (0.13)	B	16
	13.5	4.4 (0.14)	A	8.2 (1.49)	AB	7.7 (0.14)	AB	25.0 (1.94)	A	14.0 (1.58)	AB	9.3 (0.17)	A	17
	15	4.2 (0.12)	AB	8.7 (0.55)	AB	7.1 (0.18)	B	21.3 (3.90)	A	7.9 (1.58)	BC	9.8 (0.11)	A	18
	16.5	4.1 (0.06)	AB	10.2 (1.07)	A	8.0 (0.20)	A	24.6 (2.91)	A	18.4 (1.58)	A	9.8 (0.07)	A	18

[*1] Column 'Switch count' contains the number of times that parameter p_2 was identified in a population of n = 18 (Fig. 5.2).

Table 5.2: Final number of leaves and parameter values of eqn 5.2 and 5.5 for four rice cultivars at five different temperature regimes. Means per cultivar with the same letter are not significantly different ($p \leq 0.05$). Note that no significant differences within cultivar were found for the final leaf numbers and parameter L_c.

Cultivar	Temperature(°C)	p_1(d)*1		p_2(d)*2		L_c(d)*3		$t(L_c)$(d)*4		T_Δ(°C)*5 at $T_b = 10$°C*6		Final leaf no.
IR36	22	4.8(0.02)	A	10.6(0.05)	A	7.7(0.02)		31.9(0.09)	A	14.6(0.23)	A	10.5(0.50)
	24	4.4(0.10)	AB	10.0(0.42)	A	7.7(0.15)		28.2(0.55)	AB	17.2(1.88)	A	10.5(0.22)
	26	4.0(0.05)	BC	8.7(0.14)	A	7.7(0.29)		26.7(1.37)	AB	16.1(0.54)	A	10.7(0.42)
	28	3.8(0.08)	C	8.8(0.25)	AB	7.5(0.21)		24.0(0.79)	BC	23.3(1.85)	A	10.0(0.00)
	32	3.2(0.07)	D	6.4(0.90)	B	7.1(0.52)		19.2(1.94)	C	22.1(5.28)	A	10.0(1.00)
IR72	22	4.7(0.08)	A	13.0(1.00)	A	7.9(0.25)		32.2(1.53)	A	20.8(1.56)	AB	11.0(0.00)
	24	4.4(0.11)	AB	11.5(0.33)	A	7.5(0.06)		28.7(0.92)	AB	22.4(0.81)	AB	10.7(0.21)
	26	4.1(0.02)	BC	9.4(0.14)	B	7.3(0.16)		25.5(0.95)	BC	20.8(0.57)	B	11.2(0.31)
	28	3.7(0.00)	CD	7.9(0.30)	B	7.7(0.01)		24.4(0.05)	CD	20.3(1.45)	AB	11.0(0.00)
	32	3.5(0.04)	D	7.8(1.05)	B	7.5(0.00)		21.1(0.08)	D	30.5(7.11)	A	11.0(0.00)
Nippon	22	5.6(0.00)	A	12.0(0.00)	A	6.9(0.00)		32.0(0.00)	A	7.6(0.00)	A	8.0(0.00)
	24	5.0(0.07)	A	4.9(0.98)	B	3.6(0.78)		11.9(2.74)	A	4.4(0.98)	A	7.5(0.34)
	26	4.1(0.14)	B	5.6(1.29)	B	4.6(1.30)		14.5(5.07)	B	6.4(2.04)	A	7.7(0.42)
	28	3.6(0.05)	BC	5.0(0.00)	AB	5.8(0.23)		16.8(1.17)	BC	7.4(0.36)	A	8.0(0.00)
	32	3.1(0.33)	C	4.8(0.20)	AB	5.9(0.59)		14.6(2.64)	C	12.4(1.75)	A	9.5(0.50)
IR64616H	22	4.9(0.15)	A	12.6(1.15)	A	7.7(0.06)		31.3(0.54)	A	20.0(3.41)	A	11.5(0.50)
	24	4.3(0.04)	B	11.4(0.52)	A	7.6(0.05)		28.5(0.34)	B	21.9(1.54)	A	10.8(0.31)
	26	4.1(0.06)	C	10.7(0.28)	AB	8.2(0.27)		28.9(1.27)	C	25.9(1.28)	A	11.0(0.26)
	28	3.4(0.14)	D	8.2(0.05)	BC	7.5(0.17)		22.7(0.40)	D	23.3(0.87)	A	11.0(0.00)
	32	3.4(0.10)	D	7.8(0.75)	C	8.1(0.51)		23.6(2.24)	D	25.7(0.34)	A	11.0(0.00)

*1 p_1 first phyllochron (°Cd) (eqn 5.1)
*2 p_2 second phyllochron (°Cd) (eqn 5.1)
*3 L_c number of leaves at the time of the phyllochron switch (eqn 5.1)
*4 $t(L_c)$ time at L_c(eqn 5.1)
*5 T_Δ theoretical difference between air and soil temperature (°C) to equalize p_1 and p_2 (eqn 5.5)
*6 T_b base temperature (°C) (eqn 5.3)

Table 5.3: Final number of leaves and parameter values of eqn 5.2 and 5.5 for wheat (n = 16). For symbol explanation see footnote Table 5.2

Cultivar	P_1(d)	P_2(d)	L_c(d)	$t(L_c)$(d)	T_Δ(°C) at $T_b = 0$°C	Final leaf no.
Minaret	64.8(0.22)	131.2(9.80)	5.9(0.10)	321.5(6.10)	11.8(1.70)	8.3(0.10)

Table 5.4: Parameter values for four rice cultivars of the linear thermal time model (eqn 5.3) and beta model (eqn 5.4) used in Fig. 5.5.

	Parameter p_1 as a function of temperature (Linear model)			**Parameter p_2 as a function of temperature (Linear model)**		
Cultivar	r^2	θ_T [*1]	T_b [*2]	r^2	θ_T [*1]	T_b [*2]
IR72	0.806	146.4	10.9	0.814	106.3	0.00
IR36	0.701	188.2	4.74	0.872	102.9	0.49
IR64616H	0.661	197.1	6.65	0.879	92.3	2.97

				Parameter p_2 as a function of temperature (Beta model)			
				r^2	α [*3]	T_o [*4]	p_o [*5]
Nipponbare	0.883	62.9	10.9	0.623	3.45	29.2	0.222

[*1] θ_T phyllotherm (°Cd) (eqn 5.3)
[*2] T_b base temperature (°C) (eqn 5.3)
[*3] α shape parameter (eqn 5.4)
[*4] T_o optimal temperature (°C) (eqn 5.3)
[*5] p_o phyllochron, p, at T_o (eqn 5.3)

Table 5.5: Parameter values of linear model (eqn 5.6) for teff, used in Fig. 5.2.

	Parameter p_1 as a function of day length			**Parameter p_2 as a function of day length**		
Cultivar	[*1]$\sum$(residuals)^2	β_1 [*2]	β_0 [*3]	[*1]$\sum$(residuals)^2	β_1 [*2]	β_0 [*3]
Gibe	1764	0	46.6	25298	-2.1	139.2
Ziquala	1109	0	45.8	33505	0	111.6
Ayana	1184	0	44.7	45060	0	99.7
04T19	1295	0	46.9	37868	0	96.8

[*1] Note that sum of squared residuals are given instead of r^2 because in most cases there was no parameter value for slope of change β_1
[*2] β_1 slope of change (°Cd·h^{-1}) (eqn 5.6)
[*3] β_0 intercept (°Cd) (eqn 5.6) * Note that if $\beta_1 = 0$ then $\beta_0 = \theta_T$

Discussion

The results of this study show that for the cereals teff, rice and wheat there are two consecutive developmental phases differing in phyllotherm. Within the two phases the phyllotherm is constant for each leaf. Therefore we can describe time to leaf appearance for all leaf ranks using two phyllotherms plus two parameters representing the moment and leaf rank at the switch between the two phyllotherms (eqn 5.1). We hypothesize that our model for time to leaf appearance (eqn 5.1) can fill the systematic gap between data and model predictions of Streck *et al.* (2008). Re-examining the data of Yin and Kropff (1996) for the higher range of sub optimal rice temperatures revealed, furthermore, for rice that phyllochron can be normalised using a linear thermal time model (eqn 5.3). In more detail, the linear 2-parameter thermal time concept (eqn 5.3) is the most parsimonious way of accurately describing phyllochron as a function of temperature, except for phyllochron 2 of Nipponbare, where the more extensive 3-parameter beta model (eqn 5.4) was justified on the basis of an F-Test (Fig. 5.5 and Table 5.4).

It is unlikely that the difference between soil and air temperature is in itself the only explaining factor for the difference between phyllotherm 1 and 2. The temperature difference, T_Δ, needed to equalize the two phyllochrons is much higher than the measured difference between soil and air temperature in teff. Moreover, in the rice experiments water temperature equalled air temperature and still our calculations indicate that large values of T_Δ are required to equalize the two phyllotherms. Although choosing a higher base temperature lowered the value for T_Δ, extremely high base temperatures still gave values for T_Δ that could not be explained by a soil − air temperature difference alone. Nevertheless we do still advocate accounting for the effects of both soil and air temperature on the development of the shoot system (i.e. meristem and outgrowing leaves) in experiments and models. We conclude, though, that phyllotherm differences can persist when taking such effects into account.

Day length had no significant effect on the value of phyllotherm 1 (P_1) in teff; on the other hand phyllotherm 2 (P_2) seemed to be sensitive to day length (Fig. 5.2). Nevertheless, based on our data no clear systematic relation between photoperiod and P_2 could be discerned. Detection of phyllotherm 2, i.e. establishing a parameter value P_2, depended on final leaf number (Fig. 5.4) and final leaf number, at constant temperature, is mainly determined by day length (Fig. 5.2). In other words, the alleged day length effect on phyllotherm 2 (P_2) is confounded with final leaf number.

The question arises what did cause the sudden change in phyllotherm and phyllochron? Increase in sheath length with leaf rank could be an explaining factor for the existence of two phyllochrons. Because the longer the sheath, the longer a leaf has to elongate before it visually appears. However, for leaf ranks higher than L_c, the sheath length is more or less constant in both teff and wheat (Fig. 5.7). Panicle initiation and the phyllotherm switch did not exactly coincide and we did not identify day length effects on phyllotherm 1. As argued before, the effects of day length on phyllotherm 2 are ambiguous.

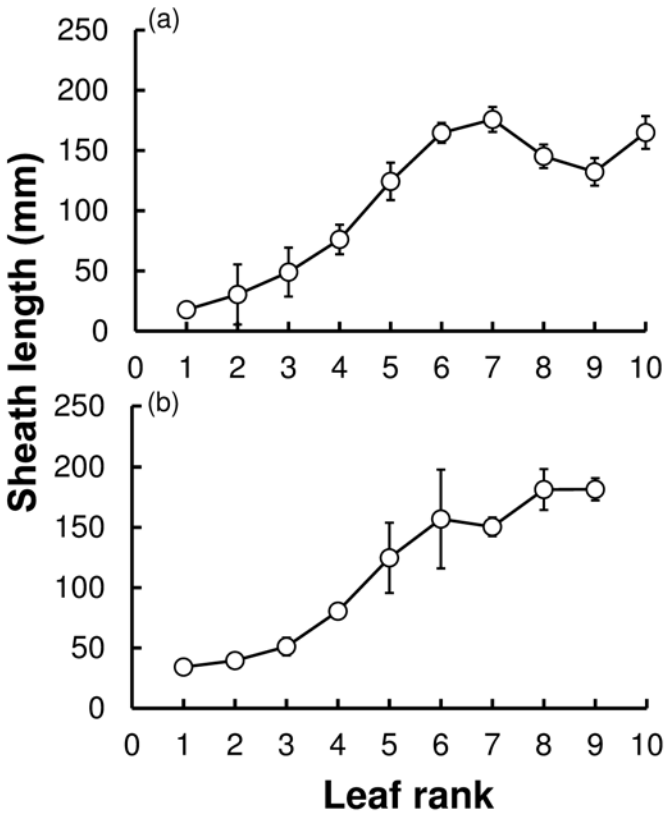

Fig. 5.7: Leaf rank plotted against sheath length of (a) teff cultivar 04T19 and (b) wheat cultivar Minaret. Data originate from an additional greenhouse experiment in containers and the wheat experiment as described in materials and methods.

In other words, our results do not pin point a particular environmental factor that explains the existence of two phyllotherms. Because the switch in phyllotherm is rather sudden, the hypothesis of a increase in phyllochron as a result of ageing is unlikely to be true for teff, rice and wheat. However, the sudden switch in phyllochron does indicate that there is a trigger signal for leaf appearance retardation at later stages of plant development.

We did find a systematic one to one relation between parameter L_c and the number of main stem leaves at PI plus one, $r^2 = 0.991$ (Fig. 5.6) indicating that from the second leaf following PI onwards, leaves appeared slower than the leaves before PI. Consequently, the timing in days (or degree days) of PI can be estimated by subtracting the value of p_1 (or P_1) from the value of $t(L_c)$. From a physiological perspective this seems logical. As at the moment of PI, the leaf in the sheath of the appearing leaf is already substantially developed. Therefore a signal that retards leaf development will not have a clear observable effect on time to leaf appearance, i.e. parameter $t(L_c)$. Such a putative signal can, however, effect

the smaller leaves that will undergo substantial cell division in the near future. Although the evidence seems rigorous, one has to bear in mind that unlike the leaf appearance data, these are data averaged over a number of plants which individually differ in L_c. The PI data is based on the observation that 3 out of 6 randomly chosen plants per cultivar per day length regime contained a generative main stem meristem. No observations on leaf appearance could be done on the plants for which the meristem switch was observed due to the destructive nature of these observations. Thus, we did not establish a direct (biological) link for individual plants between the observations of PI and an increase in time to leaf appearance.

Although, in all the cases we studied a clear phyllochron switch was present, we note that leaf appearance data from the climate chamber experiment of Jamieson *et al.* (2008) with wheat cultivar Rongotea did not demonstrate an explicit switch in phyllochron. It is possible that the absence of a phyllochron switch in cultivar Rongotea is due to the relatively small leaf size of its final leaves, i.e. factor 2 to 3 smaller than e.g. cultivar Avalon (Lawless *et al.*, 2005). On the other hand it could be that in particular wheat cultivars the signal for leaf retardation is completely absent, or that these cultivars are simply not sensitive to this signal. Apart from any putative cultivar effect, we speculate that when authors did not observe the sudden switch in phyllochron this could be for two other reasons: either the environmental conditions induced low final number of leaves (making it harder to observe the switch (Fig. 5.3), or authors bulked data originating from different individual plants. Since there is plant-to-plant variation in the timing of the switch, $t(L_c)$, the leaf number at the switch, L_c, the final leaf number and the second phyllotherm, P_2, bulking data from different plants will result in a more curvilinear smooth transition between phyllotherm 1 and 2 (Fig. 5.8).

Therefore, for a whole population of plants, it is not necessarily inappropriate to describe the appearance of successive leaves over time with a smooth curvilinear power equation (Yin & Kropff, 1996) (Fig. 5.8). However, such a model implies a gradual change in phyllotherm for an individual plant, but as this study shows the change in cereal phyllotherm is rather sudden for individual plants, hence the term phyllotherm switch.

In this chapter we provided equations to describe the phyllochron of cereals over time. We did not identify a biological mechanism that causes this switch. Based on our results we postulate that there is a feedback mechanism between the leaves and the meristem. In short day plants florigen levels are expected to be low under long day conditions. Yet, although not significant, the parameter value of phyllotherm 2 increased with day length. This is not to be expected if florigen itself is the cause of leaf outgrowth retardation. Therefore in contrast to Lifschitz *et al.* (2006) who suggested that 'floral transition and growth attenuation are two facets of the same cellular responses in tomato', we argue that there might be an additional or indirect mechanism that under pins leaf outgrowth retardation in cereals. It would be interesting to know what the underlying mechanism for the phyllotherm switch is. Is the phyllotherm switch the result of source-sink relations between leaf and meristem? Does it involve sugar signalling or is there some other active signal? And like Thiagarajah and Hunt

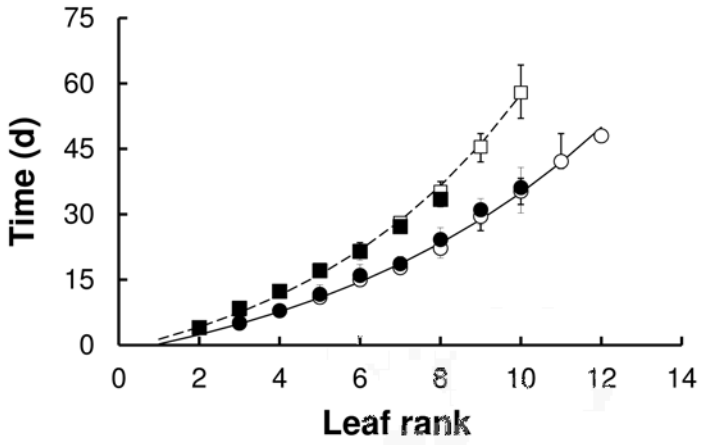

Fig. 5.8: Average time required for a leaf to appear (d) for teff plotted against leaf rank counted acropetally. Closed symbols represent a short day length (9 h); open symbols represent long day length (18 h); circles represent a high temperature (24.3 °C) and squares a low temperature (19.3 °C); error bar represent minimal and maximal time to leaf appearance (n = 1-18). The lines represent the model from Yin & Kropff (1996).

(1982) questioned for maize, what physiological reason causes phyllotherms to be more or less constant at different temperatures and day lengths?

In conclusion, the time between the appearances of successive leaves (i.e. phyllochron) is not constant. For the cereals teff, rice and wheat there are two consecutive developmental phases differing in phyllochron (i.e. phyllochron and/or phyllotherm 1 and 2). The effect of temperature on rice phyllochrons 1 and 2 can be normalised using the thermal time concept. Day length had, furthermore, no systematic effect on phyllotherm 1 and 2 in teff. The switch between phyllotherm 1 and 2 is abrupt and the difference between soil and air temperature is not the single explaining factor for the delayed outgrowth of the higher leaf ranks.

Appendix 1

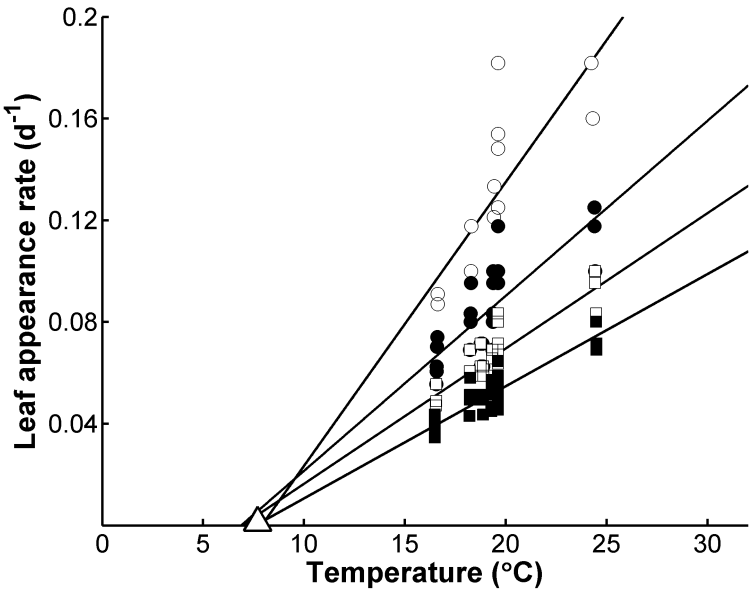

Fig. A1.5.9: Base temperature T_b (open triangle) estimated on basis of time^{-1} to appearance for leaf 2 (open circles); leaf 3 (closed circles); leaf 4 (open squares) and leaf 5 (closed squares) of cultivar Ayana plotted against the temperatures:16.3, 18.3, 18.8, 19.3, 19.8 and 24.3 °C. Lines represent simple linear regression lines used to extrapolate to a rate of zero, where $T_b = 7.8$°C

Appendix 2

Here we explain the used statistical tests in more detail. The following test statistic (Fisher's F-test) was used to test the significance of two additional model parameters (i.e. eqn 5.1a versus using both eqn 5.1a and 5.1b):

$$F = \frac{\left(\frac{SSE_{simple} - SSE_{extended}}{df_{simple} - df_{extend}}\right)}{\left(\frac{SSE_{extended}}{df_{extend}}\right)} \tag{A2.5.7}$$

where SSE_{simple} is the sum of squared error of eqn 5.1a (simple model) and $SSE_{extended}$ is the combined sum of squared error of eqn 5.1a and 5.1b (extended model); df_{simple} and $df_{extended}$ are the degrees of freedom for the simple and extended model, i.e. number of leaves minus total model parameters, for eqn 5.1a and 5.1b. Using a F probability density function the corresponding p-value was obtained. If the p value was smaller than or equal to 0.05, we concluded that the extended model was significantly better than simple model. If the p value was higher than 0.05, we concluded that there was no compelling evidence justifying the additional two of parameters of the extended model and thereby accepting the simple two parameter model.
The following test statistic (student t-test) was used to test the significance of parameter β_F (eqn 5.2).

$$t = \frac{\beta_F - 0}{se\left(\beta_F\right)} \tag{A2.5.8}$$

where β_F is a parameter of eqn 5.2 and se β_F is the standard error of the parameter calculated on basis of the regression standard error matrix. Using a student t probability density function the corresponding p-value was obtained. The null hypothesis $\beta_F = 0$ was rejected when the p-value was less than 0.05.

Appendix 3

Table A3.5.6: Final number of leaves and parameter values of eqn 5.2 and 5.5 for four rice cultivars at five different temperature regimes. Means per cultivar with the same letter are not significantly different ($p \leq 0.05$). Note that no significant differences within cultivar were found for the final leaf numbers and parameters P_2 and L_c

Cultivar	Temperature(°C)	P_1(d)[*1]		P_2(d)[*2]	L_c(d)[*3]	$t(L_c)$(d)[*4]		Final leaf no.
IR36	22	57.2(0.2)	BC	126.6(0.6)	7.7(0.0)	382.8(1.1)	A	10.5(0.5)
	24	57.9(1.6)	C	129.1(8.9)	7.0(0.4)	345.3(26.0)	A	10.5(0.2)
	26	61.9(1.0)	BC	124.0(2.8)	6.8(0.4)	357.9(27.2)	A	10.7(0.4)
	28	66.2(1.4)	AB	152.1(9.9)	7.2(0.4)	411.7(34.9)	A	10.0(0.0)
	32	70.0(1.5)	A	140.8(19.8)	7.1(0.5)	423.3(42.8)	A	10.0(1.0)
IR72	22	55.3(0.0)	C	151.2(7.2)	7.8(0.1)	373.5(5.4)	A	11.0(0.0)
	24	61.4(1.8)	BC	159.4(4.3)	7.5(0.1)	394.2(17.3)	A	10.7(0.2)
	26	65.2(1.0)	AB	149.9(2.2)	7.3(0.2)	408.0(15.2)	A	11.2(0.3)
	28	66.9(0.0)	AB	142.2(5.4)	7.7(0.0)	439.9(0.8)	A	11.0(0.0)
	32	71.5(0.0)	A	170.5(23.1)	7.5(0.0)	464.6(1.8)	A	11.0(0.0)
Nippon	22	62.4(0.0)	A	102.0(0.0)	5.7(0.0)	290.1(0.0)	A	8.0(0.0)
	24	61.8(2.2)	A	69.0(13.9)	3.8(0.8)	175.0(41.3)	A	7.5(0.3)
	26	64.0(2.0)	A	65.9(20.9)	3.9(1.2)	192.4(64.0)	A	7.7(0.4)
	28	63.9(0.9)	A	90.0(0.0)	5.8(0.2)	303.0(21.0)	A	8.0(0.0)
	32	64.9(3.3)	A	101.2(0.0)	5.5(0.2)	286.0(23.5)	A	9.5(0.5)
IR64616H	22	56.6(0.9)	D	150.6(13.8)	7.7(0.1)	375.1(6.5)	AB	11.5(0.5)
	24	60.6(0.6)	CD	155.6(7.3)	7.5(0.1)	390.2(7.5)	B	10.8(0.3)
	26	65.1(0.9)	B	170.4(4.5)	8.2(0.3)	462.6(20.3)	A	11.0(0.3)
	28	62.4(0.6)	BC	143.1(4.5)	7.2(0.4)	390.1(25.8)	AB	11.0(0.0)
	32	71.5(0.0)	A	155.1(1.1)	7.7(0.1)	473.4(4.1)	AB	11.0(0.0)

[*1] P_1 first phyllotherm (°Cd) (eqn 5.1)
[*2] P_2 second phyllotherm (°Cd) (eqn 5.1)
[*3] L_c number of leaves at the time of the phyllochron switch (eqn 5.1)
[*4] $t(L_c)$ time at L_c(eqn 5.1)

Chapter

6

General Discussion

Author

Sander H. van Delden

General discussion

The general objective of the work described in this thesis was to examine some of the major yield constraints of teff, thus helping breeders to identify targets for breeding high yielding cultivars for cultivation in the Netherlands. Together with this practical objective, I also wanted to advance current understanding of seed physiology, biomechanics and phenology of cereals and teff in particular. In the future the gained ecophysiological knowledge of teff could be used to underpin crop management measures and provide detailed breeding advice. This chapter will place the findings in this context and starts with a discussion on modelling and understanding of plants in general. Then for each research topic (germination (Chapter 2), lodging (Chapter 3), phenology (Chapters 4 and 5) the findings are summarised and future research directions towards a generic model for field-grown teff are described. This is followed by a discussion on the implications of the research findings for breeders. Finally I will discuss the best strategy for further scientific research on teff in order to increase grain yields in the Netherlands.

Modelling: a tool to conceptualise

As a plant biologist I am eager to understand the functioning of plants in relation to their environment. To help improve this understanding, a plant or a crop can be described as a system. A system is defined as a limited part of reality. This limited part of reality can be divided into interrelated elements. These elements can be further sub-divided, and this can be repeated until the elements become elementary particles. And from bottom-up, interacting, interrelated, or interdependent elements forming an integrated whole can be defined as a system. Chaitin (2006) argues "comprehension is compression." In my view, to understand (i.e. comprehend) a system, means to conceptualize its features to a certain extent. A concept can be defined as a general idea derived or inferred from specific instances or occurrences in reality (possibly represented in the form of schemes, tables, figures and equations) (American Heritage Dictionary of the English Language). According to De Wit (1924-1993) a model is simplified representation of a system capturing its essential features and serving a defined purpose. If the purpose of a model is to create understanding of system to a certain extent, a model should be a conceptualisation of a system. A model of a system can be constructed by describing correlations between system elements (e.g. seed and water potential). Model components are concepts that represent system elements, or groups of interdependent system elements, and thus often represent subsystems themselves (e.g. seed and water potential). The behaviour of the constructed model allows the modeller to generate understanding of the system in question. Comparing model behaviour and the observed reality can demonstrate the gained understanding of a system. If the model describes the system in its original environment (which was used to construct the model) adequately, but the model fails to describe the system in a new environment, then the understanding of reality is proven to be too simple. In contrast, a model is too complex (i.e. unnecessarily hinders understanding) when a

simpler model provides the same insights in the functioning of reality as a more complex model (Occam's razor). Model simulation studies can envisage new knowledge on system behaviour, without having observed such behaviour in reality.

Empirical and mechanistic models

A distinction can be made between empirical models and mechanistic models (Box & Hill, 1967; Thornley, 1976). An empirical model represents a summary of a certain system state. This system state (e.g. flowering, germination) is determined by a fixed empirical correlation with one or more system modulators (e.g. time, environmental factors). This class of models is based on observation, rather than on postulates and theory. Structuring and summarising numerous observations on system characteristics and system behaviour can lead to novel (empirical) insights on a system, i.e. the field of statistics. Empirical models are often easier to interpret than mechanistic models because empirical models generally have fewer parameters (Hunt, 1979). Empirical models reflect little of the mechanisms that underlie, or explain system behaviour.

Mechanistic models generally comprise elements that are at least one aggregation level deeper than the aggregation level of the system that is being modelled. Dynamic mechanistic models can describe multiple system states in time and space resulting from integrated feedback loops which are affected by system modulators. Mechanistic models can portray systems behaviour and emerging (not yet observed in reality) properties, which may well be unexpected and even counterintuitive (Yin & Struik, 2010). Thus, as discussed for empirical models, mechanistic models can also lead to new insights on a system. The level of understanding in mechanistic, opposed to empirical models, is generally deeper.

Biologically interpretable model parameters: a contribution to conceptualisation

A parameter in a model can be biologically interpreted if the parameter represents a particular state or concept of a biological system (e.g. minimal temperature for germination (T_b), Chapter 2). Additionally, parameters are also biologically interpretable when parameters modify the state of a biological system in a conceptual manner (e.g. growth rate). For physiologists Biologically interpretable parameters contribute to the ease and level of understanding of the system under research. The biological parameters used in this thesis are introduced to advance our understanding of cereals and teff in particular. This advanced understanding can contribute to the breeders concept of a high yielding teff cultivar, as will be discussed.

Models in this thesis

In this thesis several observations under controlled environmental conditions of the plant *Eragrostis tef* were presented. In order to summarize the data resulting from these observations, the data were compressed using empirical regression models. These models were mostly non-linear logarithmic equations. Linear models can, however, still be a useful representation of reality. For example by using broken linear functions (as used in Chapter 4) a sudden change in the system (plant) can be convincingly portrayed. In a crop situation single plants are elements (members) of a population, i.e. the crop. The crop can be regarded as a system with plants as elements. In contrast to using broken linear functions for single plants, in a crop situation the sum of the sudden changes of individual plants was best summarized using an empirical smooth continuous function (Fig.5.8). In this thesis I aimed to design and use regression models in such a way that they give an accurate and clear representation of the data at hand, by using only a limited number of parameters. I furthermore, attempted to design regression models with biologically interpretable parameters (see next paragraph).

Research findings and their limitations

In this section I will discuss some of the biological parameters that were used and computed for teff. I will summarize the findings per research topic (germination, lodging and phenology) and for each topic I make recommendations for future research to eventually create a dynamic crop model.

Germination

As discussed in Chapter 1, viable teff seed can fail to germinate as a result of sub-optimal or supra optimal temperatures and adverse water potentials in the top layer of the soil. Subsequently bad seedling establishment can reduce crop yields later in the season (Forcella *et al.*, 2000). In Chapter 2 of this thesis a model framework was presented in order to describe the ability of teff to germinate under different combinations of fixed temperature and water potential. We showed that the postulate of a normal distribution of seed germination rate can describe the time course of seed germination at any combination of temperature and water potential. Final germination percentage and lag phase are emerging from the combination of mean germination rate and its variation among seeds in a population. The biological concept that emerges from this model is that germination rate is not linearly related to temperature and that the biological parameter for minimal and maximal temperature for germination (base and ceiling temperature) depends on water availability (water potential). Conversely, the minimal water potential required for germination depends on temperature. We introduced the novel hydrothermal rate (HTR) approach, which overcomes fitting problems for teff seed germination that we encountered using the

conventional hydrothermal time (HTT) modelling approach (Alvarado & Bradford, 2002). In order to extrapolate this modelling framework to a field situation, the model needs to be extended on at least three points.

Firstly, the HTR model accurately describes the time to germination for a particular combination of water potential and temperature, but it remains to be tested whether the sum of rates in progress towards germination under fluctuating conditions follows directly from the rate of progress under static conditions. For example high temperatures could damage the seed and therefore delay or completely inhibit germination at a future moment in time. Optimal temperatures in combination with low water potentials that prevent germination can advance the progress towards germination (priming). Priming can enhance the speed of seed germination under permissive germination conditions (Dahal & Bradford, 1990). In other words, there could be some progression or regression towards germination that is not yet captured by our modelling framework.

Secondly, in Chapter 2 we discussed germination and not emergence. In a field situation seedling emergence is the prime indicator of crop establishment and seed germination is not. Germination is, however, the key component of emergence. We chose to research germination instead of emergence because seed germination is a less arbitrarily measured than seedling emergence. Another practical reason is that temperature and water potential can be better and more uniformly regulated for germinating seeds than for emerging seedlings. As recommended by the International Seed Testing Association (ISTA), we defined germination as the moment that the radicle had reached a length of 2mm instead of the moment the radical just protruded the seed. By using a radicle length of 2mm instead of radical protrusion, a germinated seed is more likely to also become a viable seedling. This makes the association between germination and emergence potentially simpler, but not necessarily simple. Sowing depth, diseases, herbivores, oxygen availability, seed reserves, and soil properties all affect the moment of and variation in seedling emergence in the field.

Thirdly, the experiments were done with one variety (Gibe Dz-Cr-255). Although this Gibe is a reasonably representative variety for Ethiopian teff, we observed in preliminary experiments that there were some differences in time to germination between varieties (and seed batches). Thus, in order to create a generic teff germination model, more varieties and seed batches have to be tested. Based on our results, there is no need to include dormancy or imbibition as separate factors in a future emergence model for teff. We did not observe any dormancy in the period from 2004 until present, and judging from the fast germination, within 1 day at higher temperatures, imbibition period in teff is negligibly short. In other words, imbibition is currently already effectively captured the modelling framework. Which is not surprising judged on the small seed size of teff.

Lodging

Analysis of the biomechanical properties of teff showed that teff is very susceptible to lodging. In contrast to the current conventional ideas of teff breeders, in Chapter 3 we assessed that not only the shoots of teff are prone to lodging but also the roots are a major factor in the lodging process on Dutch sandy soils. Furthermore we showed that water adhering to the shoots increases lodging susceptibility. To transform this model into a more dynamic mechanistic (explanatory) model under fluctuating field conditions several extensions are required.

Firstly, the location of the centre of gravity of a mouldable object (e.g. bending shoot with a drooping panicle) is not static, as suggested by the conventional lodging models developed for wheat (Crook & Ennos, 1994). Another assumption of the conventional modelling approach is that shoots can be interpreted as uniform rigid beams. However, teff shoots dimensions vary considerably (internodes taper and successive nodes are thicker than the internodes on which they're attached), and shoots are not rigid. These two observations make the assumption of a uniform rigid beam a crude interpretation of reality. In a dynamic situation with a tapering shoot, the plant's base is not necessarily the weakest point of the shoot. A future model should be able to identify the weakest point at any location along the shoot. This could be done by measuring bending properties, density (weight) and dimensions at several places along the shoot; and subsequently integrating these properties into a dynamic finite element model that is capable of handling large deflections.

Secondly, according to Lebrowski (1999) the mechanical behaviour of the stems is a result of rather complex shoot-wind interactions, where dynamic loads and resulting oscillations include the effect of the forces encountered during strong or oscillating winds. Future models should, therefore, integrate the effects of wind drag that cause small and large oscillating deflections in any cereal.

Thirdly, all measurements on the bending properties were done with 'fully turgored cells', at one sowing density and one type of fertilizer management. However, in the field drought, sowing density and fertilizer application can significantly modify the bending properties of the plant (Mulder, 1954). Incorporation of the effects of bending properties, together with wind drag, plant oscillations and a safety factor at any point along the shoot into our framework would enable us to accurately calculate the appropriate shoot dimensions for panicle bearing cereals in general. With such a model a better risk assessment can be made and trade-offs in plant resource investment can be calculated.

Fourthly, the lodging experiments were conducted on one sandy soil with low shear strength; soil shear strength is a factor that influences the risk of lodging but its effects have not been fully addressed in this thesis. Also, the roots of teff were in some occasions not strong enough to form a proper cone. Further research should include the bending properties of the roots and their relation to shear strength and cone formation in order to expand model validity to a wider range of cultivation conditions.

Phenology

In Chapter 4 we showed that day length has no systematic effect on the timing of appearance of successive leaves. Day length does, however, determine the final number of leaf ranks that is formed on the main stem. Furthermore, we identified a rather sudden switch in speed of leaf emergence. This switch seemed to be independent of temperature and might be related to the moment of panicle initiation (Fig. 5.6). Chapter 5 provides a detailed description and quantification of the response of teff to day length. We found that the Ethiopian cultivars Ziquala (DZ-Cr-358) and Gibe (DZ-Cr-255) showed a stronger photoperiod response than the two new Dutch cultivars (Ayana and 04T19). Not only panicle initiation, but also development and outgrowth of the panicle were influenced by photoperiod. Plant-to-plant variation in time to heading, the total number of phytomers per shoot, the number of elongated internodes and biomass were higher in long day than in short day treatments for all cultivars. To obtain an accurate prediction of phenology in the field for any location on the globe (under non-stress growth conditions) at least three aspects should be addressed.

Firstly, we identified the day length response to constant day length regimes. In reality day length gradually changes over time, which can be a signal per se. In other words, the response to day-length may be altered by the speed of change in day length (Constable & Rose, 1988; Bonhomme *et al.*, 1991; Clerget *et al.*, 2004; Ile *et al.*, 2007), and may differ between an increasing day length and an decreasing day length. Thus it remains to be tested whether progress towards heading under changing day length conditions follows directly from the sum of progress under static conditions, i.e. the phytotron. The response to day length, moreover, differs during the course of plant development. Reciprocal transfer experiments that are not published in this thesis (Van Delden *et al.*, 2009), showed that teff cultivars differ in their 'photo period sensitive phase (PSP)' and photo period insensitive phases: 'basic vegetative phase (BVP)' and 'post photo period phase (PPP)'. Whether a model based on constant day length could become an accurate enough model describing phenology in the field for teff remains to be researched.

Secondly, we tested the response to day length by using light as a signal. In our experiments photosynthetically active radiation (PAR) of the day length extending light was low. This was done in order to prevent a possible confounded effect of PAR with the day length extending signal. In the field changes in day length coincide with changes in PAR. The combined effect of the day length signal and PAR may result in a different day length response than the one we established in our phytotrons. Especially because there are indications of interaction between day length and light use efficiency regarding biomass accumulation (Adams & Langton, 2005).

Thirdly, in a field situation temperature will also affect time to heading. This thesis did not discuss the relation between temperature and time to heading or leaf emergence. We did, however, perform some experiments involving temperature treatments. These experiments showed that under short day conditions all four tested teff cultivars required approx. 1.5 times more time to flowering at 16 °C compared to 19 °C. Under long day conditions, however, cultivar Ayana only required approx.1.1 times more time to flowering,

and the other three teff cultivars required approx. 1.3 times more time to flowering at 16 °C compared with 19 °C. One of the concepts can be used to explain this different effect of temperature under long days compared to short days is the change in photoperiod sensitivity during plant development. It has been shown that different photoperiod sensitive phases also differ in their sensitivity to temperature (Yin, 1996). Under different day lengths the duration of these phases will change and thus also the effect of temperature on the progress towards flowering will change. If temperature effects of teff are to be determined, the experimental design should comprise a large number of temperature treatments above a large number of replicates per treatment be on replicates at more temperatures and day lengths instead of more replicates within one temperature environment. Time to flowering (heading) is often found to have a different response to temperature compared with germination and leaf appearance (Yin & Kropff, 1996; Porter & Gawith, 1999). Therefore the temperature response of germination cannot be used in modelling time to heading, but according to Parent *et al.* (2010) might be useful in modelling leaf appearance.

Research implications for breeders

In the previous sections research findings were summarized and recommendations for future research were provided per specific research topic. The general objective of this thesis was helping breeders to identify targets for breeding high-yielding cultivars for cultivation in the Netherlands. Working with teff for several years and being involved in brainstorming sessions with the teff breeders provided me the opportunity to identify these breeding targets. Although all breeding efforts listed below are in my opinion required and most of them are interrelated, I attempted to list them in order of priority:

I: Time to flowering should be reduced. Flowering should occur before the end of June, to allow a proper seed filling and ripening period. Breeding for earliness should be done in the Netherlands. We showed a strong relation between earliness and short days. Therefore early teff cultivars identified in the Netherlands (long days) are most likely also early in Ethiopia (short days), while the inverse is unlikely.

II: Lodging is a major problem in the Netherlands. It reduces grain quantity and quality. Breeding for shorter, thicker shoots and stronger roots with a wider root plate would significantly reduce the lodging problem. The current model, despite the above highlighted simplicity, provides an adequate tool to assess progress towards this goal with any new accession. The lodging metre is an excellent tool to quantify lodging resistance.

III: Grain shedding should be reduced; teff grains should be longer and stronger attached to the panicle.

IV: Base temperature of teff phenology should be lowered in order to decrease time to flowering (I), achieve a good seedling establishment early in the season, and be more competitive with weeds.

V: Crop uniformity should be increased. That is plant-to-plant, shoot-to-shoot and grain-to-grain variation should be reduced. Uniform seedling emergence, less extensive tillering, no axillary branches and somewhat more condensed panicles, result in a more uniform crop with higher yields.

VI: Grain quality of teff should be preserved. The mistake of aiming only for a quantitative increase in yield has been made previously in e.g. hemp (Ranalli, 2004) and wheat (Fan *et al.*, 2008). This mistake should not be repeated, so markers for good baking quality, protein and nutrient composition should be identified. Marker identification for backing quality is not very simple, because I suspect a strong Genotype by Environment by Management Interaction (GxExM). This can result in different markers between different growth environments.

According to Assefa *et al.* (2011) rusts are an increasing problem in Ethiopia. In our experiments in the Netherlands incidence of pathogens damage was higher in the lodged plots than in the plots with supported plants. Susceptibility to nematodes could cause problems in some rotation schemes and reduce anchorage strength to some degree. But at the moment breeding against these diseases is not a priority as it is unlikely that these factors currently form a major yield constraint in the Netherlands.

Breeding for a high yielding teff cultivar

An accurate dynamic model, based upon the empirical models in this thesis could be used for more detailed prototyping or ideotyping[1]. Ideotyping could further detail the 'breeders'concept of a high-yielding cultivar. However, the breeding targets listed in the previous section can already contribute to breeding for a high yielding teff cultivar in the Netherlands. Although I am not a plant breeder, based on literature and experience I have some ideas on how to achieve the identified breeding targets. Here I will provide a brief summary of approaches to reach these breeding targets. In the past 10 years Dutch breeders already have obtained a wide range in germplasm by mass selection from landraces. The straightforward mass selection techniques of repeated resowing early flowering mass-harvested plants resulted in several early cultivars, as exemplified by cultivar Ayana (Chapter 3-5). In other words, the breeding target for early heading (I) has already been reached to a great extent. Currently even earlier cultivars are tested in field trials. Early heading is breeding priority number one, because it is related to all of the listed breeding targets. In regard to lodging (II), early heading plants produce fewer elongated internodes (Chapter 4), resulting in shorter plants. Shorter plants are potentially less susceptible to lodging. Early heading also circumvents adverse weather conditions later in the season and thereby reduces seed shedding (III) and increases grain quality (VI). Aside from photoperiod response, early heading can also result from a lower base temperature (IV). A lower base temperature can increase the growing season, by making early sowing possible and can herewith increase

[1] An 'ideotype' of teff is a description of the genetic traits that optimize yield for a given set of constraints.

the yield potential. Simple breeding techniques, like screening for seeds that are able to germinate at low temperatures, can contribute to early heading (I) and better seedling establishment resulting in a more uniform crop (V).

Berhe (1975) discovered that cross pollination in teff is possible and detailed breeding techniques for intra-specific hybridisation. Currently the early heading cultivars have thin shoots, a narrow root plate and are extensively tillering. Identifying markers for earliness could assist hybridisation between early cultivars (e.g. Ayana) and tall, robust cultivars (e.g. 04T19). The introduction of early heading genes to cultivar 04T19 will most likely shorten this cultivar in height.

In TILLING, traditional chemical mutagenesis is followed by high-throughput screening for particular point mutations (Henikoff *et al.*, 2004). This novel non-transgenic PRC-based method is designed to identify and introduce hereditable genetic variation in genes that affect the relevant traits (Syngenta-Foundation, 2011). TILLING is already successfully used to improve crops such as maize (Till *et al.*, 2004), barley (Comai & Henikoff, 2006), rice (Till *et al.*, 2007) and wheat (Slade *et al.*, 2005). Breeding material of Dutch breeders could serve as excellent starting material for these techniques. Köhler *et al.* (2003) identified a genetic mechanism that is related to seed abortion in Arabidopsis. Screening for these genetic mechanisms in teff, combined with the TILLING approach could result in teff cultivars with seeds that are firmly attached to the panicle Assefa *et al.* (2011) reported that the TILLING technic resulted in the cloning of two dwarfing genes for teff. However, as we argue in Chapter 3, not only improving shoot dimensions but also improving the root system is important in breeding for lodging resistance. Phenotypic screening for resistance to root lodging could be done by simply measuring crown root diameter, root plate diameter and counting crown root number. In later breeding stages the lodging meter (Fig. 3.1) could assist in finding varieties that are even more resistant to root lodging. I like to stress, however, that during early breeding stages these screenings should be done in a crop situation. High yielding solitary plants do not necessarily result in a high yielding crop (Donald, 1967). Plant morphological characteristics like height and root plate diameter may differ between solitary plants and plants in a crop.

Having summarised these breeding technics, the question arises whether research time and energy is best spent by making more complex dynamic models? Although crop models can help creating an ideotype, these models are not able to physically create the desired plant. In my opinion, however, creating (mechanistic) dynamic models will be beneficial to breeders, in the sense that breeders will know in more detail what to breed for. Breeding for earliness, for example, can cause that plant become too short (Berry *et al.*, 2004), flower too early or produce too few leaves. This can reduce grain yields. Estimating the optimal shoot dimensions, time of heading and leaf number for a non-lodging high-yielding cultivar is most effectively done using dynamic crop models. Additionally these models could be used as management support tools in teff cultivation.

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Summary

Teff (*Eragrostis tef* (Zuccagni) Trotter) is a C_4 annual grass species (*Poaceae*) originating from Ethiopia. Teff cultivation in the Netherlands is thought to be economically interesting because teff grains and flour are rich in iron and do not contain gluten. Gluten is a multi-protein complex in seeds that can cause coeliac disease in genetically predisposed humans. The absence of gluten in teff grains, make teff a desirable ingredient in health products, particularly for coeliac disease patients. Teff can replace gluten containing cereals in products such as pasta, bread, beer, cookies and pancakes.

This project was funded by the Dutch technology foundation (STW) with the objective to gain scientific knowledge on teff, and facilitate a successful introduction of teff into Dutch agriculture. The research topics in this thesis were chosen in consultation with breeders, farmers, food technologists, crop physiologists and agronomists. At the start of this project Dutch teff yields were modest (1.0 - 1.5 Mg·ha^{-1}). The sowing and harvest date were (too) late in the season and the crop is sensitive to lodging. For teff to be economical feasible in the Netherlands yield have to be in the order of 2.5 - 3.0 Mg·ha^{-1}.

The general objective of the work described in this thesis was to detail some of the major yield constraints of teff, thus helping breeders to identify targets for breeding high yielding cultivars for cultivation in the Netherlands. Together with this practical objective, I also wanted to use the research described in this thesis to advance current understanding of seed physiology, biomechanics and phenology of cereals and teff in particular. Therefore, although the main model species in this thesis is teff, for several plant traits an explicit comparison was made to wheat (*Triticum aestivum* L.) and rice (*Oryza sativa* L.), using published and newly gathered data.

In the introduction (Chapter 1) I postulate several constraints for high teff grain yields in the Netherlands. On the basis of these constraints we chose four research topics for this thesis. We studied seed germination (Chapter 2), lodging resistance (Chapter 3), day length response (Chapter 4), and the pattern of leaf appearance over time (Chapter 5).

Seed germination (Chapter 2)

Viable teff seed can fail to germinate as a result of sub-optimal or supra optimal temperatures and lack of free available water (i.e. low water potential) in the top layer of the soil. In chapter 2 we discuss a study on teff germination in response to temperature and water potential. Experimental data on seed germination were obtained at 17 temperatures and 5 water potentials, using a complete factorial design with 3 replications. The experiments resulted in a highly discriminating data set. Hydrothermal time models are commonly used to describe such a data set. Hydrothermal time models can describe germination at the permissive range of temperatures and water potentials resonably well. These models may,

however, fail at the extremes of the range of conditions that allow germination. Therefore we presented a modified framework to describe teff seed germinaton. In this modified model framework we postulate a normal distribution of seed germination rate. This postulate can explain the time course of seed germination at any combination of temperature and water potential. Final germination percentage and lag phase are emerging properties of the modelled mean germination rate and its variation among seeds in a population. Cardinal temperatures in this framework are a smooth function of water potential and, conversely, the cardinal water potential is a smooth function of temperature. Numerous authors encountered difficulties when parameters for cardinal temperatures or cardinal water potentials contained a spread. To circumvent these difficulties we simply modelled the spread in germination as a function of both temperature and water potential. The newly developed framework gives better predictions of seed germination than alternative models. It uses smooth continuous functions and, moreover, describes several biological interactions that are not captured by the conventional hydrothermal time models. Unlike the hydrothermal time models, the framework evades statistical problems of degree of freedom inflation.

Lodging (Chapter 3)

Teff is susceptible to lodging, in both Ethiopia and the Netherlands. Lodging is the permanent displacement of crop plants from their vertical due to root or shoot failure. Lodging is believed to be a major yield constraint in teff. The causes of lodging are analysed and discussed in Chapter 3 of this thesis. The analysis of lodging was done by using, modifying and validating conventional biomechanical models. The model parameters were obtained from a field trial with two contrasting teff cultivars (Ayana and 04T19). During this field trial we used novel in situ and laboratory measurements under wet and dry conditions. We showed that teff is more susceptible to root lodging than to shoot lodging; although the data indicated that shoot strength is also insuffcient. Hence, simultaneously breeding for both improved root anchorage and shoot strength is advocated. The study showed that the conventinal lodging model, derived for the spike-bearing cereal wheat, needed modifcations in order to be able to deal with panicle-bearing plants like teff and rice. Water adhering to plants due to rain or dew increased calculated lodging susceptibility. To prevent underestimation of lodging susceptibility, future lodging research should be done under completely wet conditions (water saturated soil and wetted shoots). Cross species model validation for shoot lodging was done with rice and showed similar results.

Time to flowering (heading) (Chapter 4)

The long day length in the Netherlands may increase the time to flowering in teff. As a consequence of late flowering the grain ripening period partly takes places under adverse weather conditions later in the season. Teff plants are mainly self-pollinating and heading and pollination almost coincide. From here on we will use the term heading instead of

flowering. Heading was defined as emergence of the tip of the inflorescence from the sheath of the flag leaf. We conducted several phytotron and greenhouse experiments to describe the day length response of teff regarding: time to panicle initiation, time to heading, number of phytomers, plant height and biomass. In these experiments, two Ethiopian cultivars, Gibe and Ziquala, and two cultivars from a Dutch breeding programme, Ayana and 04T19, were exposed to day lengths of 9, 10.5, 12, 13.5, 15, 16.5 and 18 h. Our results showed that heading in teff was significantly delayed by long days. Teff is therefore a short day plant; not only panicle initiation, but also development and outgrowth of the panicle were influenced by photoperiod. Plant-to-plant variation in time to heading, the total number of phytomers per shoot, the number of elongated internodes and biomass were higher in long day than in short day treatments, for all cultivars. In this chapter we provide a detailed description and quantification of the response of teff to day length. We presented a smooth logistic function with biologically intrepretable parameters. This function is generally applicable in short day cereals as shown for rice. Our findings suggest that it is feasible to breed for a teff genotype which is well adapted to northern latitudes of e.g. the Netherlands.

Phyllochron (Chapter 5)

The relatively low average temperatures and long day lengths in the Netherlands compared to Ethiopia could prolong the developmental stages of the crop. Phyllochron, defined as the time interval between the appearance of two successive leaves, is a widely used indicator of the rate of crop development before heading. In Chapter 5 we analysed the response of teff phyllochron to day length and the response of rice phyllochron to temperature. The theory on the phyllochron under constant diurnal temperature and day length conditions is still controversial. Many studies have highlighted inaccuracies in predictions of the timing of appearance between two successive leaves (i.e. phyllochron) in the field. This chapter provides an accurate description of the fundamental concepts on the timing of leaf appearance in teff, rice and wheat. We grew four teff cultivars under constant temperature conditions at six different day lengths. To assess the effect of temperature on rice phyllochron, we re-analysed literature data on four rice cultivars grown at five temperatures. Additionally, newly gathered data on timing of leaf appearance of outdoor-grown wheat was analysed. There are two consecutive phases differing in phyllochron, phyllochron 1 (p_1) and phyllochron 2 (p_2), with $p_1 < p_2$. The effect of temperature on p_1 and p_2 can be normalised with the linear thermal time concept. Day length has no systematic effect on the values of p_1 and p_2. The switch from p_1 to p_2 is abrupt and the difference between soil and air temperature can not account entirely for difference between p_1 to p_2. After re-evaluation of literature data this abrupt increase in phyllochron seemed to be also present in both wheat and rice. The sudden increase in phyllochron might be related to the moment of panicle initiation and the switch *per se* is most likely independent of temperature.

Discussion (Chapter 6)

In Chapter 6 the research findings are placed in the context of the general objective of this thesis. This chapter begins with a discussion on modelling and understanding of plants in general. Then for each research topic (germination (Chapter 2), lodging (Chapter 3), phenology (Chapters 4 and 5) the findings are summarised and future research directions towards a generic model for field-grown teff are described. This is followed by a discussion on the implications of the research findings for breeders. Six breeding targets emerged from this discussion: **I**: Early flowering; **II**: Reduction of lodging susceptibility; **III**: Reduction of grain shedding; **IV**: Lowering of the base temperature; **V**: Increasing crop uniformity; **VI**: Maintaining grain quality. Breeding advice on how to reach these targets is provided. Aside from classical breeding techniques, which are still very useful for teff, using molecular breeding techniques (like TILLING, i.e. chemical mutagenesis followed by high-throughput screening for particular point mutations) is the best strategy to increase grain yields in the Netherlands. Although crop models can help creating an ideotype, these models are not able to physically create the desired plant. In my opinion, however, creating (mechanistic) dynamic models will be beneficial to breeders, in the sense that breeders will know in more detail what to breed for. Breeding for earliness, for example, can cause plants becoming too short, flower too early or produce too few leaves. This can reduce grain yields. Estimating the optimal shoot dimensions, time of heading and leaf number for a non-lodging high-yielding cultivar is most effectively done using dynamic crop models. Additionally these models could be used as management support tools in teff cultivation.

Samenvatting

Eragrostis tef is een eenjarige C_4 grassoort (*Poaceae*), die zijn oorsprong in Ethiopië vindt. Teffteelt in Nederland wordt geacht economisch haalbaar te zijn omdat, teffgraan en -meel rijk zijn aan ijzer en geen gluten bevatten. Gluten is een multi-eiwit complex, dat coeliakie kan veroorzaken bij mensen die hier (genetisch) vatbaar voor zijn. Het ontbreken van gluten in teffgraan, maakt teff een geschikt ingrediënt in gezondheidsproducten, in het bijzonder voor coeliakie-patiënten. Granen, die gluten bevatten, kunnen worden vervangen door teff en wel in producten als pasta, brood, bier, koekjes en pannenkoeken.

Dit project werd gefinancierd door de Nederlandse Technologiestichting STW. STW wilde naast het ontwikkelen van wetenschappelijke kennis over teff, ook via dit onderzoek de introductie van teff als nieuw gewas voor de Nederlandse landbouw faciliteren. De onderwerpen van dit promotieonderzoek werden gekozen in overleg met veredelaars, boeren, voedingstechnologen, plantfysiologen en agronomen. De Nederlandse teff-opbrengsten aan het begin van dit project waren bescheiden (1.0-1.5 $Mg{\cdot}ha^{-1}$). De zaai- en oogstdatum waren (te) laat in het seizoen. Daarnaast bleek teff zeer gevoelig voor legering. Legering is het omvallen van stengels in een gewas als gevolg van wortel- of stengelfalen. Om een winstgevend gewas in Nederland te worden, dient de opbrengst van teff in de orde van 2.5 - 3.0 $Mg{\cdot}ha^{-1}$ te zijn.

De overkoepelende doelstelling van de werkzaamheden -beschreven in dit proefschrift- was het identificeren en het nauwkeurig beschrijven van de factoren, die limiterend zijn voor de graanoogst van teff. Inzicht in de oogstlimiterende factoren zou veredelaars kunnen helpen bij het identificeren van concrete veredelingsdoelen voor teff. Het behalen van deze veredelingsdoelen zou uiteindelijk kunnen leiden tot een hoogproductief teff gewas onder Nederlandse teelt omstandigheden. Samen met dit praktische doel wilde ik de fenologische (i.e. de relatie tussen een periodiek biologisch fenomeen en klimatologische omstandigheden), zaad fysiologische en biomechanische kennis van granen in het algemeen en teff in het bijzonder uitbreiden. Daarom heb ik naast teff, wat diende als belangrijkste modelsoort, ook onderzoek gedaan naar verscheidene planteigenschappen van tarwe (*Triticum aestivum* L.) en rijst (*Oryza sativa* L.). Hiervoor heb ik gebruik gemaakt van zowel gepubliceerde als zelf vergaarde gegevens. In de inleiding (hoofdstuk 1) heb ik de voornaamste beperkingen gepostuleerd die hoge opbrengsten voor teff in Nederland in de weg staan. Op basis van deze beperkingen hebben wij vier onderzoeksonderwerpen gekozen. De onderwerpen van dit proefschrift zijn: zaadkieming (hoofdstuk 2), legering (hoofdstuk 3), daglengterespons (hoofdstuk 4) en het patroon van bladverschijning in de tijd (hoofdstuk 5).

Zaadkieming (Chapter 2)

Als de temperatuur sub- of supra-optimaal is en/of wanneer de waterbeschikbaarheid (waterpotentiaal) in de toplaag van de bodem niet toereikend is, dan kiemen teffzaden niet. In dit hoofdstuk bestudeerden we de kiemkracht van teff in relatie tot temperatuur en waterpotentiaal. Onze gegevens zijn afkomstig uit een experiment met een volledig factoriëel proefontwerp, betreffende 17 temperaturen en 5 waterpotentialen met 3 herhalingen. Dit experiment resulteerde in een zeer uitgebreide set met kiemgegevens. Een dergelijke gegevensset wordt doorgaans beschreven met hydrothermale tijdmodellen. Hydrothermale tijdmodellen kunnen kieming in het voor kieming geschikte bereik van temperaturen en waterpotentialen redelijk goed beschrijven. Echter, deze modellen falen doorgaans in het beschrijven van kieming in de extremen van het permissieve bereik van temperaturen en waterpotentialen. Daarom hebben we een aangepast modelraamwerk voor zaadkieming van teff ontwikkeld. In dit gewijzigde model postuleren we een normale verdeling van de kiemsnelheid van het zaad. Door gebruik te maken van dit postulaat kunnen we het tijdsverloop van kieming onder alle gebruikte combinaties van temperatuur en waterpotentiaal beschrijven. De duur van de periode tot de eerste zaden kiemen en het uiteindelijke kiempercentage volgen uit de gemodelleerde gemiddelde kiemsnelheid en de variatie in kiemsnelheid tussen de zaden in een populatie. Kardinale temperaturen (i.e. optimum-, basis- en plafondtemperatuur voor kieming) zijn in ons modelraamwerk een continue functie van de waterpotentiaal. Omgekeerd is de kardinale waterpotentiaal (basis waterpotentiaal) een continue functie van temperatuur. Verschillende wetenschappers ondervonden moeilijkheden, toen ze een spreiding toekende aan parameters voor de kardinale temperaturen en/of aan de kardinale waterpotentiaal. Om deze moeilijkheden te omzeilen, hebben we de spreiding in tijd tot kieming gemodelleerd als een functie van zowel de temperatuur als de waterpotentiaal. Dit nieuwe modelraamwerk geeft een betere beschrijving van zaadkieming dan alternatieve modellen. Het raamwerk maakt gebruik van een flexibele continue functie en beschrijft bovendien een aantal biologische interacties die niet worden beschreven door de conventionele hydrothermale tijdmodellen. In tegenstelling tot de conventionele hydrothermale tijdmodellen, bezit ons modelraamwerk wel een statistisch correcte analyse van de gegevens.

Legering (hoofdstuk 3)

Zowel in Ethiopië als in Nederland is teff gevoelig voor legering. Legering wordt beschouwd als een belangrijke opbrengstbeperkende factor in teff. Door het aanpassen en valideren van conventionele biomechanische modellen hebben we een analyse van legering uitgevoerd. In dit hoofdstuk wordt deze analyse besproken. De modelparameters werden verkregen uit een veldproef met twee contrasterende teff cultivars (Ayana en 04T19). In deze veldproef maakten we gebruik van nieuwe in situ - en laboratoriummetingen onder natte en droge omstandigheden. We toonden aan, dat teff vatbaarder is voor wortelfalen dan voor stengelfalen. Echter, onze gegevens toonden ook duidelijk aan dat de stengels

van teff niet sterk genoeg zijn. Vandaar ons advies aan veredelaars om gelijktijdig op zowel wortelstelsel- als stengelsterkte te veredelen. De conventionele modellen die legering beschrijven, zijn afgeleid zijn uit experimenten met tarwe, een gewas met een rechtstandige aar. Deze conventionele legeringsmodellen hebben grote aanpassingen nodig om de juiste beschrijving te geven van pluim-dragende planten zoals teff en rijst. Water, dat aan planten hangt als gevolg van regen of dauw, verhoogt significant de vatbaarheid voor legering. Om een structurele onderschatting van legering te voorkomen, moet toekomstig onderzoek worden gedaan onder volledig natte omstandigheden: zowel een water verzadigde bodem als bevochtigde scheuten. Intersoortelijke modelvalidatie is gedaan met rijst (*Oryza sativa*). De resultaten van rijst waren vergelijkbaar met de resultaten van teff.

Tijd tot bloei (pluimverschijning) (hoofdstuk 4)

Het Nederlandse groeiseizoen kent lange dagen, in vergelijking tot het Ethiopische groeiseizoen. Deze langere dagen kunnen de tijd tot bloei in teff drastisch verlengen. Als gevolg van deze latere bloei vindt de graanrijping deels plaats onder slechte weersomstandigheden aan het einde van het groeiseizoen. Teffplanten zijn voornamelijk zelfbestuivend; de verschijning van de pluim en de bloei vallen vrijwel samen. De definitie van pluimverschijning is: het verschijnen van de pluim uit de schede van het vlagblad. Omdat de pluimverschijning beter waar te nemen is dan de bloei, wordt in de rest van deze tekst de term pluimverschijning in plaats van bloei gebruikt. Door middel van verschillende fytotron- en kasexperimenten hebben we de daglengte-reactie van teff beschreven voor: tijd tot pluiminitiatie, tijd tot pluimverschijning, het aantal fytomeren, de planthoogte en de bovengrondse plantmassa. In deze experimenten stelden we twee cultivars (Gibe en Ziqual) uit Ethiopië en twee cultivars (Ayana en 04T19) afkomstig uit een Nederlands veredelingsprogramma, bloot aan daglengtes van: 9, 10.5, 12, 13.5, 15, 16.5 en 18 uur. Onze resultaten lieten zien, dat de tijd tot het verschijnen van de pluim aanzienlijk werd verlengd onder invloed van lange dagen. Teff is dus een 'kortedagplant'. Niet alleen pluimverschijning maar ook de ontwikkeling en uitgroei van de pluim werden sterk beïnvloed door de fotoperiode. Plant-tot-plant variatie in de tijd tot pluimverschijning, het totale aantal phytomeren per scheut, het aantal gestrekte internodiën en de bovengrondse plantmassa namen toe onder invloed van lange dagen ten opzichte van korte dagen. In dit hoofdstuk geven we een gedetailleerde beschrijving en kwantificering van de daglengte-respons van teff. We presenteren een continue logistische functie met biologisch interpreteerbare parameters. Deze functie is waarschijnlijk algemeen toepasbaar in de 'kortedaggranen' zoals we aantoonden in rijst. Onze bevindingen suggereren dat het mogelijk is, om via veredeling te komen tot een teffgenotype dat aangepast is aan de noorderbreedte van Nederland.

Phyllochron (hoofdstuk 5)

In vergelijking met Ethiopië zijn de temperaturen in Nederland laag en zijn de daglengtes lang. Hierdoor kan het Nederlandse groei seizoen de eerste ontwikkelingsstadia van teff

significant vertragen. Phyllochron, gedefinieerd als het tijdsinterval tussen het verschijnen van twee opeenvolgende bladeren, is een veel gebruikte indicator voor het ontwikkelingstempo voor de bloei. In dit hoofdstuk analyseren we de reactie van teff-phyllochron op daglengte en de reactie van rijst-phyllochron op temperatuur. De theorie over de lengte van het phyllochron onder constante dagtemperatuur en daglengte is nog steeds controversieel. Veldstudies laten onjuistheden zien in de huidige manier, waarop de chronologie van bladverschijning (phyllochron) wordt beschreven. Dit hoofdstuk geeft een nauwkeurige beschrijving van de fundamentele concepten met betrekking tot bladverschijning in teff, rijst en tarwe. We hebben vier teff cultivars blootgesteld aan zes verschillende daglengtes bij een constante dagtemperatuur. Om het effect van temperatuur op rijst phyllochron te onderzoeken hebben we literatuurgegevens van vier rijst cultivars bij vijf temperaturen geanalyseerd. Bovendien hebben we gegevens over het phyllochron van in de buitenlucht (in containers) geteelde tarwe geanalyseerd. Het bleek dat in alle drie de granen twee opeenvolgende fasen in phyllochron waar te nemen zijn: phyllochron 1 (p_1) en phyllochron 2 (p_2), met $p_1 < p_2$. Het effect van de temperatuur op p_1 en p_2 kan worden genormaliseerd met het lineaire 'thermisch- tijdsconcept'. Daglengte heeft geen systematisch effect op de waarden van p_1 en p_2. De omschakeling van p_1 naar p_2 is abrupt en het verschil tussen bodem- en luchttemperatuur verklaart niet volledig het verschil tussen p_1 en p_2. De plotselinge toename van phyllochron kan worden gerelateerd aan het moment van pluiminitiatie. De plotselinge verandering is op zichzelf waarschijnlijk onafhankelijk van de temperatuur.

Discussie (hoofdstuk 6)

In dit hoofdstuk worden de resultaten van het onderzoek geplaatst in de context van de algemene doelstelling van dit proefschrift. Het hoofdstuk begint met een discussie over het modelleren en begrijpen van planten in het algemeen. Dan worden de bevindingen voor elk onderzoeksonderwerp, te weten kiemkracht (hoofdstuk 2), legering (hoofdstuk 3) en fenologie (hoofdstukken 4 en 5), samengevat. Hierna worden potentiele onderzoekslijnen uiteengezet om tot een generiek teffteelt-model te komen. Deze uiteenzetting wordt gevolgd door een discussie over de implicaties van de onderzoeksresultaten voor veredelaars. Uit deze discussie komen zes veredelingsdoelstellingen voort: **I**: Vroege bloei; **II**: Het reduceren van legeringsvatbaarheid; **III**: Reduceren van zaaduitval uit de pluim; **IV**: Verlaging van de basistemperatuur; **V**: Verhogen van gewasuniformiteit; **VI** : Handhaving van graankwaliteit. Over de manier waarop deze veredelingsdoelen te bereiken zijn, wordt in dit hoofdstuk gediscussieerd. Afgezien van de klassieke veredelingstechnieken -die nog altijd zeer nuttig voor teff zijn- kunnen moleculaire veredelingstechnieken (zoals TILLING, dwz chemische mutagenese, gevolgd door high-throughput screening voor interessante puntmutaties) zeer behulpzaam zijn bij het verhogen van de huidige teff-graanopbrengst in Nederland. Hoewel gewasgroeimodellen kunnen helpen bij het creëren van een ideotype, zijn deze groeimodellen logischerwijs niet in staat om de gewenste plant fysiek te creëren. Echter, naar mijn mening, zal het creëren van (mechanistische) dynamische modellen vere-

delaars wel degelijk kunnen helpen bij het fysiek creëren van de gewenste plant. Modellen kunnen veredelaars in groter detail een concept van de ideale plant onder verschillende groeicondities voor ogen stellen. Veredeling voor vroegheid kan er bijvoorbeeld toe leiden dat planten te kort worden, te vroeg bloeien en te weinig bladeren produceren. Dit kan de graanopbrengst negatief beïnvloeden. Om tot een hoog renderende cultivar te komen, kunnen de optimale plantafmetingen, optimale tijd tot pluimverschijning en het optimale aantal bladeren op de scheuten, het best worden geschat met behulp van (mechanistische) dynamische gewasgroeimodellen. Bovendien kunnen deze modellen worden gebruikt bij het ontwikkelen van teeltadviezen voor teff.

Curriculum Vitae

Sander Hermanus van Delden was born on 30 Juni 1979 in Leeuwarden, the Netherlands. Because of his dyslexia he initially attended the MAVO 'Nijehove College', in Leeuwarden from which he graduated in 1995. That same year he started at the 'Friesland College' and received both: a HAVO diploma and MBO degree in informatics in 1997. After his graduation he started a BSc program in communication of natural sciences (biology, physics and chemistry) named 'Natuurwetenschappen & Milieu' at the 'Noordelijke Hogeschool' in Leeuwarden. As part of this education he was a high school teacher in biology and temporary (substitute) teacher in health, physics, chemistry and mathematics at: the 'Zernike College' in Zuidlaren (2000-2001). After graduating for his BSc in 2001 he did several different skilled and unskilled jobs. He worked for instance as a caterer, a protocol developer at the institute of Food Safety 'Rikilt' and as highschool tutor for beta courses. In 2002 he started a study in Biology at Wageningen University. After completing a two year BSc program, he conducted two MSc theses. His first thesis was entitled 'Optimising seed priming by hormone addition to induce cross-tolerance' at the chair group of Plant Physiology. His second thesis was entitled: 'Flowering and secondary fibre content of hemp' at the chair of Crop Physiology. Additionally he did a molecular internship entitled: 'Engineering cold stress tolerant plants' at Enza Zaden Research & Development B.V. in Enkhuizen. He graduated in 2006, which was the same year he started his PhD study at the Centre for Crop System Analysis in Wageningen.

He has a natural interest in technology. He, furthermore, enjoys exploring the possibilities and functions of software packages (e.g. Matlab, SAS, Excel, Latex and Genstat). At his leisure he likes to swim, fitness or cycle. The combination of sports, yoga and meditation allow him to think clear and stay open-minded.

PE & RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE & RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4 ECTS)

- Designing a high-yielding 'ideotype' for the facilitation of the introduction of Teff in the Netherlands

Post-graduate courses (8.5 ECTS)

- Advanced statistics; PE & RC (2008)
- The art of modelling; PE & RC (2008)
- Organization and leadership for PhD students; Utrecht school of Governance (2009)

Laboratory training and working visits (1 ECTS)

- The biomechanics of lodging; University of Manchester (2008)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- Basic statistics (2008)

Competence strengthening / skills courses (7.5ECTS)

- Project and time management; WGS (2007)
- Competence assessment; WGS (2007)
- Professional communication strategies; WGS (2008)
- Techniques for writing and presenting a scientific paper; WGS (2009)
- Philosophy and Ethics of Food Science and Technology (2009)
- Career orientation; WGS (2010)

PE & RC Annual meetings, seminars and the PE & RC weekend (2.4 ECTS)

- PE & RC Weekend (2006)
- PE & RC Day (2006-2010)
- EPS Career day (2010)

Discussion groups / local seminars / other scientific meetings (5.6 ECTS)

- FLOP: Frontier Literature in Plant Physiology (2006-2010)

International symposia, workshops and conferences (8 ECTS)

- RYLA: Rotary Youth Leadership awards (2006)
- Gene-Plant-Crop Relations (2006)
- Xth Congress of the European Society for Agronomy; Bologna, Italy (2008)
- SEB: Society for Experimental Biology; Glasgow, Scotland (2008)

Lecturing / supervision of practicals / tutorials (18.3 ECTS)

- Soil-Plant Relations (2007-2010)
- Research Methods in the Life Sciences (2007)
- Populatie Ecologie (2007)
- Research Methods in Crop and Weed ecology (2007-2008)
- Crop Ecology (2008)

Supervision of MSc student

- Seed germination of teff (Erwin Boogaard)

Statement on conflicts of interest

It is not common practice to have a 'Statement on conflicts of interest' in a PhD thesis conducted at Wageningen University. Personally, however, I think it should be common practice. As John Lubbock (1834-1913) put it: 'What we see depends mainly on what we look for'. I would like to add: 'What we see also depends on how we look' (e.g. Ball, 2007; Bach & Hinton, 2005; and after Carl Gustav 1875 - 1961; Katan, 2007). It is well known that industry funding may bias conclusions of scientific work in favour of the sponsors (Ioannidis, 2005; Katan, 2007). By choosing which scientific projects are to be funded, or are not to be funded, sponsors already determine 'where the scientist should look'. And thus, to some extend, determine 'what the scientist sees'. Or more importantly what the scientist does not see. 'How the scientist looks', i.e. perceives reality, not only depend on the physical state of the scientist. It also depends on personal interests, financial interests and the social and theoretical background of the scientist. For that reason I included the next paragraph in this thesis.

As crop physiologist, I have a fundamental interest in the phenology and morphology of crop plants. For my career as a scientist it is important to publish in renowned scientific journals. For a short summary of my social background, I refer to my curriculum vitae at page 179. During this project I was under contract of Wageningen University, this University received expenses for my employment by STW. STW is the Dutch Technology Foundation, which is the applied science division of NWO, and the Technology Programme of the Ministry of Economic Affairs in the Netherlands (project 07328). During the project I had no paid secondary jobs or responsibilities. I furthermore did not receive fees or gifts, other than germplasm in the form of seeds to conduct my research, and I discussed the research progress during two or three dinners paid by the Share Foundation. I do not own stocks or have other interests in companies involved in breeding or in the production of flour. The research in this thesis is performed according to high scientific standards in order to generate unbiased results.

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This research is supported by the Dutch Technology Foundation (STW), which is the applied science division of NWO, and the Technology Programme of the former Ministry of Economic Affairs (project 07328). STW assigned a user committee to this project consisting of breeders (Share Foundation), farmers, a farmers' cooperation (Agrifirm), a food technologist (Soil & Crop Improvement BV), crop physiologists and agronomists (Wageningen UR). Together we identified the yield constraints that are the most limiting for growing the crop teff in the Netherlands. The detailed examination of these yield constraints could facilitate a successful introduction of teff to Dutch agriculture and will advance our scientific knowlegde on teff.

Teff, a new source for gluten free food (project 07328)