Genotypic variation in energy efficiency in greenhouse crops: underlying physiological and morphological parameters

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Chapter 1

General introduction

Energy efficiency in Dutch greenhouses

Greenhouse horticulture in The Netherlands is a highly sophisticated form of crop production. High-tech modern greenhouses enable a refined control of production through regulation of the greenhouse environment (e.g. temperature, light and CO_2 concentration) (Bakker and Challa, 1995). This results in high production levels and good product quality. However, it also requires high inputs, especially in labor, capital and energy. Energy accounts for 19% of the production costs in tomato (Peet and Welles, 2005) and for 16% of the production costs in cut chrysanthemum (Spaargaren, 2002). The Dutch greenhouse horticulture accounts for 7% of the total energy used in the Netherlands and for about 80% of the energy used in agriculture (Oude Lansink and Bezlepkin, 2003). Moreover, this high energy use also results in environmental pollution due to the emission of CO_2. For these reasons, the government and the greenhouse sector have made an agreement to decrease energy efficiency index (primary fuel use per unit product relative to the base-year 1980 at which the index is set to 100) in 2010 by 65% compared to 1980 (GLAMI, 2007). Energy efficiency index can be decreased either by a decrease in use of fossil fuel or by an increase in yield. More recently, an agreement was reached on the reduction of the emission of CO_2, which is only possible by an absolute reduction in fossil fuel use (GLAMI, 2007).

In 2003 energy efficiency index had decreased by 49% relative to 1980 (Table 1). This enhanced efficiency can to a great extent be attributed to a large increase in production, which in turn is the result of the continuous release of higher yielding cultivars, improvements in cultivation techniques (e.g. soilless cultivation, CO_2 enrichment) and improvements in greenhouse structure (e.g. improved light transmissivity) (Ruijs *et al.*, 2001). Between 1980 and 2003 tomato yield more than doubled (Table 1). Especially between 1980 and 1990 there was a large production increase. In the 1990's, as a consequence of the 'Wasserbombe-effect', many growers switched from round tomato cultivars to truss tomato cultivars, emphasizing the need for higher quality tomatoes (Ruijs *et al.*, 2001). Chrysanthemum production shows a more gradual yield increase in time (Table 1). This is partly due to an increase of the production area equipped with assimilation lights and to the higher radiation level of these lamps (Ruijs *et al.*, 2001). In contrast to the increase in production, the absolute amount of energy used per m^2 has hardly changed (Table 1). Furthermore, the area used for greenhouse cultivation has increased slightly, by 7% in 2003 compared to 1990 (Van der Knijff *et al.*, 2006). All in all, CO_2 emission in 2003 was almost equal to that in 1990.

In the short term, energy efficiency index can be further improved by investing in energy saving technologies or by improving the current production potential (Oude Lansink and Bezlepkin, 2003). On the other hand, greenhouse cultivars that are better adapted to temperatures below the current economical optimum greenhouse temperature could also contribute significantly to an improvement in energy efficiency and a decrease in CO_2 emission. Lowering set-point temperature in the greenhouse by 2ºC would lead approximately

Table 1: Progress in the productivity of tomato and cut chrysanthemum (KWIN, 2002; CBS/LEI, 2006), and the use of fossil fuel and energy efficiency index in the whole greenhouse sector (Van der Knijff *et al.*, 2006) between 1980 and 2003.

	1980	1985	1990	1995	2000	2003
Tomato (kg m^{-2})	18	25	38	43	44	47
Chrysanthemum (stems m^{-2})	104	141	153	162	186	202
Fossil energy (m^3 m^{-2})	41	31	45	45	42	41
Energy efficiency index [z]	100	60	67	60	56	51

[z] primary fuel use per unit product relative to the base-year 1980 at which the index is set to 100

to a 16% reduction of energy in tomato and a 12% reduction in chrysanthemum (Dueck *et al.*, 2004). This strategy is a more long-term solution since it would imply the development of new cultivars which takes between 10 to 15 years. It is therefore worthwhile and urgent to investigate the possibilities for breeding less temperature sensitive cultivars.

Tomato breeding

Tomato (*Lycopersicon esculentum*) originates from southern America and its genetic background probably includes the tropical *L. cerasiforme* Dun. (Rick, 1995). The Spanish introduced tomato into Europe in the early 16th century, but European acceptance came slowly as the fruits were first considered poisonous. By the end of the 18th century they became wide-spread cultivated in north-western Europe (Harvey *et al.*, 2002). Tomatoes are now one of the most widely eaten vegetables in the world with a global production that is estimated at 110 million tonnes in 2003 (Costa and Heuvelink, 2005). Tomato production in The Netherlands is characterized by its high intensification and productivity. In general, plants are transplanted into the greenhouse in December, harvesting starts in March and continues till November but on about 10% of the area growers use assimilation lights, making year-round production possible (Costa and Heuvelink, 2005). There are many cultivars, varying in several aspects including color, taste and size, ranging from the small sweet-tasting cherry to the large beefsteak tomato (Van de Vooren *et al.*, 1986; Dorais *et al.*, 2001).

For tomato growers it is important to grow a high yielding crop with high quality fruits with minimal production costs. Therefore, the main breeding goals focus on characteristics that ensure reliable and high yields, reduce production costs and result in high quality fruits (Lindhout, 2005). The widely-spread use of tomato as a model plant for research in plant physiology, pathology and genetics has helped to improve breeding and cultivation methods (Ho, 1996a). Breeders have been very successful in transferring disease resistances into modern greenhouse varieties (Lindhout, 2005), with positive effects on yield. Breeding for higher quality and better tasting fruits became more important during the 1990's. Consequently, many breeders are also focusing on improving sugar and acid levels in tomato fruits (Dorais *et al.*, 2001), but breeding for a higher sugar content in the fruits is hampered by the negative relationship between yield and sugar content (Stevens and Rick, 1986).

In a predominantly self-pollinating species such as tomato, even without selection,

variation tends to decrease (Lindhout, 2005). New varieties are usually derived from crosses between high yielding but genetically related varieties. This has led to increased productivity but it has narrowed the genetic variability further, thus limiting the breeding potential (Tanksley and McCouch, 1997). Fortunately, wild related species present a genetic resource which can broaden the genetic basis of cultivated tomato. The variation present in cultivated tomato is only a fraction of the genetic variation present in cross compatible landraces and wild species (Miller and Tanksley, 1990).

Chrysanthemum breeding

First reports about wild chrysanthemums appeared in literature in China by about 200 B.C (Fukai, 2003). The current chrysanthemum cultivars (*Chrysanthemum morifolium* Ramat., syn. *Dendranthema* × *grandiflorum* (Ramat.) Kitam.) are complex hybrids of species that originate from China and Japan (Spaargaren, 2002) and its genetic background probably includes *C. indicum* var. *procumbens* Kitam. × *C. zawadskii* var. *latilobum* Kitam but the true origin is still unclear (Fukai, 2003). From China cultivated (garden) chrysanthemum have gradually spread across the world. Cut chrysanthemum growing in The Netherlands only became important after 1900, but year-round cultivation, which started in 1960 (Spaargaren, 2002), was a big step forward. Chrysanthemum is a short-day plant that will not initiate flowers when day-length is above a critical (cultivar dependent) value (Post, 1948). Therefore, natural flowering in The Netherlands only occurs in autumn (Kofranek, 1992) and the year-round cultivation is only possible due to an efficient control of the photoperiod. In autumn and winter, supplementary light is needed to extend the natural photoperiod to long day conditions in order to enhance vegetative growth. Black-out screens are used in the spring and summer to shorten the photoperiod and promote flower induction. Today, chrysanthemum is the second largest floricultural crop in the world market (Fukai, 2003) and growers can choose from more than 300 cultivars, differing in color, form, size, reaction time, labor input, etc. (Trip *et al.*, 2000).

Consumer demands have a large impact on the breeding goals of chrysanthemum, although there are many different market segments which all have their own specific demands, mainly with regards to flower characteristics. One of the main objectives of chrysanthemum breeders is therefore to develop new flower shapes and colors. In general, breeding companies keep a large number of flower shapes and colors in their assortment, so that they can adapt quickly to changes in the market (Spaargaren, 2002). Additionally, growers have cultivation specific demands, like shorter cropping cycles, disease resistance, enough weight during winter, etc. Hence, reduction of reaction time (number of short-days till flowering) is another important breeding goal, as it reduces the cultivation period and therefore increases the number of cropping cycles that can take place in a year.

New cultivar development takes place by hybridization, selection and mutagenesis

(Chatterjee *et al.*, 2006). Once a new variety has been produced, breeders will try to develop a whole line of flower-color mutants (Broertjes *et al.*, 1980). Spontaneous (color) mutations occur frequently and therefore they play a major role in chrysanthemum breeding, but mutations can also be induced by applying X- or γ-ray (Schum, 2003).

Integrating crop physiology into plant breeding

Fruit and flower growth are part of the integrated growth and development of the whole plant. Yield is determined by the interaction between growing conditions and morphological and physiological characters of the whole plant (Ho, 1992). Therefore, yield is an incredible complex trait that is determined by dry matter production, partitioning and dry matter content, each of them also influencing product quality (Marcelis *et al.*, 1998). The importance of each of these parameters may depend on the environment (e.g. light) and cultivation techniques (e.g. application of CO_2).

Dry matter production depends on the light interception and on the efficiency of turning the intercepted light into dry matter (light use efficiency, LUE). Light interception is mostly determined by the leaf area per unit ground area index (leaf area index; LAI), while LUE mainly depends on photosynthesis and respiration (Wallace and Yan, 1998).

Only the dry matter allocated to the harvestable organs contributes to yield. In tomato only the fruits are harvested and therefore dry matter partitioning has a direct effect on production. However, as greenhouse tomato shows an indeterminate growth pattern and is grown almost year-round in the greenhouse, it is important that a certain balance between vegetative (to maintain sufficient light interception) and generative (production) growth is maintained. In cut chrysanthemum the whole plant is harvested (with the exception of the roots), but partitioning can have a large influence on external quality. Since horticultural products are usually sold on a fresh weight basis, yield is also determined by the water content (Marcelis *et al.*, 1998).

Breeding mostly focuses on one or a few components of this complex system, aiming at maximizing the yield (Wallace and Yan, 1998). To improve energy efficiency it is of utmost importance to understand the morphological and physiological processes that influence yield at different growth conditions. This information would help breeders to focus on specific plant characteristics when aiming at a given target, for instance the adaptation to sub-optimal temperature conditions.

Aim and outline of the thesis

This thesis aims at quantifying and understanding the possibilities for improving energy efficiency of greenhouse crops by plant breeding. Tomato and chrysanthemum are used as

model crops, being an important greenhouse vegetable and flower crops, respectively, that require relatively large energy inputs. The main emphasis is on exploring possible differences in growth, development and ultimately yield within tomato and chrysanthemum cultivars in response to sub-optimal temperature, but attention is also given to improving yield at optimal temperatures. The ultimate goal of this study is to give breeders some guidelines concerning the most important traits on which they should concentrate when breeding for sub-optimal temperature tolerant cultivars. For both crops, the focus is on biomass production, but since chrysanthemum is a cut flower also a number of external quality attributes is dealt with.

This study is split in two parts. The first section focuses on increasing energy efficiency in chrysanthemum, while the second part deals with tomato. In **Chapter 2.1** a literature review on the effects of temperature on chrysanthemum cultivars is presented. This chapter gives an overview of the response to temperature in chrysanthemum, with special emphasis on the variation between cultivars. Furthermore, it identifies gaps in knowledge in this field of research. In **Chapter 2.2** the variation in temperature response for yield and quality related traits in 25 cultivars is reported. Furthermore, to study this genetic variation in temperature response in more detail, an in-depth analysis with four contrasting cultivars was carried out, covering a wider range of temperatures. Additionally, the effects of temperature applied during different phases in the cultivation period were quantified.

Besides breeding for cultivars with a shorter reaction time at sub-optimal temperature, an alternative approach would be to exploit cultivars that are heavier at sub-optimal temperature so that they could be grown at a higher plant density, enhancing the production per m^2. Therefore, **Chapter 2.3** provides a detailed study on the combined effects of temperature and plant density on four chrysanthemum cultivars.

Chapter 3.1 has a similar structure as **Chapter 2.1** but it refers to tomato cultivars. In **Chapter 3.2** the role of tomato breeding in increasing yield between 1950 and 2002 is studied, with special emphasis on the underlying physiological and morphological processes behind increasing yield. Additionally, there is a focus on the variation in temperature response between cultivars. As this variation in temperature response between commercial cultivars is limited, thus hampering breeding more energy efficient tomato cultivars, there is a need for alternative ways to increase energy efficiency.

The variation in cold tolerance that exists among related wild *Lycopersicon* species, which originate from South America, could potentially be useful for the development of new cultivars with increased energy efficiency. This strategy is addressed in **Chapter 3.3** where we examined the effects of temperature on growth of young vegetative tomato plants of the cultivar Moneymaker and two wild relatives (*L. hirsutum* and *L. pennellii*). The physiological and morphological parameters which underlie interspecific differences in growth response to sub-optimal temperatures are analysed. Recently a series of BILs that contain small introgressions of *L. pennellii* in the greenhouse cultivar Moneymaker background were developed. In **Chapter 3.4** we investigated the possibilities of using *L. pennellii*

introgressions for improved energy efficiency in greenhouse tomato.

In **Chapter 4** the main achievements and limitations of this study are discussed. Furthermore, the prospects for breeding more energy-efficient greenhouse cultivars are discussed on the basis of the present results and suggestions for future research are presented.

Chapter 2

Chrysanthemum

Chapter 2.1

The influence of temperature on growth and development of chrysanthemum cultivars: a review

Abstract

The effects of temperature, especially in the sub-optimal temperature range, on growth and development of chrysanthemum (*Dendranthema grandiflorum* syn. *Chrysanthemum morifolium*) are reviewed with special emphasis on cultivar differences. The developmental aspects analysed in this paper are leaf unfolding rate, stem elongation, time to flowering, and the number and sizes of flowers. Growth is studied as biomass production and partitioning to different plant organs. Temperature has a significant effect on development, especially on leaf unfolding rate and time to flowering, both of which show an optimum response to temperature. The optimum for time to flowering is cultivar-dependent and lies between 17º - 22ºC. Also, for the other developmental traits, there are clear differences between cultivars in their response to temperature. The effect of temperature on biomass production is less clear. When leaf area index is low, sub-optimal day temperatures decrease biomass production due to the formation of thicker leaves. Biomass produced up to flowering is highly variable and depends on cultivar and the interaction between temperature and other growing conditions. More research is required to determine whether differences in biomass produced between cultivars are related to differences in the duration of the cultivation period, growth rate, or both. The possibilities for breeding for low energy demand are also discussed.

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Introduction

Chrysanthemum is one of the most important ornamental crops worldwide and is produced both as cut flower and as pot plant. Year-round production can be achieved in greenhouses by controlling climate conditions. In the winter months, especially in more northern latitudes, this means that high energy inputs are required because the greenhouses must be heated in order to maintain good plant quality and production levels. The optimal temperature range in greenhouses in winter lies between 18º - 20ºC (Spaargaren, 2002). In The Netherlands, in 2000, annual heating costs represented 16% of the total cost for cut chrysanthemum cultivation (Spaargaren, 2002). An agreement was made between the Dutch government and the horticultural sector to increase energy efficiency (Ruijs *et al.*, 2001). There are two ways in which this can be achieved. One is by increasing the yield m^{-2}, while the other method inclines producing the same yield at a lower temperature set-point. Energy efficiency has been increased considerably in recent years, mainly due to increased production m^{-2} (Ruijs *et al.*, 2001). But as the combustion of natural gas results in the emission of carbon dioxide, which contributes to global warming the absolute amount of energy used should decrease. Therefore it is important to study the possibilities of breeding chrysanthemum cultivars that can be grown at lower temperatures, but reaching equal production levels.

In cut chrysanthemum cultivation, two different phases can be distinguished. First a period of long-day (LD; day-length more then 12 h) is maintained so that the plants grow vegetatively. Depending on the season, this period will last between 10 - 25 days (Carvalho, 2003). Then plants are grown under short-day (SD; day-length less then 12 h) conditions, leading to flower induction and development. The period between the start of the SD period and flowering under optimal conditions (reaction time) can vary between 6 - 11 weeks, although breeders apply a strong selection pressure on shorter reaction times, so that the cultivation period can be shortened (Spaargaren, 2002). During each of these phases, growth (e.g., the production and partitioning of biomass) and development (e.g., leaf appearance rate, time to flowering, stem elongation, number and sizes of flowers) can be influenced by temperature (Wilkins *et al.*, 1990; Carvalho *et al.*, 2005).

For certain greenhouse crops (e.g., tomato), the variation between different cultivars in developmental and growth related traits is limited (Smeets and Garretsen, 1986b). But, for ornamental crops like chrysanthemum, selection not only takes place on the basis of rapid growth and high yield, but also on flower, stem and leaf characteristics and vase-life. Therefore, it is likely that there are larger cultivar differences in growth and development-related traits. Breeders can use this variation to produce a more energy efficient crop.

Here we provide an overview of the influence of (sub-optimal) temperature on growth and development in chrysanthemum, to achieve a better understanding of the underlying components that cause certain cultivars to grow better at lower temperatures. By sub-optimal temperatures, we mean temperatures below the optimum for flowering. Furthermore, in this

review we analyse the presence of variation within chrysanthemum acessions for growth and development-related traits. The first part of this paper describes the influence of sub-optimal temperatures on developmental aspects, then the production and distribution of dry matter are described. Finally the options for breeding more energy efficient chrysanthemums are discussed.

Effects of temperature on developmental aspects

Number of leaves

Leaf unfolding rate shows an optimum response (optimum around 25ºC) to 24 h average temperatures (AT) between 12º - 28ºC (Larsen and Hiden, 1995; Carvalho *et al.*, 2002), but this optimum response was shown to be stronger when considering day temperature (DT) alone (Carvalho *et al.*, 2002). Another report using 17 cultivars suggests a linear rise with increasing DT and night temperature (NT) (De Jong and Smeets, 1982). However, the temperature range in that experiment was smaller (10º - 17ºC) and well below the optimum temperature reported.

The development of leaf primordia is terminated when the apical flower is initiated and, therefore, leaf number at flowering is highly dependent on the duration of the LD period and on the cultivar. This might explain apparently conflicting results. For instance, plants grown at the optimal temperature during the LD period can produce more leaves during this phase than plants grown at sub-optimal temperatures, but, when the SD period starts, flower initiation begins earlier (see Time to flowering section, below) and the benefit could, if the advantage at the end of the LD period was not too large, be annulled. This might explain why, in plants to which temperature treatments were applied in both the LD and SD periods, equal leaf numbers at 10ºC and 16ºC NT have been reported for the cultivar `May Shoesmith´ (Bonaminio and Larson, 1980), while for three other cultivars an increase in NT from 13ºC to 22ºC led to only a small increase in leaf number (Heij and De Lint, 1987). The LD period in the latter experiment was longer, but it could also be that cultivar effects play a significant role here, as the temperature response of flower initiation is highly cultivar dependent (see Time to flowering section, below). For plants that started their temperature treatment at the start of the SD period, equal final leaf numbers (Lepage *et al.*, 1984; Larsen and Hiden, 1995), or a quadratic increase in leaf numbers have been reported as temperatures increased from 10ºC to 30ºC (Karlsson *et al.*, 1989c).

Stem elongation

Total stem-length is determined both by the number of internodes (i.e., number of leaves) and by internode length (Pearson *et al.*, 1995). During the LD period, stem elongation is reduced at lower AT, due to a decreased number of internodes and decreased internode length (De Jong and Smeets, 1982). It is unclear whether this response is the same for all cultivars. De

Jong (1982) concluded, for 25 cultivars grown at 14ºC and 20ºC AT, that stem length was influenced by the interaction between AT and cultivar. However, this author did not show the extent of this interaction.

Stem-length at flowering decreased with decreasing AT (Bonaminio and Larson, 1980; Lepage *et al.*, 1984; Heij and De Lint, 1987; Whealy *et al.*, 1987). This must result from decreased internode length as the final leaf number is only slightly affected or unaffected by temperature (see Number of leaves section, above). As a consequence of delayed flower initiation at sub-optimal temperatures (see Time to flowering section, below), differences in the final stem-length are small (Cockshull *et al.*, 1995). As the response of flower initiation to temperature is highly cultivar-dependent, it is likely that the effect of temperature on final stem-length is also cultivar-dependent.

Internode length is dependent on the difference between DT and NT (i.e., DIF). Plants grown under a negative DIF have shorter internodes compared to plants grown under a positive DIF, at the same AT (Karlsson *et al.*, 1989c; Bertram, 1992; Cockshull *et al.*, 1995; Carvalho *et al.*, 2002). However, Langton and Cockshull (1997) concluded that internode length of `Bright Golden Anne´ was not explained by DIF, but their experiment lasted only 10 days, such that internodes had not yet fully elongated. This can explain why their findings are conflicting with the majority of other authors, as shown by Carvalho *et al.* (2002). Langton and Cockshull (1997) stated also that DIF has no real biological meaning and that internode length depends on actual DT and NT. This is in agreement with Carvalho *et al.* (2002), who clearly demonstrated that, within a range of temperatures (18º - 24ºC, in their case), the positive effect of a higher DT on internode length is compensated by a similar negative effect of a higher NT on internode length. Such an effect of DT and NT, i.e. opposite but equal in magnitude, can occur when the optimum NT is much lower than the optimum DT (Carvalho *et al*. 2002), and in such situations DIF can accurately predict final internode length. Note that in most experiments the day-length is not equal to the length of the night. This could explain why internodes are shorter after SD with decreasing AT. During the LD period, increased internode length could be expected with increasing AT. However, De Jong and Smeets (1982) counted the day extension treatment as NT, which could explain the shorter internodes found in this experiment. Based on the DIF concept, the manipulation of DT and NT can be used to control plant height (Erwin *et al.*, 1989). Another method of controlling plant height with temperature is achieved by applying a short-term drop in temperature (DROP treatment; reviewed by Carvalho and Heuvelink, 2001). The effectiveness of DROP treatment is dependent on the timing, duration and the magnitude of the treatment. The effect of DROP treatment is largest in the early hours of the light period (Cockshull *et al.*, 1995).

Time to flowering

Time to flowering is determined mainly by the number of days between the start of the SD period and flowering. Although growers vary the length of the LD period with the season, the

time from the start of the SD period to flowering strongly determines the length of cultivation. Temperature has a significant influence on this trait, especially in winter conditions when light levels are low. In general, time to flowering shows an optimum response to temperature usually between 17ºC and 22°C, depending on the cultivar (Figure 1; De Jong, 1978; De Lint and Heij, 1987; Karlsson *et al.*, 1989a; Hiden and Larsen, 1994; Adams *et al.*, 1998; Persson and Larsen, 1998). Large differences exist between cultivars in the extent of the delay in flowering at sub-optimal temperatures. Fifteen cultivars showed flowering delays between 5 - 56 d when cultivated at 13ºC compared to 17ºC (De Jong, 1984). Furthermore, the effect of DT and NT is not the same in all cultivars. Some cultivars respond as strongly to DT as to NT, and the response can be taken as a response to AT (Cockshull *et al.*, 1981), while other cultivars respond more strongly to DT than to NT (Cockshull *et al.*, 1981; Karlsson and Heins, 1986) even when the night period is longer than the day period.

Some researchers have investigated the effect of temperature applied at different phases of the cultivation period. Temperature during the LD period has no influence on time to flowering (De Jong and Smeets, 1982; Wilkins *et al.*, 1990). The SD period can be divided into a period of flower initiation and a period of flower development (Karlsson *et al.*, 1989a), and both are sensitive to temperature. Although the time to visible flower bud appearance shows an optimum response to temperature (Cockshull, 1979; De Lint and Heij, 1987; Karlsson *et al.*, 1989a) the very early stages of flower bud initiation, judged by the increase in apex size viewed with a microscope is delayed at lower temperatures, but not at temperatures above 20°C (Horridge and Cockshull, 1979; Van Ruiten and De Jong, 1984; Karlsson and McIntyre, 1990; Adams *et al.*, 1998). Further flower development reacts according to an optimum response (Karlsson *et al.*, 1989a; Adams *et al.*, 1998) where the optimum temperature becomes lower as the plants mature (Karlsson *et al.*, 1989a).

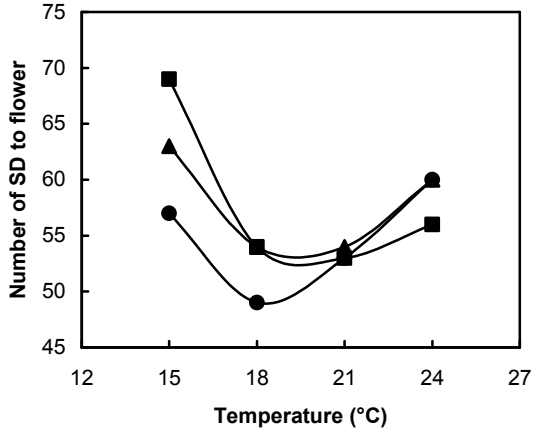

Figure 1: The number of short days until flowering for three cut chrysanthemum cultivars (▲ 'Beverly', ■ 'Grand Pink', ● 'Mundial') grown at four temperatures (Van der Ploeg, unpublished data). Deviation maximum two days.

Irradiance also has an influence on time to flowering. Plants flowered earlier at higher irradiance, although the absolute response per unit increase in irradiance declined as irradiance increased (Karlsson and Heins, 1986; Karlsson *et al.*, 1989b). In part, the effect of irradiance may be an indirect effect of temperature. The temperature of chrysanthemum leaves does increase with increasing radiation and the temperature of the flowers, which do not have stomata to regulate their temperature, is expected to increase even more with increasing radiation (Langton *et al.*, 2000). Furthermore plant density has only a very small effect or no effect on time to flowering (Langton *et al.*, 1999; Lee *et al.*, 2002a), while density also affects the amount of light received by each plant.

Number of flowers

Several studies report higher numbers of flowers at higher temperatures (Cathey, 1954; Lepage *et al.*, 1984; Carvalho *et al.*, 2005), mainly because more flower buds are produced (Carvalho *et al.*, 2005). This increase in flower number is mainly due to the influence of NT (Cathey, 1954; Lepage *et al.*, 1984), although cultivar-specific deviations from this trend have been observed (Parups and Butler, 1982; Lepage *et al.*, 1984). In a study with six cultivars, the cultivar `Accent´ showed an increasing number of flowers with increasing DT between 10ºC and 18ºC, while the cultivar `Boston´ showed decreasing number of flowers with increasing DT. In the other four cultivars, DT had no effect on the number of flowers (Lepage *et al.*, 1984). Moreover, the influence of temperature was not the same during the whole cultivation period. Increasing temperature in the last phase of the SD period (i.e., from visible terminal flower bud up to harvest) has a promoting effect on the number of flowers, while the temperature during the bud initiation period has no influence (Carvalho *et al.*, 2005).

There is also an interaction between light and temperature. In Spring, a higher number of lateral shoots was found with increasing temperature, while in winter no effect of temperature was found on lateral shoot number (Schoellhorn *et al.*, 1996).

Flower size

Over a large range of cultivars and temperatures (13º - 26ºC) the diameter of individual flowers on plants that grew at lower temperatures was higher (Vince, 1960; De Jong, 1978; Bonaminio and Larson, 1980; Tsujita *et al*., 1981; Willits and Bailey, 2000; Nothnagl *et al*., 2004; Carvalho *et al*., 2005). However, there were differences between cultivars in the extent of this response. Reducing the NT from 15°C to 10°C increased flower size in eight cultivars between 3 - 34% (Vince, 1960). Flower size in chrysanthemum depends on the number of florets and the size of the florets (Machin and Scopes, 1978). The floret initation rate was reduced at 10°C compared to 15°C AT (Karlsson and McIntyre, 1990); however, a longer initation period can neutralise the possible impact of this effect on the final number of ray florets. Indeed, decreasing the NT from 15ºC to 10°C did not result in a decreased number of ray florets, therefore the increase in flower size must be due to an increase in ray floret length

(Vince, 1960). The effect of sub-optimal temperatures on individual flower size is mainly a result of the extended growth period of the flower, as the growth rate is only marginally affected (Nothnagl *et al*., 2004). It is therefore likely that, under higher light conditions when the cultivation period is not as much extended by temperature, flower size is also less affected by temperature. In fact, Carvalho *et al*. (2005) found an increased flower size at 17°C compared to 21°C in a winter greenhouse experiment, where the growth period was extended by 7 d, while in a climate room experiment with higher light conditions, no effect of temperature on flower size was found between 15ºC and 24°C. In this latter experiment, the cultivation period was extended by only 2 - 3 d.

Total flower area, however, shows an optimum response with the optimum temperature around 18ºC to 20ºC for both DT and NT depending on the light level (Karlsson and Heins, 1986; Karlsson *et al*., 1989b). As individual flower size increases with decreasing temperature, the decreased total flower area at sub-optimal temperature must be the result of a lower flower number (see Number of flowers section, above).

Biomass production and partitioning

Biomass production during LD

In order to study growth at lower temperatures, plant growth analysis can be used. Plant growth analysis is an explanatory, holistic and integrative approach to interpret plant form and function (Hunt *et al.*, 2002). It makes use of simple primary data, such as weights and leaf area. Plant growth analysis can be performed according to a classical approach, measured across one harvest interval, or a functional approach, using data from many harvests and fitted curves (Hunt *et al.*, 2002).

The relative growth rate (RGR) describes the rate of increase in plant mass per unit plant mass already present. Differences in RGR can be explained by differences in leaf area per unit plant mass (LAR; leaf area ratio), or by differences in the rate of increase in plant mass per unit leaf area (NAR; net assimilation rate or ULR; unit leaf rate; Hunt, 1990; Figure 2). LAR is the product of specific leaf area (SLA; total leaf area per unit leaf mass) and leaf weight ratio (LWR; total leaf mass per unit total plant mass). NAR represents the carbon gain in photosynthesis minus the carbon use in respiration.

Within chrysanthemum accessions there is a large variation in RGR (Parups and Butler, 1982; De Jong and Jansen, 1992), which was found to be correlated with LAR and not with NAR. The increase in LAR resulted from an increase in SLA (De Jong and Jansen, 1992). The correlation of RGR with LAR holds for most variation within plant species. In general, an inherently higher RGR can be explained for 80 - 90% by a higher LAR, and only for 10 - 20% by a higher NAR (Poorter, 1989).

There are few publications on the RGR of chrysanthemum at different temperatures. The RGR of nine cultivars grown under a split NT (4 h at 16ºC/ 10 h at 10ºC) increased compared

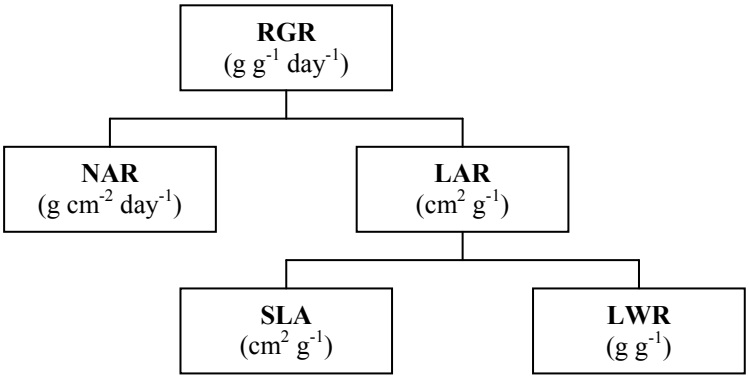

Figure 2: The relative growth rate (RGR) and its components and units. Abbreviations: NAR= net assimilation rate, LAR= leaf area ratio, SLA= specific leaf area, LWR= leaf mass ratio.

to the RGR of plants grown at a constant NT (14 h at 16ºC) due to an increase in NAR. This increase in NAR is probably caused by lower respiration rates during the night (Parups and Butler, 1982).

Hughes and Cockshull (1972) reported that there was no increase of LAR and SLA with increasing DT between 18.3ºC and 23.9ºC, while Acock *et al*. (1979) reported an increasing LAR due to increasing SLA with increasing AT between 10ºC and 30ºC. An explanation for these contradictory results could be that NT plays a more prominent role than DT, but cultivar differences may also have a role. The increase in SLA observed by Acock *et al*. (1979), however, did not result in an increase in RGR, as the total mass was not affected by the AT. This is probably due to the high initial leaf area index (LAI) in the experiments (2.7 - 3.5) and therefore most of the light was intercepted from the start of the experiment. Above a critical LAI (around 3), temperature has no effect on dry mass gain (Kohl and Thigpen, 1979). In contrast, De Jong and Smeets (1982) reported that decreasing DT from 17ºC to 10°C during the LD had a negative effect on fresh mass. This could well be the result of a decrease in SLA, as the initial LAI (0.94) in this experiment was well-below the critical LAI level. A reduced RGR, due to a reduced SLA at sub-optimal temperatures, was also found in other plant species, such as tomato (Paul *et al.*, 1984; Smeets and Garretsen, 1986b) and daisy (Stirling *et al.*, 1998). On the other hand, NTs between 10ºC and 22ºC did not have an effect on the fresh mass produced during the LD period (De Jong and Smeets, 1982; Heij and De Lint, 1987), even though in the experiment of De Jong and Smeets (1982), NT was applied for a longer time than DT.

Photosynthetic activity per unit leaf dry matter increased with increasing temperature between 10º - 30ºC (Acock *et al.*, 1979), while the net photosynthetic rate measured on the fifth leaf showed an optimum response to temperature that was dependent on both CO_2 concentration and light level (Heins *et al.*, 1986). In a crop stand, however, the optimum

temperature for photosynthesis is determined by the integrated temperature effect of different leaf layers in the canopy, which causes a drastic flattening of the response (Schapendonk and Brouwer, 1985). For chrysanthemum crop photosynthesis a rather flat temperature optimum was also detected, where the optimum was dependent on the light level and CO_2 concentration (Körner, 2003).

Biomass production during SD

In the period between the start of the SD treatment and the time when the terminal flower bud became visible, the total aerial dry mass was found to be most sensitive to temperature. Increasing temperature during this period from 16ºC to 24ºC resulted in a positive quadratic response of total dry mass at flowering (Carvalho *et al.*, 2005). During the SD period, plants grown at sub-optimal AT and measured at the same time have slightly lower dry mass than plants grown under optimal AT regimes (De Lint and Heij, 1987). Increasing the DT between 18.3ºC and 29.4°C had no effect on biomass (Hughes and Cockshull, 1972). Lowering the NT for part of the night (4 h at 16ºC, 10 h at 10ºC) resulted in a significant increase in total dry mass for some cultivars while for other cultivars no effect on total dry mass was found (Parups and Butler, 1982).

Biomass at flowering

Records on the effects of temperature on total biomass at flowering are contradictory (Table 1). Total plant mass was found to decrease (Karlsson and Heins, 1992), remain constant (Tsujita *et al.*, 1981; Carvalho *et al.*, 2005) or to increase (Tsujita *et al.*, 1981; Lepage *et al.*, 1984) with decreasing temperatures. Part of the reason for these differences might be that not all cultivars react in a similar manner to temperature. Of the three cultivars grown by Tsujita *et al*. (1981), two had a higher plant mass at 13ºC than at 16ºC NT, while one produced equally heavy plants at both NTs. On the other hand, Lepage *et al*. (1984) did not find any differences in fresh mass between six cultivars in response to temperature. Interactions with other growth conditions, such as light intensity, might also play a role in explaining contradictory results from different experiments.

Lepage *et al*. (1984) concluded that fresh mass at flowering decreased only with increasing NT and that DT had no effect. However, the nights were longer than the days and when the data were replotted against AT it appears that final plant fresh mass was negatively linearly related to AT (Figure 3A).

Final biomass at flowering is determined both by the growth rate and by the duration of the cultivation period. None of the papers on biomass production in chrysanthemum mentioned which of these factors cause increases or decreases in biomass with increasing temperature. Plotting total plant fresh mass values that were found in the experiment of Lepage *et al*. (1984) against days to flower, shows a positive linear relationship between these two traits, when the 10º/10ºC DT/NT treatment was removed (Figure 3B). Growth rate, on the other

Table 1: Summary of the effects of temperature on chrysanthemum fresh (F) or dry (D) mass production.

Time of measurement	Temperature combinations (°C)	Reaction to	Total mass (g plant^{-1}) [a]		Additional information
End of the LD period					
Acock *et al*. (1979)	AT: 10; 15; 20; 25; 30	AT	D	0	
De Jong and Smeets (1982)	DT: 10; 14; 17	DT	F	-	day 9 h
	NT: 10; 14; 17	NT		0	
Heij and De Lint (1987)	DT 19	NT	F	0	day 16 h
	NT 13; 16; 19; 22				
During the SD period					
Hughes and Cockshull (1972)	DT 18; 21; 24; 29	DT	D	0	day 8 h
	NT 15.6				
De Lint and Heij (1987)	DT 14; 17; 21	DT	F	-	day 8 h
	NT 14; 17; 21; 25	NT		-	
At flowering					
Tsujita *et al*. (1981)	NT 13; 16	NT	F	+	2 cultivars
		NT		0	1 cultivar
Lepage *et al*. (1984) [b]	DT: 10; 14; 18	AT	F	+	
	NT: 10; 14; 18				
Karlsson and Heins (1992) [b]	DT: 10; 14; 20; 26; 30	DT	D	-	day 10 h
	NT: 10; 14; 20; 26; 30	NT		-- [c]	
Carvalho *et al*. (2005) [d]	AT: 17; 21	AT	D	0	
	AT: 15; 18; 21; 24	AT	D	- (minimum at 15.9°C)	LD to visual bud

[a] + increase with decreasing temperature; - decrease with decreasing temperature; 0 not affected by temperature

[b] In these experiments the temperature treatment was applied only during the SD period.

[c] To point out that the response to NT was greater than the response to DT.

[d] Two separate experiments.

hand showed a small increase with increasing AT. The increase in biomass with decreasing AT in this experiment must therefore be a result of the increased growth period alone. Although Karlsson and Heins (1992) reported the opposite effect of temperature on total dry mass, replotting their data shows that total plant dry mass was positively linearly related to cultivation period between 14ºC and 26ºC (Figure 4A). Because, in that experiment, plants were measured every 10 d no difference was recorded for time to flowering at 14ºC and 20ºC AT, although supra-optimal temperatures did delay flowering. This relationship is stronger at higher light intensity. It should be mentioned that, for other light intensities used in the same paper, this relationship does not hold, probably as a result of the more extreme temperature range used at these light intensities (10º - 30ºC). However, contrary to the data of Lepage *et al*. (1984), in the experiment of Karlsson and Heins (1992) there also appeared to be a linear relationship between growth rate and final plant biomass, which seemed to be independent of the light level (Figure 4B). Decreases in biomass with decreasing temperatures, in the experiment of Karlsson and Heins (1992), are therefore a combined effect of a decreased cultivation period and a decreased growth rate. In the experiment of Tsujita *et al*. (1981), differences in response to temperature between different cultivars can be explained by differences in growth rate. The two cultivars that were heavier at 13°C NT compared to 16°C NT had a higher growth rate during the SD at 13°C, while the cultivar that showed equally heavy plants at both NTs showed no difference in growth rate between the two NTs.

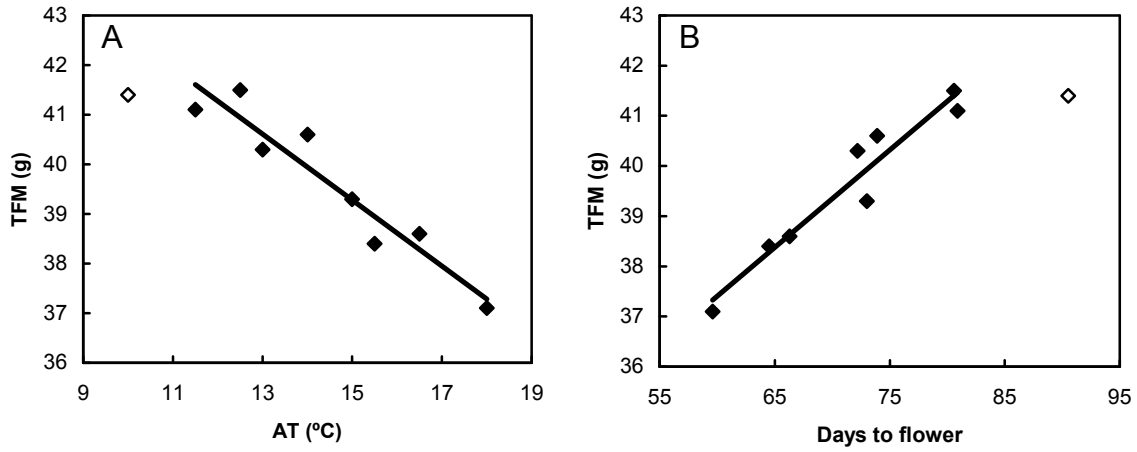

Figure 3: The effect of AT (A) and days to flower (B) on total fresh mass (TFM) of whole plants. Data are the average values for six cultivars. The solid lines represent linear regressions where: (A) y = -0.67x + 49.3 (r^2 = 0.91) and (B) y = 0.19x + 25.7 (r^2 = 0.93). In both regressions the treatment 10ºC/10ºC DT/NT (◊) was omitted. Data replotted from Lepage *et al.* (1984).

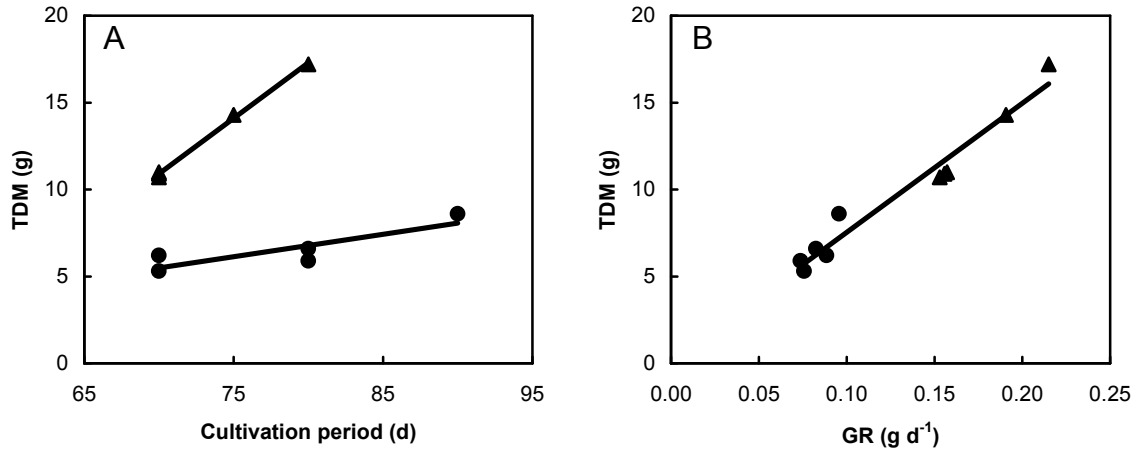

Figure 4: Effect of length of the cultivation period (A) and growth rate (GR; B) on final total dry mass (TDM) of the cultivar 'Bright Golden Anne' grown under photosynthetic photon flux (PPF) levels of 5.8 (●) or 17.6 (▲) mol m^{-2} d^{-1}. The solid lines represent linear regressions where: (A, ●) y = 0.13x – 3.56 (r^2 = 0.74); (A, ▲) y = 0.64x – 33.9 (r^2 = 0.997); and (B) y = 74.2x + 0.11 (r^2 = 0.96). Data replotted from Karlsson and Heins (1992). Data from the other PPF levels were omitted as the DT and NT ranges for these light levels were too extreme.

Partitioning

Because temperature influences plant development, it also influences partitioning of assimilates to different plant organs. This should be reflected in the effect that temperature has on the mass of individual plant organs. However, similar to data on total biomass production, records on the mass of each plant organ are contradictory.

During the LD period, partitioning towards the leaves was independent of AT, while

partitioning towards the roots increased with increasing AT at the expense of partitioning towards the stem (Acock *et al.*, 1979). Increasing stem and leaf mass at higher DT (De Jong and Smeets, 1982) should thus be a result of the increase in total biomass, although it could also be that the effect of temperature on partitioning could differ for different cultivars or growing conditions. Leaf dry matter was used to create a larger leaf area at higher temperatures (Bonaminio and Larson, 1978; Acock *et al.*, 1979; Whealy *et al.*, 1987), resulting in thinner leaves. The increased leaf area with increasing temperatures was a result of increased leaf number and increased leaf size (Whealy *et al.*, 1987).

In contrast to the effect of temperature on assimilate partitioning during the LD period, partitioning towards the leaves was enhanced by AT in the SD period at the expense of assimilates going to the roots (Karlsson and Heins, 1992). This is supported by the fact that leaf mass increases with increasing AT (Khattak and Pearson, 1997). However, another report suggested that leaf mass decreases with increasing AT (Lepage *et al.*, 1984) but, in this experiment, total biomass also decreased at higher AT. The percentage of dry matter partitioned to the stems was positively related to DIF (Karlsson and Heins, 1992). The mass of the stem increased with decreasing NT (Lepage *et al.*, 1984). It is unclear whether this response is the same for all cultivars. De Jong (1982) concluded, for 25 cultivars grown at 14ºC and 20ºC AT, that stem weight was influenced by the interaction between AT and cultivar. However, this latter study does not show the extent of this interaction.

Dry matter partitioning towards the flowers in chrysanthemum was not influenced by AT over the whole growth period (Karlsson and Heins, 1992). Nevertheless, it was found that lowering the AT between the start of the SD period and the time the bud became visible, increased the partitioning towards the flowers (Carvalho *et al.*, 2005). In a temperature range from 10º - 30°C, flower mass showed an optimum response to DT, where the optimum was dependent on available light (Karlsson and Heins, 1992). Over a much smaller temperature range (10º - 18ºC), it was found that flower mass increased with increasing AT (Lepage *et al.*, 1984). As in this experiment, total biomass production also increased partitioning towards the flowers was not affected by temperature.

Options for energy saving

In Dutch greenhouses, decreasing the temperature set-point from 20ºC to 18°C reduces energy consumption by 20% on an annual basis (Körner, 2003). One obvious character to look for when breeding a more energy efficient chrysanthemum cultivar is no delay or a reduced delay in flowering, at sub-optimal temperatures under low light conditions. There is a large variation between the chrysanthemum accessions for this trait, which makes it possible to select cultivars in which the cultivation period at sub-optimal temperatures is less delayed. In the 1980s, when oil prices were high, research focused on reducing fuel use by reducing (night) temperatures in the greenhouse. For chrysanthemum, this research was focused mainly

on developing cultivars with an improved flowering capability at lower temperatures. Within a series of clones selected at low temperature, reduced sensitivity to temperature accounted for most of the ability to flower at low temperature (De Jong, 1989). This research has resulted in cultivars that were capable of flowering at lower temperatures (Larsen and Persson, 1999), but the optimum temperature for flowering was not changed in these cultivars, therefore growers have not changed the temperature set-points in their greenhouses. Breeding chrysanthemum cultivars with a lower temperature optimum might therefore be a better solution. However, in summer this could lead to problems with delayed flowering at higher temperatures. A combination with a broader temperature optimum would be a solution to this problem (De Jong, 1989).

Some cultivars have a higher biomass at flowering when grown under sub-optimal temperatures compared to optimal temperatures. Higher biomass at the end of the cultivation period might mean that more plants can be cultivated m^{-2} or a shorter LD period is needed at lower temperatures. This could compensate, in part, for a lower number of stems produced on an annual basis, caused by cultivation at sub-optimal temperatures. Breeding for energy efficient chrysanthemums therefore does not necessarily have to focus on reducing flowering time at sub-optimal temperature alone, but could also focus on cultivars with a high biomass production at sub-optimal temperatures. However, it is unclear how this will affect quality.

Another option to save energy would be to reduce the temperature during only part of the cultivation period. The optimum temperature for flower development decreases with plant age (Van Ruiten and De Jong, 1984; Karlsson *et al.*, 1989a), therefore temperatures during the later phases of cultivation could be decreased. At these later stages of the cultivation period, when LAI is sufficiently high, somewhat lower temperatures will not affect dry matter production. At present in cut chrysanthemum production, reducing the temperature for part of the cultivation period causes practical problems, as different phases are grown in soil in the same greenhouse. However, practical trials are underway to develop a mobile cultivation system for chrysanthemum (Neefjes, 2004), making it possible to adjust the temperature in each phase of the cultivation period.

The variation within the chrysanthemum accessions is large, also in temperature response of the traits studied in this paper. Interactions between temperature and cultivar occurred for every developmental trait, biomass production and assimilate partitioning. The high variability between chrysanthemum cultivars is supported by RAPD analysis which has shown that even within only 15 chrysanthemum cultivars, a high level of genetic variability exists (Martin *et al.*, 2002).

Conclusions and needs for further research

Temperature has a significant influence on all developmental aspects in chrysanthemum (e.g., leaf appearance rate, stem elongation, time to flowering, and numbers and sizes of flowers). Especially under low light conditions, flower initiation and development are delayed at sub-

optimal temperatures, but the extent of this delay depends on the cultivar. Temperature also indirectly influences leaf number and stem length as apical flower initiation during the SD period stops the formation of new leaves sooner in plants grown at optimal temperature. A longer period of flower development causes individual flowers to be larger under sub-optimal temperatures.

Compared to the effects of temperature on development, the effects of temperature on biomass production are smaller but still significant. Growth is reduced at lower DT if the LAI is below a critical level (approximately 3). This is possibly related to a lower SLA (i.e., thicker leaves) at lower temperatures. Thicker leaves lead to less light interception per unit leaf mass. For crops with a high LAI leaf thickness hardly influences light interception as all the light is already intercepted. At the end of the cultivation period, the effect of temperature on biomass production is highly variable and depends on the cultivar and interactions with other growth conditions (e.g., light levels). Within certain temperature ranges part of the difference in biomass results from differences in cultivation period, due to differences in flower initation and development. However, in several experiments, the growth rate was also changed. More research is needed to explain differences in the temperature response of biomass production between cultivars and growth conditions.

Breeders can use the variation between the chrysanthemum cultivars to breed more energy efficient cultivars. One option is to breed a cultivar with a lower, or preferably a broader, temperature optimum for flowering. Another possibility is to breed a cultivar with higher biomass production at sub-optimal temperatures. This would mean that chrysanthemums can be grown at lower temperatures with a shorter LD period, or at a higher plant density (with a delay in the cultivation period). Further research is needed to see whether, in this way, it is feasible to produce the same number of stems on an annual basis and how this will affect quality.

Chapter 2.2

Variation between cut chrysanthemum cultivars in response to sub-optimal temperature

Abstract

To breed for more energy efficient cut chrysanthemum (*Chrysanthemum morifolium* Ramat.) cultivars it is important to know the variation of the temperature response existing in modern cultivars. In a greenhouse experiment with 25 chrysanthemum cultivars, a significant variation was observed in temperature response (16 or 20ºC) for reaction time, total dry mass produced, stem length and flower size and number. To study this genetic variation in temperature response over a larger range of temperatures (15 - 24ºC), four contrasting cultivars ('Annecy', 'Delianne', 'Reagan', and 'Supernova') were selected in a second experiment. Furthermore, a third experiment was performed in which the cultivation period was split into three phases and the influence of temperature in each of these phases was studied for the four selected cultivars. Dry mass production in all cultivars was very sensitive to temperature during the long day period. Relative growth rate showed an optimum response to temperature with the optimum around 24ºC. Net assimilation rate also showed an optimum response to temperature, while leaf area ratio increased linearly with temperature. Compared to these temperature effects during the long day, the effect of temperature on absolute growth rate during the short day was, depending on the cultivar, relatively small or even absent. The reaction time, on the other hand, was very temperature sensitive, showing an optimum that was cultivar dependent. The temperature response of the total dry mass production during the whole cultivation period was therefore very cultivar dependent. Furthermore depending on the cultivar stem length increased with temperature, especially during the LD, due to both increasing internode number and average internode length. The response of both flower size and number to temperature were also highly cultivar specific. The possibilities of using this genetic variation for breeding are discussed.

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Introduction

Several crop species, e.g. cut chrysanthemum, can only be grown year-round in colder climates when cultivated in heated greenhouses. This implies that production during autumn and winter will require high energy inputs and therefore energy efficiency, defined as the number of marketable stems produced per unit of energy input, is low. Lowering the temperature set-point in the greenhouse could be a way to increase this energy efficiency. This may, however, have adverse effects on crop growth and quality. It is therefore important that new cultivars are better adapted to cultivation at lower temperatures. Chrysanthemum cultivation can be divided into a long day (LD) period, in which the plants grow vegetatively, and a short day (SD) period. In this latter period flower initiation and further development takes place. The number of days from the start of SD to harvest is referred to as the reaction time and this trait is very sensitive to temperature, showing a definite temperature optimum (De Jong, 1978). Adaptation to lower temperatures can be achieved by breeding cultivars with a broader temperature optimum for reaction time. Another option is to create cultivars with a higher biomass production at sub-optimal temperatures, so that a longer reaction time can be compensated by a higher plant density or shorter LD period. For breeding new, more energy efficient cultivars, genetic variation for these traits in the present cultivar range is extremely valuable.

The genetic variation in temperature response for time to flowering has been well studied in cut chrysanthemum (e.g. De Jong, 1978; Cockshull *et al.*, 1981; De Jong, 1984). This research has resulted in cultivars that were capable of flowering at lower temperatures (Larsen and Persson, 1999), while the temperature optimum of these cultivars did not change. Therefore, growers did not reduce temperature set-points in order to guarantee a shorter cultivation period. In spite of the importance of the response of plant growth to temperature, relatively few studies have focused on the genetic variation within chrysanthemum accessions for this trait. It is also important to know how chrysanthemum growth and development react to temperature in different stages of the cultivation period. For example, temperature is known to be a critical factor for flower initiation (Wilkins *et al.*, 1990), while for further flower development the optimum temperature becomes lower as the plants mature (Karlsson *et al.*, 1989a). Although a higher temperature during the LD period increased the number of flowers in the cultivar Reagan, most flower characteristics (e.g. size, color) were more sensitive to temperature during the SD period (Carvalho *et al.*, 2005). However, as these studies were done with a single cultivar, it is difficult to generalize these results. More information is also fundamental for a proper analysis of possible differences between cultivars.

The aim of the present study was to evaluate the variation in growth and development for temperature responses among chrysanthemum cultivars and to obtain a better insight into the underlying physiological processes. Furthermore, we tried to identify phases in the cultivation period in which temperature is crucial. In order to study the variation for temperature response

on various characteristics related to growth performance an experiment was carried out in which 25 cut chrysanthemum cultivars were grown at two temperature regimes. From these 25 cultivars four contrasting cultivars were selected for an in depth study in a second experiment under a wider range of temperatures (15 - 24ºC). Finally, a third experiment was carried out in which different temperatures were applied to three different phases of the cultivation period for the four selected chrysanthemum cultivars.

Material and Methods

Experimental set-up

Exp. 1 (Table 1) was carried out in four compartments (12.8 × 12.0 m) of a multispan Venlo-type greenhouse (Wageningen University, The Netherlands, lat 52ºN). Each compartment contained eight parallel soil beds (1.125 × 10.25 m) of which the outer two were used as border. Rooted cuttings in peatblocks (4.2 × 4.2 cm) of 25 chrysanthemum cultivars were obtained from two breeding companies (Fides Goldstock Breeding, Maasland, The Netherlands: 'Feeling Green', 'Grand Pink', 'Greenbird', 'Mundial', 'Reagan Improved', 'Shining', 'Spoetnik', 'Supernova', 'Tiger', 'Universe', 'Voyager', 'Woodpecker' and from Deliflor, Maasdijk, The Netherlands: 'Anastasia', 'Annecy', 'Beverly', 'Biarritz', 'Bradford', 'Cayenne', 'Delianne', 'Dublin', 'Granada', 'Hastings', 'Managua', 'Orinoco', 'Zembla'). These cuttings were planted on 27 Nov. 2002 at a density of 48 plants/m^2. The heating set-point was 16ºC (low temperature; LT) in two compartments and 20ºC (high temperature; HT) in the other two compartments. Ventilation temperature set-points were set 1ºC above the heating set-point. During the first 3 weeks of the cultivation period the plants were grown under LD conditions, followed by SD until flowering. The apical flower bud was removed in an early stage. Supplementary irradiance was provided by high pressure sodium lamps (HPS, Philips SON-T Agro, 44 µmol m^{-2} s^{-1} photosynthetic active radiation, *PAR*; Philips, Eindhoven, The Netherlands), which were kept continuously on during the day period of the LD (19 h) and SD (9 h 30 min) period. Plants were grown under ambient CO_2. Greenhouse climate was automatically recorded every 5 min using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands).

Table 1: Basic information on three cut chrysanthemum experiments described in this paper.

Exp.	Location	Number of cultivars	Realized temperature (ºC)	Plant density (plants/m^2)	Incident *PAR* (mol m^{-2} d^{-1})
1	Greenhouse	25	16.5 (LT); 20.1 (HT)	48	4.2 – 5.6 [x]
2	Climate room	4	15.0; 18.0; 21.0; 24.0	69	10.9
3	Climate room	4	16.0 (LT); 20.0 (HT) [y]	69	10.9

Realized actual air temperature and incident *PAR* are averages over the whole growing period.

LT = low temperature, HT = high temperature

[x] Indicates range of variation between cultivars

[y] Cultivation is divided in three phases [phase I – long day period (LD); phase II - start of short day (SD) to visible bud (VB); phase III - VB to flowering] and at the end of each phase, one-half of the plants were moved to the other temperature.

Exp. 2 and 3 (Table 1) were conducted in four artificially lit climate rooms (4.50 m × 3.25 m × 2.20 m). Cuttings of four chrysanthemum cultivars ('Annecy', 'Delianne', 'Reagan Improved' and 'Supernova') were planted in 14-cm-diameter pots containing peat-based commercial potting compost (Lentse Potgrond nr. 4; 85% peat, 15% clay; Lentse Potgrond, Lent, The Netherlands) at a plant density of 69 plants/m^2. Plants were placed on side by side trolleys. During the first 2 weeks plants were grown under LD conditions, followed by SD until flowering. Assimilation lights (HPI-T plus and HPS SON-T Agro, Philips, 1:1, 380 µmol m^{-2} s^{-1} *PAR*) were continuously on for 8 h followed by 11 h (LD) or 3 h (SD) of incandescent light (12 µmol m^{-2} s^{-1} *PAR*; purely photoperiodic). Plants were grown under ambient CO_2 and at constant vapor pressure deficit (VPD = 0.58 kPa). Plants were watered by hand as required. Fertilization was done on a weekly basis (Kristalon; Hydro Agri, Vlaardingen, The Netherlands). In Exp. 2 the temperatures in the four climate rooms were set at 15, 18, 21 and 24ºC. Within each climate room there were two blocks of each cultivar. This experiment was repeated. In Exp. 3 the temperatures in two climate rooms were set at 16ºC (LT) and in the other two at 20ºC (HT). Both at the start of SD and at the moment the apical bud became visible (visible bud; VB), plants from half of the trolleys were placed in the other temperature treatment and the other half remained at the same temperature, dividing the cultivation period in three phases (Phase I – LD; Phase II – start SD till VB; Phase III – VB till final harvest). This resulted in a total of 32 treatments (i.e. eight temperature treatments for each of the four cultivars).

Measurements

In all experiments destructive measurements were carried out at planting, the start of the SD, and at final harvest. In Exp. 2, one more harvest was conducted during the LD and four more destructive measurements were conducted during SD at approximately equal intervals (10-12 d). In Exp. 1 at each destructive measurement five plants per plot were harvested, leaving two rows of border plants between cultivars and harvests. In Exp. 2 and 3, three plants per plot were measured at each harvest, except for the final harvest when six plants per plot were taken. Final harvest of all plants occurred when at least three plants had at least three flowers fully open. This stage was reached at different times depending on the cultivar and temperature. Within a given treatment, data were collected on all plants at the same time. After each destructive measurement the plants were rearranged such that plant density remained equal and a row of border plants around the measurement plants was maintained. Stem, leaf and flower fresh and dry weight (ventilated oven, 105ºC for at least 15 h), number of leaves on the main stem, number of flowers (including flower buds > 5 mm) and stem length were determined. Total plant leaf area and individual flower area of the first lateral flower (model 3100 area meter; LI-COR, Lincoln, Nebr.) were determined. Internode appearance rate (IAR) was determined as the slope of the regression line between leaf number and time for observations during the LD and the first measurement in SD.

With the dry matter and leaf area observations collected in Exp. 2, relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) were calculated over the LD period according to the "classical approach" described by Hunt (1990), using the measurements at planting (leaf area index (LAI) > 0.31) and at the end of the LD period (LAI < 1.9). Furthermore the absolute growth rate (AGR) was calculated as the slope of total dry matter (TDM) against time during the SD period (for all treatments $R^2 > 0.95$; LAI > 2.2).

Statistical design and analysis

Exp. 1 and 2 had a split-plot design with temperature as the main factor and cultivar as the split factor. Exp. 3 was analyzed as a complete randomized design with two replications. Normality of data was checked, using the 'Kolmogorov-Smirnov' test from the SPSS 12.0.1 package (SPSS Inc.; Chicago, USA). When data were not normally distributed a square root transformation was obtained and normality of these transformed data was checked. In all cases normality was produced by this transformation. An analysis of variance was conducted and treatment effects were tested at 5% probability level, except for the main temperature effects in Exp. 3 which were tested at a 1% probability level (high degree of freedom for residual). Mean separation was done by Student's *t*-test ($P = 0.05$). The statistical software package Genstat 8 (VSN International Ltd; Herts, UK) was used. In Exp. 2, the effect of temperature was separated in a linear and a quadratic component. Based on the outcome of the ANOVA, a linear regression model was built with Temperature, $(\text{Temperature})^2$ and cultivar as regressors. If a quadratic effect of temperature was found the linear component was also put in the model.

Results

Temperature variation among 25 cultivars

In Exp. 1 a significant interaction was observed between cultivar and temperature for all analyzed characteristics, which reflects the contrasting behavior of these 25 cut chrysanthemum cultivars (Table 2). Reaction time was always longer at LT but the magnitude of this effect varied greatly between cultivars. Flowering in 'Supernova' was delayed by only 4 days while flowering for 'Reagan' was delayed by 13 days (Table 2). Eleven cultivars had a significantly higher total aerial plant dry mass (TDM) at flowering when cultivated at LT compared to HT, while for the other 14 cultivars there was no significant difference between TDM produced under HT and LT (data not shown). For quality attributes, such as flower number and size, a large variation in temperature response was observed (Table 2).

From the 25 cultivars studied in Exp. 1, four cultivars ('Annecy', 'Delianne', 'Reagan' and 'Supernova') were selected based on their contrasting behavior in response to temperature in reaction time and biomass production. Additionally, these cultivars showed differences in

Table 2: Range of variation for temperature response among 25 cut chrysanthemum cultivars grown at low temperature (LT; 16.5ºC) and high temperature (HT; 20.1ºC) for reaction time, total dry mass (TDM), leaf area (LA), number of internodes (NoI), stem length, individual flower area (FA), number of flowers (NoF) and flower mass ratio (FMR) in Exp. 1.

	Difference (LT – HT) [y]						
	Median	Maximum			Minimum		
Reaction time (d)	8	Reagan	13	(21)	Supernova	4	(7)
TDM (g /plant)	0.84	Granada	2.64	(44)	Annecy	-0.58	(NS)
LA (cm^2/plant)	23	Granada	192	(17)	Grand Pink	-165	(-12)
NoI (no./plant) [z]	-1.4	Reagan	3.3	(9)	Grand Pink	-5.8	(-11)
Stem length (cm)	-10	Anastacia	13.8	(19)	Shining	-30	(-35)
FA (cm^2/flower)	5.2	Bradford	12.7	(35)	Feeling Green	-0.8	(NS)
NoF (no./plant) [z]	-1.3	Reagan	2.3	(19)	Annecy	-4.0	(-23)
FMR (g/g)	0.009	Spoetnik	0.051	(34)	Feeling Green	-0.028	(NS)

Numbers in parentheses are the relative increase at LT (as a percentage) when the differences were found to be significant.
NS Difference between LT and HT nonsignificant.
[y] F-probability of cultivar × temperature interaction (< 0.009 for all parameters)
[z] ANOVA based on square root transformed data.

temperature response to leaf area, leaf number, stem length, flower number and flower size (Table 2). In general, 'Delianne', which reacted similar to 'Granada', and 'Reagan' were more sensitive to temperature. On the other hand, 'Annecy' and 'Supernova' were less sensitive and only flower number was significantly decreased at LT, although reaction time was more affected by temperature for 'Annecy' (11 days delayed at LT compared to HT).

Temperature response in four selected cultivars

Reaction time

The minimum of the reaction time (Fig. 1A) was cultivar dependent ($P < 0.001$). The cultivar 'Supernova' had the lowest temperature minimum and the shortest reaction time at 15 °C, while 'Reagan' had the highest optimum and the longest reaction time at 15 °C. The overall regression model could explain 98% of the variation in reaction time.

When grown at LT during phase II and III all cultivars showed a delay in flowering, varying between 2 to 4 d (Table 3). However, for all four cultivars the delay at LT was similar in both phases of the SD, even though phase III was about twice as long.

Table 3: Duration of each of the cultivation phases and the delay in reaction time measured at final harvest at low temperature (LT; 16ºC) compared to high temperature (HT; 20ºC) during different phases of the cultivation period for four chrysanthemum cultivars in Exp. 3.

Phase		Duration (d)	Delay in final harvest LT compared to HT (d)			
			Annecy	Delianne	Reagan	Supernova
I	LD period	14	0	0	0	0
II	Start SD to VB	16 – 21	3	3	4	2
III	VB to harvest	36 – 42	3	3	4	2

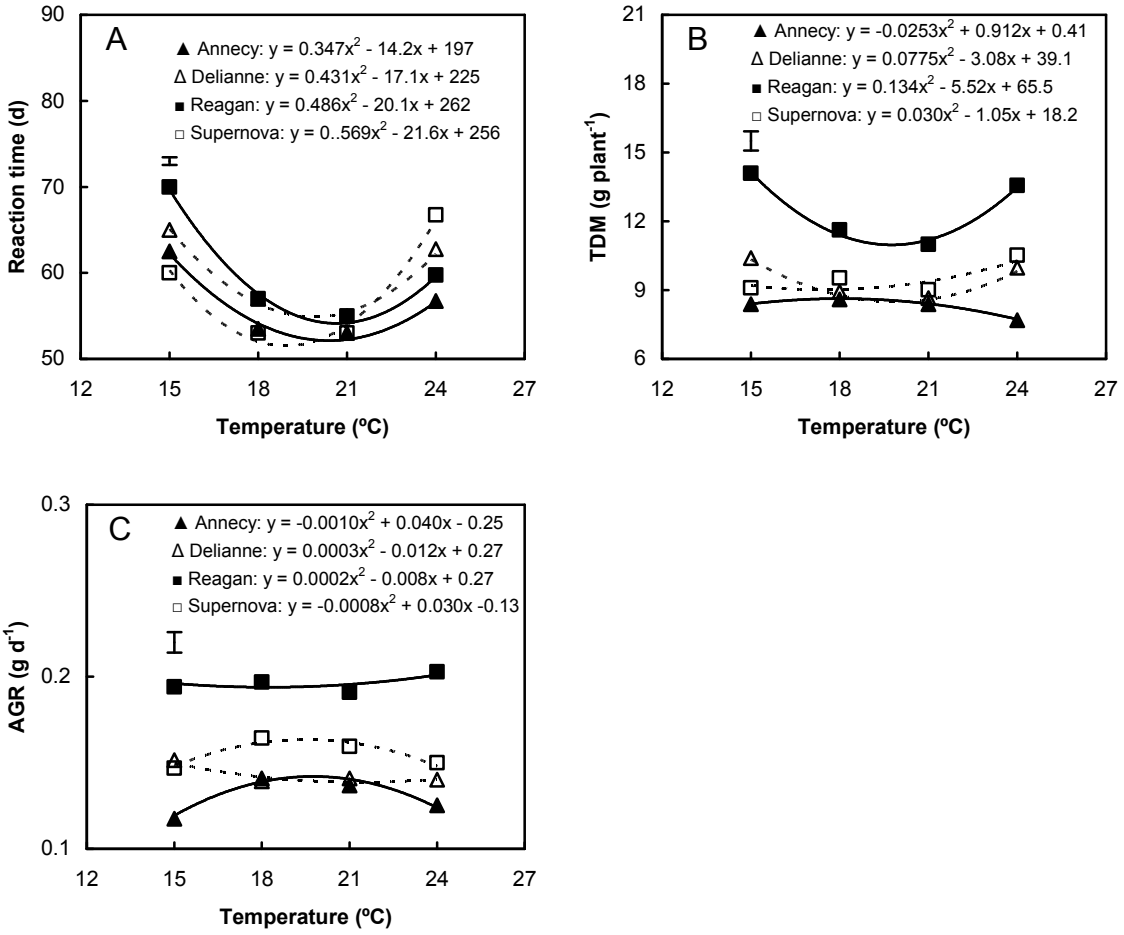

Figure 1: Reaction time (A), total plant dry mass (TDM) (B) and absolute growth rate (AGR) during the short day period (C) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an R^2adj = 0.98 (A), R^2adj = 0.84 (B), R^2adj = 0.83 (C). Vertical bars denote SE of regression= 0.90 (A), SE of regression = 0.82 (B) and SE of regression = 0.012 (C).

Total dry mass (TDM)

TDM showed a significant interaction (P = 0.002) between cultivar and temperature (Fig. 1B). 'Reagan' was very sensitive to temperature, showing a minimum around 20ºC. 'Delianne' responded similarly, although less pronounced. In contrast, for 'Supernova' the TDM was only significantly increased at 24 °C and 'Annecy' did not show any significant response to temperature within the studied temperature range (15-24ºC). An overall regression model showed that temperature could explain 84% of the variation observed in TDM for the four cultivars.

The temperature effect on TDM depends on the phase of the cultivation (Table 4). During the LD period (phase I) plants were most sensitive to temperature. Plants grown at HT during phase I (Table 4) were heavier than plants grown at LT during this phase (P < 0.001). Furthermore, the effect of temperature during phase II was dependent on the temperature

Table 4: The effect of a temperature increase from low temperature (LT; 16º) to high temperature (HT; 20ºC) during three phases of the cultivation period in four cut chrysanthemum cultivars on total dry matter (TDM), stem length, number of internodes (NoI), average internode length (IL), number of flowers (NoF) and individual flower area (FA) measured at final harvest stage in Exp. 3.

	Temperature effect (LT-HT)		
	Phase I [x]	Phase II [x]	Phase III [x]
TDM (g/plant)	+1.3 [y]	+0.58 LT phase I [z] NS HT phase I	+0.60 Annecy NS Delianne -0.65 Reagan NS Supernova
Stem length (cm)	+4.2 [y]	+4.3 Annecy NS Delianne +4.5 Reagan NS Supernova	NS
NoI (no./plant)	+1.6 Annecy +1.9 Delianne +0.9 Reagan +2.1 Supernova	NS	NS
IL (cm/internode)	NS Annecy NS Delianne +0.11 Reagan NS Supernova	+0.09 Annecy NS Delianne +0.17 Reagan NS Supernova	NS
NoF (no./plant)	+4.8 Annecy +2.9 Delianne +3.5 Reagan +1.9 Supernova	+1.0 LT phase I [z] NS HT phase I	+1.7 [y]
FA (cm^2/flower)	NS	NS	NS Annecy -4.0 Delianne NS Reagan -6.2 Supernova

NS Difference between LT and HT is nonsignificant

[x] Phase I = long day period (LD); Phase II = start of short day (SD) to visible bud (VB); Phase III = VB to flowering

[y] Same temperature effect for all cultivars

[z] Same temperature effect for all cultivars but interaction between temperature in Phase I and temperature in Phase II

during the previous phase ($P = 0.023$). When plants were cultivated at HT during phase I, the temperature during phase II did not have a significant influence on TDM. However, when plants were grown at LT during phase I, plants had a higher TDM when cultivated at HT during phase II than plants cultivated at LT during phase II. The effect of temperature in phase III was dependent on the cultivar ($P = 0.008$), varying from a significant negative effect of higher temperature for 'Reagan' up to an increase in TDM with temperature in phase III for 'Annecy'.

Growth characteristics

Although there were clear differences present between the cultivars in Exp. 2, when it comes to the growth characteristics during the LD period, all cultivars responded in a similar way to

Table 5: Effect of temperature and cultivar on relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) of cut chrysanthemum during the LD period in Exp. 2.

		RGR ($g\ g^{-1}\ d^{-1}$)	NAR ($g\ m^{-2}\ d^{-1}$)	LAR ($cm^2\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LWR ($g\ g^{-1}$)
Temp (°C)						
15		0.093 a	3.70 a	223	315	0.712 c
18		0.110 b	4.37 b	224	334	0.675 b
21		0.119 c	4.63 b	231	359	0.646 a
24		0.122 c	4.62 b	245	373	0.659 ab
F_{pr} [z]	Lin	< 0.001	0.006	**0.047**	**0.005**	0.004
	Quadr	**0.008**	**0.036**	0.267	0.677	**0.017**
Cultivar						
Annecy		0.110 b	3.91 a	248 c	343 b	0.726 b
Delianne		0.114 b	4.48 b	226 ab	319 a	0.709 b
Reagan		0.114 b	4.93 c	216 a	347 b	0.623 a
Supernova		0.105 a	3.98 a	234 b	371 c	0.633 a
F_{pr} [z]		**0.005**	**0.001**	**0.009**	**0.002**	**< 0.001**

[z] F probability (significant levels < 0.05 presented in bold). Different letters indicate significant differences between treatments based on Student's *t*-test ($P = 0.05$).

temperature (Table 5). RGR increased quadratically with temperature till an optimum around 24 °C. NAR also showed an optimum response to temperature but was only significantly lower at 15 °C. LAR and SLA showed a linear increase with temperature, while LWR showed a quadratic response with a minimum around 22 °C. 'Supernova' had a lower RGR than the other three similar cultivars. Furthermore 'Supernova' had a low NAR. Contrary, 'Reagan' had the highest NAR but this was combined with a low LAR, due to a low LWR.

Absolute growth rate (AGR) during SD showed an interaction between cultivar and temperature ($P = 0.003$) (Fig. 1C). In both 'Annecy' and 'Supernova' temperature had a quadratic effect on AGR with a similar optimum around 19º - 20ºC but the later cultivar having a higher AGR. 'Delianne' and 'Reagan' did not show significant differences in AGR between the different temperature treatments. At all temperatures the AGR of 'Reagan' was clearly higher than for the other cultivars.

Stem length, internode appearance rate (IAR), leaf number and average internode length

In all four cultivars stem length increased linearly with temperature, but this increase was larger for 'Reagan' ($P < 0.001$) compared to the other three (Fig. 2A). An overall regression model could explain 96% of the variation observed in stem length. For all cultivars temperature during phase I had a positive effect on stem length, while temperature during phase II only affected stem length in 'Annecy' and 'Reagan'. In the last part of the SD period (phase III) temperature had no effect on stem length (Table 4).

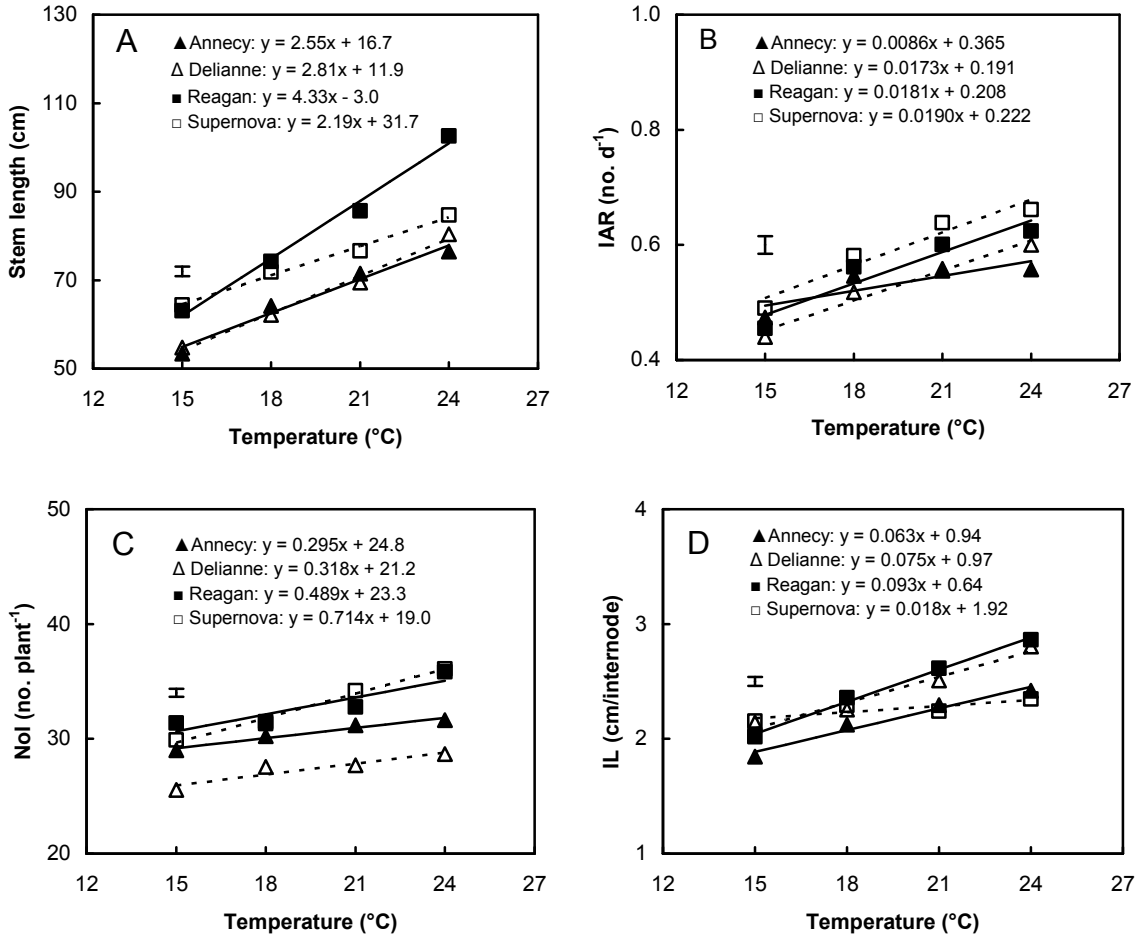

Figure 2: Stem length (A), internode appearance rate (IAR) (B), final leaf number (NoI) (C) and average internode length (IL) (D) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an R^2adj = 0.96 (A), R^2adj = 0.81 (B), R^2adj = 0.93 (C), R^2adj = 0.92 (D). Vertical bars denote SE of regression = 2.22 (A), SE of regression = 0.0309 (B), SE of regression = 0.695 (C) and SE of regression = 0.0779 (D).

IAR (Fig. 2B) and final leaf number (Fig. 2C) increased in all cultivars with temperature but this effect was less strong in 'Annecy' compared to the other cultivars. The overall regression model could explain 81% of the variation present in IAR and 93% of the variation present in leaf number. 'Delianne' had the lowest leaf number at all temperature treatments. For all cultivars leaf number only increased with temperature during phase I (Table 4), with 'Reagan' significantly less sensitive ($P = 0.026$).

Similarly to leaf number, average internode length was increased linearly with temperature in all cultivars (Fig. 2D) and an overall regression model could explain 92% of the variation present. The increase in internode length with temperature was only marginal in 'Supernova', while it was greatest in 'Reagan'. Higher temperature during phase I increased internode length of 'Reagan', while the average internode length of the other three cultivars was

unaffected by temperature during phase I (Table 4). Higher temperature during phase II increased average internode length of 'Annecy' and 'Reagan', while the other two cultivars were unaffected by temperature during phase II. Temperature during phase III had no effect on internode length for any of the cultivars.

Flower characteristics

The number of flowers per plant (Fig. 3A) showed a significant interaction between cultivar and temperature (P =0.015). For instance, in 'Annecy' no significant effect of temperature on flower number was found, while in 'Delianne' increased temperature had a negative effect on number of flowers. Contrary, 'Reagan' and 'Supernova' showed increase in flower number with temperature from 15ºC to 24ºC. In all cultivars higher temperature during phase I and III resulted in an increase in flower number, especially during phase I for the cultivars 'Annecy' and 'Reagan' (Table 4). The effect of temperature during phase II was dependent on the temperature during the previous phase (P = 0.002). When plants were cultivated at HT during phase I, the temperature during phase II did not have a significant influence on flower number, whereas plants cultivated with LT during phase I showed a small increase in flower number with temperature during phase II.

Individual flower size showed a significant interaction (P = 0.047) between cultivar and temperature (Fig. 3B). Temperature had no significant influence on the flower size of 'Reagan', while for the other three cultivars flower size showed a quadratic response, with a minimum that was cultivar dependent. Furthermore, 'Delianne' was more sensitive to temperature than 'Annecy' and 'Supernova'. Temperature during phase I and II did not influence flower size in any of the cultivars, whereas flower size was reduced at higher temperature during phase III for 'Delianne' and 'Supernova' (Table 4).

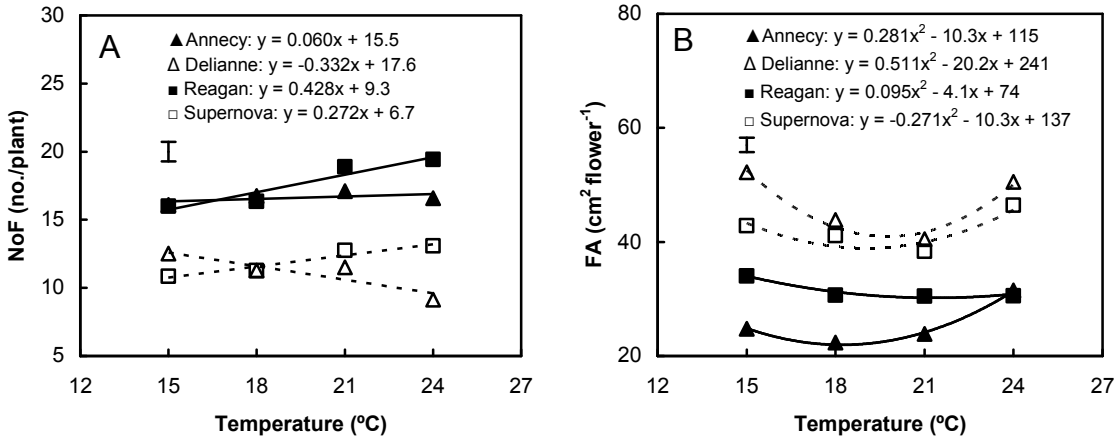

Figure 3: Number of flowers (NoF) per plant (A) and individual flower area (FA) of the first basipetal flower (B) of four chrysanthemum cultivars as a function of temperature. Formulas indicate regression model for each cultivar, with an R^2adj = 0.85 (A), R^2adj = 0.93 (B). Vertical bars denote SE of regression = 1.45 (A) and SE of regression = 2.54 (B).

Discussion

This paper clearly demonstrates that cut chrysanthemum genotypes differ in their response to temperature for growth and quality aspects (e.g. Table 2). As in chrysanthemum flower shape and color are important selection criteria, flowers with special shapes and colors may result in higher market prices, which might compensate for lower growth rates. Therefore, the selection pressure in cut chrysanthemum has not only been in the direction of higher growth rates, resulting in a large variation for growth related traits.

Reaction time, TDM and growth characteristics

Reaction time showed a quadratic response to temperature and the minimum was cultivar dependent, varying from around 19 to 21ºC (Fig. 1A). An even wider range of variation had been established previously (De Jong, 1978). Especially early stages of flower initiation and development are known to be temperature sensitive (Van Ruiten and De Jong, 1984). Also in this study temperature during the bud initiation phase had a relatively strong influence on reaction time (Table 3).

The response of TDM to temperature depended on the cultivar (Fig. 1B). This might be an explanation for varying responses in previous studies (reviewed in Chapter 2.1). However, different temperature ranges and interactions with several other growing conditions, e.g. light level, might also play a role. Contrary to our results, Lepage *et al*. (1984) found that fresh weight of six cultivars all showed the same temperature (10 – 18ºC) response. The difference might originate from the way in which the cultivars were selected in our study; the four chrysanthemum cultivars in this study were not selected solely based on the temperature response for reaction time, but growth responses were also taken into account.

TDM in chrysanthemum showed an exponential increase over time followed by a linear growth phase (Lee *et al.*, 2002a). The effects of temperature were clearly different in each phase and depended on genotype (Table 4 and 5, Fig. 1C). Especially higher temperature during the LD period increased TDM for all cultivars (Table 4), as a result of an increase in RGR. The optimum temperature for RGR (Table 5) in our study was slightly lower than optimum temperatures reported for young tomato plants (Hussey, 1965) and pansy (Adams *et al.*, 1997), both showing a maximum around 25ºC. In this study rooted cuttings (initial LAI between 0.31 and 0.57) were used and internal shading resulted in a lower temperature optimum for RGR (Adams *et al.*, 1997). In order to understand differences observed in RGR, RGR was separated into an assimilatory (NAR) and a morphological (LAR) component. LAR showed a linear increase with temperature, as a result of an increase in SLA. This is in agreement with Acock *et al*. (1979), although in their research no effect on dry weight gain was found, probably due to the high initial LAI (2.7) so that most light was already intercepted. In our study, lower RGR at 15ºC was also caused by reduced NAR. However, between 18 and 24ºC temperature had no effect on NAR. This is in line with Körner (2003)

who reported that chrysanthemum crop photosynthesis also shows a rather flat optimum response to temperature. Temperature during the beginning of the SD period only affected TDM if the temperature during LD was low (Table 4). Reduced dry mass production during the LD and decreased SLA (thicker leaves), resulted in a lower LAI at LT. When LAI is low, not all the light is intercepted by the canopy and therefore morphological changes, like decreased SLA, will affect light interception. As canopy growth proceeds, gradually more internal shading will occur and morphological changes will have less effect on plant growth.

The cultivar with the lowest RGR in this study, 'Supernova', also had a low NAR (Table 5). However, this NAR did not differ from the NAR of 'Annecy', but in the latter cultivar a low NAR was compensated by a high LAR. On the other hand, the cultivar with the highest NAR, 'Reagan', had the lowest LAR. Therefore in this study no clear relationship could be found between RGR and NAR or between RGR and LAR to explain differences between cultivars. This is in conflict with De Jong and Jansen (1992) who found RGR of 15 cultivars to be correlated with LAR but this could be related to the lower number of cultivars used for our growth analysis.

Compared to the effect of temperature on RGR during the LD, the effect of temperature on AGR during the SD is relatively small or even absent (Fig. 1C). Furthermore, the optimum for 'Annecy' and 'Supernova' was located between 19 and 20ºC, and therefore, far below the optimum for RGR. In the linear growth phase the crop is closed and therefore most of the light is intercepted. Kohl and Thigpen (1979) also reported that above a LAI of 3, temperature did not affect dry weight gain.

Differences in TDM at flowering (Fig. 1B) can be explained by differences in reaction time, growth rate or a combination of both. For instance in 'Delianne' and 'Reagan' the quadratic response of TDM to temperature was solely caused by a similar response in reaction time as AGR was not affected by temperature (Fig 2C), while in 'Annecy' the effect on reaction time was counteracted by an opposite effect on AGR. The negative influence of higher temperature during phase III on TDM in 'Reagan' (Table 4) must be a result of a shorter cultivation period. 'Reagan' had the longest extension of cultivation period at LT compared to HT (Table 2, 3). Furthermore, 'Reagan' had a higher AGR than the other cultivars and therefore differences will show up sooner. On the contrary, the positive effect of temperature during phase III on 'Annecy' is a result of a reduced AGR which could not be compensated for by a longer growth period.

Stem length, IAR, final leaf number and average internode length

Stem length increased with temperature as a result of increasing leaf (internode) number and average internode length (Fig 2; Table 4). In previous research an optimum around 25ºC for IAR has been established (Larsen and Hiden, 1995; Carvalho *et al.*, 2002). The temperature range in this study was below the optimum, which might explain why a linear response was found. Chrysanthemum starts to initiate flowers soon after start of the SD period (Van Ruiten

and De Jong, 1984) and once the plant has induced the apical flower bud, it loses the ability to initiate new internodes. Not surprisingly, only temperature during the LD period influenced final leaf number (Table 4). After the flower buds have been initiated, stem elongation is solely due to internode elongation. Although internode length is generally highly dependent on the difference between day and night temperature (i.e. DIF) (Karlsson *et al.*, 1989c; Carvalho *et al.*, 2002) and not 24-h average temperature, in this study average internode length increased with temperature even though DIF was 0 (Table 4, Fig 3D). However, as in these experiments several internodes were already present when the experiment started and the final number of internodes differed between cultivars and temperature treatments it is not possible to directly compare these results to measurements on individual internodes. Internode elongation in 'Reagan' follows a sigmoidial time course that, depending on temperature, takes between 17 and 24 days to complete (Carvalho *et al.*, 2002). Therefore, most internodes have fully elongated by the time phase III starts and temperature during this last phase had thus no influence on internode elongation and subsequently stem length.

Flower characteristics

The effect of temperature on flower characteristics depended on cultivar (Fig 3). This genetic variation in temperature response has been well established in previous research for both flower number (Lepage *et al.*, 1984) and individual flower size (Vince, 1960; Willits and Bailey, 2000). Individual flower size of three cultivars showed a minimum ranging between 18 and 20ºC, depending on the cultivar (Fig. 3B). The increased flower size at sub- and supra-optimal temperatures might be related with increasing reaction times. However, this can not explain why flower size in 'Reagan' did not increase at sub-optimal temperatures. Therefore, different processes could be involved in contrasting cultivars.

A significant positive effect of temperature during phase I and III on number of flowers was previously reported for 'Reagan' (Carvalho *et al.*, 2005) and was here confirmed for other chrysanthemum cultivars (Table 4). Additionally, a small increase in flower number with temperature during phase II was reported, but only if temperature during phase I was low. The positive effect of temperature on number of flowers during phase I and phase II is likely to be related with the increase in TDM, since a higher assimilate availability will result in a higher number of flowers (Carvalho and Heuvelink, 2003). The larger increase in flower number for 'Annecy' and 'Reagan' is probably associated with the smaller flower size in these two cultivars. Even though the formation of the apical flower bud takes place during phase II, lateral and second order flowers continue to be formed during phase III, as a consequence of the basipetal progression of flower formation in chrysanthemum. Higher temperature during this phase mainly increases the number of flowers due to an increase in the percentage of flower buds (Carvalho *et al.*, 2005). Furthermore, for 'Delianne' and 'Supernova' higher temperature during phase III increased the size of the first lateral flower, while flower size of the other two cultivars did not respond to temperature in any of the

phases (Table 4). As ‘Delianne’ and ‘Supernova’ were also the cultivars with the largest flowers, differences could probably be detected more easily.

Concluding remarks

Temperature influence on chrysanthemum varied greatly between cultivars and temperature sensitivity depended on the phase of the cultivation period. This provides several opportunities for breeding more energy efficient cultivars. The genetic variation for temperature response during the LD period is limited but breeders could exploit the variation in growth parameters between cultivars to construct new lines with a higher RGR. This will have the result that the crop closes faster, at which stage temperature has less influence on crop growth. A higher RGR could be achieved by combining a high partitioning towards the leaves (high LWR), thin leaves (high SLA) and a high NAR. Genotypic differences were present for all these traits. During the SD temperature influenced mainly the rate of development. As the influence of temperature on development is highly cultivar specific there are plenty of opportunities to select less temperature sensitive genotypes. Furthermore it is important that these genotypes do not show a reduced AGR at low temperature.

Furthermore, changes could be applied during the cultivation period. For instance a more dynamic heating strategy could be applied. It is then important to keep the temperature during the LD period high so that enough biomass is formed. For growth, temperature during the SD is less important but quality is influenced by temperature during the SD (Carvalho *et al.*, 2005). At present this will run into practical problems in continuous chrysanthemum production as in the same greenhouse compartment, plants at different stages of development are cultivated (Carvalho *et al.*, 2005). However, as at this moment practical trials are performed for a mobile cultivation system, in future might be feasible to adapt temperature better to stage of cultivation.

Chapter 2.3

Higher energy efficiency in cut chrysanthemum using genotypic variation in temperature response

Abstract

Energy efficiency of greenhouse cut chrysanthemum can be increased by breeding. Besides breeding for cultivars with a shorter reaction time at sub-optimal temperature, an alternative approach would be to develop cultivars that are heavier at sub-optimal temperature so that they could be grown at a higher plant density enhancing the production per m^2. Therefore, the combined effect of temperature and plant density on growth and development of four cut chrysanthemum cultivars was investigated in three greenhouse experiments, carried out in different seasons. For growth related traits no interactions between temperature and cultivar were found, limiting the possibilities for breeding. At suboptimal temperature, growth rate early in the cultivation period decreased as a consequence of a lower light interception resulting from a lower specific leaf area. Therefore, a higher dry mass production at lower temperature could be only explained by a longer cultivation time. Temperature also influenced external quality, but these effects were cultivar dependent. For instance, temperature affected the slope of the positive linear relationship between total dry mass and number of flowers, reducing number of flowers at low temperature for the same plant dry mass. The implications of these results on the possibilities for breeding for sub-optimal temperature tolerant cultivars are discussed.

Submitted as:

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Introduction

In The Netherlands greenhouse industry is an important user of energy as it accounts for 7% of the national energy use (Oude Lansink and Bezlepkin, 2003). Most of this energy is used in colder winter months for heating up greenhouses to maintain high production levels year-round. Cultivars that are better adapted to lower temperatures could thus contribute significantly to a reduction in energy use and consequently in CO_2 emission. For breeding for more energy efficient cultivars genotypic variation in temperature response is necessary.

Cut chrysanthemum is an important ornamental crop grown year-round in heated Dutch greenhouses. Lowering the cultivation temperature results in a longer cultivation period, due to a delay in flowering time (Lepage *et al.*, 1984). Therefore on an annual basis a reduced number of stems per m^2 can be harvested. One possibility for breeding more energy efficient chrysanthemum cultivars could be to create cultivars with less or no delay in flowering time at sub-optimal temperatures. Within the chrysanthemum assortment a large variation in temperature response for flowering time has been observed (e.g. De Jong, 1978; Chapter 2.2). Additionally, as some cultivars also produce heavier plants at sub-optimal temperature, another strategy is to breed for heavier plants at sub-optimal temperature regime (Chapter 2.2). This would allow a higher planting density at lower temperature, thus making up for the increase in cultivation time by producing more stems per m^2. However, this strategy would only be feasible if growth rate of the crop is not decreased at sub-optimal temperature. Plant growth can be enhanced either by increasing the efficiency in which light is converted into dry mass (i.e. light use efficiency; LUE) and/or by increasing light interception, due to a higher leaf area index (LAI). So far, little is known about the mechanisms responsible for increasing plant mass at sub-optimal temperature, which has been observed in some cultivars.

Besides the effect of temperature on growth, both temperature and plant density also affect several external quality aspects. For example, increasing plant density results in a decrease in the number of flowers per plant (Lee *et al.*, 2002b; Carvalho and Heuvelink, 2003), while the effect of temperature on number of flowers is highly cultivar dependent (Lepage *et al.*, 1984; Chapter 2.2). Furthermore, for the cultivar Reagan Improved a clear relationship was established between number of flowers and total biomass per plant over a wide range of growing conditions (Carvalho and Heuvelink, 2003), but it is still unclear whether and how temperature affects this relationship. In contrast, flower size is hardly influenced by plant density and the effect of temperature on flower size is cultivar dependent (Vince, 1960; Chapter 2.2). It is therefore important to also examine exactly how a lower cultivation temperature combined with a higher plant density would affect these quality aspects.

The scope of this paper is to study the combined effects of temperature and plant density on growth and external quality aspects of cut chrysanthemum cultivars. To obtain a better insight into the variation in temperature response between cultivars, a detailed analysis was performed, focusing on the underlying morphological and physiological aspects.

Material and Methods

Experimental set-up

Three experiments were conducted between September 2003 and February 2005 (Table 1). Each experiment was carried out in four greenhouse compartments (12.8m × 12.0m) that were part of a multispan Venlo-type greenhouse (Wageningen University, The Netherlands, lat 52˚N). Each compartment contained eight parallel soil beds (1.125m × 10.25m) of which the outer two beds acted as borders. Block rooted cuttings of three or four cultivars (Table 1) were obtained from two breeding companies in The Netherlands (Fides Goldstock Breeding and Deliflor) and were planted at two or three plant densities on the dates indicated in Table 1. The selection of the four cultivars was based on their contrasting response to temperature, which was observed in a previous study for reaction time and biomass production (Chapter 2.2). Single stem plants were grown supported by a wire mesh, connected to a moveable frame that included the heating pipes.

Heating set-point was 16˚C (low temperature; LT) in two compartments and 20˚C (high temperature; HT) in the other two compartments. Ventilation temperature set-points were one degree above the heating set-point. Plants were initially grown under long day (LD) conditions followed by short days (SD) until final harvest (Table 1). High pressure sodium lamps (HPS, Philips SON-T Agro, 44 μmol m^{-2} s^{-1} photosynthetically active radiation, PAR) were kept on continuously during the day hours of the LD (05:00 till 24:00) and SD period (7:30 till 17:00). Plants were grown under ambient CO_2. Greenhouse climate was automatically recorded every 5 minutes using a commercial computer system. Irrigation was provided when needed and plant protection was applied according to an integrated pest management scheme using both biological and chemical agents. No growth regulators were applied to the crop. The terminal flower bud was pinched as soon as it separated from the other crown buds (<5mm).

Measurements

In all experiments destructive measurements were carried out at planting, start of SD and at final harvest, using five plants per experimental unit. Besides these three destructive measurements some additional measurements were performed in different experiments. In Exp. 1 and 2, two extra periodic destructive measurements were conducted at approximately equal intervals during the SD. In Exp. 3 periodic destructive measurements were carried out every four to six days during the LD and every 10 – 14 days during the SD, resulting in three extra measurements during the LD and five during the SD. Between plots used for destructive measurements always at least two rows of border plants were left, to avoid disturbance of the light distribution. Final harvest of all five plants from the same experimental unit occurred when at least three plants had at least three flowers fully open. This stage was reached at different times depending on the cultivar and temperature. Within a given treatment, data

Table 1: General information on three greenhouse experiments. Dates are expressed as day of the year (day 1 = 1 Jan.). Abbreviations: HT = high temperature; LD = long day; LT = low temperature; PAR = photosynthetically active radiation; SD = short day

	Exp. 1	Exp. 2	Exp. 3
Year	2003	2004	2004 – 2005
Planting date	253	16	307
Duration of LD (d)	14	14	14
Duration of SD (d)	54 – 63	54 – 67	61 – 80
Outside global radiation [x] (MJ m^{-2} d^{-1})	7.0 – 7.6	4.9 – 6.1	2.1 – 2.5
Incident PAR LD [y] (MJ m^{-2} d^{-1})	3.5	1.1	1.3
Incident PAR SD [y] (MJ m^{-2} d^{-1})	1.6 – 1.8	1.6 – 1.9	0.8 – 0.9
Plant density (plants m^{-2})	32; 48; 64	32; 48; 64	32; 64
Temperature [z] (ºC)	18.0; 21.1	16.9; 20.5	16.2; 20.2
Cultivars	Annecy	Annecy	
	Delianne	Delianne	Delianne
	Reagan Improved	Reagan Improved	Reagan Improved
	Supernova	Supernova	Supernova

[x] Averaged over the complete cultivation period

[y] Averaged over the complete LD or SD period

[z] Realized 24-h temperatures averaged over the complete cultivation period for LT and HT treatment, respectively

were collected on all plants at the same time. Stem, leaf and flower fresh and dry mass (ventilated oven, 105˚C for at least 15 h) and number of flowers (including flower buds > 5mm) were determined. Total plant leaf area and individual flower area of the first lateral flower (LI-COR Model 3100 Area Meter, Lincoln, NE, USA) were determined.

Growth analysis and light use efficiency (LUE)

The dry mass and leaf area observations collected during the LD period in Exp. 1 and 2 were used to calculate relative growth rate (RGR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) according to the "classical approach" described by Hunt (1990). In Exp. 3 the growth analysis was conducted according to the "functional approach" as more measurements were available. The best fitting polynome for the relation between the natural logarithms of total dry mass, leaf dry mass and leaf area with time was calculated by using the ordinary "least squares estimate". In all cases polynomials of degree 2 were found to be necessary and sufficient. To exclude ontogenetic effects all growth parameters in Exp. 3 were compared on the basis of a TDM interval instead of a time interval. The TDM interval is 0.23 – 0.87g, because this was the largest possible interval in this experiment not requiring extrapolation of data.

Furthermore data collected during the SD were used to calculate the absolute growth rate (AGR). In Exp. 1 and 2, AGR was calculated between each of the four destructive harvests, but the AGR over last destructive harvests (i.e between the before last and final harvest) was not taken into account as final harvest date differed between cultivars and temperature treatments, resulting in different light intensities. In Exp. 3, AGR was calculated over the first 20 days of SD and subsequent 23 days of SD as the slope of total dry mass per m^2 against time.

A time course of LAI, based on linear interpolations between destructive leaf area

measurements, was calculated for each treatment. Daily intercepted PAR (Photosynthetical Active Radiation, 400-700nm) was based on measured daily global radiation, assuming 47% PAR in global radiation, a greenhouse transmissivity of 49% and a light extinction coefficient for a chrysanthemum crop of 0.72 (Lee *et al.*, 2002a). Light use efficiency (LUE) was defined as the slope of accumulated dry mass production during the SD (i.e. dry mass at the destructive measurements minus dry mass at the start of the SD period) and accumulated intercepted PAR over the SD period.

Statistical design and analysis

All experiments had a split-plot design with temperature as the main factor and cultivar×density as the split factor. Normality of data was checked, using the 'Kolmogorov-Smirnov' test from the SPSS 12.0.1 package (SPSS Inc.; Chicago, USA). Transformation of data was not necessary as all data were normally distributed. An analysis of variance was conducted and treatment effects were tested at 5% probability level. Mean separation was done by Student's *t*-test. The statistical software package Genstat 8 (VSN International Ltd; Herts, UK) was used.

To establish the effect of temperature and cultivar on the relationship between total biomass per plant and number of flowers a linear regression model was build using Genstat's General Linear Regression menu with cultivar, total dry mass per plant (TDM_p) and realized temperature as input. Due to the limited temperature range in these experiments we chose not to use a quadratic temperature term. The terms of the model were chosen via stepwise regression.

Results

Climate data

The difference between realized temperature and set point temperature was rather large in Exp. 1 (on average 2˚C higher throughout the cultivation period; Table 1). Especially during the first weeks it was difficult to maintain set-point temperatures in this experiment. In contrast, in Exp. 2 desired temperatures were more difficult to maintain towards the end of the experiment, while in Exp. 3 set-point temperatures could be maintained over the complete cultivation time. Consequently, the difference between HT and LT treatment varied between 3.1 and 4.0˚C depending on the experiment (Table 1).

Reaction time

Reaction time varied greatly between experiments, temperature and cultivar (Table 2), whereas plant density effects were relatively small; doubling the density from 32 plants m^{-2} to 64 plants m^{-2} resulted in only a 2 - 4 days longer reaction time. In Exp. 3 reaction time was longer than in the other two experiments for all cultivars. Furthermore, LT increased reaction

Table 2: The effect of temperature and cultivar on the reaction time (days) of cut chrysanthemum in three experiments. Abbreviations: HT = high temperature; LT = low temperature

	Exp. 1			Exp. 2			Exp. 3		
Cultivar	LT	HT	LT - HT	LT	HT	LT - HT	LT	HT	LT - HT
Annecy	59	54	5	63	54	9	---	---	---
Delianne	62	56	6	62	57	5	73	63	10
Reagan Improved	63	57	6	67	60	7	80	63	17
Supernova	59	55	4	60	55	5	65	61	4

--- not present in experiment

time, by 4 to 17d compared to HT, and made the difference between cultivars larger. 'Reagan Improved' was relatively temperature sensitive, especially in Exp. 3 where plants grown at LT were harvested 17d later than plants grown at HT. On the other hand, 'Supernova' was in all experiments the least temperature sensitive.

Dry mass production

Total dry mass

The effect of temperature on total dry mass per m^2 (TDM_a; Fig. 1) and total dry mass per plant (TDM_p; Table 6) differed between experiments. In Exp. 1 no significant effect of temperature on TDM_a and TDM_p was found, while in Exp. 2 and 3 TDM_a and TDM_p were higher at LT (Fig. 1B and 1D; Table 6). However, in Exp. 3 this temperature effect was strongest in 'Reagan Improved', which showed a 23% increase in TDM_p at LT, while TDM_p in 'Delianne' only increased by 8% (Table 6). Plant density had a strong positive effect on TDM_a in all experiments, resulting in up to 36% higher TDM_a (Fig. 1C). In contrast, increasing plant density decreased TDM_p for all cultivars, but in Exp. 2 and 3 this negative effect was stronger in 'Reagan Improved' compared to the other cultivars (Table 7). Except for 'Reagan Improved', that showed a significantly higher yield, the other three cultivars did not differ from each other in terms of TDM_a and TDM_p (Fig. 1A; Table 6).

Growth rates

To have a better insight into the factors that influence TDM_a, the growth rates were analyzed taking into account different phases of the cultivation period (Table 3). During the LD period RGR was affected by temperature, plant density and cultivar. Both LT and higher plant density decreased RGR up to around 10 %. In all experiments 'Supernova' had a significantly lower RGR than the other three cultivars.

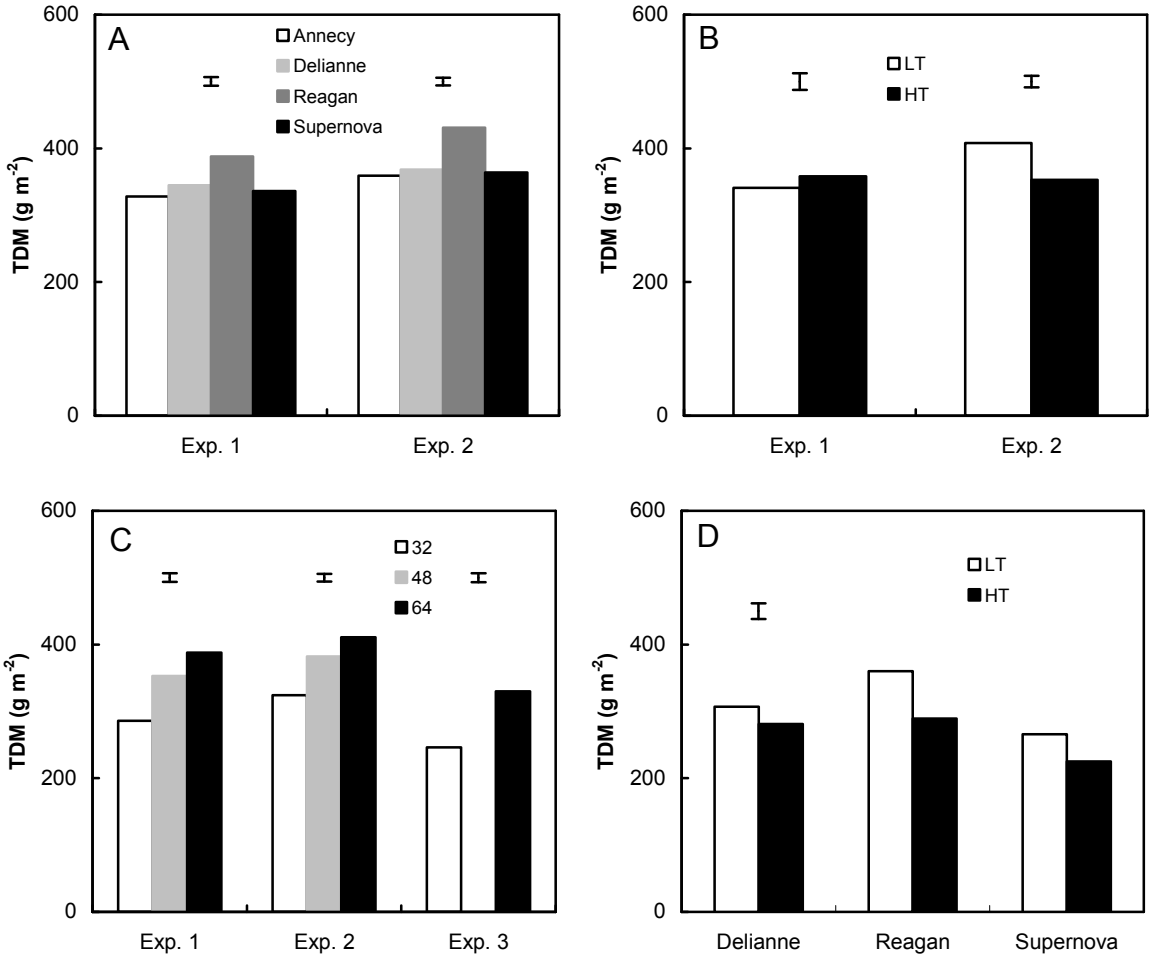

Figure 1: The effect of cultivar (A; D), temperature (B; D) and plant density (C) on cut chrysanthemum in three experiments. In Exp. 3 there was an interaction between cultivar and temperature (D). Vertical bars indicate LSD for each experiment. (A) $LSD_{Exp.\ 1}$ = 15; $LSD_{Exp.\ 2}$ = 13; (B) $LSD_{Exp.\ 1}$ = 25; $LSD_{Exp.\ 2}$ = 17; (C) $LSD_{Exp.\ 1}$ = 13; $LSD_{Exp.\ 2}$ = 11; $LSD_{Exp.\ 3}$ = 14; (D) LSD = 23.

The effect of temperature on AGR was not constant during the SD. In the first part of the SD period AGR was significantly lower at LT, while in the second part of the SD period temperature did not affect AGR (Table 3). In contrast, plant density showed a consistently positive effect on AGR throughout the SD period, but this effect was less strong in the second part of the SD. Some significant differences in AGR were found among cultivars but these were not consistent between experiments and over the SD period.

Table 3: The effect of temperature, plant density and cultivar on relative growth rate during the LD period (RGR) and absolute growth rate during the first (AGR SD_1) and second (AGR SD_2) part of SD period of cut chrysanthemum in three experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5 % level. Abbreviations: C = cultivar; D = density; HT = high temperature; LD = long day; LT = low temperature T = temperature; SD = short day

	RGR LD [w] ($g\ g^{-1}\ d^{-1}$)			AGR SD_1 [x] ($g\ m^{-2}\ d^{-1}$)			AGR SD_2 [y] ($g\ m^{-2}\ d^{-1}$)		
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 3
Temperature									
LT	**0.104**[a]	**0.082**[a]	**0.076**[a]	**5.39**[a]	**3.29**[a]	**2.40**[a]	5.24	7.05	3.57
HT	**0.111**[b]	**0.088**[b]	**0.083**[b]	**6.46**[b]	**3.63**[b]	**2.84**[b]	5.42	6.87	3.58
Density(pl m^{-2})									
32	**0.110**[b]	**0.086**[b]	**0.083**[b]	**5.01**[a]	**2.81**[a]	**2.36**[a]	**4.64**[a]	6.57	**3.13**[a]
48	**0.109**[ab]	**0.085**[ab]	---	**5.92**[b]	**3.42**[b]	---	**5.46**[b]	6.87	---
64	**0.105**[a]	**0.082**[a]	**0.077**[a]	**6.30**[b]	**3.84**[c]	**2.87**[b]	**5.58**[b]	6.81	**3.90**[b]
Cultivar									
Annecy	**0.110**[bc]	**0.099**[c]	---	6.02	**3.69**[b]	---	**4.93**[a]	6.60	---
Delianne	**0.113**[c]	**0.093**[c]	**0.084**[b]	5.79	**3.22**[a]	2.70	**4.92**[a]	7.04	3.62
Reagan Improved	**0.108**[b]	**0.083**[b]	**0.082**[b]	6.06	**3.64**[b]	2.64	**6.28**[b]	6.94	3.74
Supernova	**0.101**[a]	**0.075**[a]	**0.072**[a]	5.83	**3.30**[a]	2.52	**5.17**[a]	7.26	3.36
F-prob [z]									
T×D×C	*0.876*	*0.938*	*0.320*	*0.571*	*0.695*	*0.508*	*0.818*	*0.820*	*0.246*
T×C	*0.114*	*0.222*	*0.957*	*0.864*	*0.833*	*0.577*	*0.861*	*0.356*	*0.445*
D×C	*0.988*	*0.547*	*0.224*	*0.722*	*0.106*	*0.307*	*0.875*	*0.367*	*0.730*
T	***0.023***	***0.009***	***0.011***	***0.026***	***0.043***	***0.039***	*0.239*	*0.568*	*0.933*
D	***0.014***	***0.021***	***<0.001***	***<0.001***	***<0.001***	***0.002***	***0.002***	*0.115*	***<0.001***
C	***<0.001***	***<0.001***	***<0.001***	*0.711*	***0.002***	*0.592*	***0.001***	*0.251*	*0.319*

[w] Calculated according to classical approach in Exp. 1 and 2 and according to the functional approach in Exp. 3

[x] Absolute growth rate calculated over the first 20 days of the SD period.

[y] Absolute growth rate calculated over the period between 20 days after start of SD till 47, 41 or 43 days after start of SD for Exp. 1, Exp. 2 and Exp. 3, respectively

[z] F-probability (significant levels <0.05 presented in bold).

--- not present in experiment

Leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR)

Both LAR and SLA decreased up to 15% at LT, while LWR showed a small but significant increase at LT in Exp. 2 and 3 (Table 4). In general, density had a small positive effect on LAR and SLA, while LWR was not affected by density. 'Annecy' had a significantly higher LAR, SLA and LWR than the other three cultivars.

Table 4: The effect of temperature, plant density and cultivar on leaf area ratio (LAR), specific leaf area (SLA), and leaf weight ratio (LWR) in three experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5 % level. Abbreviations: C = cultivar; D = density; HT = high temperature; LT = low temperature T = temperature

	LAR ($cm^2\ g^{-1}$) [y]			SLA ($cm^2\ g^{-1}$) [y]			LWR [y]		
	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3	Exp.1	Exp.2	Exp.3
Temperature									
LT	**184**[a]	**235**[a]	**238**[a]	280	**335**[a]	**349**[a]	0.656	**0.699**[b]	**0.683**[b]
HT	**194**[b]	**272**[b]	**252**[b]	287	**392**[b]	**373**[a]	0.676	**0.691**[a]	**0.676**[a]
Density (pl. m^{-2})									
32	188	**251**[a]	**243**[a]	**280**[a]	**359**[a]	**356**[a]	0.673	0.698	0.684
48	185	**250**[a]	---	**279**[a]	**358**[a]	---	0.665	0.695	---
64	193	**259**[b]	**247**[b]	**293**[b]	**374**[b]	**366**[b]	0.659	0.692	0.675
Cultivar									
Annecy	**209**[c]	**299**[b]	---	**291**[c]	**389**[c]	---	**0.721**[c]	**0.769**[d]	---
Delianne	**180**[a]	**237**[a]	**255**[b]	**260**[a]	**341**[a]	**356**[b]	**0.691**[b]	**0.694**[c]	**0.716**[c]
Reagan Improved	**172**[a]	**241**[a]	**222**[a]	**279**[b]	**364**[b]	**339**[a]	**0.617**[a]	**0.664**[b]	**0.656**[a]
Supernova	**193**[b]	**237**[a]	**258**[b]	**306**[d]	**362**[b]	**387**[c]	**0.631**[a]	**0.654**[a]	**0.667**[b]
F-prob [z]									
T×D×C	*0.829*	*0.595*	*0.135*	*0.963*	*0.836*	*0.145*	*0.803*	*0.082*	*0.289*
T× C	*0.149*	*0.488*	*0.077*	*0.874*	*0.475*	*0.138*	*0.105*	*0.152*	*0.832*
D×C	*0.927*	*0.193*	*0.341*	*0.913*	*0.160*	*0.377*	*0.998*	*0.639*	*0.507*
T	***0.038***	***0.002***	***0.002***	*0.741*	***0.001***	***<0.001***	*0.179*	***0.008***	***0.022***
D	*0.142*	***0.025***	***0.019***	***0.010***	***0.002***	***<0.001***	*0.108*	*0.179*	***<0.001***
C	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***	***<0.001***

[y] Calculated according to classical approach in Exp. 1 and 2 and according to the functional approach in Exp. 3

[z] F-probability (significant levels <0.05 presented in bold).

--- not present in experiment

Leaf area index (LAI)

Differences in the development of LAI occurred between temperatures, plant densities and cultivars (Fig. 2). During LD and the first part of SD, LAI was lower at LT but differences between temperature treatments gradually disappeared during the SD period. In some cases LAI was even higher at LT at the end of the experiment (Fig. 2). Furthermore, increasing plant density resulted in a higher LAI, throughout all the cultivation period, as a consequence of a higher LAI at planting. This higher initial LAI resulted in a significantly higher light interception especially during the LD and beginning of SD. 'Annecy' was the cultivar with the higher LAI during the complete cultivation period in both experiments.

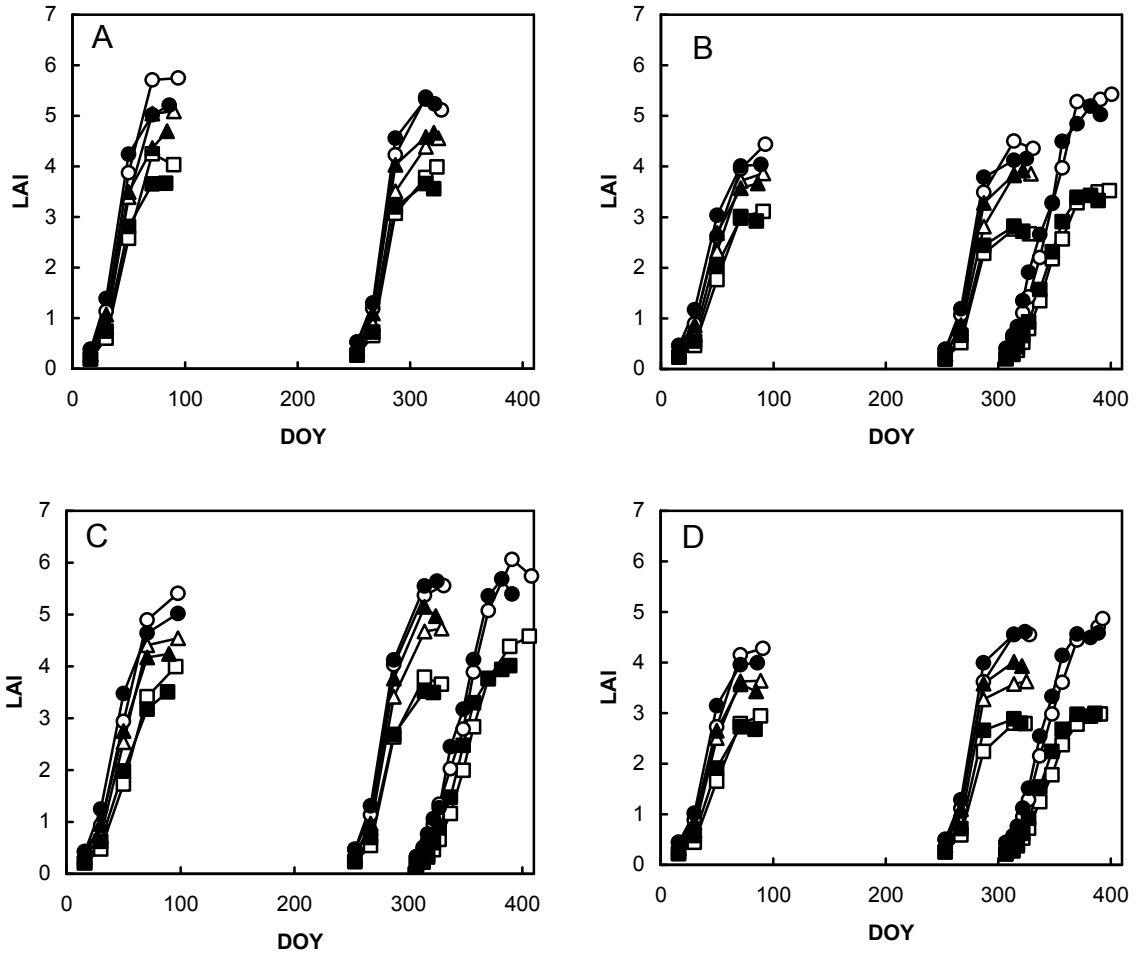

Figure 2. Time patterns of leaf area index (LAI) for Annecy (A), Delianne (B), Reagan (C) and Supernova (D) grown at three plant densities (32 pl m^{-2} (■,□), 48 pl m^{-2} (▲,Δ), 64 pl m^{-2} (○,●)) and at two temperatures (LT (□,Δ,○), HT (■,▲,●))in three greenhouse experiments. Dates are expressed as day of the year (DOY; 1 = 1 january) except for Exp. 3 which continues in the next year (Exp. 1 = DOY 253 - 330; Exp. 2 = DOY 16 -97; Exp. 3 = DOY 307 - 401).

Light use efficiency (LUE)

Temperature did not influence LUE and only a marginal plant density effect was present (< 6%; Table 5). However, LUE was significantly affected by cultivar: 'Annecy' had the lowest LUE while highest LUE was recorded for 'Reagan Improved'. Moreover, LUE differed considerably among experiments reaching its higher values in Exp. 3.

Table 5: The effect of temperature, plant density and cultivar on light use efficiency during the SD period of cut chrysanthemum in three experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5 % level. Abbreviations: C = cultivar; D = density; HT = high temperature; LT = low temperature T = temperature; SD = short day

	LUE (g MJ^{-1}) [y]		
	Exp.1	Exp. 2	Exp. 3
Temperature			
LT	3.42	4.00	5.66
HT	3.60	4.10	5.50
Density(pl m^{-2})			
32	**3.32[a]**	3.94	5.47
48	**3.69[b]**	4.23	---
64	**3.53[ab]**	3.98	5.69
Cultivar			
Annecy	**3.24[a]**	**3.87[a]**	**---**
Delianne	**3.54[b]**	**4.08[b]**	**5.67[b]**
Reagan Improved	**3.83[c]**	**4.18[c]**	**5.79[b]**
Supernova	**3.43[b]**	**4.06[b]**	**5.28[a]**
F-prob [z]			
T×D×C	*0.892*	*0.173*	*0.847*
T×C	*0.498*	*0.194*	*0.124*
D×C	*0.620*	*0.064*	*0.102*
T	*0.063*	*0.072*	*0.279*
D	***0.010***	*0.342*	*0.065*
C	***<0.001***	***<0.001***	***0.002***

[y] calculated for the SD period

[z] F-probability (significant levels <0.05 presented in bold).

--- not present in experiment

External quality

Flower characteristics

The effect of temperature on number of flowers (NoF) differed between experiments and between cultivars (Table 6). In most cases NoF was not significantly affected by temperature, the only exceptions being 'Reagan Improved' in Exp. 2 and 'Supernova' in Exp. 3, where NoF was significantly lower at LT. In contrast, in all experiments and for all cultivars increasing plant density decreased the NoF (Table 7). However, the magnitude of this effect was cultivar dependent. For instance, 'Reagan Improved' was especially sensitive to plant density: increasing density from 32 to 64 plants m^{-2} decrease NoF by up to 52%.

Table 6: The effect of temperature and cultivar on total dry mass per plant (TDM_p), number of flowers (NoF) and flower size in three experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5 % level. Abbreviations: C = cultivar; D = density; HT = high temperature; LT = low temperature T = temperature

Exp.		TDM_p (g)			NoF			flower size (cm^2)		
1		LT	HT		LT	HT		LT	HT	
	Annecy	7.0	7.3	**7.2^{a}**	16.5	17.3	**16.9^{c}**	**19.3^{a}**	**18.2^{a}**	18.8
	Delianne	7.4	7.5	**7.5^{a}**	11.8	12.4	**12.1^{b}**	**50.5^{e}**	**40.1^{d}**	45.3
	Reagan Improved	8.2	8.8	**8.5^{b}**	15.3	18.0	**16.7^{c}**	**29.8^{b}**	**29.4^{b}**	29.6
	Supernova	7.1	7.5	**7.3^{a}**	10.3	11.8	**11.0^{a}**	**37.0^{c}**	**31.8^{b}**	34.4
		7.4	7.8		13.5	14.9		34.1	29.9	
	F-prob[x]									
	T ×D×C	*0.703*			*0.910*			*0.244*		
	T×C	*0.619*			*0.076*			***<0.001***		
	T	*0.101*			*0.089*			*0.016*		
	C	***<0.001***			***<0.001***			*<0.001*		
2		LT	HT		LT	HT		LT	HT	
	Annecy	8.5	7.2	**7.8^{a}**	**16.9^{b}**	**17.8^{bc}**	17.4	**28.9^{b}**	**20.4^{a}**	24.6
	Delianne	8.6	7.6	**8.1^{a}**	**12.9^{a}**	**12.1^{a}**	12.5	**52.2^{g}**	**44.8^{e}**	48.5
	Reagan Improved	10.1	8.9	**9.5^{b}**	**17.4^{b}**	**18.5^{c}**	17.9	**35.1^{c}**	**35.1^{c}**	35.1
	Supernova	8.6	7.3	**7.9^{a}**	**12.3^{a}**	**12.6^{a}**	12.4	**48.2^{f}**	**39.6^{d}**	43.9
		8.9^{b}	**7.7^{a}**		14.9	15.2		41.1	35.0	
	F-prob[x]									
	T ×D×C	*0.822*			*0.088*			*0.175*		
	T×C	*0.784*			***0.015***			***<0.001***		
	T	***0.002***			*0.292*			*0.005*		
	C	***<0.001***			*<0.001*			*<0.001*		
3		LT	HT		LT	HT		LT	HT	
	Delianne	**6.8^{d}**	**6.3^{bc}**	6.5	**11.0^{b}**	**10.4^{b}**	10.7	**48.5^{c}**	**39.6^{b}**	44.0
	Reagan Improved	**8.1^{e}**	**6.6^{cd}**	7.3	**14.0^{c}**	**14.9^{c}**	14.4	**30.0^{a}**	**29.3^{a}**	29.6
	Supernova	**5.8^{b}**	**5.0^{a}**	5.4	**7.3^{a}**	**10.6^{b}**	8.9	**43.6^{bc}**	**33.4^{a}**	38.5
		6.9	5.9		10.7	12.0		40.7	34.1	
	F-prob[x]									
	T ×D×C	*0.508*			*0.071*			*0.102*		
	T×C	***0.008***			***<0.001***			***0.001***		
	T	*0.003*			*0.037*			*0.038*		
	C	*<0.001*			*<0.001*			*<0.001*		

[x] F-probability (significant levels <0.05 presented in bold). Plant density effects are shown in table 7.

The influence of temperature on flower size also differed between experiments and cultivars (Table 6). Flower size of 'Delianne' and 'Supernova' was significantly increased at LT in all experiments. In contrast, in 'Reagan Improved' temperature did not affect flower size. Furthermore, in most cases plant density showed a significant negative influence on flower size (Table 7), but this effect was less strong than the one described for NoF. Interestingly, flower size in 'Reagan Improved' was the least affected by plant density.

Table 7: The effect of plant density on total dry mass per plant (TDM_p), number of flowers (NoF) and flower size for four cultivars in three experiments. Different letters within columns and treatments indicate significant differences between treatments based on LSD at 5 % level. Abbreviations: C = cultivar; D = density; T = temperature

Exp.		TDM_p (g)			NoF			flower size (cm^2)		
1		32	48	64	32	48	64	32	48	64
	Annecy	8.5	7.4	5.6	21.2	17.0	12.5	**21.8^{b}**	**17.9^{a}**	**16.5^{a}**
	Delianne	8.6	7.7	6.1	14.1	12.6	9.7	**47.6^{f}**	**45.5^{f}**	**42.7^{e}**
	Reagan Improved	10.1	8.6	6.7	22.3	16.8	10.9	**29.6^{c}**	**30.2^{c}**	**29.0^{c}**
	Supernova	8.5	7.4	5.9	13.6	11.2	8.3	**35.4^{d}**	**33.4^{d}**	**34.3^{d}**
		8.9^{c}	**7.8^{b}**	**6.1^{a}**	**17.8^{c}**	**14.4^{b}**	**10.4^{a}**	33.6	31.8	30.6
	F-prob[x]									
	D×C	*0.310*			*0.809*			***0.007***		
	C	***<0.001***[y]			***<0.001***[y]			*<0.001*[y]		
	D	***<0.001***			***<0.001***			*<0.001*		
		32	48	64	32	48	64	32	48	64
2	Annecy	**9.4^{d}**	**7.9^{c}**	**6.3^{a}**	**20.9^{e}**	**17.1^{d}**	**14.1^{c}**	**25.4^{a}**	**24.2^{a}**	**24.2^{a}**
	Delianne	**9.9^{d}**	**8.2^{c}**	**6.1^{a}**	**14.7^{c}**	**12.4^{b}**	**10.4^{a}**	**51.3^{f}**	**47.8^{e}**	**46.3^{de}**
	Reagan Improved	**11.9^{e}**	**9.6^{d}**	**7.1^{b}**	**24.1^{f}**	**17.7^{d}**	**12.0^{b}**	**35.5^{b}**	**35.6^{b}**	**34.1^{b}**
	Supernova	**9.4^{d}**	**8.2^{c}**	**6.2^{a}**	**14.8^{c}**	**12.7^{b}**	**9.8^{a}**	**46.2^{d}**	**44.1^{d}**	**41.3^{c}**
		10.1	8.5	6.4	18.6	15.0	11.6	39.6	38.0	36.5
	F-prob[x]									
	D×C	***<0.001***			***<0.001***			***0.027***		
	C	*<0.001*[y]			*<0.001*[y]			*<0.001*[y]		
	D	*<0.001*			*<0.001*			*<0.001*		
		32	48	64	32	48	64	32	48	64
3	Delianne	**7.7^{d}**	**---**	**5.4^{b}**	**12.5^{d}**	**---**	**8.9^{b}**	46.3	**---**	41.8
	Reagan Improved	**9.1^{e}**	**---**	**5.6^{b}**	**18.5^{e}**	---	**10.4^{c}**	30.6	---	28.7
	Supernova	**6.4^{c}**	**---**	**4.5^{a}**	**11.4^{c}**	---	**6.5^{a}**	40.8	---	36.2
		7.7	---	5.1	14.1	---	8.6	**39.2^{b}**	---	**35.5^{a}**
	F-prob[x]									
	D×C	***<0.001***			***<0.001***			*0.468*		
	C	*<0.001*[y]			*<0.001*[y]			***<0.001***[y]		
	D	*<0.001*			*<0.001*			***<0.001***		

[x] F-probability (significant levels <0.05 presented in bold).

[y] Cultivar effects are shown together with temperature effects in table 6.

--- not present in experiment

Relationship between NoF and TDM_p

An overall linear regression model including cultivar and TDM_p as regressors could explain 85% of the variation observed in NoF (Table 8). However, in Exp. 2 and 3, despite a higher TDM_p at LT, NoF was unaffected or even lower at LT (Table 6), suggesting that this relationship is influenced by temperature. Therefore, realized temperature was further included into the regression model, as it improved the model significantly (P <0.001) (Table 8; Fig 3). Lower temperature significantly reduced the slope of the relationship between

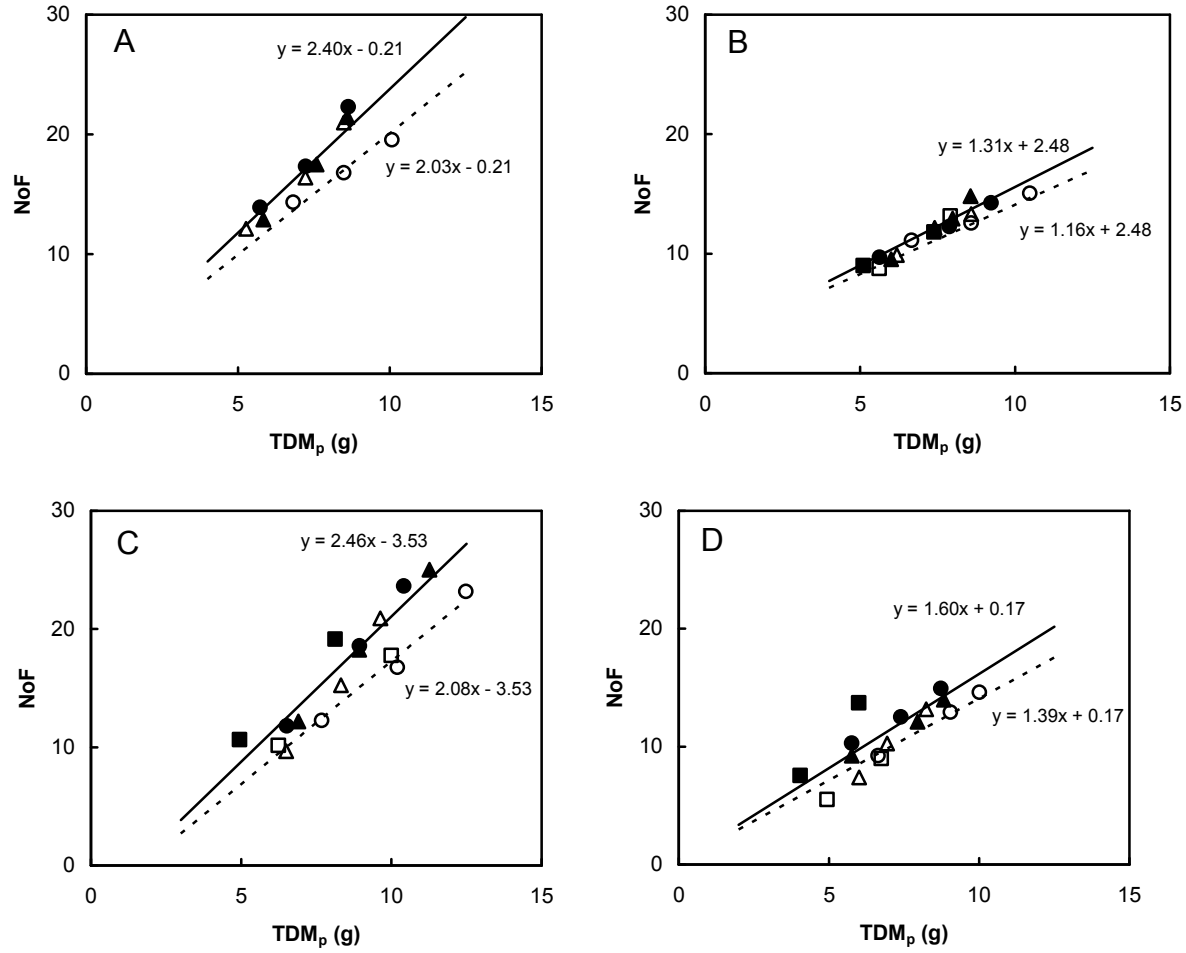

Figure 3: Relationship between total number of flowers per plant (NoF) and total dry mass per plant (TDM_p) for four cut chrysanthemum cultivars (Annecy (A), Delianne (B), Reagan (C) and Supernova (D)) grown at HT (closed symbols) and LT (open symbols) in Exp. 1 (▲Δ), Exp. 2 (●○) and Exp. 3 (■□). Lines represent prediction by the regression model for 16°C (dotted line) and 20°C (solid line) realised temperature.

TDM_p and NoF, especially in 'Reagan Improved' and 'Annecy' whereas 'Delianne' and 'Supernova' were clearly less temperature sensitive.

Discussion

Dry mass production

TDM_a in cut chrysanthemum is influenced by both the length of the cultivation period (namely reaction time) and by the growth rate. However, higher TDM_a at lower temperatures (e.g. Exp. 2 and 3; Fig. 1), was merely the result of a longer reaction time, as growth early in the cultivation period was even reduced at LT. The increase in reaction time at sub-optimal

Table 8: Outcome of the general linear regression model (A) and of the adjusted regression model (B) for number of flowers (NoF) of four chrysanthemum cultivars, using as regressors: total dry mass per plant (TDM_p) and realized temperature (T).

	A	**B**
Cultivar	cultivar*TDM_p	Cultivar*TDM_p*T
Annecy	$NoF = 2.05TDM_p + 1.80$	$NoF = (0.555 + 0.092T)TDM_p - 0.21$
Delianne	$NoF = 1.32TDM_p + 2.09$	$NoF = (0.555 + 0.038T)TDM_p + 2.48$
Reagan Improved	$NoF = 2.22TDM_p - 2.45$	$NoF = (0.555 + 0.095T)TDM_p - 3.53$
Supernova	$NoF = 1.44TDM_p + 0.87$	$NoF = (0.555 + 0.052T)TDM_p + 0.17$
R^2adj [z]	0.85	0.92

[z] Adjusted R^2

temperature (Table 2) is probably due to both delayed flower initiation and slower flower development (Karlsson *et al.*, 1989a; Chapter 2.2). Furthermore, plants flower later at lower light levels (Karlsson and Heins, 1986; Karlsson *et al.*, 1989b), which explains our findings where Exp. 3 showed the longest reaction time and also the lowest light levels (Table 1 and 2). Since plant density only increased reaction time slightly, it would not cause a further delay in harvesting at sub-optimal temperature.

The lower RGR during LD and lower AGR during the first part of the SD observed in plants grown at LT (Table 3) were related with a reduced LAI (Fig 2), resulting in a lower light interception. This reduction in LAI was caused by a lower LAR, due to a lower SLA (Table 4). The increase in leaf thickness (i.e. lower SLA) was previously reported for cut chrysanthemum (Chapter 2.2) and it is a common response to lower temperatures, which has been observed in several plant species including tomato (Smeets and Garretsen, 1986b; Chapter 3.3). However, since chrysanthemum has a determinate growth pattern (Pearson *et al.*, 1995), delayed flower initiation at LT resulted in a longer time for the formation of new leaf area. This explains why the difference in LAI between temperature treatments gradually disappeared during the SD. Therefore, final LAI was either equal at both temperature levels or even higher at LT, depending on the experiment and the cultivar (Fig. 2). LUE was not affected by temperature (Table 5). This agrees with previous findings where chrysanthemum crop photosynthesis shows a rather flat temperature optimum (Körner, 2003). Therefore, LUE is not a limiting factor for growing chrysanthemum under lower temperature regimes. Nevertheless, LUE was higher in Exp. 3, confirming that at lower light levels (Table 1) plants convert light more efficiently into biomass (Karlsson *et al.*, 1987; Lee *et al.*, 2002a).

In all experiments the longer cultivation period at LT compensated at least partly for the lower growth rate during early cultivation. However, TDM_a only increased significantly at LT in Exp. 2 and 3 (Fig 1). In Exp. 1 light levels at the end of experiment were relatively low and an extension of the cultivation period thus only contributed for a relatively small part to TDM_a. On the other hand, in Exp. 2 and 3 light levels increased towards the end of the cultivation period. Extra cultivation time at the end of the experiment did thus contribute

strongly to TDM_a. Another aspect that might explain differences between experiments is the difference in realised temperatures as in Exp.1 there was only 3.1ºC between the LT and HT compartments, whereas for Exp. 2 and 3 this increased to 3.6 and 4.0ºC, respectively.

External quality

As expected, reducing temperature or increasing plant density, in order to improve energy efficiency had consequences on the flower characteristics but these effects were strongly cultivar dependent. In most cases NoF was hardly influenced by temperature, whereas plant density strongly reduced NoF in all cultivars. This negative effect of plant density was previous observed and it is related to a reduction in assimilate availability (Carvalho and Heuvelink, 2003). A positive linear relationship between NoF and TDM_p was described in the past for 'Reagan Improved' (Carvalho and Heuvelink, 2003). This study goes further showing that this relationship is not only influenced by temperature but also extremely cultivar dependent (Table 8; Fig. 3). At a lower temperature, the same TDM_p (which can be obtained by manipulating plant density) resulted in a lower NoF. Furthermore, the extent to which temperature affects this relationship is cultivar dependent. For instance, in 'Reagan Improved' temperature showed a strong influence on the relationship between NoF and TDM_p. Fortunately, this relationship was less sensitive to lower temperature in other cultivars (e.g. 'Delianne'), offering good prospects for breeding. The temperature effect on the relationship between TDM_p and NoF might be an indirect effect of a difference in temperature sensitivity between flower induction and flower development. Flower induction is relatively more temperature sensitive than flower development (Van Ruiten and De Jong, 1984; Karlsson *et al.*, 1989a; Chapter 2.2). The difference in response between the cultivars might be related both with a different optimum temperature for flower initiation (Van Ruiten and De Jong, 1984) as well as with flower type. The less temperature sensitive cultivars 'Delianne' and 'Supernova' formed less second-order lateral flowers, both at HT and LT, therefore they did not benefit from an extension in the cultivation period at LT, that resulted in higher assimilate availability and consequently in higher TDM_p.

Lowering temperature can be beneficial for 'Delianne' and 'Supernova' which responded with larger flowers (Table 6), while flower size in 'Reagan Improved' was unaffected by temperature. The positive effect of lower temperature on flower size is probably mainly an effect of a longer flower growth period at lower temperatures (Nothnagl *et al.*, 2004). Strangely, this did not happen in 'Reagan Improved' in spite of being the cultivar with the largest temperature effect on reaction time. However, the large increase in reaction time in Exp. 3 at LT (17d) was largely a result of delayed flower initiation (data not shown). In general, the effect of plant density on flower size was considerably smaller than the effect on NoF (Table 7). Flower size in 'Reagan Improved' was the least sensitive for plant density. This is in agreement with Carvalho *et al*. (2005) who observed that flower size in 'Reagan Improved' was only influenced by assimilate supply at very low light levels.

Implications for breeding for sub-optimal temperature tolerance

Lowering the temperature to reduce energy use in cut chrysanthemum cultivation results in a longer duration of the cultivation period, which is a drawback in the annual yield since these extra days could be used to start a new crop. On other hand, from the growth point of view LT could be applied during the second phase of SD period as AGR during the last part of the cultivation is not affected by temperature. Since, flower development is less temperature sensitive than flower initiation (Van Ruiten and De Jong, 1984; Karlsson *et al.*, 1989a; Chapter 2.2), we expect that lowering the temperature during the last phase of cultivation would only increase reaction time slightly. This small increase in reaction time can be compensated by a bit higher plant density. However, currently it is still not feasible to adjust the temperature to the stage of the cultivation because in the same greenhouse compartment different stages of development are cultivated simultaneously (Carvalho *et al.*, 2005). Though, we expect that in the future this might be possible if mobile cultivation systems are implemented.

Besides the negative aspects of growing plants at a lower temperature combined with a higher plant density on reaction time and yield, some adverse effects on plant quality were also found. For instance, even if the same TDM_p could be reached, this would result in a lower number of flowers at LT. On the contrary, lowering the temperature can increase flower size in some cultivars, although higher plant density could reduce this effect slightly. Therefore, these counteractive effects of temperature on the flower characteristics asks for a need of knowing the market demands, in order to adjust the growth conditions accordingly. Another disadvantage of growing cut chrysanthemum at higher plant densities is the reduction of the crop uniformity in terms of plant weight, especially under low light (winter) conditions (Langton *et al.*, 1999).

Reaction time is one of the traits that showed the largest differences in temperature response between cultivars. It is therefore obviously an important characteristic to be used by breeders. However, this study underlines the importance of taking growth and quality aspects into account as well. We expect that even if breeders would produce cultivars with a reduced cultivation time at sub-optimal temperature, growers will not grow these cultivars at a lower cultivation temperature unless equal yield and quality could be reached. To improve yield at LT the breeders should focus on reducing the temperature effect on SLA. In this study SLA and consequently growth in the first half of the cultivation were reduced at sub-optimal temperatures (Table 3 and 4). Unfortunately, no variation in temperature response was found for growth and underlying components within these four cultivars. Though, it could be possible that within a wider range of cultivars more variation is present. Nevertheless, it is important to keep in mind that these four cultivars were already selected out of a sample of 25 cultivars, based on the widest range of temperature response for several characteristics including growth. An alternative option for breeding less temperature sensitive cultivars would be to counteract the lower SLA by increasing partitioning to the leaves at lower

temperatures, thus compensating for the negative effects of SLA on LAR. In these experiments some small but significant increases were found on LWR at LT (Table 4), but a larger increase is necessary to compensate the negative effect of SLA completely. However, it is also important to consider the possible implications that this strategy would have on other plant aspects, like external quality attributes.

Chapter 3

Tomato

Chapter 3.1

Influence of suboptimal temperature on tomato growth and yield: a review

Abstract

The effects of temperature on growth, development and yield of tomato (*Lycopersicon esculentum*) are reviewed with special emphasise on cultivar differences. The focus is on suboptimal temperatures above the level where chilling injury occurs. Temperature has a large effect on all developmental aspects. Leaf and truss initiation rates decrease linearly with decreasing temperature. Although these rates may be different for different cultivars their response to temperature is the same. Young plants grown at suboptimal temperature produce thicker leaves, so that they catch less light and therefore have a lower relative growth rate. There was no interaction between temperature and cultivar for relative growth rate and related traits. In a crop producing fruits this aspect is of less importance as most of the light is intercepted anyway. At suboptimal temperature, fruit set is reduced as a result of poorer pollen quality. The period between anthesis and ripening of the fruit increases and as the growth rate of the fruit at a certain development stage is independent of temperature, fruits become larger at suboptimal temperature. Higher temperature leads to an increased early yield at the cost of vegetative growth but may also cause a delay in later trusses. Total yield over a whole season might be equal at lower temperatures, but higher tomato prices early in the season do not make it economically profitable to reduce the temperature in the greenhouse. Short-term effects might thus be different from long-term effects. In the literature the link between yield and whole plant growth is often missing, limiting the possibilities of studying the underlying processes that contribute to changes in yield. Breeding for cultivars with equal production at lower temperatures is hampered by the limited variation for temperature response in cultivated tomato. Therefore breeders have to look for other sources of variation, like related wild *Lycopersicon* species.

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Introduction

Tomato, like many horticultural crops, originates from subtropical areas. In more northern areas this crop is grown in greenhouses where the temperature can be kept higher than outside. Hence, high energy costs are incurred for climate control. In The Netherlands, 50 to 60 m^3 gas per m^2 are used annually for heating. With increasing public concern about environmental problems including CO_2 emission from the use of fossil fuel, the greenhouse sector will have to improve its energy efficiency. Over the past two decades energy efficiency (kg tomatoes MJ^{-1} energy input) in Dutch greenhouses has already been largely improved, almost exclusively as a result of higher production levels. In 2003, production was almost twice as high as in 1980, while the annual energy input per m^2 was similar to that in 1980 (Van der Knijff *et al.*, 2004). However, the absolute amount of energy input will have to be reduced as well. Part of the reduction of energy use can be reached by technical measures (e.g. the material covering the greenhouse or the use of thermal screens; Bot, 2001; Van der Knijff *et al.*, 2004) or by 'temperature integration', using the ability of a tomato crop to compensate temperature within a certain period (e.g. a higher temperature during daytime can compensate for a lower temperature during the night and *vice versa*; De Koning, 1988). Another option is decreasing the temperature in the greenhouse. This could be achieved by breeding for cultivars that are better adapted to lower temperatures. A reduction of the temperature set-point by 2ºC can lead to a reduction of about 16% in energy costs (Elings *et al.*, 2005).

Below 12°C almost no growth is expected for tomato (Criddle *et al.*, 1997). Chilling injury occurs when tomato plants are exposed to these temperatures for a long period of time. Depending on the intensity and duration of exposure to the chilling temperatures, photosynthesis, respiration, membrane integrity, water relations and hormonal balance of the plants may be affected (Graham and Patterson, 1982). In the end, the plant may die (Brüggemann *et al.*, 1992). In this paper we focus on growth at suboptimal temperatures that are above the chilling temperature. Each aspect of growth and development has its own temperature optimum and this optimum might change with plant age. Suboptimal temperature in this paper is therefore defined as the temperature below the current economic optimum for Dutch greenhouse growers, which is currently around 19ºC - 20ºC, but above chilling temperatures.

Tomato yield is not an isolated characteristic and depends on the growth of the whole plant. If the tomato plant does not grow well then it will never give a high yield. Therefore yield is determined by the interaction between plant morphology, physiology and growth conditions. Decreasing the temperature in the greenhouse will have an influence on different aspects of tomato growth (e.g., biomass production and partitioning) and development (e.g., leaf and truss appearance, fruit growth period).

In order to define what we can expect from breeding, in this paper we intend to give an overview of the effects of temperature on growth, development and ultimately yield of tomato. We will also give an overview of the available genetic variation for temperature response in present day tomato cultivars. The presence of variation is important to reduce energy use via plant breeding. The effect of temperature during different stages of (plant) development will be discussed, starting from the vegetative plants to a crop that is producing fruit.

Vegetative growth

The growth of young vegetative tomato plants can be characterised by their relative growth rate (RGR). The RGR describes the rate of increase in plant mass per unit plant mass already present. Differences in RGR can be explained by differences in leaf area per unit plant mass (LAR; leaf area ratio) or by differences in the rate of increase in plant mass per unit leaf area (NAR; net assimilation rate or ULR; unit leaf rate), as RGR is the product of LAR and NAR (Hunt, 1990). LAR is the product of specific leaf area (SLA; total leaf area per unit leaf mass) and leaf weight ratio (LWR; leaf biomass per unit total plant mass). NAR is proportional to net leaf photosynthetic rate.

The RGR during the first 9 days after emergence shows an optimum response to temperature, where the optimum day temperature (DT) is 25°C, independent of the night temperature (NT); whereas the optimum NT is dependent on the DT and ranges between 18ºC and 25°C (Hussey, 1965). The optimum temperature for dry matter accumulation decreases with plant age (Went, 1945). At high temperatures, the RGR is initially high but also decreases rapidly with time (ontogenetic decrease), while for lower temperatures the RGR is initially low, but the decline is also slower than at higher temperatures (Adams *et al.*, 1997), because of faster internal shading at higher temperature. This also causes limitations when comparing RGRs at the same time point for plants grown at different temperatures. Therefore several scientists have chosen to compare RGR at a fixed plant weight (Lindhout *et al.*, 1991), over a weight interval (Heuvelink, 1989) or at the same stage of development (Venema *et al.*, 1999).

In the suboptimal temperature range, the RGR (Table 1) of tomato plants was reduced at lower average temperatures (Paul *et al.*, 1984; Hoek *et al.*, 1993; Venema *et al.*, 1999) as well as when plants were grown only at a lower NT (Smeets and Garretsen, 1986b; Franco, 1990; Nieuwhof *et al.*, 1991). The effect of DT is even larger, as plant growth is reduced with an inversed temperature regime (NT higher than DT; Calvert, 1964; Hussey, 1965; Heuvelink, 1989). Tomato plants that are grown under an inversed temperature regime are also reduced in length, due to shorter internodes (Calvert, 1964; De Koning, 1988; Heuvelink, 1989; Langton *et al.*, 1997). This effect of temperature regime is well-known in many horticultural crops as the "DIF effect" (difference between day and night temperature; Erwin *et al.*, 1989). Plant

Table 1: An overview of papers on the effect of *temperature* on RGR and its components

	Temperature treatment (ºC)	Number of cultivars used	RGR	NAR	LAR	SLA	LWR
Average temperature							
Paul *et al.*, 1984	25; 20; 15; 12.5	5	--[ni]	-[i]	--[ni]	--[ni]	
Hoek *et al.*, 1993	18; 15; 12	4	-[ni]				
Venema *et al.*, 1999	25/20; 16/14	2	-[ni]		-	-[ni]	+
Night temperature							
Smeets and Garretsen, 1986a	14; 10; 6	16	-[ni]	+/-[i]	-[i]	-[i]	+[i]
Nieuwhof *et al.*, 1991	14; 10; 6	15	-[nm]	+/-	-	-	+
Franco, 1990	15; 10; 7.5; 5	1	-	+/-	-	-	
Inversed temperature regime							
Calvert, 1964	15.5/13.3; 15.5; 15.5/17.8; 17.8/15.5; 17.8; 17.8/20; 20/17.8; 20; 20/22.2	1	-				
Hussey, 1965	All combinations with DT/NT 10; 15; 20; 25; 30	1	-				
Heuvelink, 1989	26/16; 24/18; 22/20; 20/22; 18/24 16/26; 24/12; 18/18; 24/24	1	-	+/-	-	-	+/-

- = reduced at suboptimal temperature or inversed temperature regime; +/- = not influenced by temperature; + = increased at suboptimal temperature or inversed temperature regime

To differentiate a strong response from a weaker response (--) and (-) are used

[i]) Indicates when an interaction was found between growth parameter and cultivar.

[ni]) Indicates when no interaction was found between growth parameter and cultivar.

[nm]) Indicates when presence or absence of an interaction between growth parameter and cultivar was not mentioned

height is also reduced at lower average temperature (Hurd and Graves, 1985; Khayat *et al.*, 1985; Nieuwhof *et al.*, 1997), but that reduction in plant height is caused by a reduction in the number of leaves (internodes) formed.

The decrease in RGR at suboptimal NT is caused (Table 1) by a decrease in LAR, while NAR is not affected by NT (Smeets and Garretsen, 1986b; Franco, 1990; Nieuwhof *et al.*, 1991). When both DT and NT are reduced, the reduction in RGR is correlated with both a reduction in LAR (Paul *et al.*, 1984; Venema *et al.*, 1999) and to a lesser extent in NAR (Paul *et al.*, 1984). This, however, conflicts with the effect of an inversed temperature regime (low DT with high NT), where the reduction in RGR could be explained solely by a reduction in LAR (Heuvelink, 1989). An explanation for this could be the relatively low temperature (12.5ºC) used by Paul *et al*. (1984), just above the level where chilling injury is expected. The LWR was slightly higher at lower (night) temperature (Smeets and Garretsen, 1986b; Nieuwhof *et al.*, 1991; Venema *et al.*, 1999). The reduction in LAR at lower (night) temperatures is thus due to a reduction in SLA (Paul *et al.*, 1984; Smeets and Garretsen, 1986b; Franco, 1990; Nieuwhof *et al.*, 1991; Venema *et al.*, 1999). Also, the lower LAR at an inversed temperature regime is caused by a lower SLA, resulting in less light interception (Heuvelink, 1989). The increase in leaf thickness at lower temperatures is a result of larger cells, mainly in the transverse direction (Hoek *et al.*, 1993) that store more starch (Venema *et al.*, 1999).

The decrease in NAR, which is small compared to the decrease in LAR, at lower temperature could be confirmed by the lower rate of net photosynthesis. Photosynthesis decreased when DT was reduced from 25ºC to 16ºC (Venema *et al.*, 1999). A reduction in the NT from 14ºC to 6°C combined with a DT of 19ºC did not affect net photosynthesis on a leaf area basis, while photosynthesis on a fresh leaf weight basis decreases under lower NTs due to the increasing leaf thickness (Van de Dijk and Maris, 1985). Photosynthesis in these experiments was measured on one leaf and hence the effect of temperature could be different at the canopy level. For the leaf there is an optimum response, while at the canopy level, each leaf receives a different radiation level and has therefore a different temperature optimum. Consequently, the temperature optimum for crop photosynthesis is rather flat (Schapendonk and Brouwer, 1985).

RGR differs between cultivars (Smeets and Garretsen, 1986b; 1986a; Lindhout *et al.*, 1991; Hoek *et al.*, 1993; Janssen *et al.*, 1995), but this genotypic variation in RGR (lowest = 82% of highest) was small compared to the genotypic variation in NAR (lowest = 62% of highest), LAR (lowest = 67% of highest) and SLA (lowest = 69% of highest) reported by Smeets and Garretsen (1986a; b) and Janssen *et al*. (1995). Breeding for cultivars with a higher RGR is hampered by a strong negative correlation between NAR and LAR and between NAR and SLA (Smeets and Garretsen, 1986a; b; Nieuwhof *et al.*, 1991; Nieuwhof *et al.*, 1993; Janssen *et al.*, 1995). Differences in net photosynthesis rate (lowest = 87% of highest) and dark respiration (lowest = 68% of highest) were present between cultivars(Van de Dijk and Maris, 1985; Van de Dijk, 1987; Janssen *et al.*, 1995).

'Temperature x cultivar' interactions (Table 1) were absent for RGR (Paul *et al.*, 1984; Smeets and Garretsen, 1986b; Hoek *et al.*, 1993), but were present for NAR (Paul *et al.*, 1984; Smeets and Garretsen, 1986b). For LAR, interaction was found in one study (Smeets and Garretsen, 1986b), but was absent in another study (Paul *et al.*, 1984). The difference between these experiments is probably due to the smaller number of cultivars used in the experiment by Paul *et al*. (1984).

Temperature also affects dry matter content. In young vegetative plants the dry matter content of leaves was increased at suboptimal (16ºC/14ºC DT/NT) compared to the dry matter content of leaves of plants grown at 25ºC/20ºC DT/NT (Venema *et al.*, 1999). In mature plants the dry matter content of the above ground parts of the plant was increased at both low (14°C) and high (26°C) temperatures compared to intermediate temperatures (Adams *et al.*, 2001).

Flowering and fruit set

A lower (night) temperature during the first 10 days after cotyledon expansion reduced the number of leaves before the first truss appeared (reviewed by Dieleman and Heuvelink, 1992) and this effect is stronger at low light intensities (Calvert, 1959). But since low temperature

also reduces the rate of leaf appearance, low temperature does not reduce the time to first flowering, which is longer at lower temperature (Hurd and Graves, 1985). Heating the flower buds hastens flower opening (Adams *et al.*, 2001).

The flower number in the first truss increases with decreasing DT (Rylski, 1979), NT (Calvert, 1957; Wittwer and Teubner, 1957) and average 24 hrs temperature (Charles and Harris, 1972; Ercan and Vural, 1994). Another report suggests that air temperature had no effect on flower number in the first truss but a lower root temperature increased this number (Phatak *et al.*, 1966). As the other previously mentioned authors did not control root temperature separately it is possible that the effect of air temperature on flower number is actually an effect of root temperature.

Fruit set is optimal between 18ºC and 20°C (Charles and Harris, 1972; De Koning, 1994). The reduced fruit set at suboptimal temperatures does not result from effects on stigma level or ovule viability (Fernandez-Munoz and Cuartero, 1991), but is a result of the formation of poorer quality pollen (reviewed by Picken, 1984).

The rate of development (leaf and truss appearance rate) is decreased at lower (night) temperature (Hurd and Graves, 1985; Khayat *et al.*, 1985; Nieuwhof *et al.*, 1997; Adams *et al.*, 2001). The truss appearance rate tends to respond linearly to average temperature between 17ºC and 27°C (De Koning, 1994). The truss appearance rate differed between cultivars, but 'temperature x cultivar' interactions were absent (De Koning, 1994).

Fruit growth

The period between anthesis and maturity of a fruit decreases with increasing average temperature between 14ºC and 26°C (Figure 1A; Hurd and Graves, 1985; Heuvelink and Marcelis, 1989; De Koning, 1994; 2000; Adams *et al.*, 2001). This effect is greater at lower temperatures (De Koning, 2000). Large differences between DT and NT increased the growth period only slightly (De Koning, 1994). When the rate of progress to maturity is plotted against average temperature, a linear response is found (Adams *et al.*, 2001). However, temperature sensitivity is not the same for the whole fruit growth period. Higher temperatures during the first week after anthesis shortened the time to maturity (De Koning, 1994). During this period, the RGR of the tomato fruit is high and, in this interval, cell division and elongation take place (Monselise *et al.*, 1978). Then there is a period in which a higher temperature hardly increases the development rate of a tomato fruit (De Koning, 1994; Adams *et al.*, 2001). In that period, when cell elongation takes place, the RGR steadily decreases (Monselise *et al.*, 1978). In the last 1- 2 weeks, when the fruit is close to maturity, increasing temperature again has again a large influence in decreasing the time to maturity of the fruit (De Koning, 1994; Adams *et al.*, 2001). In that period the RGR is almost zero, and it is likely that processes involved in the ripening of the tomato (e.g. autocatalytic ethylene production) are affected by temperature (Monselise *et al.*, 1978).

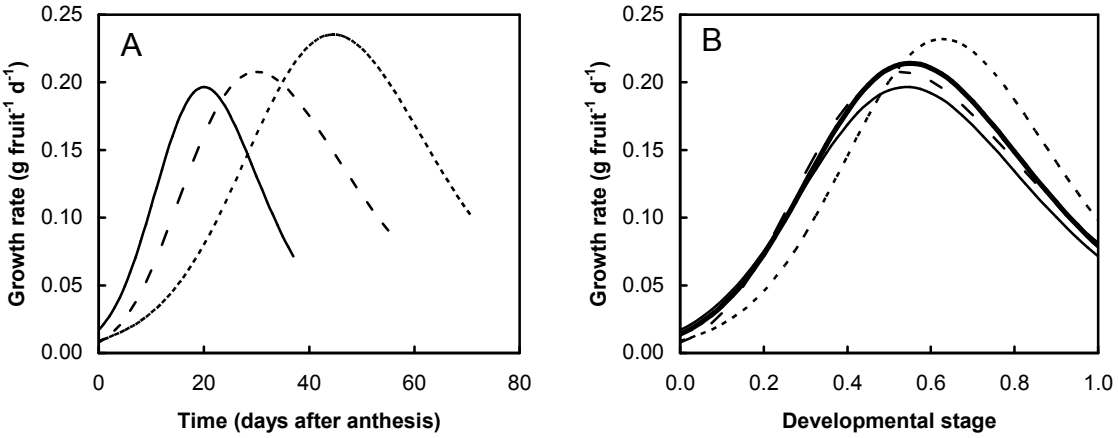

Figure 1: Growth rate of tomato fruits at 17ºC (- - -), 21ºC(— — —) and 25ºC (——) as a function of time after flowering (A) and as a function of developmental stage (time from flowering/ time from flowering until final harvest) of fruit (B). The dark solid line (——) in panel B represents the average curve (Reprinted with permission from Heuvelink and Marcelis, 1989).

The relation between growth rate and developmental stage of a tomato fruit (calculated as the time after anthesis divided by the time from flowering until maturity) is almost independent of temperature between 17ºC and 25ºC (Figure 1B; Heuvelink and Marcelis, 1989; De Koning, 1994). However, longer growth periods with decreasing temperatures between 17 and 26°C, cause fruits to become larger when grown at a lower temperature (Sawhney and Polowick, 1985; De Koning, 1994; Newton *et al.*, 1999; Adams *et al.*, 2001). This was also observed when only the NT was reduced from 16ºC to 11°C, while DT remained at 20ºC (Hurd and Graves, 1985). This difference in size was related to the number of locules the fruit contained, and was probably related to modifications in ovule structure (Sawhney and Polowick, 1985). More locules probably also mean more seeds. The weight of a tomato fruit was found to be positively correlated with seed number (Imanishi and Hiura, 1975), but this relationship is different for different temperature regimes (Rylski, 1979).

When the temperature was increased for a short time during the growth period of a tomato fruit, the absolute fruit growth rate increased (Pearce *et al.*, 1993; Kitano *et al.*, 1998; Thompson *et al.*, 1999). This increase could also be achieved when only the fruits were heated (Kitano *et al.*, 1998; Araki *et al.*, 2000; Adams *et al.*, 2001). Thus a discrepancy exists between the long-term and short-term effects of temperature on fruit growth rate. This difference between short- and long-term temperature response of fruit growth could possibly be explained by acclimation of the amount of enzymatic machinery responsible for the uptake and processing of assimilates. The catalytic activity of the enzymatic machinery will increase immediately with short increases in temperature, while the amount of enzymatic machinery will only be adjusted over a longer period (De Koning, 1994).

The dry matter content of fruits increases slightly with increasing temperatures between

17ºC and 23°C (De Koning, 1994), which might, therefore have a positive influence on flavour. Consumers also preferred the flavour of tomatoes produced at 23ºC compared to tomatoes produced at 17ºC (Buitelaar and Janse, 1990).

Yield

Tomato yield is primarily determined by the amount of intercepted light (Newton *et al.*, 1999) and assimilate partitioning (Ho, 1996b). Temperature significantly affects the partitioning of assimilates between the vegetative and generative parts (Khayat *et al.*, 1985; De Koning, 1989; Adams *et al.*, 2001). The effect of temperature on partitioning is probably not a direct effect (Heuvelink, 1995), but is an indirect effect of temperature on the rate of development, fruit set and abortion. At a higher temperature, trusses appear faster (Hurd and Graves, 1985; Khayat *et al.*, 1985; Nieuwhof *et al.*, 1997; Adams *et al.*, 2001) and therefore, initially, there are more fruits on the plant at a higher temperature. These will grow at the expense of vegetative growth, but may also cause a delay in the growth of newly set fruit and might even lead to flower or fruit abortion (De Koning, 1989), as developing and flowering trusses are weaker sinks than fruiting trusses (Ho and Hewitt, 1986). Despite instantaneous deviations, the ratio between vegetative and generative growth over a longer growth period seems to be independent of average temperature between 17ºC and 23°C (De Koning, 1989). Unfortunately, in many papers on the effect of temperature on yield, only fruit mass and fruit characteristics were measured. This limits the possibilities of studying the underlying processes that contribute to a yield increase or decrease.

Because of the shorter fruit growth period and a faster initiation of new trusses at higher temperatures, early yields are higher at higher temperatures (Hurd and Graves, 1985; De Koning, 1989; Adams *et al.*, 2001). However, this increase is not unlimited with increasing temperature. For plants grown at 26°C, fruit set was poor and many fruits were parthenocarpic, resulting in lower production compared to plants grown at 18ºC or 22°C (Adams *et al.*, 2001). Attempts to overcome the delay in harvesting at suboptimal air NTs by heating the roots, led to a reduction in the quality of early produced fruits and early yield (Hurd and Graves, 1985).

Later yield was equal when NT set-point was reduced from 16ºC to 11°C (Hurd and Graves, 1985). Although when temperatures fall below the level where fruit set is affected, yields are lower than at temperatures where fruit set is not affected (Nieuwhof *et al.*, 1997; Adams *et al.*, 2001). At 14°C, fruits were parthenocarpic, small, hard and had no marketable value (Adams *et al.*, 2001). The yield of cultivar ‘Moneymaker’ was reduced when NT set-point was lowered from 18ºC to 12°C, while the yield of the cultivar ‘Cherry 35070E Danmark’ was equal at both NTs (Khayat *et al.*, 1985). This reduction in yield for the cultivar ‘Moneymaker’ at a NT set-point of 12°C was due to a reduction in fruit size. From this paper it remains unclear whether also total biomass production and partitioning were affected

differently for the two cultivars at the lower NT treatment.

Temperatures can also be reduced for part of the growth period. Lowering the NT set-point from 15ºC to 11°C from anthesis until picking of the first fruits resulted in a 6% increase in yield after 18 weeks of fruit picking, caused by an increase in mean fruit weight. Lowering the NT after the first fruits were picked resulted in a similar increase in yield (Hurd and Graves, 1985). However, the reduction in energy use was greater when the set-point temperature was reduced in the early part of the season, when outside temperatures were lower. Increasing the temperature for short periods during the growing season increased yield in the week following the increase in temperature, but had no influence on the overall yield (Adams and Valdes, 2002). This is probably due to the speeding-up effect of temperature on fruit ripening, while other fruits are unaffected, leading to a lower yield in the following weeks (Adams and Valdes, 2002).

Three regimes between which the temperature patterns differed, but with approximately equal average temperatures, did not result in differences in yield (Hurd and Graves, 1984). This may lead to the conclusion that the yield is independent of temperature regime. However, although early yield is not affected by temperature regime, final yield was higher at higher NT (smaller DIF) as a result of larger fruits (De Koning, 1988). On the other hand, when a treatment with much higher DT than NT (14 DIF), was compared to a treatment with a smaller difference between DT and NT (5 DIF), the yield was higher with the larger DIF treatment due to an increase in individual fruit mass (Gent and Ma, 1998). Temperature integration over several days, where the temperature over an integration period is kept constant, but within that period temperature can be adjusted according to the outside climate (up to 12 days with an amplitude of 6ºC), does not lead to a decrease in yield when the temperature integration starts when plants have acquired a sufficiently high leaf area index (De Koning, 1990).

Discussion

Temperature has a significant influence on many aspects of growth and development in tomato (Table 2). Biomass production in young plants is heavily influenced by temperature because, at suboptimal temperatures, the plants produce thicker leaves resulting in less total light interception. Also the rate of photosynthesis is slightly lower at suboptimal temperatures, although this effect may be counteracted by a lower respiration rate. In a fruit-producing crop these effects are likely to be less important as most light is intercepted anyway; however, at that stage temperature has a large influence on partitioning of assimilates as a result of different rates of development. At higher temperatures, the initial fruit load is higher as there are more trusses on each plant, which results in less assimilates being available for vegetative growth; later this could even lead to a limited supply of assimilates available for developing and flowering trusses, resulting in reduced fruit set and early fruit abortion. Short-term effects

Table 2: Overview of the most import effects of suboptimal temperature on development, growth of young tomato plants and growth of fruit producing crop.

Parameter	Effect of sub-optimal temperature
Development	
Leaf initiation rate	Lower
Truss appearance rate	Lower
Fruit development rate	Lower
Young plant	
RGR	Reduced
LAR and SLA	Reduced
NAR	Slightly reduced or not affected
Fruit producing crop	
Growth rate	Hardly affected
Crop photosynthesis	Hardly affected
Partitioning	Initially more in favour of vegetative parts; over larger period equal
Fruit set	Reduced
Fruit/truss growth rate	Independent of temperature when measured at same developmental stage
Yield	Initially lower at suboptimal temperature, final yield lower or equal

could thus be different from long-term effects. Unfortunately, in yield experiments researchers rarely measure vegetative growth, leaving important questions unanswered. We can only speculate on whether decreases in yield were due to a lower production of assimilates, a lower partitioning to the fruits, or to a combination of both.

Although much research has been performed on the effects of temperature on the growth of young tomato plants, this is not the stage where the largest reduction in energy use can be expected. In the nursery, young tomato plants stand close together and therefore occupy only a limited amount of space. The largest reductions in energy can thus be reached in the months when the plants have been transferred to the production greenhouses where they are more widely spaced. In The Netherlands 40% of the annual energy is used in the first 12 weeks in the production greenhouses (KWIN, 2002). Since it is time-consuming to do yield experiments, many researchers have tried to find early and easy detectable ways to predict tomato growth and ultimately yield. Foolad and Lin (2000) tried to link cold-tolerance during seed germination with vegetative growth of tomato. They found that there was no relationship between the two, indicating that in these stages of development different genes are involved. It is therefore likely that traits determining yield at lower temperature are not necessary linked to growth in the vegetative stage. However, young plants that do not grow well at low temperatures are not expected to produce a high yield at low temperature.

Although final yields might be equal at suboptimal temperatures compared to yields under optimal conditions, the higher prices for tomatoes early in the season might not make it profitable for a grower to reduce temperatures in the greenhouse. When compared economically, taking into account that earlier produced fruits have a higher market value, and the reduction in energy costs when plants are grown at lower NT, after 10 weeks of harvesting reducing NT set-point from 16ºC to 11°C was not profitable in 1982 (Hurd and Graves, 1985). This situation might change, however, as energy prices increase or the supply of early

grown tomatoes comes from another source (e.g., countries with more light in the winter, or greenhouses equipped with winter assimilation lights). In fact, over the last 10 years this has happened increasingly. Another option is to reduce the NT set-points after picking of the first fruits which results in a small increase in yield, but the reduction in energy used for heating was only marginal (Hurd and Graves, 1985).

Temperature also affects fruit quality, as temperature has a direct influence on metabolism and, thus, affects cellular structure and other components that determine fruit quality such as colour, texture, size and organoleptic properties (Dorais *et al.*, 2001). Lower temperatures increase the time required for ripening and therefore increase the size of the fruits. However if the temperature is too low and fruit set is affected, the number of "hollow" fruits is increased.

Few authors have studied possible interactions between temperature and cultivar on yield. Although there is some variation for vegetative growth between different tomato cultivars, there seems to be no interaction between temperature and cultivar. At optimum temperature there is variation in yield between different types of tomato (e.g. "cherry", "round", "beefsteak") due to differences in assimilate partitioning (Ho, 1995; 1996b). Some differences in yield response to temperature were also found between these types (Khayat *et al.*, 1985). The limited genetic variation between cultivated tomato varieties for temperature response hampers breeding for equal production at lower temperatures. However, in wild tomato species there is much larger variation in both growth and temperature responses (Lindhout *et al.*, 1991; Foolad and Lin, 2000). These *Lycopersicon* species are native to western South-America, where they grow in a wide range of habitats, from sea level up to 3200 m a.s.l. (Rick, 1995). Some accessions that grow at high altitudes are known to be chilling-tolerant (e.g., *L. hirsutum*, *L. peruvianum*; Wolf *et al.*, 1986). Venema (1999) showed that the accumulation of starch at suboptimal temperature was much less pronounced in chilling-tolerant *Lycopersicon* species than in cultivated tomato. These accessions might therefore be an interesting resource from which to improve tomato yield at suboptimal temperatures. Through the use of molecular markers and linkage maps, it is possible to identify favorable Quantitative Trait Loci (QTLs) from wild species and to incorporate them into current cultivars (Tanksley and McCouch, 1997). A number of QTLs for cold tolerance have been identified in a set of Near Isogenic Lines (NILs), each containing a single *L. hirsutum* introgression into a cultivated outdoor tomato genome (Monforte and Tanksley, 2000). Some of these QTLs induced 25-41% higher dry mass than the isogenic control when grown at chilling temperatures (9ºC/4ºC DT/NT; Oyanedel *et al.*, 2001). This suggests opportunities to find QTLs for growth at suboptimal temperatures.

Conclusion

Temperature has a large influence on growth and development in tomato. In young vegetative tomato plants growth is reduced because the plants make thicker leaves. In mature plants this

aspect can be neglected as most of the light is intercepted anyway. Early yield is affected by temperature because less assimilate goes to the fruits at lower temperatures due to lower fruit and truss development rates early in the season. But short-term temperature effects could be different from long-term temperature effects as a high initial fruit load might lead to a limited assimilate supply for developing trusses. In the literature surveyed here, the link between yield and whole plant growth is missing. This limits the possibilities of studying the underlying processes that contribute to a yield increase or decrease.

Variation in temperature responses between currently cultivated tomato cultivars is limited, which hampers breeding for equal economic levels of production at lower temperatures. Therefore breeders must look for alternative sources of variation in the temperature response of tomato. Wild relatives of the cultivated tomato provide one option.

Chapter 3.2

Breeding for a more energy efficient greenhouse tomato: past and future perspectives

Abstract

Energy efficiency can be increased either by increasing the production per m^2 or by reducing the energy input per m^2, e.g. by reducing temperature set-points in the greenhouse. So far, in Dutch glasshouse tomatoes energy efficiency was almost exclusively raised by yield increases. To study the role of tomato breeding in this production increase, yield and underlying components of 7 cultivars released between 1950 and 2002 were studied. Furthermore, variation in temperature response between cultivars was studied. In three experiments yield and biomass production of in total 11 cultivars were evaluated at two temperature regimes (17/15˚C and 21/19˚C day/night temperature set-points). Breeding has resulted in a remarkable increase in production. Under current conditions, yield of modern cultivars was on average 40% higher than yield of 'Moneymaker', released in 1950. This increase in production resulted from a higher light use efficiency. Although the fraction of assimilates partitioned to the fruits showed small differences between cultivars, this trait was not related to year of release. Furthermore, more recently introduced cultivars produced larger fruits rather than more fruits. All cultivars responded similar to both temperature regimes for all important characteristics, limiting the possibilities of using existing cultivars in a breeding program for improved yield at lower temperatures.

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Introduction

Many horticultural crops, like tomato, originate from (sub)tropical areas. Especially at more northern latitudes high energy inputs are required to grow tomatoes in heated glasshouses. As both energy prices and public concern for environmental problems, caused by the emission of CO_2, are rising, it is important that energy efficiency (the amount of product produced per unit energy input) increases. Over the past two decades energy efficiency in Dutch greenhouses has increased significantly almost exclusively as a result of higher production levels (Van der Knijff *et al.*, 2004). Between 1980 and 2004 tomato production gradually increased from 18 kg m^{-2} to 50 kg m^{-2} (KWIN, 1998; CBS, 2006). This increase in yield is partly the result of changes in cultivation techniques (e.g. growing on substrate, supply of CO_2, extended cropping season) and technical measures (e.g. use of climate computers, higher greenhouse transmissivity) but this gain can also be partly attributed to the work of plant breeders who developed higher yielding cultivars. For breeders, to produce cultivars with further yield improvements, it is important to realize how breeding has affected yield in the past. Yield is the product of total biomass production and the partitioning of assimilate towards harvestable organs (harvest index). In most cereal crops yield increases by genetic improvement could be ascribed to an increased harvest index (Hay, 1995). For example, in barley increased yields in cultivars introduced between 1900 and 1980 were mainly the result of an increase in harvest index from 0.36 to 0.48 (Hay, 1995). However, in maize (Hay, 1995) and lentil (Whitehead *et al.*, 2000) increased yield could be ascribed to increased biomass production. To what extent and in which way breeding has contributed to increased yield in tomato is so far not known.

Another possibility to further increase energy efficiency of greenhouse tomato is by reducing the greenhouse air temperature, thus reducing the amount of energy used per m^2. Decreasing the temperature set-point by 2ºC could potentially result in an energy saving of 16% (Elings *et al.*, 2005). However, reducing air temperature has several unfavorable effects on production, e.g. a delay in harvest and lower (early) yields (Hurd and Graves, 1985; Adams *et al.*, 2001). To overcome these adverse effects of low temperature new cultivars have to be developed. Genetic variation in temperature response is essential as a basis for cultivar improvement. Previous studies on young plants have shown that the variation in temperature response between tomato cultivars is limited (Paul *et al.*, 1984; Smeets and Garretsen, 1986b) but information about the variation in temperature response on yield is scarce (Chapter 3.1). Khayat *et al*. (1985) found that the yield of 'Moneymaker' was reduced when night temperature set-point was 12ºC instead of 18º to 12ºC, while yield of the cultivar 'Cherry 35070E Danmark' was unaffected by this reduction in night temperature. However, it is not clear whether in 'Moneymaker' total biomass production, partitioning, or both were affected. If there is variation in temperature response it is important to know which underlying processes are responsible for differences between cultivars.

The aim of this paper is to determine to what extent and in which way breeding has

affected yield in tomato over the past 50 years. Furthermore several modern cultivars are evaluated for possible differences in temperature response and the underlying physiological and morphological factors, that can explain these possible differences, are studied.

Material and Methods

Experimental set-up

Three experiments (Table 1) were conducted in three successive years in two compartments (12 m × 12.8 m) that were part of a multispan Venlo-type greenhouse (Wageningen University, The Netherlands, lat. 52ºN). The cultivars used in Exp 1 and 2 were expected to respond differently to temperature based on preliminary work. In Exp 3 a selection was made from several older and more recent cultivars (Table 1). Seeds were sown in trays filled with commercial potting soil on dates indicated in Table 1. About 14 days after sowing seedlings were pricked out and transferred to rockwool cubes and placed on ebb/flood benches in another compartment of the same greenhouse. About 2 weeks before anthesis of the first truss, plants were transferred to the cultivation compartments and placed on rockwool slabs at a plant density of 2.5 plants m^{-2}. At anthesis of the first flowers the temperature in each greenhouse compartment was set at the desired level. All axillary shoots were removed weekly and plants were trained according to the high wire system (Peet and Welles, 2005). Old leaves below the lowest ripening truss were removed weekly. Plant nutrition and pest and disease control were conducted according to common practice. Flowers were pollinated by bumble bees.

Greenhouse climate

Heating set-points for day/night were 17/15ºC (low temperature treatment; LT) and 21/19ºC (high temperature treatment; HT). Ventilation set-points were 1ºC above the heating set-points. Greenhouse climate was automatically recorded every 5 minutes using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Daily global radiation outside the greenhouse was obtained from the department of meteorology at about 800 m distance. Realized average temperature and radiation are given in Table 1.

Measurements

Destructive measurements were carried out at anthesis, at the end of the experiment and two times during the experiment, resulting in more or less equal time intervals between measurements. At each destructive harvest two plants per plot were measured, except for the final harvest, when four plants per plot were taken. Fresh and dry mass (ventilated oven; at least 10h at105ºC) from leaves (including petioles), stem, fruit trusses, removed leaves and picked fruits and leaf area (LI-COR Model 3100 Area Meter) were determined. Number of leaves (>0.5cm), number of trusses (>0.5cm) and number of fruits (>0.5cm) were recorded.

Table 1: Basic information on the three greenhouse experiments. Dates are expressed as day of the year (day 1 = 1 January).

	Exp. 1	Exp. 2	Exp. 3
Year	2002	2003	2004
Sowing date	334	327	353
Start date	28	20	48
End date	155	142	172
Outside global radiation[x] (mol m^{-2} d^{-1})	53.4	51.1	63.1
Temperature[y] (ºC)	LT: 18.5 HT:21.2	LT: 18.5 HT: 20.9	LT: 19.6 HT: 21.3
Cultivars[z]	Counter (1985) Pronto(1990) Chaser (1992) Prospero (1997)	Capita (1992) Chaser (1992) Prospero (1997)	Moneymaker (1950) Extase (1960) Calypso (1982) Liberto (1988) Gourmet (1991) Chaser (1992) Encore (2002)

[x] Averaged over the whole cultivation period.

[y] 24 h average greenhouse temperature, averaged over the whole cultivation period

[z] Year of release of each cultivar is given between brackets

The plants used for destructive measurements were surrounded by guard plants. Extra side shoots were allowed to grow on guard plants to replace measured plants in order to maintain stem density and light distribution in the crop.

Greenhouse climate

Heating set-points for day/night were 17/15ºC (low temperature treatment; LT) and 21/19ºC (high temperature treatment; HT). Ventilation set-points were 1ºC above the heating set-points. Greenhouse climate was automatically recorded every 5 minutes using a commercial computer system (Hoogendoorn, Vlaardingen, The Netherlands). Daily global radiation outside the greenhouse was obtained from the department of meteorology at about 800 m distance. Realized average temperature and radiation are given in Table 1.

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Light use efficiency

For each treatment a time course of leaf area index (LAI), based on linear interpolations between destructive leaf area measurements, was calculated. Based on measured daily global radiation, a greenhouse transmisivity of 58%, assuming 47% photosynthetic active radiation (PAR) in the global radiation, and a light extinction coefficient of the canopy of 0.75 (Heuvelink and Buiskool, 1995), the daily intercepted PAR by the crop was calculated. Light use efficiency (LUE) was calculated as the dry matter production divided by the integral of intercepted PAR over a period between two destructive measurements. LUE was averaged over three periods resulting from four destructive harvests.

Statistical analysis

In all experiments a split-plot design was used, with temperature as the main plot and cultivars as the split factor. As for temperature no real replications were present, the two plots of each cultivar were considered as independent repetitions. Data were checked for normality using the 'Kolmogorov-Smirnov' test (SPSS 12.0). Analysis of variance was conducted, using Genstat 8, and treatment effects were tested at 5 % probability level, except for the temperature effect which was tested at 10 % probability level due to the low degrees of freedom. Mean separation was done by Student's t-test ($P = 0.05$).

Results

Although there were clear cultivar and temperature effects, none of the experiments showed an interaction between temperature and cultivar for any important characteristic. Therefore, the effects of temperature and cultivar on growth and yield are presented separately.

Temperature effects

In all experiments fruits grown at HT had a significantly shorter fruit growth period (Table 2) and therefore plants grown at HT produced earlier ripe fruits than plants grown at LT (Fig 1). Hence, during early phases of the cultivation cumulative yield was higher at HT (Fig. 1). However, once harvesting had started at LT, heavier fruits could be picked at LT and consequently cumulative yield increased more rapidly at LT. Therefore, in Exp 1 and 3 no differences were present in yield between the two temperature treatments at final harvest stage (Table 2). Only in Exp 2 cumulative yield was still higher at HT, but as the slope of yield against time was higher at LT (Fig. 1) it is expected that if Exp 2 would have lasted longer yield differences between HT and LT would also disappear. In all experiments the cumulative number of fruits harvested was significantly higher at HT (Table 2). Furthermore, in Exp 1 and 3 fruits produced at HT had a higher dry matter content than fruits grown at LT. In Exp 2 no effect of temperature on fruit dry matter content was observed.

Table 2: The effect of temperature in three experiments on fruit growth period (FGP; number of days between anthesis and harvesting of first fruits per truss) of the first three trusses, total cumulative yield fresh ($Yield_{FW}$) and dry weight ($Yield_{DW}$), total number of harvested fruits ($NoF_{harvest}$), average fruit mass of harvested fruits (AFM), fruit dry matter content (FDMC) of harvested fruits, total plant biomass (TDM) and the fraction of assimilates partitioned to the fruits (FF) at the final harvest stage. Values are averages over 4, 3 and 7 cultivars for Exp 1, 2 and 3, respectively.

Exp.	Temperature	FGP (d)	$Yield_{FW}$ (kg m^{-2})	$Yield_{DW}$ (kg m^{-2})	$NoF_{harvest}$ (m^{-2})	AFM (g $fruit^{-1}$)	FDMC	TDM (kg m^{-2})	FF
1	LT	75 b	9.6	0.44	148 a	3.02 b	0.050 a	1.35	0.586
	HT	54 a	8.8	0.44	183 b	2.46 a	0.055 b	1.27	0.582
	F-prob.[z]	***0.007***	*0.243*	*0.840*	***0.031***	***0.012***	***0.010***	*0.141*	*0.757*
2	LT	78 b	7.5 a	0.40 a	105 a	3.90 b	0.053	1.24	0.598
	HT	58 a	9.0 b	0.47 b	163 b	2.90 a	0.052	1.25	0.604
	F-prob.[z]	***0.005***	***0.060***	***0.094***	***0.010***	***0.019***	*0.518*	*0.937*	*0.636*
3	LT	74 b	11.9	0.56	169 a	3.34 b	0.051 a	1.54	0.578
	HT	58 a	11.3	0.58	183 b	3.12 a	0.055 b	1.50	0.568
	F-prob.[z]	***0.014***	*0.348*	*0.732*	***0.088***	***0.096***	***0.016***	*0.455*	*0.240*

[z] F-probability (significant levels < 0.10 presented in bold). Different letters within an experiment indicate significant differences between treatments based on Student's *t*-test ($P = 0.10$).

Total dry matter (TDM) production and the fraction of assimilates distributed towards the fruits over the whole cultivation period were unaffected by temperature (Table 2). Although overall partitioning was not affected by temperature, during the first six weeks after anthesis the fraction of assimilates distributed towards the fruits was significantly higher at HT (Fig 2). Contrary, during the last six weeks of the experiment distribution of assimilates towards the fruits was significantly higher at LT (Fig 2). Fruit load (measured as the number of fruits on

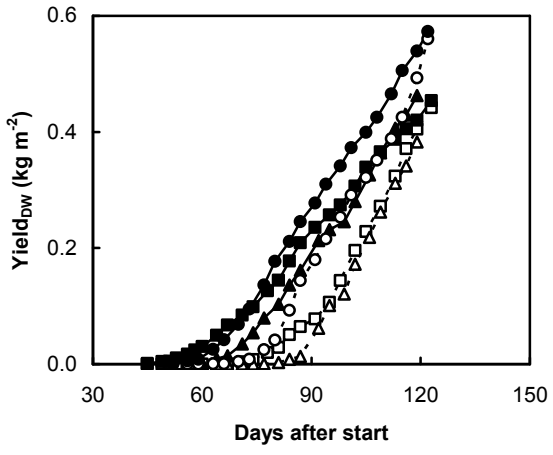

Figure 1: Cumulative yield dry weight of tomato grown at LT (--□Δ○--) or HT (—■▲●—) as a function of days after start of the temperature treatments, averaged over 4, 3 or 7 cultivars in Exp 1(■,□), Exp 2 (▲,Δ) and Exp 3 (●,○) respectively.

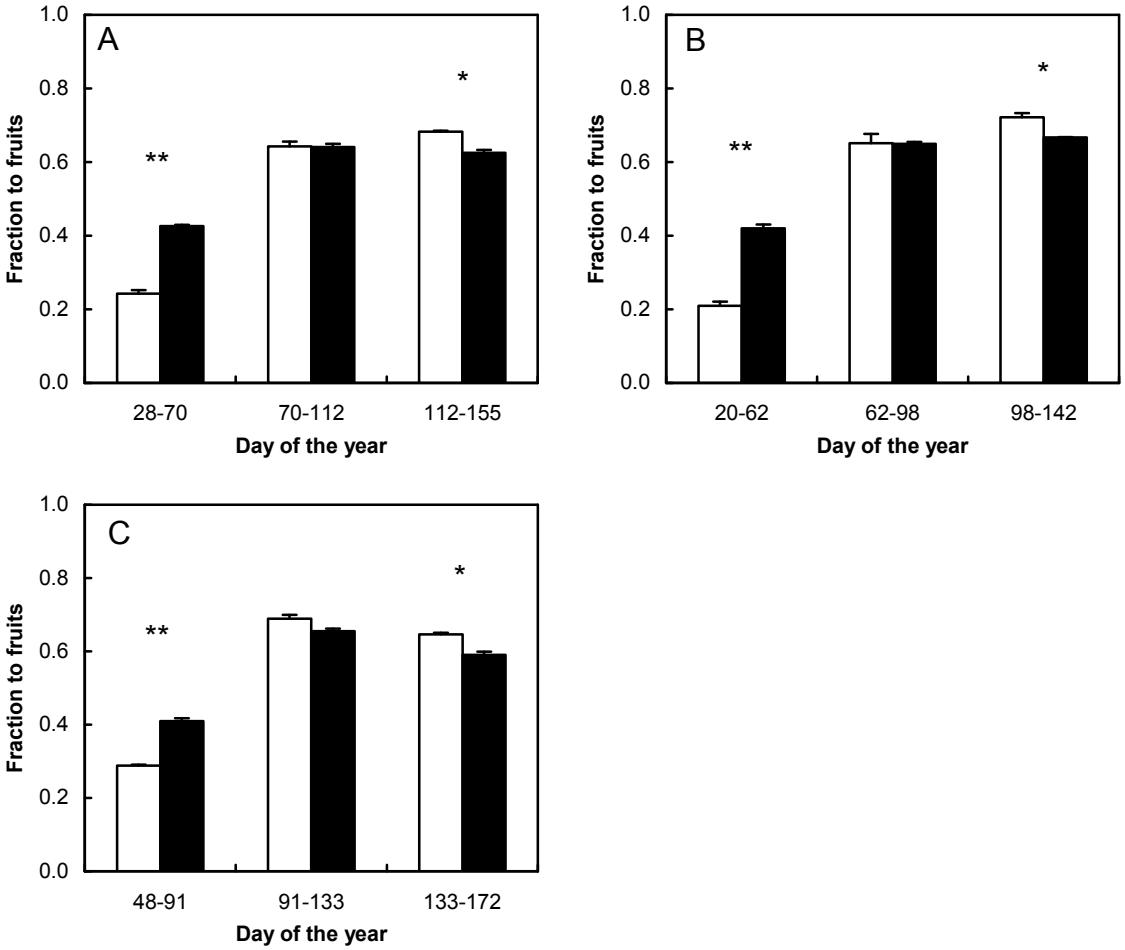

Figure 2: Fraction of total above-ground dry matter distributed to the fruits between destructive harvests of tomato grown at LT (open bars) and HT(closed bars) averaged over 4, 3 or 7 cultivars in Exp 1(A), Exp 2 (B) and Exp 3(C), respectively. Vertical bars are standard errors of mean. * indicates significant differences at $P = 0.05$ and ** $P = 0.01$.

the plant) at the second destructive measurement was higher at HT while at the third destructive harvest fruit load was equal at both temperatures in Exp 2, while in Exp 1 and 3 it was higher at LT (Fig 3). At the final destructive measurement fruit load was higher at LT in Exp 1 and 2 while it was equal for both temperatures in Exp 3.

Temperature had a strong influence on development (Table 3). At HT more leaves and trusses were produced than at LT and as a consequence stem length was also higher at HT. However, although the number of trusses was higher at HT, the total number of fruits produced was unaffected by temperature in Exp 1 and 3.

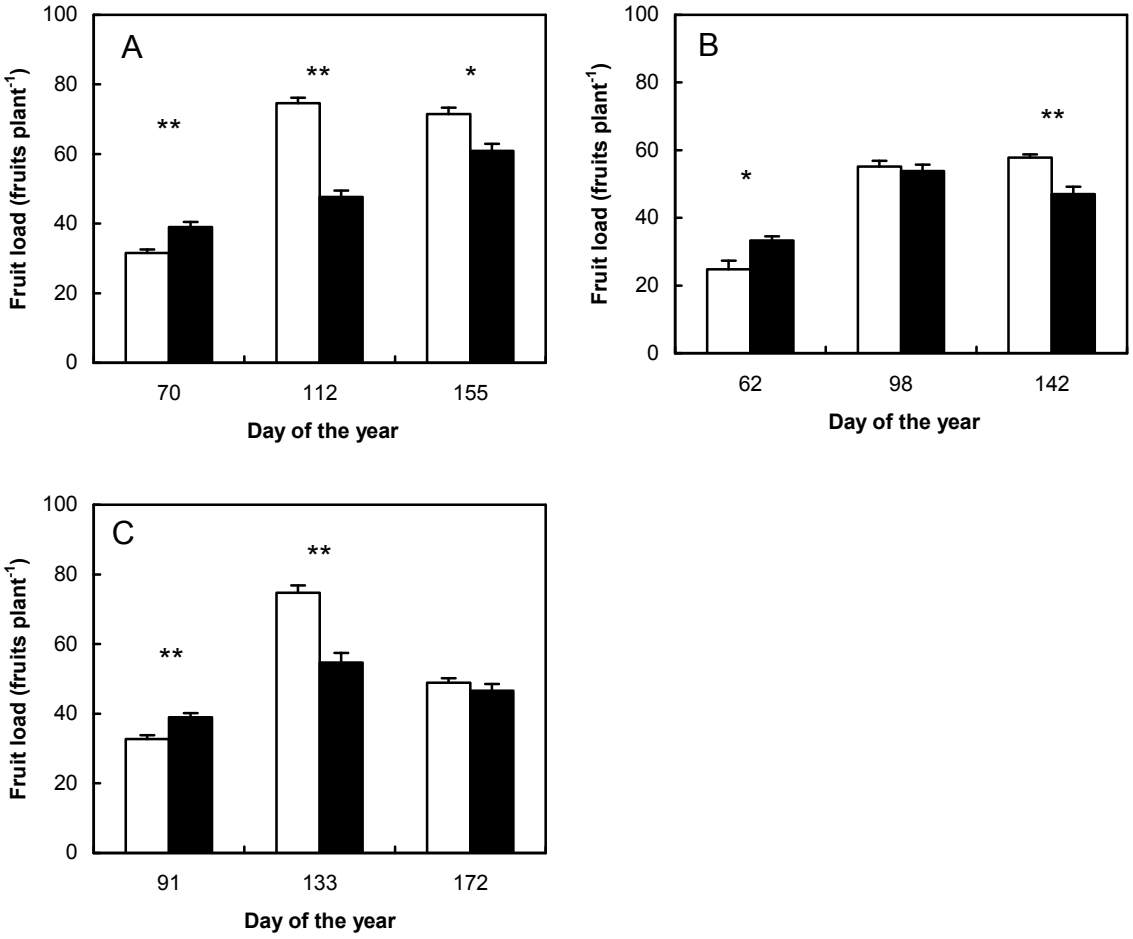

Figure 3: Fruit load (number of fruits per plant) of tomato grown at LT (open bars) and HT (closed bars) averaged over 4, 3 or 7 cultivars in Exp 1(A), Exp 2 (B) and Exp 3(C), respectively. Vertical bars are standard errors of mean. * indicates significant differences at $P = 0.05$ and ** $P = 0.01$.

Table 3: The effect of temperature on stem length, total number of leaves produced (NoL), number of trusses (NoT) and total number of fruits produced (NoF) per plant at the final harvest stage. Values are averages over 4, 3 and 7 cultivars for Exp 1, 2 and 3, respectively.

Exp.	Temperature	Stem length (cm)	NoL (plant^{-1})	NoT (plant^{-1})	NoF (plant^{-1})
1	LT	491 a	63 a	17.0 a	130
	HT	598 b	72 b	20.5 b	133
	F-prob.[z]	***0.007***	***0.006***	***0.009***	*0.612*
2	LT	419 a	52 a	13.5 a	99 a
	HT	533 b	63 b	17.5 b	112 b
	F-prob.[z]	***0.024***	***0.016***	***0.003***	***0.020***
3	LT	492 a	63 a	16.6 a	117
	HT	550 b	69 b	18.9 b	120
	F-prob.[z]	***0.001***	***0.019***	***0.006***	*0.172*

[z] F-probability (significant levels < 0.10 presented in bold). Different letters within an experiment indicate significant differences between treatments based on Student's *t*-test ($P = 0.10$).

Cultivar effects

Cultivars differed in fruit growth period but this was not related to the year of release. Significant differences in total yield between cultivars were only present in Exp 3 (Table 4). The two oldest cultivars, 'Moneymaker' and 'Extase' had a significantly lower yield than the cultivars released after 1982. Within the five cultivars that were released after 1982 no significant differences in yield were present. On average these cultivars produced a 41% and 22% higher yield dry weight than 'Moneymaker' and 'Extase', respectively. In Exp 1 and 3 cultivars also differed in fruit size and number (Table 4). In general fruits of modern cultivars were larger than fruits of the older cultivars. The two newest cultivars 'Chaser' and 'Encore' produced the largest fruits, while the smallest fruits were produced by 'Moneymaker' and 'Extase'. Within the modern cultivars, the cultivars which produced a lower number of fruits showed a higher average fruit size. Only in Exp 3 significant differences were present between cultivars in dry matter content of harvested fruits. Dry matter content ranged between 5.0% for 'Encore' and 5.5% for 'Moneymaker' and 'Gourmet'. Dry matter content of the fruits was negatively correlated with fruit size ($r^2 = 0.72$).

Table 4: Cultivar effect in three experiments on fruit growth period (FGP; number of days between anthesis and harvesting of first fruits per truss) of the first three trusses, total cumulative yield fresh ($Yield_{FW}$) and dry weight ($Yield_{DW}$), total number of harvested fruits ($NoF_{harvest}$), average fruit mass (AFM), dry matter content of harvested fruits (FDMC), total plant biomass (TDM) and the fraction of assimilates in the fruits (FF) at final harvest stage. Values are the averages for 2 temperature regimes.

Exp.	Cultivar	FGP (d)	$Yield_{FW}$ (kg m^{-2})	$Yield_{DW}$ (kg m^{-2})	$NoF_{harvest}$ (m^{-2})	AFM (g $fruit^{-1}$)	FDMC	TDM (kg m^{-2})	FF
1	Counter	61 a	9.4	0.46	185 c	2.54 a	0.053	1.32 b	0.600 b
	Pronto	63 b	8.9	0.43	169 b	2.53 a	0.052	1.23 a	0.598 b
	Chaser	66 c	9.2	0.45	151 a	3.02 b	0.053	1.37 b	0.570 a
	Prospero	67 c	9.4	0.44	155 ab	2.88 b	0.051	1.33 b	0.568 a
	F-prob.[z]	***<0.001***	*0.435*	*0.145*	***0.004***	***<0.001***	*0.075*	***0.032***	***0.002***
2	Capita	66 a	8.3	0.45	140	3.33	0.054	1.28	0.606
	Chaser	67 a	7.9	0.41	123	3.43	0.052	1.23	0.589
	Prospero	70 b	8.6	0.45	133	3.48	0.053	1.21	0.607
	F-prob.[z]	***0.002***	*0.497*	*0.234*	*0.065*	*0.534*	*0.643*	*0.308*	*0.110*
3	Moneymaker	65 ab	8.5 a	0.43 a	163 ab	2.64 a	0.055 d	1.29 a	0.579 cd
	Extase	69 cd	10.2 b	0.50 b	175 bc	2.87 ab	0.054 c	1.39 a	0.570 abc
	Calypso	66 bc	12.2 c	0.61 c	178 bc	3.44 de	0.053 c	1.55 b	0.572 bc
	Liberto	62 a	12.6 c	0.61 c	188 cd	3.23 cd	0.052 b	1.54 b	0.598 d
	Gourmet	63 ab	12.3 c	0.62 c	199 d	3.12 bc	0.055 d	1.59 b	0.586 cd
	Chaser	66 bc	13.1 c	0.63 c	175 bc	3.60 ef	0.052 b	1.68 b	0.557 ab
	Encore	70 d	12.5 c	0.57 c	155 a	3.71 f	0.050 a	1.61 b	0.550 a
	F-prob.[z]	***0.001***	***0.001***	***0.001***	***0.005***	***<0.001***	***<0.001***	***0.001***	***0.003***

[z] F-probability (significant levels < 0.05 presented in bold). Different letters within an experiment indicate significant differences between treatments based on Student's *t*-test ($P = 0.05$).

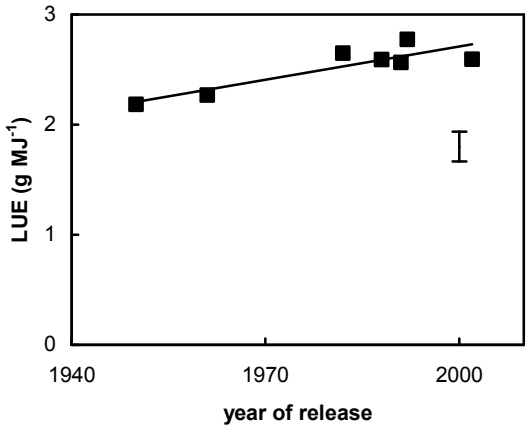

Figure 4: Light use efficiency (LUE) over the whole growth period as a function of the year of release of tomato cultivars. Values are averaged over 2 temperature regimes (Exp 3). Line represent linear regression: y = 0.010x - 17.4, r^2 = 0.78. Vertical bar represents LSD = 0.27

At the end of Exp 1 there were differences in TDM and distribution of assimilates towards the fruits. For 'Pronto' TDM was lower than for the other cultivars. Larger differences in TDM were present in Exp 3, 'Moneymaker' and 'Extase' produced 19% and 13% less TDM than the five cultivars released after 1982. Yield was positively correlated with TDM (r^2 = 0.86). To determine whether the increase in TDM was a result of increased light interception or whether light was used more efficiently, the light use efficiency (LUE) was calculated. Both 'Moneymaker' and 'Extase' had a lower LUE than the more recently released cultivars. Moreover, LUE showed a linear increase with year of release (Fig. 4). Small, but significant differences were also present between cultivars in partitioning towards the fruits but these differences were unrelated to year of release. In 'Encore' 55% of the assimilates were distributed towards the fruits while in 'Liberto' almost 60% of the assimilates went to the fruits. Assimilate partitioning was negatively correlated with fruit growth period (r^2 = 0.73).

Clear differences were also present between cultivars in stem length and number of trusses in Exp 1 and 3 (Table 5). However, the cultivars with longer stems were not always the cultivars with the higher number of leaves (internodes), thus clearly showing that differences between cultivars were related both to leaf number and internode length. Small but statistically significant differences were also present in the total number of fruits produced by each cultivar.

Discussion

Cultivar improvement over the past 50 years

Tomato breeding has contributed substantially to increased yield and biomass production. The five cultivars released after 1982 produced 32 to 47 % higher yields (dry weight) than

Table 5: Cultivar differences in stem length, total number of leaves produced (NoL), number of trusses (NoT) and total number of fruits produced per plant (NoF) at final harvest stage in three experiments. Values are the averages for 2 temperature regimes.

Exp.	Cultivar	Stem length (m plant^{-1})	NoL (plant^{-1})	NoT (plant^{-1})	NoF (plant^{-1})
1	Counter	5.27 a	70 b	19.7 b	142 b
	Pronto	5.71 c	71 b	19.3 b	132 a
	Chaser	5.30 ab	66 a	18.3 a	125 a
	Prospero	5.49 ab	64 a	17.8 a	128 a
	F-prob.[z]	***0.007***	***0.006***	***< 0.001***	***0.005***
2	Capita	4.86	60	15.8	111
	Chaser	4.57	57	15.3	103
	Prospero	4.84	56	15.5	102
	F-prob.[z]	*0.215*	*0.070*	*0.592*	*0.161*
3	Moneymaker	4.71 a	67	16.8 a	118 ab
	Extase	4.95 b	63	17.9 bc	126 b
	Calypso	5.08 bc	64	17.6 ab	111 a
	Liberto	5.33 d	69	18.5 bc	125 b
	Gourmet	5.85 e	67	18.6 c	127 b
	Chaser	5.34 d	65	17.8 abc	117 ab
	Encore	5.19 cd	66	16.9 a	107 a
	F-prob.[z]	***<0.001***	*0.053*	***0.007***	***0.014***

[z] F-probability (significant levels < 0.05 presented in bold). Different letters within an experiment indicate significant differences between treatments based on Student's *t*-test ($P = 0.05$).

'Moneymaker', the oldest cultivar used in this study (Table 4). Higher yields were a consequence of an increase in TDM due to higher LUEs (Fig 4). It is therefore likely that the photosynthetic capacity of tomato has increased as well, although more research will be necessary to confirm this. Also in soybean the photosynthetic rate was increased in modern cultivars compared to older cultivars (Morrison *et al.*, 1999). Another possibility is that light interception (e.g. leaf angle distribution, light extinction coefficient) has changed.

These results illustrate the direction of tomato breeding over the past 50 years. Initially breeders mainly focused on increasing yield, but as the demand for higher quality fruit increased, the focus has shifted towards characteristics that reduce production costs or ensure reliable production of high yields of high quality fruits (Ho, 1996a; Lindhout, 2005). Breeding during the past decades has also led to the progressive introduction of resistance genes from related wild *Lycopersicon* species. For instance, the introduction of TMV resistant varieties gave a remarkable production increase in the early 1970s (Van de Vooren *et al.*, 1986) and yields of 'Moneymaker' and 'Extase', the only two cultivars susceptible to TMV in this study, would have been even lower if plants would have been infected with TMV.

Although TDM in 'Moneymaker' and 'Extase' was lower than in the modern cultivars, the total number of fruits produced (both harvested and green fruits) was similar to that of the modern cultivars (Table 4). The higher assimilate supply in modern cultivars resulted solely

in considerably larger fruits. As the fruit growth period was not related to year of release, the increase in fruit size must rely on larger cell division and/or cell expansion rates. Cell expansion rates could be influenced by the ploidy levels within the tomato fruit pericarp. Cheniclet (2005) showed that within a selection of cultivars, covering a wide range of fruit sizes, fruit mass correlates positively with mean cell size and ploidy level. Ho (1996b) suggested harvest index could be improved effectively by increasing the fruit size. Although breeding did effectively increase fruit size, harvest index was not increased. In this study only round tomato cultivars were used. Perhaps larger differences in harvest index could exist between different types of tomato (e.g. cherry, round, beafsteak). In fact, Ho (1996b) found differences in yield between different indeterminate types of tomato that were related to differences in harvest index, Ho (1996b) only measured partitioning 112 days after sowing; differences in yield and harvest index could thus be caused by to differences in development rate (earliness) between different types of tomato cultivars. In fact, dry matter partitioning in Exp 3 was negatively correlated with fruit development rate.

Although there were small differences between cultivars in partitioning towards the fruits, surprisingly harvest index was not related to year of release. This is in contrast to several temperate cereals where improvement in grain yield could be ascribed to a progressive increase in harvest index since 1900 (Hay, 1995). The introduction of dwarfing genes increased grain yield at the expense of straw biomass (Milach and Federizzi, 2001). However, in tomato the fraction of assimilates that was partitioned towards the stems was already relatively small in 'Moneymaker' (14 %, data not shown) and was not significantly affected by genotype. Moreover, the indeterminate growth pattern of greenhouse tomato cultivars necessitates that a certain amount of assimilates is partitioned towards the stem as new internodes need to support future leaves and trusses.

Temperature effects

Temperature was not constant during the experiments. At the start of the experiments clear temperature differences could be realised between the HT and LT compartments, but later in the season, due to higher solar radiation, it was more difficult to keep the temperature in the compartments at the desired level. Therefore towards the end of the experiment temperature differences were small. Especially in Exp 3, which started one month later than the other two experiments, the overall average temperature difference was rather small. This however did not prohibit profound differences between the temperature treatments in timing of yield and partitioning. De Koning (1989) also showed that clear differences in fruit growth and fruit load remained, when temperatures were kept equal after four different temperature treatments (17 – 23ºC) had been applied for 8 weeks.

Initially partitioning towards the fruits was higher at HT (Fig 3). Partitioning is not influenced by temperature directly but indirectly through the influence of temperature on development rate, flower and fruit abortion (Heuvelink, 1995). As a consequence of an

increasing truss appearance rate with temperature (De Koning, 1994; Adams *et al.*, 2001) there were initially more fruits on the plant at HT (Fig 3) and a higher number of fruits on the plant favors partitioning towards the fruits (Heuvelink, 1997). However, early fruit will grow at the expense of vegetative parts and as developing and flowering trusses are weaker sinks than fruiting trusses (Ho and Hewitt, 1986) this may also cause a delay in growth of newly set fruits and might even lead to flower or fruit abortion (De Koning, 1989). At the second and final destructive measurements the fruit load was either higher at LT or equal at both temperature regimes, resulting in increased partitioning towards the fruits in the last six weeks of the experiment (Fig 3). The overall partitioning during the experiment was not affected by temperature (Table 2). However, if temperature set-points would be decreased further, fruit and seed set could be affected. Adams (2001) found that fruits grown at 14ºC were parthenocarpic and attracted less assimilates than fruits grown at either 18 or 22ºC.

As TDM was not affected by temperature and partitioning approached a functional balance, the yield on the long run was not reduced at LT. Thus, focusing solely on the cumulative yield, there is no reason why temperature could not be reduced in the greenhouse. However, as early yield is more profitable, because of higher prices early in the season, it might not be economically feasible to reduce temperatures in the greenhouse. This, of course, depends very much on the amount of energy that can be saved and the energy and product prices. Hurd and Graves (1985) calculated that in 1980 it was not profitable to reduce greenhouse night temperature from 16ºC to 11ºC although it almost halved the energy costs.

Breeding for cultivars with a lower temperature demand

The lack of variation between cultivars in temperature response illustrates the limited genetic variation between tomato cultivars, which is typical of self-pollinating crops, where domestication and breeding took place outside the native area (Rick and Chetelat, 1995). Even with modern molecular techniques it is difficult to distinguish different tomato cultivars (Miller and Tanksley, 1990; Park *et al.*, 2004). Genetic variation is essential to plant breeders as a basis for crop improvement. Therefore, breeders will have to utilize alternative sources of variation. As the genetic variation within modern cultivars is only a fraction of the variation between *Lycopersicon* species, these wild species offer opportunities for breeding (Miller and Tanksley, 1990). Especially *Lycopersicon* species which are chilling resistant and capable of growing at high altitudes (e.g. *L. hirsutum)*, offer opportunities for the identification of favorable gene loci, connected with growth at sub-optimal temperature, and subsequent introgression of these genes into cultivated tomato (Venema *et al.*, 2005).

Conclusions

Although tomato breeding did increase yield significantly in the past 50 years, yields at present day greenhouse conditions seem to have reached a plateau level. Therefore, for

breeding to be able to increase energy efficiency it is important to study possibilities of breeding cultivars that reach similar or higher yields at sub-optimal temperatures. As variation in temperature response between elite cultivars is limited (no genotype x temperature interaction in these experiments) it is important that other resources, e.g. wild relatives, are utilized. The main effects of reduced temperature occur during early stages of crop growth, resulting in a later start of production and a lower early production. However on the long term total yield, biomass production and partitioning were not affected by temperature. Thus, one important aspect to consider when breeding for energy efficient cultivars is earliness.

Chapter 3.3

Wild relatives as a source for sub-optimal temperature tolerance in tomato

Abstract

As variation for temperature response within the current elite tomato cultivars is limited, it is important to look for other sources of variation. One option are wild relatives which originate from South America, where they grow at altitudes up to 3300m. In this study we examined the effects of temperature (12-24ºC) on growth of young vegetative tomato plants of the cultivar Moneymaker and two wild relatives (*L. hirsutum* LA 1777 and *L. Pennellii* LA 716). The aim was to elucidate the physiological and morphological parameters which underlie interspecific differences in growth response to sub-optimal temperatures. During a 28-day period five destructive measurements were carried out in which total dry weight, including root weight, leaf area and leaf dry weight were measured in order to calculate growth parameters. Even though 'Moneymaker' had a higher relative growth rate (RGR) over a large temperature range (16ºC – 24ºC), RGR of Moneymaker was severely reduced below 20ºC, while RGRs of *L. hirsutum* and *L. pennellii* were only decreased below 16ºC. At 12ºC RGR of Moneymaker was reduced by 41% compared to 20ºC, while in *L. pennellii* and *L. hirsutum* this decrease was only 27 and 18%, respectively. This decrease in RGR in Moneymaker was mainly a result of a decreased leaf area ratio (LAR), caused by a 35% decrease in specific leaf area (SLA). In contrast, the decrease in RGR in *L. pennellii* and *L. hirsutum* was a result of a decreased net assimilation rate (NAR) of 24 and 14%, respectively. This study illustrates that wild tomato species provide possibilities for the breeding of more energy-efficient tomato greenhouse cultivars.

Submitted as:

Van der Ploeg, A., Venema, J.H. and Heuvelink, E. (2007). Wild relatives as a source for sub-optimal temperature tolerance in tomato.

Introduction

The high yield level in Dutch greenhouses requires high energy inputs for heating the greenhouses during the dark and cold winter months. However, as energy prices and public concern about the environmental problems relating with the combustion of fossil fuel are increasing, it is important that energy efficiency (kg of tomatoes produced per m^3 of natural gas) is increased. Over the last 25 years there has been a large increase in energy efficiency mainly as a consequence of increasing production levels, while the absolute amount of energy per m^2 greenhouse area was hardly reduced. A lowering in set-point temperature by a few degrees could contribute significantly to a reduction in energy consumption. It is therefore important that new cultivars are developed that can be cultivated at (slightly) lower temperature set-points. But breeding for equal production and quality at lower temperatures is hampered by the limited genetic variation for temperature response between elite tomato cultivars (Chapter 3.1).

The variation in cold tolerance that exists among related *Lycopersicon* species could potentially be useful for the development of cultivars with increased energy efficiency (Venema *et al.*, 2005). These *Lycopersicon* species are native to western South America, where they grow in a wide range of habitats, from sea level up to 3300m a.s.l. (Rick, 1995) and some of the high-altitude species (e.g. *L. hirsutum*, *L. peruvianum*) are known to be chilling tolerant (Wolf *et al.*, 1986). However, they might also contain traits that make them grow better at moderately low or suboptimal temperatures: temperatures above chilling but below the current optimum for tomato growth.

The present work aims at determining possible differences in growth and development of young vegetative tomato plants (*L. esculentum*) cv. Moneymaker and two wild relatives (*L. pennellii* and *L. hirsutum*) in response to temperature (12 – 24ºC). Furthermore we elaborate on what physiological and morphological parameters could explain these possible differences, which could be useful for selection criteria during breeding programs.

Material and methods

Plant material and growth conditions

Seeds of *L. hirsutum* LA 1777, *L. pennellii* LA 716 and *L. esculentum* cv. Moneymaker were sown in seed trays in a greenhouse. Seeds of 'Moneymaker' were sown one week later than seeds of *L. hirsutum* and *L. pennellii*, because of the faster germination rate of 'Moneymaker'. Three weeks later soil was removed from the roots and the seedlings were transferred to 12 cm diameter pots containing expanded clay grit (range 6-8mm). To allow acclimation to the new root environment, the pots were placed on greenhouse benches in a 2 cm water layer for one week. At the start of the experiment, the plants were transferred to four identical growth

chambers (2.5m × 3.5m). Within each chamber there were six trolleys. Each trolley contained 16 plants of one species so that within each growth chamber there were two trolleys of each species. The pots were continuously standing in a layer of standard nutrient solution, covered with white plastic to prevent algae growth, and this solution was refreshed weekly.

Each chamber had a constant temperature (12, 16, 20 or 24ºC). Fluorescent tubes (Philips TL 58W, color 84) were used during 12 hours, providing 128 μmol m^{-2} s^{-1} photosynthetically active radiation at plant level. Plants were grown under ambient CO_2 (growth chamber continuously ventilated) and at a relative humidity of 55%.

Measurements

The first destructive harvest of 10 plants of each species was taken when the plants were transferred to the climate chambers. Subsequently, for the duration of 4 weeks, there were weekly destructive harvests of 4 plants per trolley. After each destructive measurement plants were redistributed so that they could grow without mutual shading. Leaf area (LI-COR Model 3100 Area Meter), leaf, stem and root fresh and dry weight (105ºC for at least 10 hours), stem length and number of leaves (>5mm) were determined in each destructive measurement.

A growth analysis was conducted according to the functional approach (Hunt, 1990). The best fitting polynome for the relation between natural logarithms of total (root and shoot) dry mass (TDM), leaf dry mass (LDM) and leaf area (LA) with time was calculated by using the ordinary "least squares estimate". In all cases polynomials of degree 2 were found to be necessary and sufficient. To exclude ontogenetic effects the growth parameters relative growth rate (RGR), net assimilation rate (NAR), leaf area ratio (LAR), specific leaf area (SLA) and leaf weight ratio (LWR) were compared on the basis of a TDM interval instead of a time interval. The TDM interval is 20.5 – 92.5mg, because this was the largest possible interval in these experiments not requiring extrapolation of data.

Results were statistically analysed as split-plot design with two replications (trolleys were taken as replications) for temperature. Temperature was the main factor and species the split factor. Analysis of variance was conducted. Mean separation was done by the Least Significant Difference (LSD) based on Student *t*-test ($P = 0.05$). The statistical software package Genstat 8.1 was used.

Results

Growth

At the end of the experiment, the accumulated total dry mass (TDM) was significantly influenced by the interaction between species and temperature ($P < 0.001$; Fig. 1A). In all species TDM was significantly lower at 12ºC than at 20ºC and 24ºC but the difference was much larger in 'Moneymaker' than in the two wild *Lycopersicon* species. TDM in 'Moneymaker' was reduced by 83% at 12ºC, while this reduction was 63 and 51% for *L.*

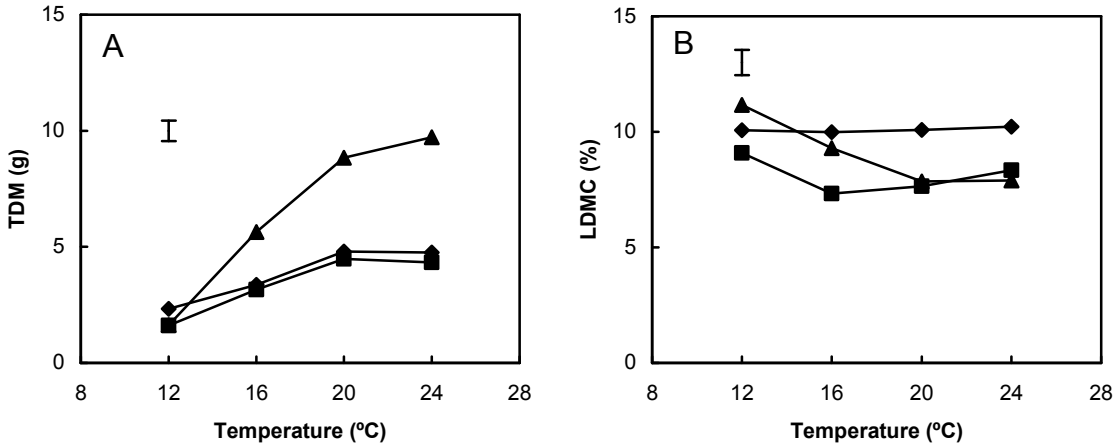

Figure 1: The effect of temperature on total dry mass (TDM) (A) and leaf dry matter content (LDMC) (B) of *L. esculentum* cv. Moneymaker (▲), *L. pennellii* (■) and *L. hirsutum* (♦) after four weeks of temperature treatment. Vertical bars indicate LSD = 0.87 (A) and LSD = 1.1 (B)

pennellii and *L. hirsutum*, respectively. *L. pennellii* and *L. hirsutum* had a significantly lower TDW than 'Moneymaker' at 16, 20 and 24ºC, while at 12ºC there were no significant differences between the species.

In order to explain differences in temperature response of biomass accumulation between species, a growth analysis was conducted (Fig. 2). At 16, 20 and 24ºC 'Moneymaker' clearly showed a higher RGR than the two wild species (Fig. 2A). However 'Moneymaker' also exhibits a severe decrease in RGR below 20ºC, while *L. pennellii* only showed a decrease in RGR below 16ºC and an even smaller decrease in RGR with temperature was found for *L. hirsutum*. At 12ºC RGR of 'Moneymaker' was reduced by 41% compared to 20ºC, while in *L. pennellii* and *L. hirsutum* this decrease was only 27 and 18%, respectively. The decrease in RGR for 'Moneymaker' was associated with a large decrease in LAR (Fig. 2B) while NAR (Fig. 2C) only showed a very small decrease with decreasing temperature. On the other hand, LAR of *L. pennellii* and *L. hirsutum* was unaffected by temperature. The decrease in RGR in *L. pennellii* and *L. hirsutum* was thus a result of a decrease in NAR. For *L. pennellii* and *L. hirsutum* NAR at 12ºC was reduced by 24 and 14%, respectively. The decrease in LAR in 'Moneymaker' is a consequence of a large decrease in SLA (Fig. 2D), which in both wild species was unaffected by temperature. In all species LWR only showed a very small decrease with decreasing temperatures.

Leaf dry matter content (LDMC) was influenced significantly by the interaction between species and temperature (P = 0.008; Fig. 1B). LDMC of *L. hirsutum* was not affected by temperature, while in *L. pennellii* LDMC showed a small increase at 12ºC but LDMC in 'Moneymaker' was even more increased at 12ºC. In contrast to both wild tomato species, LDMC of 'Moneymaker' revealed a significant increase below 20ºC.

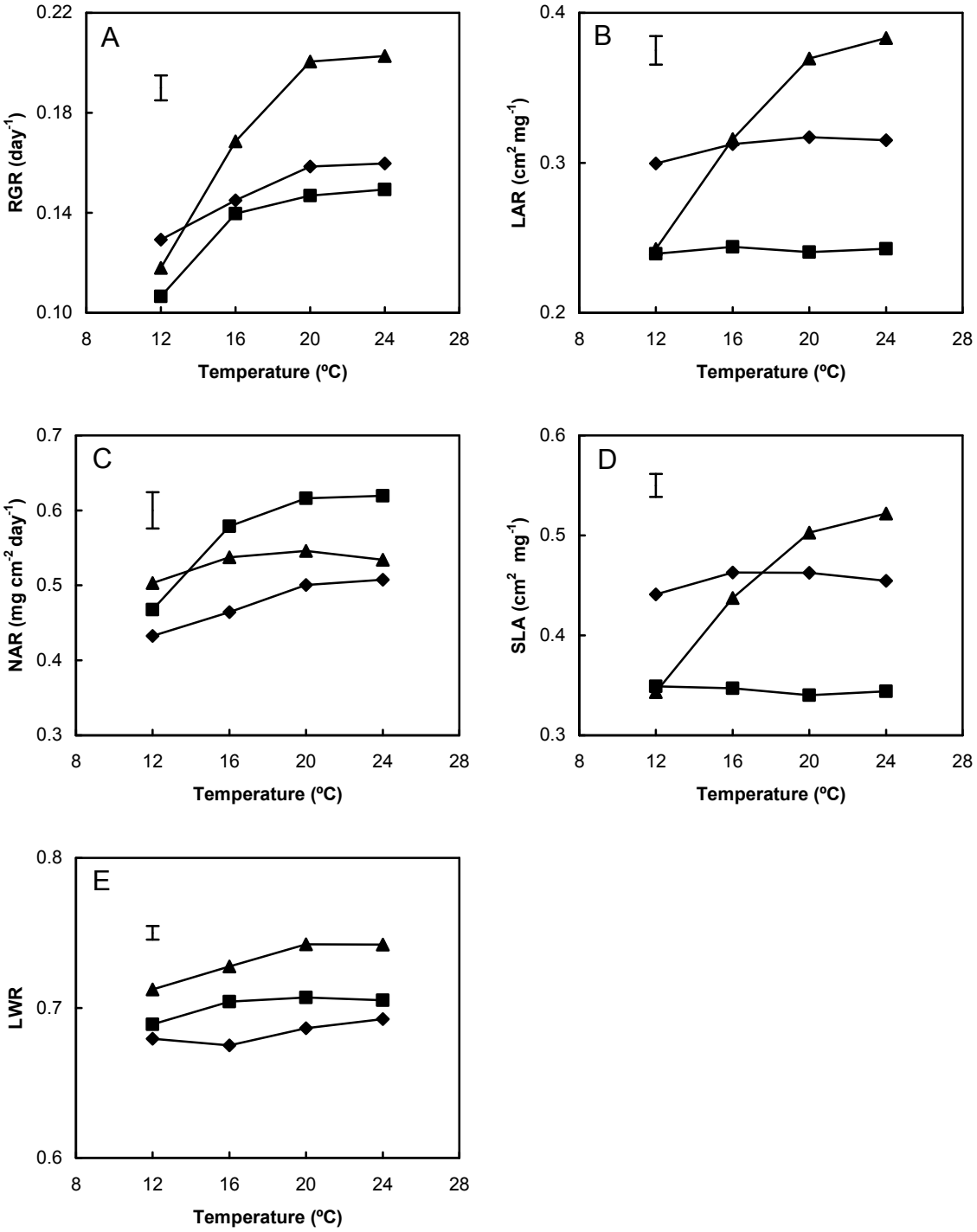

Figure 2: The effect of temperature on RGR (A), LAR (B), NAR (C), SLA (D) and LWR (E) of *L. esculentum* cv. Moneymaker (▲), *L. pennellii* (■) and *L. hirsutum* (♦). Vertical bars indicate LSD = 0.010 (A), LSD = 0.019 (B), LSD = 0.048 (C), LSD = 0.023 (D) and LSD = 0.009(E).

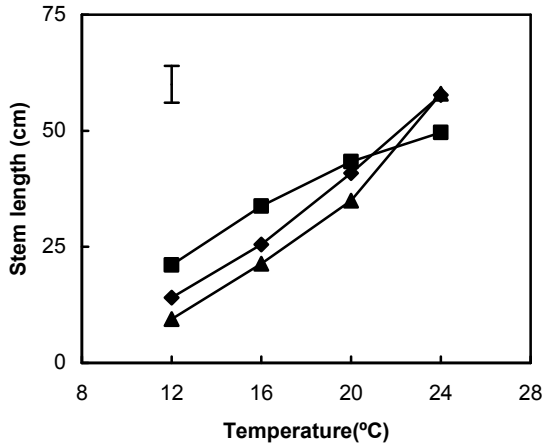

Figure 3: The effect of temperature on stem length of *L. esculentum* cv. Moneymaker (▲), *L. pennellii* (■) and *L. hirsutum* (♦). The vertical bar indicates LSD = 7.9.

Development

Stem length of all species increased with temperature but this increase was less pronounced in *L. pennellii* compared to the other two species (Fig. 3). Neither the number of leaves (P = 0.724) nor leaf appearance rate (P = 0.465) did show an interaction between temperature and species. Leaf appearance rate increased with temperature and was higher in 'Moneymaker' than in the two wild species (Table 1).

Table 1: The effect of temperature and species on leaf appearance rate. Different letters indicate significant differences for temperature (P <0.001) and species (P = 0.006), respectively.

Species	Temperature (ºC)				
	12	16	20	24	
L. esculentum cv. Moneymaker	0.181	0.303	0.358	0.391	0.308[b]
L. pennellii	0.152	0.232	0.258	0.309	0.238[a]
L. hirsutum	0.143	0.221	0.316	0.370	0.263[a]
	0.159[a]	0.252[b]	0.311[c]	0.357[d]	

Discussion

Both growth and development were influenced by temperature and species. Leaf appearance rate and therefore stem length are well known to increase with temperature, but changes in development rate in response to temperature showed a similar trend for all three species, whereas growth related traits clearly showed different temperature responses between the cultivated and the wild species.

Even though TDM accumulation was much higher for 'Moneymaker' over a large

temperature range (16 – 24ºC), the reduction in TDM production at sub-optimal temperatures (12-16°C) was much lower in both wild *Lycopersicon* species. A growth analysis can be a helpful tool in explaining differences in growth between species. RGR can be separated into an assimilatory component (NAR) and a morphological component (LAR), which in turn is the product of SLA (total leaf area per unit leaf mass) and LWR (leaf biomass per unit total plant mass). The large decrease in RGR at sub-optimal temperatures as a consequence of a decrease in SLA (increasing leaf thickness) and subsequently LAR as reported in this study for 'Moneymaker' (Fig. 1), has previously been reported for several commercial greenhouse tomato cultivars (Paul *et al.*, 1984; Hoek *et al.*, 1993; Venema *et al.*, 1999). Furthermore leaves of 'Moneymaker' showed a large increase in dry matter content at sub-optimal temperatures which suggests an inhibition of carbon translocation to sink tissues. Tomato cultivar 'Abunda' showed a large increase in starch content when grown at 16/14ºC D/NT compared to 25/20ºC D/NT, while this increase was much smaller in *L. hirsutum* (Venema *et al.*, 1999). Both *L. pennellii* and *L. hirsutum* showed a considerable smaller reduction in RGR at lower temperatures than 'Moneymaker'. Furthermore, contrary to 'Moneymaker' decreasing RGR in both wild relatives was not caused by a reduction in LAR but solely resulted from a reduction in NAR. More research is needed to elucidate the underlying mechanism(s) why carbohydrates accumulate so strongly at lower temperatures in the cultivated tomato, thereby decreasing SLA, in contrast to the wild species. As growth of *L. pennellii* and *L. hirsutum* is less temperature sensitive and and SLA is hardly affected, both species are definitely interesting sources for the breeding of new greenhouse cultivars with improved energy efficiency.

Although growth of *L. pennellii* and *L. hirsutum* stays behind on 'Moneymaker' at optimal temperatures (and even at 16°C), they still have possibilities of being used in breeding programs to improve vegetative growth rate (at decreased greenhouse temperatures). Growth and ultimately yield are complex traits, which involve several genes. Among numerous 'unfavorable' genes in the wild *Lycopersicon* species it possibly contains a few favorable genes. With molecular linkage maps it is possible to identify, map and study the effect of individual loci that control quantitatively inherited traits (Tanksley and McCouch, 1997). In fact, in an introgression line (IL) population of *L. pennellii* in a processing tomato variety a number of ILs were found that increased yield by 7-13% compared to the same variety without introgressions (Eshed *et al.*, 1996). An ideal energy-efficient tomato cultivar, with a broad temperature optimum, would combine the level of growth at optimal temperature of commercial elite greenhouse cultivars with the limited response to temperature of the wild *Lycopersicon* species.

Chapter 3.4

An attempt to increase energy efficiency in greenhouse tomato with *Lycopersicon pennellii* introgressions

Abstract

Small introgressions from *Lycopersicon pennellii* might help to increase energy efficiency in greenhouse tomato either by increasing yield or by reduced temperature sensitivity. Six previously developed back crossed inbred lines (BILs) with introgression of *L. pennellii*, covering 26 – 31 % of the *L. pennellii* genome, in the background of the greenhouse tomato cultivar Moneymaker were used to study the effect of the introgressions on yield and underlying components in a six month greenhouse experiment, at optimal (set point 21/19ºC day/night) and sub-optimal (set point 17/15ºC day/night) temperature. None of the BILs showed increased yield or a different temperature sensitivity compared to 'Moneymaker'. However four BILs had a significantly reduced yield. In one of the BILs this was caused by a reduction in total dry weight, due to a lower leaf area index, resulting in less light interception. Furthermore partitioning towards the fruits was reduced in four BILs. In one BIL, with introgressions on chromosome 9 and 10, harvesting started earlier than in Moneymaker and yield of this BIL was higher during the first part of the experiment. This difference in yield however gradually decreased and at the end of the experiment it had disappeared. The same BIL also showed a higher fruit dry matter content. The QTLs in this study are compared to previously reported QTLs and results are discussed in view of the potential for energy saving in greenhouse tomato.

Introduction

Increasing energy efficiency in greenhouse tomato (*Lycopersicon esculentum*), defined as the weight of tomatoes produced per unit energy input, will reduce production costs and fossil energy use. In The Netherlands, energy now accounts for about 20% of the production costs (Peet and Welles, 2005) and with the energy prices increasing, this will rise even more. In part, energy savings can be reached by technical measures (e.g. better greenhouse insulation) but plant breeders may/must also contribute. In principle there are two ways for plant breeders to help growers increase energy efficiency. Firstly, breeding for higher yields will increase production per m^2 and hence energy efficiency. In fact, over the past decades tomato breeders have made a tremendous increase in production possible (Chapter 3.2). A second, less explored, option would be by breeding for cultivars that give the same production but at a lower greenhouse temperature. For The Netherlands, reducing the greenhouse temperature set-points by 2ºC is estimated to result in a 16% energy saving (Elings *et al.*, 2005).

However, the small genetic variation within modern tomato cultivars limits the prospects of further increasing energy efficiency by plant breeding (Chapter 3.1 and 3.2). Wild related species present a genetic resource which can broaden the genetic variation of cultivated tomato. RFLP marker analysis showed that cultivated tomato varieties contain less than 5% of the genetic variation that is available in cross-compatible landraces and wild species (Tanksley and McCouch, 1997). Complex traits, like yield, have a continuous distribution, implying that many genes (Quantitative Trait Loci; QTLs) are involved. Wild relatives may contain (hidden) alleles, which can be introduced in modern cultivars for improved performance. Previous studies have demonstrated that introducing small introgressions of wild species genome into cultivated tomato can enhance agronomical favorable traits, in spite of the overall inferior appearance of the wild species (Eshed *et al.*, 1996; Bernacchi *et al.*, 1998a). Advances in molecular marker technologies have made it easier to localize agronomically favorable QTLs (Tanksley and McCouch, 1997).

Backcrossed inbred lines (BILs) can be a powerful tool in detecting QTLs, as all phenotypic variation between BIL and the cultivar can be attributed to the introgressed segment. Recently, Owona (2005) developed a series of BILs that contain small introgressions of *Lycopersicon pennellii* in the greenhouse cultivar Moneymaker background. *L. pennellii* may be an interesting source for increasing energy efficiency in greenhouse tomato. Relative growth rate (RGR) of young vegetative growing *L. pennellii* plants was less temperature sensitive than RGR in 'Moneymaker', although at optimal temperatures 'Moneymaker' performed better (Chapter 3.3). Furthermore, in open-field tomato QTLs were detected in *L. pennellii* that could increase several agronomically important traits, including yield (Eshed and Zamir, 1995; Eshed *et al.*, 1996; Frary *et al.*, 2004). Although field tomato has a different growth habit, determinate in contrast to the indeterminate greenhouse tomato cultivars, it could also increase yield in greenhouse tomato cultivars. In fact, one of these

BILs increased yield by 16% (Owona, 2005). However, the cumulative yield during that experiment showed one large jump and the underlying physiology behind this yield increase is not clear. Yield is one of the most complex traits, integrating most of the plant physiological and morphological functions and therefore it is important to know the underlying processes. In principle, yield is the product of biomass production and partitioning, which are both influenced by numerous processes (e.g. light interception, photosynthesis, leaf initiation rate, fruit set, fruit sink strength). Although several QTLs for yield have been detected, information about the underlying processes is still lacking.

This study aims at looking into the possibilities of using *L. pennellii* introgressions for improved energy efficiency in greenhouse tomato. Therefore, we will not only focus on increased yield at an optimal temperature regime but yield under sub-optimal temperature regime is studied as well. Furthermore we try to explain which processes are responsible for possible differences in yield and temperature response between each BIL and 'Moneymaker'.

Material and Methods

Plant material

Owona (2005) developed a number of BILs with an *L. pennellii* introgression in a 'Moneymaker' background. A complete description of the process is given in his thesis. The most relevant details are listed here (Table 1). The wild relative *L. pennellii* LA716 was crossed with *L. esculentum* cv. Moneymaker. A single F_1 was backcrossed with 'Moneymaker'. Each selection was preceded by marker aided genotyping based on AFLP markers. From BC_4 plants with a single introgression were selected for a generation of selfing. This resulted in a selection of 12 BILs, all but one containing a single homozygous introgression. A set of six BILs representing the widest genetic variation was used in this experiment for more detailed analysis. They covered 26-31% of the genome of *L. pennellii* LA716. The size of each introgression was estimated to range between 31 and 53 cM.

Experimental set-up

The experiment was conducted in two compartments (12 m × 12.8 m) that were part of a multispan Venlo-type greenhouse (Wageningen University, The Netherlands, lat. 52ºN) in

Table 1: The selected BILs with specification of the *L. pennellii* introgressions according to Owona (2005).

	Chromosome number	Introgression locus (cM)	Introgression size (cM)
BIL 1.2	1	35-75	40
BIL 2.3	2	80-133	53
BIL 5.3	5	63-103	40
BIL 8.2	8	18-49	31
BIL 9.1/10.2[1]	9 & 10	20-63 & 27-56	43 & 29
BIL 12.2	12	16-56	49

[1] contained two introgressions on two chromosomes

2006. Seeds were sown in trays filled with commercial potting soil on 14 November. Three weeks after sowing seedlings were pricked out and transferred to rockwool cubes and placed on ebb/flood benches in another compartment of the same greenhouse. Two months after the seeds were sown, plants were transferred to the cultivation compartments and placed on rockwool slabs at a plant density of 2.5 plants m^{-2}. At anthesis of the first flowers the temperature in each greenhouse compartment was set at the desired level (30 January). Leaves below the lowest ripening truss and all axillary shoots were removed weekly. Plants were trained according to the high wire system (Peet and Welles, 2005). Plant nutrition and pest and disease control were conducted according to common practice. Flowers were pollinated by bumble bees. The experiment finished on 6 June.

Greenhouse climate

Heating set-points for day/night were 17/15ºC (low temperature treatment; LT) and 21/19ºC (high temperature treatment; HT). Ventilation set-points were 1ºC above the heating set-points. Greenhouse climate was automatically recorded every 5 minutes using a commercial computer system (Hoogendoorn, Vlaardingen, the Netherlands). Daily global radiation outside the greenhouse was obtained from the department of meteorology at about 800 m distance. The average daily realized temperatures were 17.8ºC and 21.0ºC in the LT and HT compartments, respectively. The average outside global radiation was 56.6 mol m^{-2} day^{-1}.

Measurements

Destructive measurements were carried out at anthesis, at the end of the experiment and two times at approximately equal intervals during the experiment. Fresh and dry weights (ventilated oven; 105ºC) of leaves (including petioles), stem, fruit trusses, removed leaves and picked fruits and leaf area (LI-COR Model 3100 Area Meter) were determined. Number of leaves (>0.5cm), number of trusses (>0.5cm) and number of fruits were recorded. The plants used for destructive measurements were surrounded by guard plants. Extra side shoots were allowed to grow on guard plants to replace measured plants in order to maintain stem density and equal light distribution in the crop.

Light use efficiency

For each treatment a time course of leaf area index (LAI), based on linear interpolations between destructive leaf area measurements, was calculated. Based on measured daily global radiation, a greenhouse transmisivity of 58%, assuming 47% PAR in the global radiation, and a light extension coefficient of the canopy of 0.75 (Heuvelink and Buiskool, 1995) the daily intercepted PAR by the crop was calculated. Light use efficiency (LUE) was determined as the dry matter production divided by the integral of intercepted irradiance over a period between two destructive measurements. LUE was averaged over three periods resulting from four destructive measurements.

Statistical analysis

A split-plot design was used, with temperature as the main factor and genotype as the split factor. Data were checked for normality using the 'Kolmogorov-Smirnov' test from SPSS package. Analysis of variance was conducted, using Genstat 8 and treatment effects were tested at 5 % probability level (P = 0.05), except for the temperature effect which was tested at a 10 % probability level (P = 0.10) motivated by the low degree of freedom for the residuals. When significant differences for genotypes were found Dunnett's test was used for mean separation between 'Moneymaker' and each of the BILs (P = 0.05).

Results

Yield

For all genotypes harvest started earlier at HT compared to LT (Fig. 1). Both at DOY 115 and 157 (dates of destructive measurements) there was no significant interaction between temperature regime and BIL for yield. Although the differences in yield between HT and LT became less during the experiment, at the end of the experiment yield was still reduced at LT by 20 % and 25 %, for fresh and dry weight, respectively (Table 2). In contrast, plants grown at LT had more fruit weight on the plant at the last destructive measurement. The total fruit weight, of both harvested and green fruits, was not affected by temperature regime.

Several BILs differed in yield from 'Moneymaker' (Table 2, Figure 1). BIL 9.1/10.2 initially gave a higher yield. At DOY 115 yield of this BIL was increased by 37 % compared to 'Moneymaker'. However the difference in yield with 'Moneymaker' gradually decreased and had disappeared at final harvest. At the end of the experiment there was no BIL with a significantly higher yield than 'Moneymaker'. BIL 1.2, 5.3, 8.2 and 12.2 all had a significantly lower final yield than 'Moneymaker'. At the last destructive measurement a higher green fruit weight was measured for BIL 1.2, while in BIL9.1/10.2 green fruit weight was less than 'Moneymaker' (Table 2). The total fruit weight (both green and harvested red fruits) was significantly reduced for BIL 8.2, 9.1/10.2 and 12.2 (Table 2).

Fruit characteristics

BIL 2.3 produced significantly smaller fruits than 'Moneymaker', and this BIL had the highest number of harvested fruits (Table 3). For BIL 1.2 significantly less fruits were harvested during the experimental period. The other BILs did not show an effect on either fruit size or fruit number. Dry matter content was significantly increased in BIL 9.1/10.2, while for the other BILs it was not significantly different from 'Moneymaker'.

LT reduced the number of harvested fruits and increased the individual fruit weight. Furthermore it decreased the dry matter content of fruits.

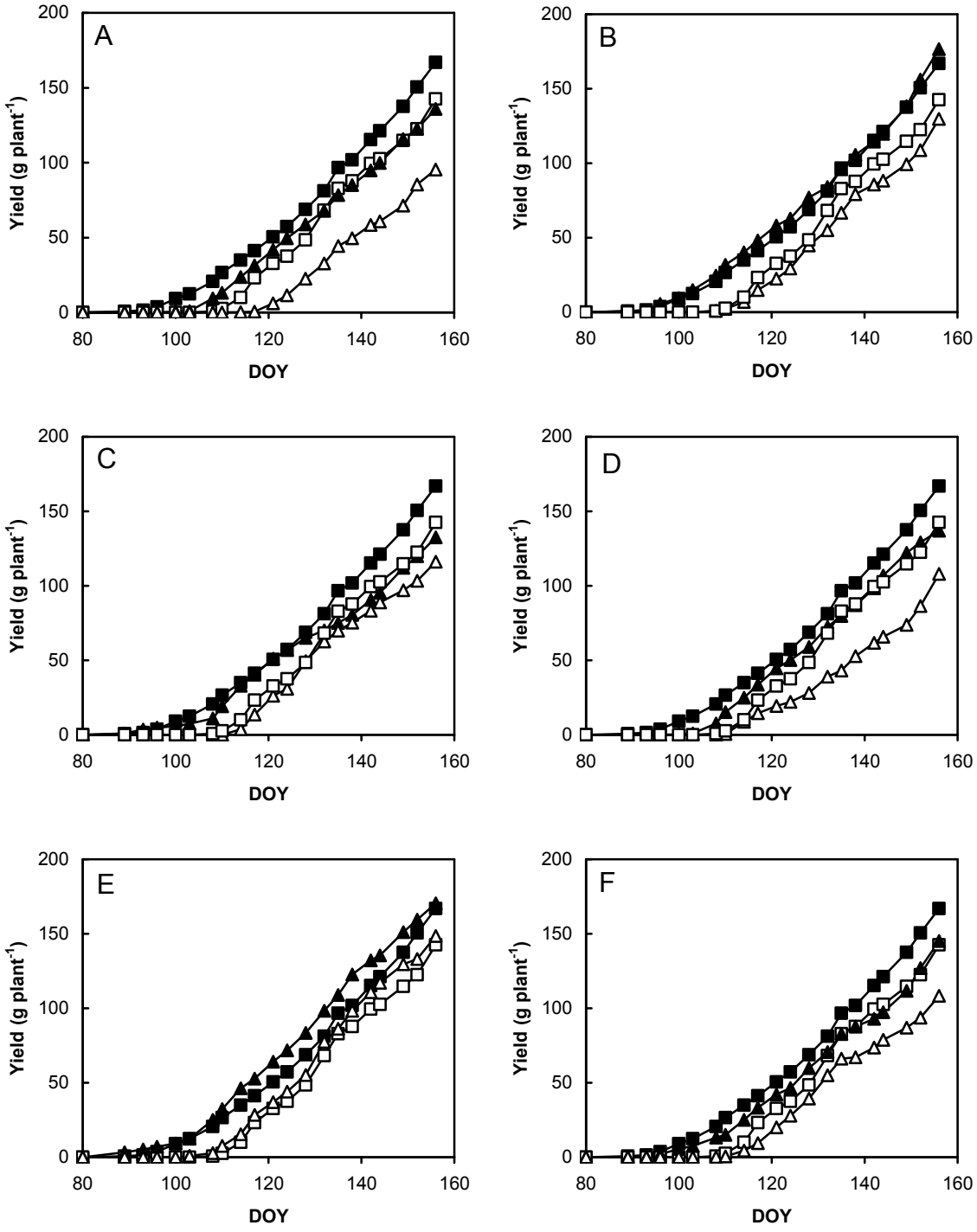

Figure 1: The effect of HT (closed symbols) and LT (open symbols) on yield (dry weight) of each BILs (▲Δ) and 'Moneymaker' (■□) pattern (dates are expressed as day of year; 1 January = DOY 1). BIL 1.2 (A), BIL 2.3 (B), BIL 5.3 (C), BIL 8.2 (D), BIL 9.1/10.2 (E) and BIL 12.2 (F).

Table 2: Cumulative yield fresh weight ($Yield_{FW}$) on DOY 157, cumulative yield dry weight ($Yield_{DW}$) on DOY 115 and 157, dry weight of green fruits at DOY 157 (FDW_P) and total fruit dry weight (FDW_T). Dates are expressed as day of the year (1 january = DOY 1).

	$Yield_{FW}$ (kg plant^{-1}) DOY 157	$Yield_{DW}$ (g plant^{-1}) DOY 115	$Yield_{DW}$ (g plant^{-1}) DOY 157	FDW_P (g plant^{-1}) DOY 157	FDW_T (g plant^{-1}) DOY 157
Moneymaker	2.15	22	152	113	265
BIL 1.2	1.79*	15*	129*	127**	256
BIL 2.3	2.21	24	148	113	261
BIL 5.3	1.94	18	132*	112	244
BIL 8.2	1.97	18	131*	107	238*
BIL 9.1/10.2	1.95	31**	156	85*	241*
BIL 12.2	1.90*	15*	129*	111	240*
F-prob[y]	**0.040**	**0.002**	**0.001**	**0.019**	**0.008**
HT	2.15	36	159	88	247
LT	1.74	8	119	132	251
F-prob[z]	**0.080**	**0.030**	**0.071**	**0.010**	**0.696**

[y] F probability (significant levels < 0.05 presented in bold)

[z] F probability (significant levels < 0.10 presented in bold)

* significantly lower than 'Moneymaker' according to Dunnett ($P = 0.05$)

** significantly higher than 'Moneymaker' according to Dunnett ($P = 0.05$)

Fruit set on the first truss was low for all BILs and 'Moneymaker' at both temperature regimes (Table 4). Even 'Moneymaker' had less than two fruits on the first truss. Both BIL 1.2 and BIL 5.3 did not have any fruit set on the first truss. BIL 12.2 had the highest number of fruits on the first truss. For all BILs and 'Moneymaker' the number of fruits on the second truss was higher than on the first truss, although the fruit number on the second truss was lower at HT. BIL 1.2 clearly stayed behind on 'Moneymaker' while all the other BILs had a similar number of fruits on the second truss. On truss 3 and 4 fruit set was good at both temperature regimes and no BIL produced either a significantly higher or lower fruit number on these trusses.

Table 3: Average fruit size of harvested fruits, total harvested fruit number and fruit dry matter content (FDMC) of harvested fruits over the complete cultivation period.

	Fruit size (g fruit^{-1})	Fruit number	FDMC (g g^{-1})
Moneymaker	2.60	59	0.071
BIL 1.2	2.69	47*	0.072
BIL 2.3	1.98*	77**	0.067
BIL 5.3	2.63	50	0.068
BIL 8.2	2.59	51	0.067
BIL 9.1/10.2	2.37	66	0.080**
BIL 12.2	2.49	52	0.068
F-prob[y]	**0.037**	**0.006**	**0.005**
HT	2.30	69	0.074
LT	2.64	45	0.069
F-prob[z]	**0.098**	**0.067**	**0.003**

[y] F probability (significant levels < 0.05 presented in bold)

[z] F probability (significant levels < 0.10 presented in bold)

* significantly lower than 'Moneymaker' according to Dunnett ($P = 0.05$)

** significantly higher than 'Moneymaker' according to Dunnett ($P = 0.05$)

Table 4: Fruit development time (number of days between anthesis and harvest ripe of first fruits per truss) of truss 3 to 5 and the average number of fruits per truss for the first four trusses of six BILs and 'Moneymaker'

	Fruit development time	Fruit number per truss		
	(d)	Truss 1	Truss 2	Trusses 3-4
Moneymaker	57	1.8	5.7	8.6
BIL 1.2	55	0.0*	2.8*	8.2
BIL 2.3	55	1.0	5.7	9.0
BIL 5.3	59	0.0*	5.6	8.0
BIL 8.2	55	1.3	4.4	9.2
BIL 9.1/10.2	52*	2.2	5.1	9.8
BIL 12.2	62**	3.1**	5.5	7.4
F-prob[y]	**<0.001**	**< 0.001**	**0.005**	0.471
HT	52	1.1	3.4	8.4
LT	60	1.5	6.5	8.8
F-prob[z]	**0.004**	0.140	**0.070**	0.661

[y] F probability (significant levels < 0.05 presented in bold)

[z] F probability (significant levels < 0.10 presented in bold)

* significantly lower than 'Moneymaker' according to Dunnett ($P = 0.05$)

** significantly higher than 'Moneymaker' according to Dunnett ($P = 0.05$)

Fruit development rate was significantly increased in BIL 12.2, while fruits of BIL 9.1/10.2 had a reduced development time (Table 4). Furthermore temperature regime had a significant effect on fruit development, at LT it took 8 days longer from anthesis to harvest ripe than at HT.

Development

Total leaf number ($P = 0.360$) and truss appearance rate ($P = 0.548$) were not significantly different for the genotypes, but truss appearance rate ($P = 0.026$) and total leaf number ($P = 0.045$) were significantly higher at HT.

Total dry mass production

Total dry mass (TDM) was significantly affected by genotype ($P = 0.037$). BIL 12.2 had a 9% lower TDM production than 'Moneymaker' (Fig. 2). Temperature treatment did not significantly influence TDM ($P = 0.151$). LUE was not affected by either temperature regime or genotype (Table 5).

Leaf area index (LAI), specific leaf area (SLA) and intercepted PAR.

On two destructive measurement dates LAI was significantly influenced by genotype (Table 5). At both the first and the last destructive measurement dates BIL 8.2 had a higher LAI than 'Moneymaker', while BIL 12.2 had a significantly lower LAI at these dates. Furthermore, on DOY 73 BIL 9.1/10.2 had a significantly reduced LAI compared to the 'Moneymaker'. SLA was significantly higher in BIL 8.2 compared to 'Moneymaker', while leaves BIL 12.2 had a significantly lower SLA. Over the complete experimental period BIL 12.2 had intercepted less light than 'Moneymaker', while BIL 8.2 had intercepted more light (Table 5).

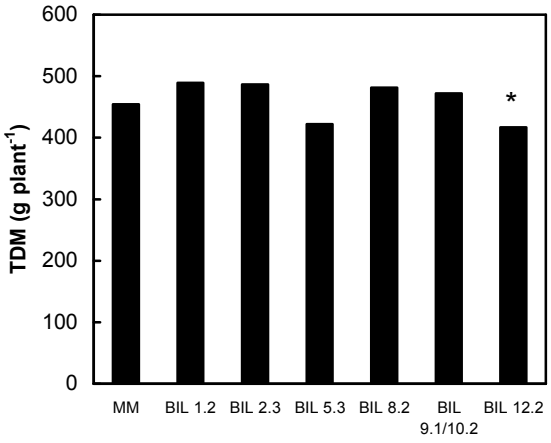

Figure 2: Total dry mass (TDM), including picked leaves and harvested fruits, for six BILs and 'Moneymaker' at the last day of the experiment (DOY 157). * indicates significant lower value than 'Moneymaker' according to Dunnett's test ($P = 0.05$).

Temperature had a significant effect on LAI only on DOY 73 ($P = 0.011$). At this date, LAI was higher at LT. SLA was significantly higher at HT. Between the two temperature regimes there was no significant difference in the amount of intercepted radiation.

Partitioning

The partitioning of assimilates cumulative for the whole experiment to fruits ($P < 0.001$), leaves ($P = 0.002$) and stem ($P < 0.001$) was affected by genotype (Fig. 3). 'Moneymaker' partitioned approximately 60% of the assimilates to the fruits. The partitioning to the fruits was significantly reduced in BIL1.2, BIL 2.3, BIL 8.2 and BIL 9.1/10.2. Partitioning to the

Table 5: Leaf area index (LAI) on DOY 73, 115 and 157 ,total intercepted PAR during the whole experimental period, light use efficiency (LUE) and specific leaf area (SLA) of six BILs and 'Moneymaker'. Dates are expressed as day of the year (1 January = DOY 1)

	LAI DOY 73	LAI DOY 115	LAI DOY 157	SLA [x] ($cm^2\ g^{-1}$)	Intercepted PAR ($MJ\ m^{-2}$)	LUE ($g\ MJ^{-1}$)
Moneymaker	3.78	3.01	2.88	324	365	2.42
BIL 1.2	3.59	2.93	2.59	308	360	2.68
BIL 2.3	3.50	2.84	3.19	321	363	2.90
BIL 5.3	3.53	2.95	2.63	318	359	2.29
BIL 8.2	4.56**	3.29	3.54**	356**	379**	2.70
BIL 9.1/10.2	3.28*	3.00	2.98	320	360	2.83
BIL 12.2	2.82*	2.64	2.36*	288*	339*	2.48
F-prob[y]	**<0.001**	0.275	**0.001**	**0.001**	**<0.001**	0.708
HT	3.32	2.89	2.93	332	358	2.59
LT	3.84	3.10	2.83	306	363	2.64
F-prob[z]	**0.011**	0.157	0.736	**0.017**	0.134	0.863

[x] SLA of removed leaves

[y] F probability (significant levels < 0.05 presented in bold)

[z] F probability (significant levels < 0.10 presented in bold)

* significantly lower than 'Moneymaker' according to Dunnett ($P = 0.05$)

** significantly higher than 'Moneymaker' according to Dunnett ($P = 0.05$)

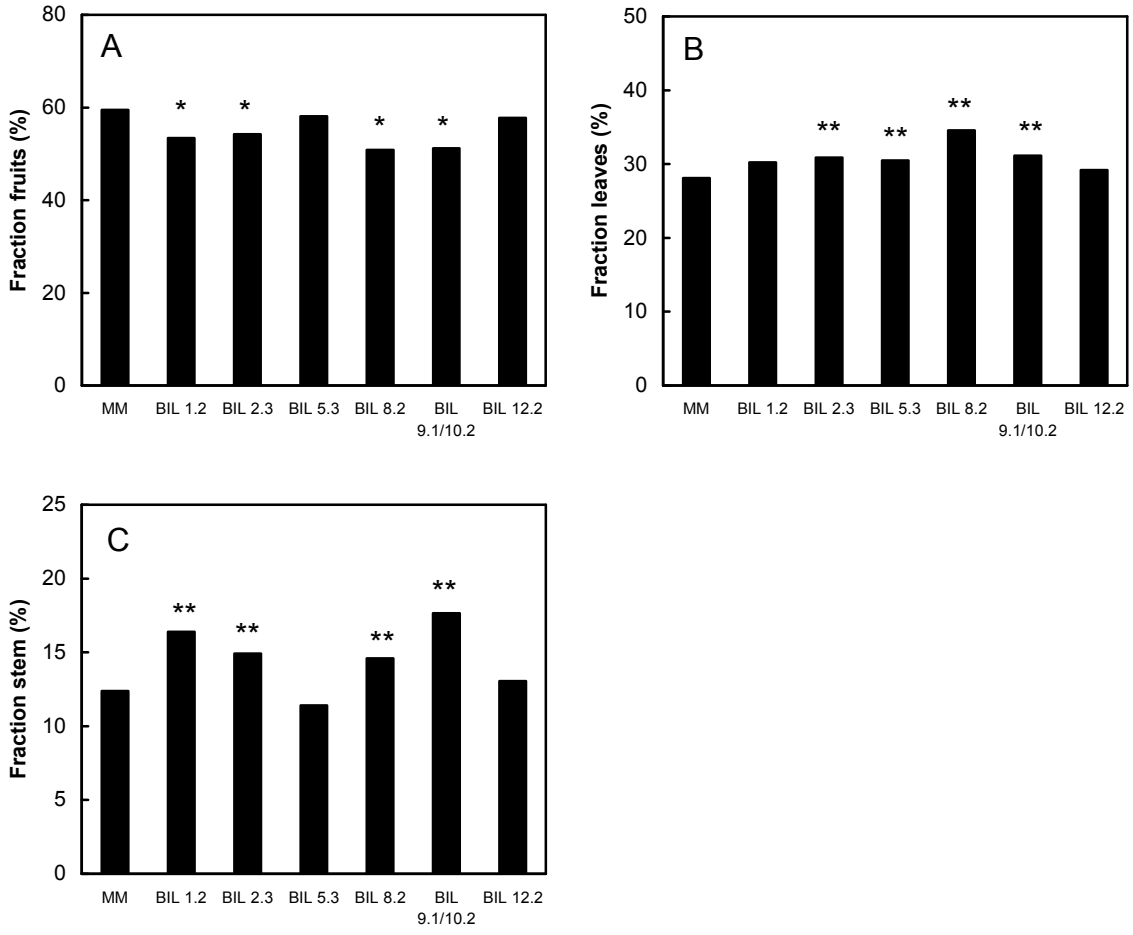

Figure 3: Dry matter partitioning to the fruits (A), leaves (B) and stem (C) cumulative over the whole experimental period for six BILs and 'Moneymaker'. Values are the averages over two temperature regimes. * indicates significantly lower values than 'Moneymaker' according to Dunnett's test(P <0.05) and ** significantly higher than 'Moneymaker' according to Dunnett's test (P <0.05).

leaves was about 28% in the 'Moneymaker', while partitioning towards the leaves was increased in BIL 2.3, BIL 5.3, BIL 8.2 and BIL 9.1/10.2. Only 13% of the dry matter was partitioned towards the stem in 'Moneymaker', while this was significantly higher in BIL 1.2, BIL 2.3, BIL 8.2 and BIL 9.1/10.2. Temperature did not affect the distribution of assimilates cumulative for the whole experiment.

Discussion

QTLs recorded in this study

None of the studied BILs resulted in a higher yield than 'Moneymaker'. This is in contrast with previous results were BIL 1.2 gave a 16% higher yield (Owona, 2005). In fact, in this

experiment BIL 1.2 gave a lower yield than 'Moneymaker' (Fig 1, Table 2). However, in this BIL bad fruit set on the lowest trusses, resulting in a delay in harvest. As more fruit weight was left on the plant (Table 2) at the final destructive measurement, the difference in yield between BIL 1.2 and 'Moneymaker' is expected to decrease when the experiment would have been continued for a few more weeks. Strangely, the higher yield for BIL 1.2 in the experiment of Owona (2005) was the result of one large raise in yield between 164 and 172 days after sowing while no explanation could be given for this peculiar boost in yield. Even so, the contradicting results between the two experiments could also arise of differences in experimental factors. Although both experiments were carried out in the same greenhouse complex, differences were present in planting date, plant density and external environmental factors (e.g. light) between different years. Previously, large differences were found in beneficial effects of *L. hirsutum* introgressions between several experimental sites (Bernacchi *et al.*, 1998b). Furthermore, *L. pennellii* introgressions that caused higher yield in an open spacing (1 plant m^{-2}) were not always the introgressions that caused yield increases in a denser (3.3 plants m^{-2}) crop spacing (Eshed and Zamir, 1994).

Four *L. pennellii* introgressions were detected that resulted in a lower yield (Fig. 1, Table 2). The lower yield of each of these BILs could be explained by several underlying factors. For example, BIL12.2 had the lowest LAI in all measurements, caused by a lower SLA (thicker leaves). Therefore, BIL 12.2 intercepted less light than the other genotypes (Table 5). The decrease in yield in this BIL was therefore the result of a lower dry matter production (Fig. 2). In both BIL 1.2 and BIL 8.2 the lower yield could be explained by a lower partitioning to the fruits, in favor of stem (both BILs) and leaves (only BIL 8.2) (Fig. 3). In BIL 1.2 this low partitioning was probably related to the poor fruit set on the lowest trusses (Table 4).

Although no BIL had a higher yield than 'Moneymaker' or showed a difference in temperature response, BIL 9.1/10.2 might be a potentially interesting BIL as its yield is higher than 'Moneymaker' early in the season (Fig. 1, Table 2 and 4). Earliness is an important characteristic in greenhouse tomato production, as early-season tomatoes have a relatively high market value. One of the major effects of sub-optimal temperature is the delay in harvesting, which makes that reducing the (night) temperature by a few degrees might not be profitable, even if total yields are comparable (Hurd and Graves, 1985). However, even for BIL 9.1/10.2 first harvest is still delayed at LT and it therefore questionably whether it can help to improve energy efficiency. However, this depends highly on the energy and product prices. Furthermore at the end of the experiment this BIL had a lower green fruit weight, which if the experiment would have lasted longer would probably result in a lower yield than 'Moneymaker', resulting from a lower partitioning of assimilates to the fruits. However, BIL 9.1/10.2 had two introgressions of in total 72cM, making this the BIL with the largest *L. pennellii* introgression in our experiment. Further backcrossing with 'Moneymaker' could break the linkage between these traits.

Comparison with previously recorded QTLs

Many previous studies have demonstrated that wild species can be a source of horticultural favorable alleles despite the overall inferior appearance of these species. The number of favorable QTLs reported in wild species, differs between studies, depending on the wild species and traits under study. In *L. hirsutum* 20% of the QTLs found for 12 traits, including yield, the *L. hirsutum* allele was associated with an improved performance from a horticultural perspective (Bernacchi *et al.*, 1998a), while in *L. pennellii* for 23 traits (yield, processing and fruit quality traits) 26% of the QTLs were associated with a better performance of the *L. pennellii* allele (Frary *et al.*, 2004). In the present study only few favorable QTLs were determined and none of these QTLs could help breeders to improve energy efficiency in greenhouse tomato cultivars. However, the BILs used in this experiment only covered about 30% of the *L. pennellii* genome. Therefore it is possible that interesting traits are located on other parts of the genome. Furthermore, as the introgression segments are still fairly large, the number of QTLs identified from these results might underestimate the real number of loci regulating these characters in two ways. First multiple loci contributing similarly to a parameter in a single BIL would be diagnosed as a single QTL. Secondly, two linked QTLs with opposite effect could mask each other, resulting in one or both QTLs being missed in the analysis. A more precise estimate of the number of relevant QTLs will be achieved when these QTLs are mapped more finely, but it is questionably whether this will be worth the effort.

Some QTLs found in the present study coincide with QTLs found in previous studies. For example, a QTL was located on chromosome 2 (BIL 2.3) which affected fruit size (Table 3), resulting in smaller fruits when the *L. pennellii* introgression was present. A major QTL at approximately the same location has previously been recorded for both *L. pennellii* and *L. pimpinellifolium* (Alpert *et al.*, 1995). This QTL refers to a single gene, *ORFX*, which is expressed early in floral development and controls carpel cell number (Frary *et al.*, 2000). Interestingly, the reduced fruit size did not result in a lower total yield as it was compensated by a higher fruit number (Table 3). Furthermore, on chromosome 9 a QTL for Brix, a measure for the total soluble sugar content, was found (Eshed and Zamir, 1995). The wild-species allele increased glucose and fructose contents in cultivated tomato fruits (Fridman *et al.*, 2000) and was mapped within a flower and fruit specific invertase (Fridman *et al.*, 2004). This QTL coincides with the location of the introgression on chromosome 9 in BIL 9.1/10.2, which showed a higher fruit dry matter content.

In addition several QTLs for yield have been located in field tomato. The number and location of QTLs differs between studies, possibly due to an interaction with the environment or different backgrounds. Eshed and Zamir (1995) found ten QTLs for yield of which only one QTL, on chromosome 7, showed a positive effect of the *L. pennellii* allele. Furthermore, Frary *et al*. (2004) detected six QTLs for yield, of which the *L. pennellii* allele on

chromosome 9 had a small positive effect. However, it remains difficult to translate these results directly to greenhouse tomato as the underlying components that are responsible for these QTLs are unknown. As the growth habit, determinate versus indeterminate growth, differs between field and greenhouse grown tomato, underlying components that are related to increasing yield in field tomato are not necessarily the same as in greenhouse tomato. It is therefore important that the underlying principles behind yield increase are studied in more detail. Furthermore knowing the underlying components could help breeders in deciding to combine certain QTLs to create even better performing cultivars.

So far, to our knowledge, no studies have been performed on the effects of temperature on *L. pennellii* introgressions in tomato. Within the temperature range (optimal –suboptimal greenhouse temperatures) in this experiment no QTLs were reported that caused temperature sensitivity on yield or yield related traits. Possibly more QTLs could be detected if the temperature range would be larger, as *L. pennellii* plant growth was less temperature sensitive (Chapter 3.3). Even more promising, could be back crossed inbred lines containing *L. hirsutum* introgressions. This species, which is known to be chilling resistant (Wolf et al., 1986), was also less temperature sensitive in the sub-optimal temperature range (Chapter 3.3). Furthermore, several QTLs have been detected in *L. hirsutum* that confer chilling tolerance in tomato (Truco *et al.*, 2000; Oyanedel *et al.*, 2001; Goodstal *et al.*, 2005).

Conclusions

In this study no QTLs were found that could help increase greenhouse energy efficiency in tomato. However, as the BILs used in this study only cover about 30% of the *L. pennellii* genome, it can not be excluded that no QTLs that can increase energy efficiency of greenhouse tomato cultivars.

Chapter 4

General discussion

For optimal growth of chrysanthemum and tomato, the environment can either be adapted to the needs of the plants (e.g. by growing in heated greenhouses), or the plants can be genetically altered to fit the environment. Usually a combination of both is applied (De Jong, 1991). For example, chrysanthemum cultivars have been developed that are adapted to the low light conditions during winter months in The Netherlands (De Jong, 1986), while on the other hand assimilation lights are often used to increase the light level. Due to increasing energy prices and the impact that high energy use has on the environment, greenhouse energy efficiency should be further increased. Plant breeders are thus facing the important challenge to adapt greenhouse crops to less energy consuming cultivation methods and especially to lower temperature set-points in the winter months.

For breeding for more energy efficient cultivars a good understanding of the morphological and physiological processes related to temperature responses is necessary. Fruit and flower growth is part of the integrated growth of the whole plant. Yield is the product of biomass production and partitioning. Biomass production depends on light interception and the efficiency in which the intercepted light is turned into biomass. In Chapters 2.1 and 3.1 the gaps in knowledge on these morphological and physiological processes underlying yield responses to temperature were identified based on literature with special emphasize on cultivar differences in chrysanthemum and tomato, respectively. From Chapter 2.1 it became clear that for chrysanthemum the effect of temperature on developmental processes has been thoroughly studied but the effects of temperature on biomass production are less clear. It was concluded that more research is required to determine where genetic differences in response to temperature in biomass produced arise from. Therefore, the main focus in Chapters 2.2 and 2.3 was on the effect of temperature on biomass production and the genetic variation for this trait. However, as for chrysanthemum external quality attributes, such as flower characteristics, are of utmost importance and have a large impact on its economical value (Carvalho and Heuvelink, 2001) the effect of temperature on these attributes was also taken into account in Chapters 2.2 and 2.3.

From the literature review in Chapter 3.1 it is clear that in tomato, being an important model system for plant physiological research, the effect of temperature on growth of young vegetative growing plants has been studied rather well. However, growth responses to temperature in adult plants has been studied less frequently and the link with yield and whole plant growth was often missing. Furthermore, knowledge on differences between cultivars in response to temperature, if present, is limited. Chapter 3.2 addressed these issues in commercial cultivars but as variation between cultivars was limited in Chapters 3.3 and 3.4 focus has been on the possibilities of using wild related *Lycopersicon* species as a source to introduce more genetic variation for growth response to temperature into tomato cultivars.

Breeding for higher energy efficiency: an historic perspective

In the late 1970s and the early 1980s, when energy prices were relatively high, scientists took a considerable interest in breeding cultivars adapted to lower temperatures (Smeets and Hogenboom, 1985). From the literature study in Chapter 2.1, it became clear that for chrysanthemum the research so far was mainly focussed on developing cultivars with a shorter reaction time at lower temperatures. This research resulted in cultivars that could flower earlier at lower temperatures, but the optimum temperature for flowering did not change (De Jong, 1991; Larsen and Persson, 1999). However, as shown in Chapters 2.2 and 2.3, growth is also reduced at sub-optimal temperature and can therefore not be ignored.

So far, in Dutch glasshouse tomatoes energy efficiency was almost exclusively raised by yield increases. Chapter 3.2 shows that breeding has contributed to a tremendous increase in tomato production over the second part of the 20th century. Yield of modern cultivars was on average 40% higher than yield of 'Moneymaker', released in 1950. Analysis of the morphological and physiological basis of increases in yield potential is important as a guide for further gains, both from the changes that have already occurred and from those that have not yet happened (Evans and Fischer, 1999). However, Chapter 3.2 also indicates that growth and development of modern cultivars respond in the same way to sub-optimal temperature as the older cultivars, thus limiting the breeding potential for cultivars with a lower temperature requirement.

The high energy prices in the early 1980's resulted in a reduction of energy use in Dutch greenhouses (see Table 1 in Chapter 1). In 1985 the energy use per m^2 was reduced by almost 25% compared to 1980. However, in the second half of the 1980's energy prices decreased again rapidly, resulting in a quick increase in energy use per m^2 back to the level of 1980. As a consequence, there was temporarily less interest in developing more energy efficient cultivars. Recently, as energy prices have been rising again and reducing CO_2 emission is getting more attention, this subject is once again back on the list of priorities from greenhouse crop breeders.

Problems and opportunities in breeding for increased energy efficiency

When breeding for new cultivars it is imperative that genetic variation is present for the desired trait. In general, in the present study it was found that there is more variation in temperature response in cut chrysanthemum than in tomato (Chapter 2.2 and 3.2). However, in tomato wild relatives can be a good source to increase this genetic variation (Tanksley and McCouch, 1997). Vegetative growth of *L. hirsutum* and *L. pennellii* was clearly less temperature sensitive than growth in greenhouse cultivars (Chapter 3.3). However, if we want to study the possibilities of introgressing positive traits originating from these or other wild

species, it is of vital importance that the development of back crossed inbred lines (BILs) progresses further. The BILs available for this study only covered a limited part (26 to 31%) of the *L. pennellii* genome (Chapter 3.4). Most of the QTLs reported in Chapter 3.4 were associated with an unfavorable performance from a horticultural perspective of the *L. pennellii* introgression, but other studies have shown that despite the overall inferior performance of the wild species, they do contain QTLs that enhance performance in a cultivar background (Bernacchi *et al.*, 1998a; Frary *et al.*, 2004).

The following section describes some of the major limiting processes and processes that provide opportunities for breeding for cultivars that are better adapted to sub-optimal temperatures and higher yielding cultivars.

Delayed development: a major concern at sub-optimal temperature

Chapters 2.1 and 3.1 show that the effects of temperature on development (e.g. reaction time, leaf and truss initiation rate, fruit development time) have been well studied and characterized in literature. In general, temperature has a strong influence on all aspects of development in both chrysanthemum and tomato (Fig. 1). Growing chrysanthemum at sub-optimal temperatures causes a delay in harvest but between chrysanthemum cultivars clear differences in the extent of this delay are present (Chapters 2.2 and 2.3). Depending on the cultivar and season the delay in harvesting at sub-optimal temperatures (16ºC) compared to optimum temperature (20ºC) ranged between 4 and 17 days (Chapter 2.3), showing plenty of breeding potential. The largest differences between cultivars occurred at sub- and supra-optimal temperatures (Chapter 2.2), while under optimum temperature conditions the differences between cultivars were rather small. The latter was not surprising as for all the cultivars used in this study the breeders indicated a response time (reaction time under optimal light and temperature) of around 49 to 54 days. Furthermore, the genotypic variation in optimum temperature (19ºC -21ºC) for flowering is rather limited (Chapter 2.2), probably resulting from selection by breeders at these temperatures.

Growing tomato at sub-optimal temperature caused a severe delay in harvest. Earliness is an important characteristic in tomato, as tomatoes that are harvested early in the season receive a higher price. Some differences were found between cultivars in fruit growth period but these were small compared to the delaying effect of a lower temperature (Chapter 3.2). *L. pennellii* contained an interesting QTL on chromosome 9 or 10 that could potentially increase earliness in greenhouse tomato (Chapter 3.4). However, as this is valid under both optimal and sub-optimal temperature conditions, we expect that growers will not decrease temperature set-points in the greenhouse as this will still result in a delay in harvest. Therefore, it is not likely that this will reduce energy consumption.

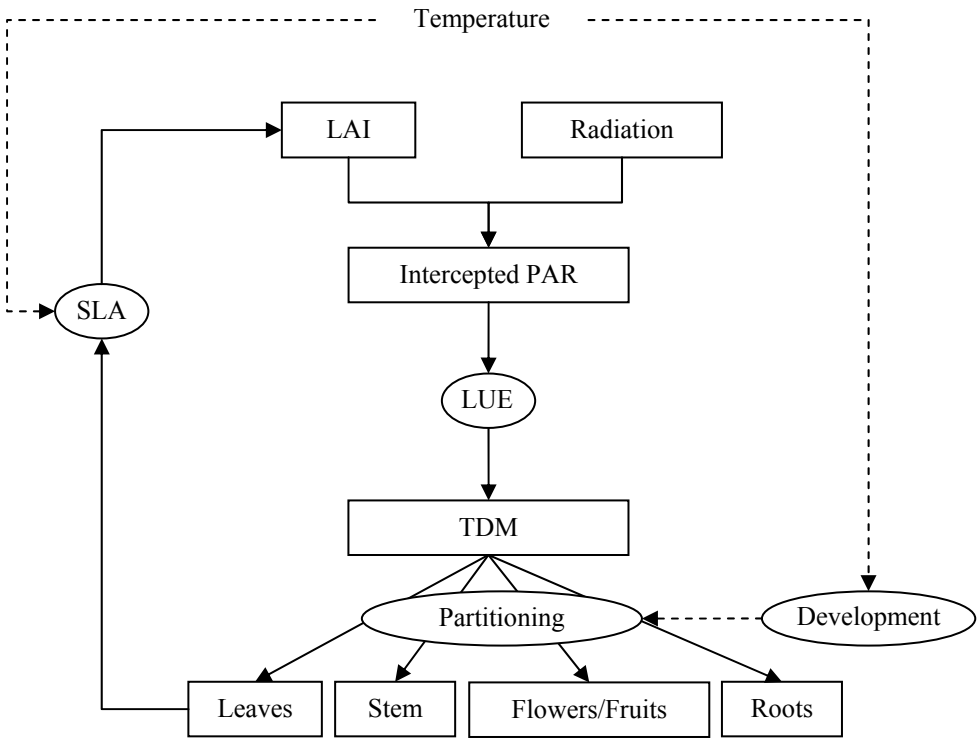

Figure 1: General overview of the processes influencing TDM and yield [in chrysanthemum the whole plant (excluding roots); in tomato fruits] and the main influences of temperature (range 16 – 20º) (dashed lines). Abbreviations: LAI = leaf area index; LUE = light use efficiency; PAR = photosynthetically active radiation; TDM = total dry mass; SLA = specific leaf area.

Increasing partitioning to harvestable organs

The delay in development at sub-optimal temperature initially resulted in a lower partitioning to flowers (chrysanthemum) and fruits (tomato). In chrysanthemum this effect is counteracted by a longer cultivation time and, therefore, after the whole cultivation period there is only a limited effect of temperature on partitioning (Chapter 2.2). In tomato, the slower truss initiation and fruit development rate at lower temperatures hampered partitioning to fruits early in the cultivation (Chapter 3.2). However, this enhanced vegetative growth and growth of the higher trusses. Thus, when taking the whole cultivation period into account no significant effect of temperature on partitioning towards the fruits was observed and, therefore, partitioning over the whole growth period is not a limiting factor for yield under sub-optimal temperature.

In chrysanthemum, the whole (aboveground) plant contributes to its economical value, but the external quality attributes such as flower characteristics are of utmost importance (Carvalho and Heuvelink, 2001). In Chapters 2.2 and 2.3 a wide range of flowers were studied. For example, ‘Delianne’ has relatively large flowers compared to ‘Reagan Improved’ but on the other hand the number of flowers per plant in ‘Delianne’ was less than in ‘Reagan

Table 1: Simulated fraction partitioned to the fruits, total dry mass (TDM), yield dry mass and average LAI, for a tomato crop planted on 10 January and ended on 26 November (Heuvelink *et al.*, 2007).

	Fraction to fruits	TDM (kg m^{-2})	Yield (kg m^{-2})	Average LAI (m^2 m^{-2})
Standard genotype	0.66	4.08	2.63	2.8
Adjusted genotype[1]	0.74	3.82	2.77	2.1
Adjusted genotype[1] + delayed leaf removal	0.74	4.01	2.91	2.6

[1] Two leaves between trusses instead of three; vegetative sink strength reduced by 1/3

Improved'. The large variation in flower types resulted in a large variation between cultivars in partitioning (ranging from 0.13 to 0.28 in Exp. 1 (25 cultivar) in Chapter 2.2).

Between tomato cultivars there was variation in partitioning, which might be used to create higher yielding cultivars (Chapter 3.2). Surprisingly, this variation was not related to year of release, as for several other crops higher yields in modern cultivars were found to be related to a higher harvest index. In most of these crops partitioning to the stems was reduced, resulting in/from semi-dwarf cultivars (Hay, 1995). However, it was observed in Chapter 3.2 that in greenhouse tomato assimilate partitioning to the stems is already rather small (10-15%) in the older cultivars. The indeterminate growth pattern of tomato makes it a necessity that at least a small part of the assimilates is continuously partitioned to the stem to guarantee year-round production. Therefore, only a limited gain can be expected from a lower partitioning to the stems (Heuvelink *et al.*, 2007). On the other hand, a higher yield gain might be expected from decreasing assimilate partitioning to leaves and hence favoring partitioning to the fruits. Potentially, this could be reached by creating cultivars with less leaves (and internodes) between trusses, i.e. cultivars with a lower vegetative sink strength (Xiao *et al.*, 2004). However, a lower number of leaves reduces LAI and may therefore reduce light interception and consequently total dry mass (Xiao *et al.*, 2004). This effect could be prevented when LAI is kept sufficiently high by appropriate crop management (e.g. delayed leaf removal). Recent model simulations have shown that in this way yield could be increased by 11% (Table 1).

Maximizing total dry mass (TDM)

Chapter 2.1 indicates that in chrysanthemum, literature reports on the effect of temperature on TDM or total fresh mass are conflicting and the underlying processes are not clear. In Chapters 2.2 and 2.3 it is shown that the effect of temperature on TDM strongly depends on the cultivar. Furthermore, the time of the year in which the experiment was conducted played an important role (Chapter 2.3). This study indicates that a large part of the increase in TDM at sub-optimal temperature can be explained by an increase in the length of the cultivation period alone (Chapter 2.3). Especially plants grown at a higher light level at the end of the cultivation period (i.e. planted after November) benefit from the longer cultivation period. Sub-optimal temperature during the LD clearly reduced growth, while during the last part of the SD temperature did not influence growth in a wide range of temperatures (Chapter 2.2 and 2.3).

Contrary to chrysanthemum, temperature did not affect TDM in any of the tomato cultivars used in this study, thus limiting the possibilities for breeding (Chapter 3.2). However, as mentioned above tomato breeding was responsible for a considerable increase in TDM, resulting in a higher yield (Chapter 3.2).

Maximizing light use efficiency (LUE)

Neither in chrysanthemum nor in tomato was LUE affected by temperature, within the range used in this study (i.e. temperature set-points between 16 and 20˚C; Chapter 2.3 and 3.2). This is probably due to the broad temperature optimum for crop photosynthesis and the compensating effect of lower maintenance respiration rates at sub-optimal temperatures. Therefore, it is concluded that LUE is not a limiting factor for production at sub-optimal temperature.

However, breeding could focus on increasing LUE in general. Between chrysanthemum cultivars differences in LUE were present (Chapter 2.3), indicating that it should be possible to select for higher LUE. So far for tomato, a higher LUE observed in the newer tomato cultivars resulted in increased TDM (Chapter 3.2). This increase in LUE was unexpected, as in most crops yield gains were generally associated with a higher harvest index (partitioning). Furthermore, selecting for net photosynthesis, photo- and dark-respiration, leaf chlorophyll content and enzymes involved in photosynthetic pathways have hardly resulted in yield gains (reviewed by Wallace *et al.*, 1993). This is due to the complexity of the processes that connect light absorption and capture, carbon dioxide fixation and carbohydrate accumulation and the many interactions that occur among them (Horton, 2000). For example, a high net assimilation rate (NAR) is commonly associated with thicker leaves (lower SLA). This negative correlation between NAR and SLA limits the possibilities for increasing RGR (Smeets and Garretsen, 1986a; Janssen *et al.*, 1995). Clearly, there is a need for more research to unravel the underlying physiology and morphology and the genetic basis for this increase in LUE in chrysanthemum and tomato cultivars.

Maximizing light interception

From this research it is concluded that for both chrysanthemum and tomato a decrease in SLA at sub-optimal temperature is a major limitation for growing these crops at sub-optimal temperature (Fig. 1). A lower SLA reduces LAI and consequently has a negative effect on light interception early in the cultivation (Chapters 2.2, 2.3, 3.1 and 3.3). Since chrysanthemum has a very low initial LAI the negative effect of sub-optimal temperature on SLA is more critical in chrysanthemum than in tomato. Although within the four cut chrysanthemum cultivars studied in detail in this thesis no variation in temperature response for SLA was present (Chapters 2.2 and 2.3), in the larger cultivar range analysed in Exp. 1 (25 cultivars; Chapter 2.2), a wider variation was found for temperature response in SLA. This variation could be used for selecting a cultivar in which SLA is less temperature sensitive.

Table 2: Total simulated crop dry weight at harvest for cut chrysanthemum grown in two seasons. Results for a standard set of parameters, or a 20 % higher SLA. Relative values given in brackets (From Heuvelink *et al*., 2007)

	Dry matter yield (g m^{-2})	
	winter	summer
Standard	315 (100%)	737 (100%)
SLA + 20%	351 (111%)	777 (105%)

Another option to increase energy efficiency is to breed for a cultivar that has a higher SLA both at optimal and sub-optimal temperature. Model simulations showed that increasing SLA is especially profitable to improve dry matter accumulation during the winter period (Table 2).

Individual tomato plants at transplanting are much larger than chrysanthemum plants, but they are grown at a much lower plant density. This explains why a decrease in SLA at sub-optimal temperatures also hampers (early) tomato production. Fortunately, it was found that, in contrast to the cultivated tomato species, SLA of the wild relatives was hardly affected by temperature (12 – 20ºC; Chapter 3.3), offering good prospects for breeding.

Product quality should not be ignored

Although product quality was not the main focus of this thesis, the manipulation of temperature and/ or plant density in order to increase energy efficiency has an impact on the external quality of chrysanthemum that should not be neglected. Chapter 2.3 shows that the positive linear relationship between total dry mass per plant (TDM_p) and flower number previously described by Carvalho and Heuvelink (2003) is cultivar specific and clearly temperature dependent (within the range 16 to 21ºC). Especially for 'Annecy' and 'Reagan Improved', the same TDM_p resulted in a lower number of flowers when cultivated at sub-optimal temperature. Attention should be paid when using these regression modules to predict number of flowers over a wider temperature range. Especially at supra-optimal temperatures, which was not the focus of this study, the model strongly over-estimated the number of flowers recorded at 24°C (Chapter 2.2). Therefore, more experiments are needed to adapt these modules to a wider temperature range. Possibly, a quadratic relationship with temperature, or a saturating type of response would fit better in the module.

Furthermore, Chapters 2.2 and 2.3 show that some cultivars produce significantly larger flowers at sub-optimal temperature. Therefore, when considering flower quality at sub-optimal temperature it depends on the market demand whether this is desirable. Chapter 2.2 also showed that sub-optimal temperature reduced stem length due to both a decrease in internode length and a decrease in the number of internodes. More compact plants are desirable as it can reduce the use of chemical growth retardants, which are costly and environmental unfriendly (Langton, 1998).

Tomato plants produced significantly larger fruits with a lower dry matter content at sub-

optimal temperature, possibly resulting in a less tasty fruit. As dry matter content in tomato fruits is generally inversely proportional to fruit size (Ho, 1996b), it could well be that the decreased dry matter content in fruits grown at sub-optimal temperature is related to this larger fruit size. To obtain the same fruit size at sub-optimal temperature as under the current temperature conditions, cultivars with smaller fruit size are required. However, this should not be a problem as a large genetic variation in tomato fruit size exists (Dorais *et al.*, 2001). Furthermore, *L. pennellii* contained interesting QTLs for both fruit size and dry matter content (Chapter 3.4). Adapting cultivation practice (e.g. by less fruit thinning) can be another option to obtain similar fruit size at sub-optimal temperature as under the current temperature conditions, as increasing the number of fruits on each truss decreases the individual fruit size (Cockshull and Ho, 1995).

Practical application

The temperature in the second half of the SD period (after flower bud initiation) could easily be reduced with only a minimal increase in reaction time and no reduction in growth (Chapter 2.2 and 2.3). The small delay might even be compensated by a higher plant density. At present in commercial greenhouses it is still not possible to adjust temperature setpoints to the stage of development as several phases of chrysanthemum are grown together in one greenhouse. A new growing concept is being developed in which chrysanthemums are grown in mobile gutters, making it possible to adjust plant density and environmental control to the stage of development (Van Os *et al.*, 2005). In this system, energy efficiency would increase already based on a more efficient use of light, but an even larger increase in energy efficiency could result from a significant reduction in temperature during the second half of the SD period.

Based on our experiments it is not possible to judge the economical feasibility of growing chrysanthemum, using current cultivation techniques, at a lower temperature set-point combined with a higher plant density. It is however important to keep in mind that on one hand growth is reduced at sub-optimal temperature in the early growth phase, but on the other hand as the reaction time becomes longer plants might stay longer at a higher plant density in the greenhouse and thus optimizing light interception. The problem of this strategy is that this extra time can also be used to plant a new crop in the same greenhouse. To study these effects on an annual basis, crop growth models, such as TOMSIM for tomato (Heuvelink, 1999) and CHRYSIM for chrysanthemum (Lee, 2002), can be an useful tool. However, these crop growth models must be adapted to accurately predict plant growth at sub-optimal temperatures.

In tomato the overall production was not affected by temperature but a strong delay in harvest was observed (Chapter 3.2). Since early produced tomatoes get higher prices it is not profitable to reduce greenhouse temperatures. However, this might change when energy

prices keep increasing.

Another important drawback of lowering greenhouse temperatures is the increased risk of fungal diseases like *Botrytis*, due to an increase in relative humidity. Growers often try to prevent this by ventilation and heating, but this approach counteracts the energy saving that results from a lower temperature set-point.

Breeding for higher energy efficiency: a future perspective

When developing lower temperature demanding cultivars, breeders must focus on: (i) the reduction in growth at sub-optimal temperature due to a decrease in SLA; (ii) the delay in development at sub-optimal temperatures (Fig. 1).

Energy efficiency can also be increased by creating higher yielding cultivars. In chrysanthemum the whole above ground plant is harvested and therefore shifts in biomass partitioning might not be profitable unless plant quality is improved, giving it a higher economical value. In tomato changing partitioning from leaves in favour of the fruits might be possible. For both crops it is important that LUE is further increased. Furthermore, a better light interception early in cultivation, for example by increasing SLA, can be beneficial for growth of both chrysanthemum and tomato although for tomato, for a large part of the cultivation time light interception can be kept at a near maximal level with modern cultivation practices.

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Summary

Greenhouse horticulture in The Netherlands is a highly sophisticated form of crop production. This results in high production levels and good product quality. However, it also requires high energy inputs. The energy used in greenhouse horticulture accounts for 7% of the total energy use in The Netherlands. It also represents 15 to 20% of the production costs in most crops. Moreover, this high energy use also results in environmental pollution due to the emission of CO_2. Therefore, it is important that energy efficiency (amount of product produced per unit energy input) is improved. Breeding for cultivars of greenhouse crops that are better adapted to temperatures below the current economical optimum temperature would contribute significantly to an improvement in energy efficiency and thus result in a decrease in CO_2 emission. On an annual basis a 1ºC lower set-point decreases energy consumption by about 8%. It is therefore worthwhile and urgent to investigate the possibilities for breeding cultivars adapted to lower cultivation temperatures.

The work presented in this thesis aims at quantifying and understanding the possibilities for improving energy efficiency of greenhouse crops by plant breeding. Chrysanthemum and tomato are used as model systems, since they are both important greenhouse crops and they require relatively large energy input. The main emphasis is on exploring possible differences in growth, development and yield within chrysanthemum and tomato cultivars in response to sub-optimal temperature. Attention is also given to improving yield at optimal temperatures as this would also improve energy efficiency. The focus in this thesis is on biomass production, but since chrysanthemum is a cut flower also a number of external quality attributes is dealt with. The ultimate goal of this study is to give breeders some guidelines concerning the most important traits on which they should concentrate their efforts when breeding for sub-optimal temperature tolerant cultivars. This study is split in two parts. The first section focuses on increasing energy efficiency in chrysanthemum (Chapter 2), while the second part deals with tomato (Chapter 3).

Chapter 2.1 presents a literature review on the effects of temperature, especially in the sub-optimal temperature range, on growth and development of chrysanthemum, giving special attention to the variation between cultivars. Furthermore, the gaps in knowledge in this field of research are identified.

In Chapter 2.2 the variation in temperature response (16 or 20ºC) for yield and yield related traits is quantified for 25 cut chrysanthemum cultivars in a greenhouse experiment. To study in depth this genetic variation in temperature response over a larger range of temperatures (15, 18, 21 or 24ºC), four contrasting cultivars (‘Annecy’, ‘Delianne’, ‘Reagan’, and ‘Supernova’) were selected in a second experiment, which was performed in climate rooms. Furthermore, a third experiment (climate room) was performed in which the cultivation period was split into three phases and the influence of temperature (16 or 20ºC) in each of these phases was studied for the four selected cultivars. It was observed that dry mass

production in all cultivars was very sensitive to temperature during the long day period (LD). Relative growth rate increased quadratically with temperature until an optimum around 24ºC, while leaf area ratio increased linearly with temperature. In contrast, the effect of temperature on absolute growth rate during the short day (SD), besides being cultivar dependent, was relatively small or even absent. The reaction time, on the other hand, was very temperature sensitive and the optimum temperature varied between cultivars (between 19 and 21ºC). Especially temperature during the bud initiation phase had a relatively strong influence on reaction time. As a result, the temperature response of the total dry mass production during the whole cultivation period was very cultivar dependent; 'Reagan' and 'Delianne' clearly showed an optimum response to temperature, while total dry mass in 'Supernova' and 'Annecy' was hardly affected by temperature. Besides the effect of temperature on yield an effect on external quality was also observed. Stem length increased with temperature, especially during the LD, due to increasing both the internode number and the average internode length. This temperature effect on stem length was stronger in 'Reagan' than in the other three cultivars. The response to temperature of both flower size and number was also highly cultivar specific.

In addition to breeding for cultivars with a shorter reaction time at sub-optimal temperature, an alternative approach would be to exploit cultivars that are heavier at sub-optimal temperature so that they could be grown at a higher plant density enhancing the production per m^2. Therefore, in Chapter 2.3, the combined effect of temperature (16 or 20ºC set-points) and plant density (32, 48 and 64 plants m^{-2}) on growth and development of four cut chrysanthemum cultivars ('Annecy', 'Delianne', 'Reagan' and 'Supernova') was investigated in three greenhouse experiments. At sub-optimal temperature, growth rate early in the cultivation period decreased for all cultivars as a consequence of a lower light interception resulting from a lower specific leaf area. A higher dry mass production at lower temperature could be explained by a longer cultivation time. From the growth point of view, sub-optimal temperature could be applied during the second phase of SD period as absolute growth rate during the last part of the cultivation is not affected by temperature. Since, flower development is relatively less temperature sensitive compared to flower initiation, lowering the temperature during the last phase of cultivation would only increase reaction time slightly. Furthermore, this small increase in reaction time can be compensated by a higher plant density. However, currently it is still not feasible to adjust the temperature to the stage of the cultivation because in the same greenhouse compartment different stages of development are cultivated simultaneously. Though, we expect that in the future this might be possible if mobile cultivation systems are implemented. Temperature also influenced external quality, but these effects were cultivar dependent. For instance, temperature affected the linear relationship between total dry mass and number of flowers, reducing the number of flowers at low temperature for the same plant weight. The extent to which temperature affects this relationship is cultivar dependent, offering good prospects for breeding. Lowering the

temperature can increased flower size for ‘Delianne’ and ‘Supernova’, while flower size of ‘Reagan’ was unaffected by temperature. These effects of temperature on flower characteristics ask for a need of knowing the market requirements.

Chapter 3.1 reviews the effects of temperature on growth, development and yield of tomato with special emphasis on cultivar differences. The focus is on sub-optimal temperature above the level where chilling injury occurs. In literature the link between yield and plant growth is often missing, limiting the possibilities of understanding temperature effects on the underlying processes that contribute to changes in yield.

Energy efficiency can be increased either by enhancing the production per m^2 or by reducing the energy input per m^2 (e.g. by lowering temperature set-points in the greenhouse). So far, in Dutch glasshouse tomatoes energy efficiency was almost exclusively improved by yield increases. To analyse the contribution of tomato breeding to this production increase, the yield and underlying components of seven cultivars (released between 1950 and 2002) were studied in Chapter 3.2. Furthermore, variation in temperature response between cultivars was investigated. In three experiments yield and biomass production of a total of 11 cultivars were evaluated at two temperature regimes (17/15˚C and 21/19˚C day/night temperature set-points). It was concluded that breeding has resulted in a remarkable increase in production. Under current conditions, yield of modern cultivars was on average 40% higher than yield of ‘Moneymaker’, released in 1950. This increase in production resulted mainly from a higher light use efficiency. Although the fraction of assimilates partitioned to the fruits showed small differences between cultivars, this trait was not related to year of release. Additionally, it was shown that more recently introduced cultivars produced larger fruits rather than more fruits.

Breeding for cultivars with equal production at lower temperatures is hampered by the limited variation for temperature response existing between tomato cultivars. Therefore, breeders have to look for other sources of variation. One option are wild relatives which originate from South America, where they grow at altitudes up to 3300m. In Chapter 3.3 we examined the effects of temperature (12, 16, 20 and 24ºC) on growth of young vegetative tomato plants of the cultivar Moneymaker and two wild relatives (*L. hirsutum* LA 1777 and *L. pennellii* LA 716). The aim was to elucidate the morphological and physiological parameters which underlie interspecific differences in growth response to sub-optimal temperatures. During a 28-day period five destructive measurements were carried out in which total dry weight (including root weight), leaf area and leaf dry weight were measured in order to calculate growth parameters. Even though ‘Moneymaker’ had the highest relative growth rate over a large temperature range (16ºC – 24ºC), relative growth rate of ‘Moneymaker’ was severely reduced below 20ºC, while relative growth rate of *L. hirsutum* and *L. pennellii* were only decreased below 16ºC. This decrease in relative growth rate observed in ‘Moneymaker’ was mainly a result of a decreased leaf area ratio, caused by a 35% decrease in specific leaf area. In contrast, the decrease in relative growth rate in *L. pennellii* and *L. hirsutum* was a result of a decreased net assimilation rate. This study illustrates that wild tomato species

provide possibilities for breeding more energy-efficient tomato greenhouse cultivars.

Small introgressions from *L. pennellii* might also help to increase energy efficiency in greenhouse tomato either by enhancing yield or by lowering temperature sensitivity. In Chapter 3.4, six previously developed back crossed inbred lines (BILs), with introgression of *L. pennellii* (covering about 31 % of their genome) in a 'Moneymaker' background, were used to study the effect of the introgressions on yield and underlying components. A six month greenhouse experiment, at optimal (set point 21/19ºC day/night) and sub-optimal (set point 17/15ºC day/night) temperature was carried out. None of the BILs showed an increased total yield or a different temperature sensitivity compared to 'Moneymaker' and in four BILs a significantly reduced yield was found. In one of the BILs this was caused by a reduction in total dry weight, due to a lower leaf area index, resulting in less light interception. Furthermore, partitioning towards the fruits was reduced in four BILs. In one BIL, with introgressions on chromosome 9 and 10, harvesting started earlier than in 'Moneymaker' and the yield of this BIL was higher during the first part of the experiment. However, this difference in yield gradually decreased and at the end of the experiment it had disappeared. The same BIL also showed a higher fruit dry matter content. This QTL coincides with a QTL recorded in previous studies for Brix, a measure for total soluble sugar content, on chromosome 9. In this study no QTLs were found that could help increase energy efficiency in tomato. However, as the BILs in this study only cover about 30% of the *L. pennellii* genome, it is possible that *L. pennellii* contains QTLs that can increase energy efficiency of greenhouse tomato cultivars. Therefore it is important that the development of BILs continues.

The main achievements and limitations of this study are discussed in Chapter 4. In general, in the present study it was found that there is more variation for temperature response in cut chrysanthemum than in tomato. It is concluded that when trying to breed for lower temperature demanding cultivars it is important to take into account the reduction in growth at sub-optimal temperature due to a decrease in SLA. Furthermore, attention should be paid to the delay in development at sub-optimal temperatures. Energy efficiency can also be increased by creating higher yielding cultivars. In chrysanthemum the whole above ground plant is harvested and therefore shifts in biomass partitioning might not be profitable unless plant quality is improved, giving it a higher economical value. In tomato changing partitioning from leaves in favour of the fruits might be possible. For both crops it is important that LUE is further increased. Furthermore, a better light interception early in the cultivation, for example by increasing SLA, can be beneficial for growth of both chrysanthemum and tomato although for tomato, for a large part of the cultivation period light interception can be kept at a near maximal level with modern cultivation practices.

Samenvatting

De Nederlandse glastuinbouw is een van de meest geavanceerde vormen van plantenteelt. Dit resulteert in hoge opbrengsten en producten van goede kwaliteit. Het betekent echter ook dat een hoge energie-input nodig is. De glastuinbouwsector is goed voor 7% van het totale energieverbruik in Nederland. Voor de meeste gewassen beslaan de energiekosten 15 tot 20% van de totale productiekosten. Daarnaast resulteert het hoge energieverbruik in milieuproblemen door de uitstoot van CO_2. Het is daarom belangrijk dat de energie-efficiëntie (de hoeveelheid geproduceerd product per eenheid energie-input) verbeterd. Door middel van veredeling zouden voor verschillende kasgewassen nieuwe cultivars kunnen worden ontwikkeld die beter zijn aangepast aan temperaturen onder de huidige economische optimale temperatuur. Dit zou leiden tot een verbetering van de energie-efficiëntie en resulteert daarnaast in een reductie van CO_2-uitstoot. Op jaarbasis resulteert een verlaging van de teelttemperatuur met 1°C in een energiebesparing van ongeveer 8%. Daarom is het belangrijk om de mogelijkheden te onderzoeken voor de veredeling van cultivars die beter aangepast zijn aan een lagere teelttemperatuur.

Het werk dat in dit proefschrift wordt beschreven, heeft als doel de mogelijkheden te onderzoeken om door middel van plantenveredeling de energie-efficiëntie van kasgewassen te verbeteren. Hiervoor zijn tomaat en chrysant als modelgewassen gebruikt, omdat beide belangrijke kasgewassen zijn, die een relatief hoge energie-input nodig hebben. De nadruk ligt vooral op het onderzoeken van mogelijke verschillen in groei, ontwikkeling en opbrengst tussen tomaten- of chrysantencultivars in reactie op suboptimale temperaturen. De nadruk ligt in dit proefschrift vooral op de drogestofproductie, maar aangezien chrysant een snijbloem is, is ook een aantal externe kwaliteitsaspecten meegenomen. Het uiteindelijke doel van dit proefschrift is om de veredelaars een overzicht te geven van de gewaseigenschappen waarop zij zich moeten richten als ze willen veredelen voor cultivars die een suboptimale temperatuur tolereren. Dit proefschrift is verdeeld in twee delen. In het eerste deel kijken we naar de energie-efficiëntie in chrysant (hoofdstuk 2), terwijl het tweede deel gericht is op tomaat (hoofdstuk 3).

In hoofdstuk 2.1 wordt een literatuuronderzoek beschreven naar de effecten van temperatuur, vooral in de suboptimale zone, op groei en ontwikkeling in chrysant, waarbij de nadruk ligt op verschillen tussen cultivars. Verder worden hiaten in kennis in dit onderzoeksveld blootgelegd.

In hoofdstuk 2.2 wordt de variatie in opbrengst en opbrengstgerelateerde eigenschappen in temperatuurgevoeligheid (16 en 20°C) van 25 chrysantencultivars onderzocht in een kasexperiment. Om de genetische variatie voor temperatuurrespons nader te onderzoeken over een breder temperatuurtraject (15, 18, 21 en 24°C) werden vier cultivars (‘Annecy’, ‘Delianne’, ‘Reagan’ en ‘Supernova’) geselecteerd voor een tweede experiment, uitgevoerd in klimaatkamers. Daarnaast is een derde experiment uitgevoerd waarbij de teeltperiode was

onderverdeeld in drie fases en de invloed van temperatuur (16 of 20°C) op ieder van die fases werd onderzocht voor de vier cultivars. De drogestofproductie tijdens de lange dag-periode (LD) was heel gevoelig voor temperatuur in alle cultivars. De relatieve groeisnelheid nam quadratisch toe met temperatuur. In tegenstelling daarmee was het effect van temperatuur op de absolute groeisnelheid tijdens de korte dag klein of helemaal afwezig, afhankelijk van de cultivar. Aan de andere kant was de reactietijd heel temperatuurgevoelig en de optimale temperatuur wisselde tussen cultivars (tussen de 19 en 21°C). Vooral tijdens de ontwikkeling van de bloemknop had de temperatuur een relatief grote invloed op de reactietijd. Alles bij elkaar genomen is het effect van temperatuur op de totale drogestofproductie over de gehele periode erg cultivarafhankelijk; 'Reagan' en 'Delianne' laten een duidelijk optimum voor temperatuur zien, terwijl in 'Supernova' en 'Annecy' bijna geen effect van temperatuur te zien was. Naast het temperatuureffect op de opbrengst was er ook een duidelijk effect op de externe productkwaliteit te zien. De lengte van de stengel nam toe met stijgende temperaturen, vooral tijdens de LD, door een hoger aantal internodia en een grotere gemiddelde internodiumlengte. Dit temperatuureffect op de stengellengte was sterker in 'Reagan' in vergelijking met de andere drie cultivars. Daarnaast was ook het effect van temperatuur op zowel bloemgrootte als op het aantal bloemen erg cultivarafhankelijk.

Naast het veredelen van cultivars met een kortere reactietijd bij een suboptimale temperatuur, is een alternatieve aanpak mogelijk door cultivars te gebruiken die zwaarder worden als ze geteeld worden bij suboptimale temperatuur zodat de plantdichtheid verhoogd kan worden om op die manier de productie per vierkante meter te verbeteren. Daarom kijken we in hoofdstuk 2.3 in drie kasexperimenten naar het gecombineerde effect van temperatuur (16 of 20ºC setpoint) en plantdichtheid (32, 48 en 64 planten per m^2) op de groei en ontwikkeling van vier chrysantencultivars ('Annecy', 'Delianne', 'Reagan' en 'Supernova'). Bij suboptimale temperatuur was de groeisnelheid van alle cultivars lager als gevolg van een lagere lichtonderschepping, die het resultaat was van een lager specifiek bladoppervlakte (specific leaf area; SLA). Een hogere drogestofproductie bij de lagere temperatuur was daarom alleen het gevolg van een langere teeltperiode. Vanuit het groei-oogpunt, kan tijdens het tweede gedeelte van de korte dag een suboptimale temperatuur worden toegepast, omdat tijdens deze laatste fase van de teelt de absolute groeisnelheid niet beïnvloed wordt door temperatuur. Daarnaast zou het lager instellen van de temperatuur tijdens deze fase maar een klein effect hebben op de reactietijd, omdat bloemontwikkeling minder temperatuurgevoelig is in vergelijking met bloemknopinitiatie. Daarnaast kan een kleine verlenging van de reactietijd eventueel gecompenseerd worden door een hogere plantdichtheid. Met de huidige teeltwijze is het echter niet mogelijk om de temperatuur aan te passen in de verschillende teeltfases, omdat in hetzelfde kascompartiment tegelijkertijd verschillende teeltfases staan. We verwachten echter dat dit in de toekomst wel mogelijk is als er meer mobiele teeltsystemen gebruikt zullen worden. Temperatuur heeft ook een invloed op de externe productkwaliteit, maar deze is cultivarafhankelijk. Temperatuur beïnvloedt bijvoorbeeld de

lineaire relatie tussen totale biomassa en het aantal bloemen, waarbij het aantal bloemen lager ligt bij een lagere temperatuur bij hetzelfde plantgewicht. De mate waarin temperatuur invloed heeft op deze relatie is temperatuurafhankelijk, wat goede uitgangspunten voor de veredeling verschaft. Een lagere temperatuur zorgt bij 'Delianne' en 'Supernova' voor grotere bloemen, terwijl bij 'Reagan' temperatuur geen invloed heeft op de bloemgrootte.

Hoofdstuk 3.1 beschrijft een literatuuronderzoek naar de effecten van temperatuur op groei, ontwikkeling en opbrengst van tomaat met een speciale nadruk op de verschillen tussen cultivars. De focus ligt vooral op suboptimale temperatuur boven het niveau waarbij de planten koudeschade (chilling injury) oplopen. In de literatuur ontbreekt vaak het verband tussen opbrengst en groei, waardoor het moeilijk is om de effecten van temperatuur op de onderliggende processen te begrijpen.

De energie-efficiëntie kan verhoogd worden door of de productie per m^2 te verbeteren of door de energie-input per m^2 te verlagen (bijvoorbeeld door de setpoint-temperaturen in de kas te verlagen). Tot nu toe is de energie-efficiëntie van Nederlandse kastomaten vooral verbeterd door een verhoging van de productie. In hoofdstuk 3.2 wordt onderzocht hoe de veredeling heeft bijgedragen aan deze productieverhoging. Daarvoor worden de opbrengst en de onderliggende componenten van zeven cultivars (op de markt gebracht tussen 1950 en 2002) bestudeerd. Daarnaast is de variatie in reactie op temperatuur tussen cultivars onderzocht. In drie experimenten is de opbrengst en biomassaproductie van in totaal 11 cultivars onderzocht bij twee temperaturen (17/15ºC en 21/19ºdag-/nachttemperatuursetpoint). Uit de resultaten kan opgemaakt worden dat de veredeling verantwoordelijk is voor een grote productiestijging. Onder de huidige teeltwijze is de opbrengst van de moderne cultivars gemiddeld genomen 40% hoger dan van 'Moneymaker', een ras dat in 1950 op de markt gebracht werd. Deze productiestijging was vooral het gevolg van de hogere efficiëntie waarmee licht wordt gebruikt (light use efficiency; LUE). Alhoewel er kleine verschillen in de verdeling van assimilaten tussen de cultivars waren, was deze eigenschap niet gerelateerd aan het jaar waarop de cultivars op de markt gebracht werden. Daarnaast produceren de nieuwere cultivars grotere vruchten in plaats van meer vruchten.

Het veredelen van cultivars met een zelfde opbrengst bij een lagere temperatuur wordt bemoeilijkt door de beperkte genetische variatie in reactie op temperatuur tussen bestaande tomatencultivars. Daarom moeten de veredelaars zoeken naar andere variatiebronnen. Een mogelijkheid is om gebruik te maken van wilde verwante soorten afkomstig uit Zuid-Amerika, waar zij tot een hoogte van 3300 m groeien. In hoofdstuk 3.3 kijken we naar de effecten van temperatuur (12, 16, 20 en 24ºC) op de groei van jonge vegetatieve planten van 'Moneymaker' en twee verwante soorten (*L. hirsutum* LA 1777 en *L. pennellii* LA 716). Het doel was om de morfologische en fysiologische parameters te ontrafelen die ten grondslag liggen aan de verschillen in groei tussen de soorten bij suboptimale temperatuur. Gedurende 28 dagen werden 5 metingen verricht, waarbij de totale drogestofproductie (inclusief wortels), bladoppervlakte en drooggewicht van het blad werden bepaald. Ondanks dat 'Moneymaker'

over een groot temperatuurtraject (16 - 24ºC) de hoogste relatieve groeisnelheid had, was de relatieve groeisnelheid van 'Moneymaker' erg verlaagd onder de 20ºC, terwijl de relatieve groeisnelheid van *L. hirsutum* en *L. pennellii* pas lager werden onder de 16ºC. Deze afname in relatieve groeisnelheid in 'Moneymaker' was vooral het gevolg van een lagere bladoppervlakteratio (leaf area ratio; LAR), terwijl bij *L. hirsutum* en *L. pennellii* de netto assimilatiesnelheid (net assimilation rate; NAR) omlaag ging. Deze studie laat zien dat wilde verwante soorten van tomaat goede mogelijkheden bieden voor het veredelen van meer energie-efficiënte cultivars van kastomaten.

Het invoegen van kleine stukjes van het genoom van *L. pennellii* in het genoom van kastomaat kan helpen om de energie-efficiëntie van kastomaten te verhogen door of de opbrengst te verhogen of de temperatuurgevoeligheid te verlagen. In hoofdstuk 3.4 worden zes lijnen die een stukje *L. pennellii*-genoom bevatten (in totaal ongeveer 31 % van het totale *L. pennellii*-genoom) in een achtergrond van 'Moneymaker' (back crossed inbred lines; BIL's) gebruikt om het effect van de invoegingen op opbrengst en de onderliggende componenten te onderzoeken. Gedurende een kasproef van zes maanden wordt het effect van optimale (setpoint 21/19ºC dag/nacht) en suboptimale (setpoint 17/15ºC dag/nacht) temperatuur bestudeerd. Geen van de BIL's had een hogere totale opbrengst of was minder temperatuurgevoelig dan 'Moneymaker', terwijl in vier van de BIL's de opbrengst lager lag dan in 'Moneymaker'. In een van de BIL's was dit het resultaat van een lager totaal drooggewicht, veroorzaakt door een lagere bladoppervlakte-index (leaf area index; LAI) waardoor minder licht onderschept kon worden. Daarnaast was de verdeling van assimilaten naar de vruchten lager in vier BIL's. In een van de BIL's, met twee *L. pennellii*-invoegingen op chromosoom 9 en 10, kon het oogsten eerder beginnen dan in 'Moneymaker' en gedurende de eerste weken van de proef was de opbrengst van deze BIL hoger dan die van 'Moneymaker'. Het verschil in opbrengst met 'Moneymaker' werd echter geleidelijk minder en was op het eind van de proef niet meer aanwezig. Dezelfde BIL had ook een hoger drogestofgehalte in de vrucht. Deze QTL (quantitative trait loci) komt overeen met een voorheen beschreven QTL voor Brix, een maat voor de totale hoeveelheid oplosbare suiker, op chromosoom 9. In deze proef werden geen QTL's gevonden die kunnen helpen de energie-efficiëntie te verhogen, maar omdat de BIL's die gebruikt werden voor deze proef maar ongeveer 30% van het totale *L. pennelli*-genoom bevatten, is het mogelijk dat *L. pennellii* QTL's bevat die de energie-efficiëntie van kastomaat zouden kunnen verhogen. Daarom is het van belang dat de ontwikkeling van de BIL's wordt voortgezet.

De belangrijkste uitkomsten en beperkingen van dit onderzoek worden besproken in hoofdstuk 4. Over het algemeen was er in dit onderzoek meer variatie voor temperatuurgevoeligheid bij chrysant dan bij tomaat. Bij het veredelen van gewassen die beter bij een lage temperatuur kunnen groeien, is het belangrijk om te letten op de verminderde groei bij suboptimale temperatuur als het gevolg van een lagere SLA. Daarnaast moet aandacht worden besteed aan de tragere ontwikkeling bij suboptimale temperatuur. Bovendien

kan de energie-efficiëntie ook worden verbeterd door cultivars met een hogere opbrengst te creëren. In chrysant worden alle bovengrondse plantendelen geoogst en daarom is het misschien niet voordelig om de verdeling van assimilaten te veranderen, tenzij de kwaliteit verbetert, wat leidt tot een hogere economische waarde. Bij tomaat kan het mogelijk voordelig zijn om de verdeling van de assimilaten naar vruchten te verbeteren ten koste van de verdeling van de assimilaten naar het blad. Voor beide gewassen is het belangrijk dat de LUE verder verhoogd wordt. Daarnaast kan een verbeterde lichtonderschepping in het begin van de teeltfase, bijvoorbeeld door het verhogen van de SLA, bevorderlijk zijn voor de groei van zowel chrysant als tomaat, alhoewel voor tomaat de lichtonderschepping met moderne teeltmethodes voor een groot gedeelte van de teelt bijna maximaal gehouden kan worden.

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Anke

Curriculum Vitae

Anke van der Ploeg werd op 23 november 1976 geboren in Rotterdam. In 1993 ging ze voor een uitwisselingsjaar naar de Little Rock, Arkansas (USA). Na deze onderbreking vervolgde ze haar VWO-opleiding aan de Regionale Scholengemeenschap Hoekse Waard, waar ze in 1996 haar diploma behaalde. In datzelfde jaar begon zij haar studie Biologie aan de toenmalige Landbouwuniversiteit Wageningen (Wageningen Universiteit), waarbij zij zich specialiseerde in plantenbiologie. Voor haar afstudeervakken heeft zij onderzoek gedaan naar het functioneren van het Cf9 resistentiegen in tomaat bij de vakgroep fytopathologie en de groei en enzymactiviteit bij Arabidopsis bij de vakgroep plantenfysiologie. Tijdens haar stage bij het Scottish Crop Research Institute (Dundee, UK) heeft ze zich bezig gehouden met het bestuderen van de kiemrustbreking in aardappel. Na haar afstuderen in 2001, is zij begin 2002 begonnen als assistent in opleiding bij de vakgroup tuinbouwproductieketens. Dit onderzoek, wat deel uitmaakt van het project Rassen onder Glas met minder Gas, resulteerde in dit proefschrift.

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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 (5.6credits)

- The influence of temperature on growth and development of chrysanthemum cultivars
- Influence of sub-optimal temperature on tomato growth and yield

Post-Graduate Courses (5.4 credits)

- Plant spectroflourmetry: applications and basic research; PE&RC (2002)
- The art of modelling; PE&RC (2004)
- Plant ecophysiology; PE&RC (2005)

Deficiency, Refresh, Brush-up and General courses (2.8 credits)

- General principles of greenhouse horticulture (2002)

Competence Strengthening / Skills courses (4.2 credits)

- Techniques for writing and presenting scientific papers; Sense (2002)
- Career orientation; PE&RC (2004)
- Presentation skills; CENTA (2004)

Name of the discussion group, local seminars and other scientific meetings (7 credits)

- Agrotechnology and- physics, the agro-production chain and protected cultivation (2002-2003)
- Regular discussion with “Rassen ander glas met minder gas” PhD students (2003-2005)
- Frontier literature of plant physiology (FLOP) (2002-2006)

PE&RC Annual Meetings, Seminars and Introduction Days (1.2 credits)

- Ethics in Science (2002)
- Global climate change and biodiversity (2003)
- Biological disasters (2004)
- The truth of science (2005)

International Symposia, Workshops and Conferences (7.2 credits)

- International workshop on models for plant growth and control of product quality in horticultural production; Potsdam, Germany (2003)
- International conference on sustainable greenhouse systems; Leuven, Belgium (2004)
- International horticultural congres; Seoul, Korea (2006)
- Hortimodel; Wageningen (2006)

Laboratory Training and Working Visits (4.3 credits)

- Greenhouse horticulture in China: situations & prospects; China (2003)

Courses in which the PhD candidate has worked as a teacher

- Practicum bio-organische chemie; ORC, 10 days

Supervision of MSc student(s)

- The effect of temperature on growth and development in chrysanthemum or tomato, 60 days, 6 students